ABSTRACT

FARRER, DIANNE CARTER. Optimizing Nitrogen Management for Soft Red Winter Wheat Yield, Grain Protein, and Grain Quality Using Precision Agriculture and Remote Sensing Techniques. (Under the directions of Randy Weisz).

The purpose of this research was to improve the management of soft red winter wheat (*Triticum aestivum* L.) in North Carolina. There were three issues addressed; the quality of the grain as affected by delayed harvest, explaining grain protein variability through nitrogen (N) management, and developing N recommendations at growth stage (GS) 30 using aerial color infrared (CIR) photography.

The impact of delayed harvest on grain yield, test weight, grain protein, and 20 milling and baking quality parameters was studied in three trials in 2002 and three trials in 2003. Yield was significantly reduced in three out of five trials due to dry, warm environments, possibly indicating shattering. Test weights were significantly reduced in five out of six trials and were positively correlated to the number of precipitation events and to the number of days between harvests, indicating the negative effects of wetting and drying cycles. Grain protein was not affected by delayed harvest. Of the 20 quality parameters investigated, flour falling number, clear flour, and farinograph breakdown times were significantly reduced due to delayed harvest, while grain deoxynivalenol (DON) levels increased with a delayed harvest.

Grain protein content in soft red winter wheat is highly variable across years and environments. A second study examined the effects of different nitrogen (N) fertilizer rates and times of application on grain protein variability. Seven different environments were utilized in this study. Though environment contributed about 23% of grain protein variability, the majority of that variability (52%) was attributed to N management. It was found that as

grain protein levels increased at higher N rates, so did overall protein variability as indicated by the three stability indexes employed. In addition, applying the majority of total N at growth stage (GS) 30 decreased grain protein stability. The concluding recommendations to reduce grain protein variability in the southeastern USA are: to reduce the range in N fertilizer rates used across the region, to avoid over application of N beyond what is required to optimize yield and economic return, and to apply spring N at GS 25.

Site-specific N management systems using remote sensing techniques can potentially improve nitrogen use efficiency (NUE) in winter wheat. The objectives of the last study were to determine if in-season agronomic optimum N rate recommendations in soft red winter wheat at growth state (GS) 30 could be developed using spectral bands and indexes obtained from aerial color infrared (CIR) photography and to determine if and how biomass at GS 30 affected these relationships. Experiments were conducted in six site-years. Relationships between optimum N rates and three spectral bands and 39 indexes were weak, with only approximately half the variability explained by the models. After separating the data into two GS 30 biomass classes (low, < 1000 kg ha^{-1} and high, > 1000 kg ha^{-1}), relationships between optimum N rates and spectral bands and indexes improved substantially. Spectral indicators consisting of the Red and the Green bands minus the Red or Green bands of a high N-status reference plot (relative Red and relative Green, respectively) had the best quadratic relationships with optimum N rates (R^2 = 0.80 and 0.81, respectively) for the high biomass class. These results indicate that agronomic optimum N rates at GS 30 can be estimated using aerial CIR photographs if areas of low and high biomass can be determined.

OPTIMIZING NITROGEN MANAGEMENT FOR SOFT RED WINTER WHEAT YIELD, GRAIN PROTEIN, AND GRAIN QUALITY USING PRECISION AGRICULTURE AND REMOTE SENSING TECHNIQUES

by
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BIOGRAPHY

Dianne Carter Farrer was born in Provo, Utah and raised in Pleasant Grove, Utah by Robert Vaughn and Louise Nelson Carter. She has three sisters, Alaine Richards, Kristin Shaw, and Janise Carter, and two brothers, James Robert Carter and David Vaughn Carter. She received her Bachelors of Science with an emphasis in Horticulture Science and Masters of Science with an emphasis in Soil Fertility from Brigham Young University in 1998 and 2001, respectively. While completing her education at Brigham Young University, she was employed at Utah Valley State College as the Botany Laboratory Manager from 1997 to 2000. As she was completing her doctoral degree at North Carolina State University, she married Steven Hal Farrer, the son of Hal Duane and Edna Gamero Farrer.

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CHAPTER ONE: Delayed Harvest Effect on Yield and Quality of Southeastern Winter Wheat

Delayed Harvest Effect on Yield and Quality of Southeastern Winter Wheat

ABSTRACT

Harvest of soft red winter wheat (*Triticum aestivum* L.) in the southeastern USA can be delayed because of inclement environmental conditions, machinery failure or other unforeseen problems. Our objectives were to determine the impact of delayed harvest on grain yield, test weight, grain protein, and 20 milling and baking quality parameters, and to determine if these impacts were correlated with environmental conditions occurring between grain ripeness and harvest. Three trials were conducted in 2002 and three trials in 2003.Treatments consisted of a timely harvest at grain ripeness and a delayed harvest, 8 to 19 d after grain ripeness. Delayed harvest reduced yield in three out of five trials and test weight in five out of six trials. In multiple regression analysis, grain yield decreases due to delayed harvest were negatively related to total precipitation and positively related to minimum daily temperatures ($R^2 = 0.99$), indicating that dry, warm environments increased yield differences, possibly due to shattering of the grain head. Reductions in test weight were linearly related to the number of precipitation events ($r^2 = 0.93$) and to the number of days between harvests ($r^2 = 0.84$), probably reflecting the negative effects of wetting and drying cycles. Grain protein was not affected by delayed harvest. Delayed harvest reduced flour falling number across all trials and grain falling number in one trial where total precipitation was high. Delayed harvest reduced clear flour in all trials and farinograph breakdown times in two trials. Delayed harvest increased grain deoxynivalenol (DON) levels in all but one trial. Overall, delaying harvest of winter wheat in the southeast reduced yield, test weight, and negatively impacted milling and baking quality factors related to carbohydrate levels in the grain.

INTRODUCTION

Harvest of soft red winter wheat in the southeastern USA can be delayed because of inclement environmental conditions, machinery failure, or other unforeseen problems. Delaying harvest after grain ripeness (135 g kg^{-1} grain moisture content after grain physiological maturity) can negatively impact grain yield, test weight, grain protein, and milling and baking quality of soft red winter wheat in other parts of the USA and other countries (Bracken and Baily, 1928; Christensen and Legge, 1984; Czarnecki and Evens, 1986; Edwards et al., 1989; Pool et al., 1958). High wind and low humidity increase the probability of yield reduction from shattering in the grain head (Clarke and De Pauw, 1983; Schlehuber and Tucker, 1967). Schlehuber and Tucker (1967) reported a 2.5% yield loss caused by lodging and/or shattering when harvest was delayed by 21 d. Clarke (1981) reported greater yield reductions with irrigated winter wheat compared to rain fed winter wheat grown in Canada, due to larger seed size in the irrigated plots than rain fed plots resulting in an increase the chance for yield reductions due to shattering.

Test weight, determined by measuring the weight of grain in a given volume, is a crude measure of grain quality with a high test weight associated with well formed kernels that developed without biotic or abiotic stresses and low test weight associated with poorly formed, shriveled or weathered kernels (Gooding and Davis, 1997; Whitcomb and Johnson, 1928). A test weight of 747 kg m^{-3} or above is considered representative of good soft wheat grain quality (USDA/ARS Soft Wheat Quality Lab., 2004) and producers can be penalized financially when test weights fall below this critical value. High compared to low test weight grain of the same weight will result in a larger volume of grain due to higher grain

carbohydrate in the well formed kernels of high test weight grain, which is of greater worth to the milling and baking industry (Gooding and Davis, 1997).

Two components of test weight are the packing efficiency and density of the grain (Yamazaki and Briggle, 1969). Milner and Shellenberger (1953) and Swanson (1941) reported that an increase in the separation and roughening of the bran coat of mature wheat grain resulted in decreased test weight or change in packing efficiency following repeated wetting and drying of the grain. Czarnecki and Evans (1986) and Pushman (1975) also found that wetting and drying cycles caused a decrease in test weight, but increasing the moisture content had a more negative effect on test weight than did drying. Lloyd et al. (1999) found that when soft red winter wheat harvest was delayed, grain test weight decreased by 1.4 to 5.4% depending on cultivar and in proportion to the time between harvests. Delaying harvest by 7 to 49 d and exposing the grain to wetting and drying cycles decreased test weights by 41 to 50 kg m^{-3} in Canadian spring wheat (Gan et al., 2000).

A change in grain protein and/or carbohydrates associated with delayed harvest could have important milling and baking consequences because many end-use quality factors are directly linked to these components (Orth and Shellenberger, 1988). Increased temperatures and reduced precipitation after physiological maturity have the potential to increase grain protein content relative to a reduction in grain carbohydrate (Christensen and Legge, 1984; Gooding and Davies, 1997), though it was found that grain protein composition was not adversely affected by a delayed harvest or by wetting and drying cycles (Christensen and Legge, 1984; Pool et al., 1958; Whitcomb and Johnson, 1929). It was found that wetting and drying cycles occurring after grain ripeness decreased the vitreous condition of the flour, increased the development time of the rheology of the dough, and reduced grain falling

number indicating changes in carbohydrate proportions of the grain (Christensen and Legge, 1984; Hagemann and Ciha, 1987; Swanson, 1941). Falling number is a measure of α-amylase enzyme activity. A lower falling number indicates higher α-amylase enzyme activity and a change in grain carbohydrates (Gooding and Davies, 1997) and a potential for sprouting. It is theorized that high humidity after grain ripeness can elevate the level of deoxynivalenol (DON) toxin produced by *Fusarium graminearum* (Schwabe) in grain and milled products (Murray et al., 1998).

The impacts of wetting and drying cycles and other environmental effects associated with delayed harvest on many grain milling and baking quality variables have not been investigated in the southeastern USA. The primary objective of this study was to determine the impact of delayed harvest on grain yield, test weight, grain protein, and 20 milling and baking quality variables of soft red winter wheat in the southeastern USA. A second objective was to determine if changes to the grain and grain quality caused by delayed harvest could be correlated with environmental conditions occurring after grain ripeness.

MATERIALS AND METHODS

A total of six trials were conducted with three trials in 2002 and three trials in 2003. Trial locations included Circle Grove Seed Farm near Belhaven, NC in 2002 (B-1, see Table 1), the Cunningham Research Station near Kinston, NC in 2002 and 2003 (C-1 and C-2, respectively), the Piedmont Research Station near Salisbury, NC in 2003 (P-2), and the Tidewater Research Station near Plymouth, NC in 2002 and 2003 (T-1 and T-2, respectively). Soils at the trial locations included a Ponzer muck (loamy, mixed, dysic, thermic Terric Medisaprists) at B-1, a Hiwassee clay loam (fine, kaolinitic, thermic Typic Rhodudults) at P-2, a Lynchburg sandy loam (fine, loamy, siliceous, thermic, Aeric

Paleaquults) at C-1, a Goldsboro loamy sand (fine, loamy, siliceous, thermic Aquic Paludults) at C-2, and a Cape Fear loam (clayey, mixed, thermic, Typic Umbraquults) at T-1 and T-2 (Table 1).

One factor was employed in this study; a treatment that consisted of different harvest dates, a timely and delayed harvest. Timely harvest occurred after the grain reached physiological maturity and first reached 135 g kg^{-1} moisture and delayed harvests ranged from 8 to 19 d later (Table 1 and Fig. 1). This factor was arranged in a randomized complete block design with five (B-1, C-1 and T-1), six (T-2), 12 (C-2), and 13 (P-2) replications.

Two soft red winter wheat cultivars, 'Pioneer 26R61' (P 26R61) and 'Coker 9704' (C 9704) were sown at optimal planting dates and seeding rates (Weisz, 2004). Cultivar C 9704 is rated as a medium maturing wheat with moderate resistant to powdery mildew (*Blumeria graminis* DC Speer) and moderate susceptibility to leaf rust (*Puccinia triticina* Eriks.), while P 26R61 is a medium-early maturing wheat that is moderately resistant to both powdery mildew and leaf rust (Bowman, 2004 and 2001; Table 1). There is no current evidence for cultivar differences in glume blotch (*Stagonospora nodorum* (Berk.)) or DON resistance. Cultivar C 9704 was grown in C-1 and T-2 and P 26R61 was grown in all other trials. Conventional tillage practices common to the southeastern region were used in each trial except P-2 where a no-tillage system was used.

Standard lime, K, and P fertilizer rates for North Carolina were applied to all trials, based on soil tests (Hardy et al., 2002). All trials received 34 kg ha^{-1} of N pre-plant as N-P-K: 10-13.2-24.9% ($N\text{-}P_2O_5\text{-}K_2O$: 10-30-30%), N source unknown. At growth stage (GS) 25 (Zadoks et al. 1974) 112 kg ha^{-1} of N fertilizer was applied in the form of urea ammonium

nitrate (UAN: 30% N) to all trial except P-2, where 112 kg ha^{-1} ammonium nitrate (NH_4NO_3: 34% N) form was applied.

Air temperature, total precipitation, number of precipitation events (Fig. 1), relative humidity, and wind speed data for days between the timely and delayed harvest at each trial were obtained at each trial from the State Climate Office of North Carolina website (http://www.nc-climate.ncsu.edu, verified March 2005). Thirty-year and trial-year growing season (October to July) mean temperature and precipitation data were obtained from the same source.

Grain yield and moisture were measured using a HarvestMaster grain gauge (Juniper Systems Inc., Logan, Utah) attached to either a Massey Ferguson MF-8 or Gleaner K2 combine (AGCO Corp., Duluth, GA). Yields were adjusted to 135 g kg^{-1} moisture. Grain samples of 0.45 to 3.0 kg were taken from each plot (approximately 16 m^2 in size) for analysis of test weight, grain protein, and milling and baking quality. Grain yield data was lost from trial C-1 because of a mechanical malfunction, but a representative grain sample was taken for test weight and grain protein analyses.

Test weight was determined on a volume weight basis with a DICKEY-john Grain Analysis computer (model number GA C2000, DICKEY-john Corp., Auburn, IL). Grain samples taken at harvest were sub-sampled (approximately 85 g) for total N analysis determined by combustion using a CHN analyzer (McGeehan and Naylor, 1988) at Waters Agriculture Laboratories (Camilla, GA). Total N was converted to grain protein by multiplying by 5.83 (Kent and Evers, 1994).

Grain samples were pooled within a harvest treatment across replications to provide 5.0 kg for milling and baking quality analysis, except for C-1 where the total grain sample

was insufficient. Grain samples were pooled across all replications for B-1 and T-1. Grain samples were pooled across each half of the replications for C-2, P-2, and T-2, creating two pooled replicates in each of these trials.

Midstate Mills, Inc., Newton, NC, performed milling and baking quality analyses following the standards of the American Association of Cereal Chemists (AACC International, 2000). Quality parameters measured were: kernel weight, grain falling number, grain DON (a laboratory error resulted in loss of grain DON data from P-2), patent flour, clear flour, extractable flour, flour moisture, flour protein, flour falling number, flour DON, and cookie spread. Patent and clear flour are a part of the extractable flour, which are used to blend specialized milling grades for specific end uses (Pyler, 1952). Rheological properties of the dough were also examined. To test the physical properties of the dough, a farinograph (C.W. Brabender Inc., Hackensack, NJ) recorded the farinograph flour absorption, development time, stability time, mixing tolerance index (MTI), and breakdown time, all components of the dough structure (Bloksma and Bushuk, 1988). Additionally, an alveograph (Chopin by Seedburo Equipment Company, Chicago IL) recorded the alveograph overpressure, extensibility, curve configuration, and work, which measures the resistance and extensibility of a dough (Bloksma and Bushuk, 1988).

Analysis of variance (ANOVA) using PROC MIXED in SAS version 8 (SAS Institute Inc., Cary, NC) was used to evaluate significance of treatment and interaction effects. Trial (the combination of year, location, cultivar, and tillage system) and harvest date were treated as fixed effects and replications were treated as random effects. Least square mean separations were employed for testing differences between and among treatments. Simple linear and quadratic regression and stepwise determination of multiple regressions of

grain yield, test weight, grain protein, and milling and baking quality on environmental factors were determined using PROC REG. For multiple regression, the STEPWISE with RSQUARE option was used in SAS version 8 (SAS Institute Inc., Cary, NC), with $p = 0.05$ as the critical value for allowing new variables to enter the model.

RESULTS

Environmental Conditions

The 30-yr growing season mean temperature and total precipitation at the locations used in this study were 13.6 ºC and 85.8 cm, respectively. The 2002 growing season mean temperature and total precipitation were 13.5 ºC and 52.4 cm, respectively, which was drier than the 30-yr mean. The 2003 mean temperature and total precipitation were 10.4 ºC and 92.7 cm, respectively, which was cooler and wetter than either the 30-yr mean or the 2002-growing season. Despite all trials receiving the same management up to harvest, there was a possibility for different grain yield, test weight, and grain protein potentials between the two growing season studied due to differences in mean temperature and total precipitation between 2002 and 2003 before harvest.

The environmental conditions of interest were those that occurred between the two harvest dates at each trial (Fig. 1). Trials C-1, T-1, and T-2 had the fewest days and precipitation events between harvests, which resulted in lower total precipitation, compared to the other trials (Fig. 1). Trials B-1 and P-2 had similar number of days and precipitation events between harvests (Fig. 1), though P-2 had the highest total precipitation (Fig. 1) and the largest precipitation event between harvests (Fig. 1). Trial C-2 had the most days between harvests and most precipitation events (Fig. 1), though all precipitation events were less than 1 cm (Fig. 1).

Grain Yield, Test Weight, and Grain Protein

Trial, harvest date, and trial × harvest date had statistically significant effects on grain yield (Table 2). Grain yield for each trial at both harvest dates (Table 3) were above the state average for North Carolina in 2002 and 2003 (4.4 Mg ha^{-1} and 3.76 Mg ha^{-1}, respectively), except the delayed harvest in B-1, T-1, and T-2. Indicating that the starting conditions at each trial before the delayed harvest were not negatively impacted by contrasting environmental conditions during the two growing seasons. Trials T-1, C-2, and T-2 had statistically significant decreases in grain yield with delayed harvest, while trials P-2 and B-1 exhibited no significant difference between timely and delayed harvests (Table 3). The greatest percent yield loss occurred in T-2, followed by T-1. Both of these trials had delayed harvest of 8 d and also had the fewest precipitation events (Fig. 1 and Table 3). Trial C-2 also sustained a high percent yield loss and had the longest delay between harvests with the most precipitation events. Trials B-1 and P-2 also had long delays between harvests and a higher number of precipitation events than T-2 and T-1, but did not sustain significant yield losses (Fig. 1 and Table 3).

Simple linear and quadratic regressions were performed for yield differences between harvests (averaged across replications) versus environmental conditions that occurred between harvests: mean daily maximum and minimum temperature, total precipitation, total number of precipitation events, mean daily wind speed, mean daily relative humidity, and days between harvests. A significant linear relationship ($r^2 = 0.90$, slope = -0.27) was found only between yield differences and total precipitation (Fig. 2), indicating that yield reductions were lowest in trials with the highest total precipitation between harvests. Based on a stepwise multiple regression analysis of yield differences versus the environmental variables,

a significant relationship between yield differences, total precipitation (partial $R^2 = 0.90$), and minimum daily temperature (partial $R^2 = 0.09$) was found ($R^2 = 0.99$) such that:

$$y = -0.25P_{tot} + 0.13T_{min} - 1.36 \quad \text{[Eq.1]}$$

where y was the yield loss (Mg ha^{-1}), P_{tot} the total precipitation (cm), and T_{min} the mean minimum daily temperature (^oC) between harvests, respectively. Equation 1 is consistent with warmer and drier conditions after grain ripeness increasing yield differences, most likely due to shattering in the grain head.

Trial, harvest date, and trial × harvest date were significant for test weight (Table 2). The interaction between trial and harvest date was marked by varying decreases in test weight due to delayed harvest across all trials with the exception of T-2 (Table 3). Trial C-2 had the greatest reduction in test weight and also had the greatest number of days and precipitation events between harvests. Trial T-2 had no significant reduction in test weight, despite having similar days between harvests and number of precipitation events compared to C-1 and T-1, where significant reductions in test weight did occur. Trial C-1 test weights at each harvest date were above the 747 kg m^{-3} standard, while the B-1, T-1, and C-2 test weights were above the 747 kg m^{-3} standard at the timely harvest, but fell below at the delayed harvest (Table 3). Trials P-2 and T-2 were conducted in the wetter and cooler 2003 growing season and exhibited test weights consistently below the minimum standard across harvest dates.

There were no significant linear or quadratic relationships between reductions in test weight and mean daily minimum or maximum temperatures, total precipitation, mean daily

wind speed, and mean daily relative humidity. There was, however, a significant positive linear relationship ($r^2 = 0.84$, slope = 6.53) between the number of days between a timely and delayed harvest and loss in test weight (Fig. 3). There was also a significant positive linear relationship ($r^2 = 0.93$, slope = 16.32) between the number of precipitation events between harvests and reductions in test weight (Fig. 4). The correlation between the number of precipitation events and days between harvest dates ($r = 0.94$) was significant, suggesting that a longer interval between grain ripeness and harvest increased the opportunity for more precipitation events and/or wetting and drying cycles, and caused a corresponding reduction in test weight.

Mean levels of grain protein in each trial were typical for the region (Bowman, 2004), ranging from 108 g kg^{-1} in trial T-2 to 125 g kg^{-1} in trials T-1 and P-2 (Table 4). Trial was the only factor contributing variability in grain protein (Table 2). There was no evident trend to explain differences in grain protein between trials. Consistent with non-significant harvest date and harvest date × trial effects, linear, quadratic and multiple regression analyses with environmental factors were not conducted for grain protein.

Milling and Baking Quality

Trial, harvest date, and the interaction of these effects were not significant for nine of the 20 milling and baking quality parameters examined (Table 2). These included flour moisture, protein, and DON; cookie spread; farinograph flour absorption, development time, and MTI; and alveograph overpressure and work.

Only trial had a significant effect on kernel weight, patent and extractable flour, farinograph stability time, and alveograph extensibility and curve configuration (Table 2); delayed harvest did not affect these parameters. Some of the differences in kernel weight

between trials may have been due to a cultivar effect because C 9704 was only grown at T-2, but there was significant variation among the remaining trials where P 26R61 was evaluated (Table 4). The trial effect for patent and extractable flour could be explained by a year effect with the 2002 trials (B-1 and T-1) significantly different than the 2003 trials (C-2, P-2, and T-2) (Table 4). Farinograph stability, alveograph extensibility, and alveograph curve configuration effects across trials revealed no apparent trend to describe the differences among trials (Table 4).

Trial and harvest date were significant sources of variation for flour falling number (Table 2). Flour falling numbers were significantly higher in 2002 trials (B-1 and T-1) from trials in 2003 (T-2, C-2, and P-2) possibly due to wetter and cooler conditions in 2003 compared to 2002 (Table 4). The mean flour falling number for a delayed harvest (315 s) was statistically significantly reduced compared to a timely harvest (358 s). Because of the non-significant interaction between trial and harvest date for flour falling number, indicating that the measured environmental conditions between harvests at each trial had no effect, differences of flour falling number between harvests at each trial were not regressed with environmental conditions.

Harvest date was the only significant source of variation for clear flour (Table 2). Delayed harvest resulted in 18% clear flour compared to a timely harvest value of 19.8 %. Because of the non-significant interaction between trial and harvest date for clear flour, indicating that the measured environmental conditions between harvests at each trial had no effect, differences of clear flour between harvests at each trial were not regressed with environmental conditions.

Harvest date, trial, and trial × harvest date were significant for grain falling number (Table 2). There was a significant reduction in grain falling number between harvest dates only in trial P-2 (Table 5) and this was responsible for the trial × harvest date interaction and possibly the harvest date and trial effects. Trial P-2 experienced the highest total precipitation and highest mean daily relative humidity, two environmental variables that could have initiated germination in the ripe grain. Nevertheless, an analysis of the whole dataset found no significant linear, quadratic, or multiple regression relationships between differences in grain falling number between harvests and the measured environmental variables.

Harvest date and trial × harvest date were significant for grain DON content (Table 2). A significant increase in the grain DON content between the two harvest dates was observed in all trials except T-1 (Table 5). Trial B-1 had the largest increase in DON (2.31 μg g^{-1}), followed by T-2 (0.90 μg g^{-1}). Trial C-2 had the smallest increase in DON levels (0.35 μg g^{-1}). There were no significant linear, quadratic, or multiple regression relationships between increases in grain DON and the measured environmental conditions between harvests.

Trial and trial × harvest date were significant for farinograph breakdown time (Table 2). In trials B-1 and T-2, breakdown time decreased with delayed harvest (Table 5). In all other trials there were no differences in breakdown times between harvest dates. There were no significant linear, quadratic, or multiple regression relationships between reductions in farinograph breakdown time and the measured environmental conditions between harvest dates.

DISCUSSION

The objectives of this study were to determine the impact of delayed harvest on grain yield, test weight, grain protein, and milling and baking quality of soft red winter wheat in the southeastern USA, and to correlate these impacts with environmental conditions that occurred between harvests. Grain yield, test weight, and several important milling and baking quality parameters were negatively affected by harvesting after grain ripeness.

Grain Yield, Test Weight, and Grain Protein

Many determinants of potential grain yield are set early in the growth of winter wheat. Nevertheless, our data showed that grain yield can be reduced substantially (as much as 19%) by delayed harvest. The highest yield differences associated with delayed harvest occurred in trials with the lowest total precipitation between harvests (Fig. 2). Increased minimum daily temperature with a decrease in total precipitation was associated with yield differences. Hot, dry conditions can induce shattering in the grain head (Chang, 1943), and this is consistent with our finding that yield differences were greatest in warmer, drier trials.

Czarnecki and Evans (1986), Pushman and Bingham (1975), and Yamazaki and Briggle (1969) reported that packing efficiency is generally determined genetically and the density of the kernel is influenced by environmental factors. Czarnecki and Evan (1986) reported that once the density decrease reached a plateau, further decreases in test weight caused by alternating wetting and drying cycles were due to changes in the packing efficiency. In our study, delayed harvest consistently caused a reduction in test weight in all trials except T-2. Significant test weight reductions of 3.3 to 14.4 % occurred with delayed harvests of 8 to 19 d after grain ripeness. Consistent with the findings of Lloyd et al. (1999), we observed a strong positive linear relationship between the number of days between

harvests and reductions in test weight (Fig. 3). Interestingly, the strongest linear relationship between reduction in test weight and the environmental variables tested was for the number of precipitation events between harvests. This suggests that grain wetting and drying cycles were important factors contributing to reductions in test weight.

Grain protein was not affected by delayed harvest. The wheat in this study was allowed to reach grain ripeness, so the maximum quantity of protein would have already been deposited in the grain. Christensen and Legge (1984) reported that only excessively hot and dry environmental conditions after physiological maturity caused changes in grain protein content. Such conditions were not present in our study.

Milling and Baking Quality

Farinograph stability, alveograph extensibility, and alveograph curve configuration are related to grain protein amounts and types (Bloksma and Bushuk, 1964) and like grain protein these milling and baking quality parameters were not affected by delayed harvest. Kernel weight, patent flour, and extractable flour are related to proteins and carbohydrates present in the grain (Gooding and Davies, 1997; Pyler, 1952) and were also not affected by harvest date.

Of the 20 quality parameters investigated in this study, only grain and flour falling number, grain DON levels, clear flour, and farinograph breakdown time showed significant effects associated with delayed harvest. In our study, flour falling number decreased with delayed harvest and grain falling number decreased at P-2 with delayed harvest (Table 5). This indicated increased α-amylase enzyme activity, though sprouting was not visibly evident. Enzyme activity may be increased with warm temperatures (Hagemann and Ciha, 1987), but a relationship between grain or flour falling number and temperature was not

evident. As grain is exposed to increased levels of moisture, the grain can imbibe water and begin germination and activate α-amylase (Gooding and Davies, 1997). This may be what happened at trial P-2, where precipitation amounts were high and grain falling number decreased significantly between harvests. The observed decrease in flour falling number was probably the result of a complex combination of temperature, increased moisture through grain wetting, and time associated with delayed harvest.

A critical level for DON in grain is 2 μg g^{-1} according to local mill standards and 1 μg g^{-1} for flour according to the FDA Advisory Level (2005). When this toxin is present in milling and baking products, it can change the flavors of foods and pose a potential health risk (Woloshuk et al., 1995). At two out of the four trials, delaying harvest caused grain DON levels to exceed the local mill standards. As the grain remains moist and cool for long periods of time, it creates a favorable environment for fungal infections and can increase grain DON levels (Murray et al., 1998). Bushnell et al. (2003) and Miller and Young (1985) indicated that grain DON levels decrease before harvest. Snijders and Krechting (1992) presented evidence that the trend for DON levels is different after harvest. The present study confirmed the findings of Snijders and Krechting (1992) with grain DON levels increasing with a delayed harvest.

A decrease in clear flour could be an indication of a decrease in grain carbohydrates with delayed harvest. Clear flour decreased between harvests, and these reductions might be linked to increased respiration and/or α-amylase enzyme activity, which over time would result in starches being broken down and utilized by the embryo.

Farinograph breakdown time is a measure of the dough's ability to retain its structure over time in the mixing process (Bloksma and Bushuk, 1964). Decreases in breakdown time

with delayed harvest (Table 5) could indicate changes in the protein to carbohydrate ratio in the grain (Bloksma and Bushuk, 1964). Because grain protein did not change with delayed harvest, the decrease in farinograph breakdown time seen at two trials (Table 5) may have been linked to changes in grain carbohydrates.

SUMMARY

Delayed harvest of soft red winter wheat caused substantial reductions in grain yield and test weight. Reductions in grain yield were negatively correlated with total precipitation between timely and delayed harvest. Multiple regression analysis further indicated that lower total precipitation and higher mean daily minimum temperatures were associated with increased yield differences consistent with shattering in the grain head occurring in the field. Reductions in test weight were linearly and positively correlated with the number of precipitation events and the number of days between harvest dates. Grain protein was not affected by delayed harvest, and consequently the related milling and baking qualities were also not affected. The milling and baking qualities that were affected by a delayed harvest were grain and flour falling number, grain DON, clear flour, and farinograph breakdown time. Flour falling number decreased with a delayed harvest and grain falling number decreased at one trial. There was also a decrease in clear flour with delayed harvest. Grain DON levels increased with delayed harvest, consistent with increased development of Fusarium head blight caused by *Fusarium graminearum*. Farinograph breakdown time decreased, indicating a change in grain carbohydrates with delayed harvest.

REFERENCES

AACC International. 2000. Approved methods AACC, 10th ed. [Online]. Available at www.aaccnet.org/ApprovedMethods/top.htm. (accessed Aug 2004; verified March 2005). St. Paul, MN.

Bloksma, A.H., and W. Bushuk. 1964. Rheology and chemistry of dough. *In* Y. Pomeranz (ed.) Wheat chemistry and technology, vol. 2. American Association of Cereal Chemists, Inc. St. Paul, MN.

Bowman, D.T. 2001. North Carolina measured crop performance: small grains 2001. Crop Science Research Report No. 194. North Carolina State University, Raleigh, NC.

Bowman, D.T. 2004. North Carolina measured crop performance: small grains 2004. Crop Science Research Report No. 211. North Carolina State University, Raleigh, NC.

Bracken, A.F., and C.H. Bailey. 1928. Effect of delayed harvesting on quality of wheat. Agron. J. 5:128-145.

Bushnell, W.R., B.E. Hazen, and C. Pritsch. 2003. Histology and Physiology of Fusarium Head Blight. p. 44-83. *In* K.J. Leonard and W.R. Bushnell (ed.). Fusarium Head Blight of Wheat and Barley. APS Press, St. Paul.

Chang, S.C. 1943. Morphological causes for varietal differences in shattering of wheat. Agron. J. 35:435-441.

Christensen, J.V., and W.G. Legge. 1984. Effect of harvest time and drying method on the yield, quality and grade of hard red spring wheat in northwest Alberta. Can. J. Plant Sci. 64:617-623.

Clarke, J.M. 1981. Effect of delayed harvest on shattering losses in oats, barley and wheat. Can. J. Plant Sci. 61:25-28.

Clarke, J.M., and R.M. De Pauw. 1983. The dynamics of shattering in maturing wheat. Euphytica. 32: 225-230.

Czarnecki, E., and L.E. Evans. 1986. Effect of weathering during delayed harvest on test weight, seed size, and grain hardness of wheat. Can. J. Plant Sci. 66:473-482.

Edwards, R.A., A. S. Ross, D.J. Mares, F.W. Ellison, and J.D. Tomlinson. 1989. Enzymes from rain-damaged and laboratory-germinated wheat I. Effects on product quality. J. Cereal Sci. 10:157-167.

FDA Advisory Level. 2005. [Online]. FDA's Advisory Levels for Deoxynivalenol (Vomitoxin): Available at http://www.ngfa.org/toxinsPDF-1.pdf. (accessed May 2005; verified June 2005).

Gan, Y.T., T.N. McCaig, P. Clarke, R.M. DePauw, J.M. Clarke, and J.G. McLeod. 2000. Test-weight and weathering of spring wheat. Can. J. Plant Sci. 80:677-685.

Gooding, M.J., and W.P. Davis. 1997. Wheat production and utilization: systems quality and the environment. Cab International. Wallingford, UK.

Hardy, D.H., D.L. Osmond, and A. Wossink. 2002. An overview of nutrient management with economic considerations. Publication No. AG-565. North Carolina Coop. Ext. Ser., North Carolina State Univ., Raleigh, NC.

Hagemann, M.G., and A.J. Ciha. 1987. Environmental × genotype effects on seed dormancy and after-ripening in wheat. Agron. J. 79:192-196.

Kent, N.L., and A.D. Evers. 1994. Kent's technology of cereals. Pergamon Press, Oxford.

Lloyd, B.J., T.J. Siebenmorgen, R.K. Bacon, and E. Vories. 1999. Harvest date and conditioned moisture content effects on test weight of soft red winter wheat. App. Eng. Agric. 15:525-534.

McGeehan S.L., and D.V. Naylor. 1988. Automated instrumental analysis of carbon and nitrogen in plant and soil samples. Comm. Soil Sci. Plant Anal. 19:493-505.

Miller, J.D., and J.C. Young. 1985. Deoxynivalenol in an experimental *Fusarium graminearum* infection of wheat. Can.J. Plant Pathol. 7:132-134.

Milner, M., and J.A. Shellenberger. 1953. Physical properties of weathered wheat in relation to internal fissuring detected radiographically. Cereal Chem. 30:202-212.

Murray, T.D., D.W. Parry, and N.D. Cattlin. 1998. A color handbook of diseases of small grain cereal crops. Iowa State University Press, Ames IA.

Orth, R.A., and J.A. Shellenberger. 1988. Origin, production, and utilization of wheat. *In* Y. Pomeranz (ed.)Wheat: Chemistry and Technology. AACC, Inc., St. Paul, MN.

Paulsen, G.M. 2004. Growth and development. *In* Kansas State Research and Extension, Wheat Production Handbook, Manhatten KS. accessed through http://www.oznet.ksu.edu/wheatpage/. (accessed Aug. 2004; verified May 2005).

Pool, M., F.L. Patterson, and C.E. Bode. 1958. Effect of delayed harvest on quality of soft red winter wheat. Agron. J. 7:271-275.

Pushman, F.M. 1975. The effects of alterations of grain moisture content by wetting or drying on the test weight of four winter wheats. J. Agric. Sci. 84:187-190.

Pushman, F.M., and J. Bingham. 1975. Components of test weight of ten varieties of winter wheat grown with two rates of nitrogen fertilizer application. J. Agric. Sci. 85:559-563.

Pyler. E.J. (ed.). 1952. Baking science and technology. Siebel Publishing Company, Chicago, IL.

Schlehuber, A.M., and B.B. Tucker. 1967. Culture of wheat. *In* K.S. Quisenberry and L.P. Reitz (ed.) Wheat and wheat improvement. Amer. Soc. Agron., Madison, WI.

Snijders, C.H.A., and C.F. Krechting. 1992. Inhibition of deoxynivalenol translocation and fungal colonization in Fusarium head blight resistant wheat. Can. J. Bot. 70:1570-1576.

State Climate Office of North Carolina. 2004. [Online]. Available at www.nc-climate.ncsu.edu. (accessed Aug. 2004; verified March 2005). Raleigh, NC.

Swanson, C.O. 1941. Effects of moisture on the physical and other properties of wheat. Crop Sci. 18:705-729.

USDA/ARS Soft Wheat Quality Laboratories. 2004. Soft wheat quality goals. Available at http://www.oardc.ohio-state.edu/swql. (accessed Aug. 2004; verified March 2005). Wooster, OH.

Whitcomb, W.O., and A.H. Johnson. 1928. Effect of severe weathering on certain properties of wheat. Crop Sci. 5:117-128.

Weisz, R. 2004. Small Grain Production Guide 2004-05. North Carolina Cooperative Extension Service, Raleigh, NC.

Woloshuk, C., D. Scott, and D. Maier. 1995. Head scab of wheat and vomitoxin. Grain quality fact Sheet #2. Cooperative Extension Services. Purdue University, West Lafayette, IN.

Yamazaki, W.T., and L.W. Briggle. 1969. Components of test weight in soft wheat. Crop Sci. 9:457-459.

Zadoks, J.C., T.T. Chang, and C.F. Zonzak. 1974. A decimal code for the growth stages of cereals. Weed. Res. 14:415-421.

Table 1. Locations, trial identification, cultivar, tillage method, and date of timely and delayed harvests of winter wheat trials studying the effects of delayed harvest in 2002 and 2003.

Location	Trial identification	Cultivar				Tillage method	Date of timely harvest	Date of delayed harvest
		Name	Heading date[†]	Powdery mildew resistance[†]	Leaf rust resistance[†]			
Circle Grove Seed Farm (Belhaven, NC)	B-1	P 26R61	Medium-early	MR[‡]	MR	Conventional	4 June 2002	20 June 2002
Cunningham Research Station (Kinston, NC)	C-1	C 9704	Medium	MR	MS	Conventional	28 May 2002	5 June 2002
Tidewater Research Station (Plymouth, NC)	T-1	P 26R61	Medium-early	MR	MR	Conventional	12 June 2002	20 June 2002
Cunningham Research Station (Kinston, NC)	C-2	P 26R61	Medium-early	MR	MR	Conventional	6 June 2003	25 June 2003
Piedmont Research Station (Salisbury, NC)	P-2	P 26R61	Medium-early	MR	MR	No-till	23 June 2003	8 July 2003
Tidewater Research Station (Plymouth, NC)	T-2	C 9704	Medium	MR	MS	Conventional	23 June 2003	1 July 2003

[†] Information taken from Bowman (2001 and 2004).
[‡] MR = moderately resistance, MS = moderately susceptible.

Table 2. Analysis of variance results for the effects of trial, harvest date, and their interaction on grain yield, test weight, grain protein, and milling and baking quality parameters of winter wheat with associated degrees of freedom (df) and coefficients of variation (CV).

	Source of variation			
Variables	Trial	Harvest date	Trial × harvest date	CV
	df = 5	df = 1	df = 5	%
Grain yield	***	***	***	7.6
Test weight	***	***	***	1.8
Grain protein	***	NS	NS	5.1
	df = 4	df = 1	df = 4	
Grain				
Kernel weight	**	NS	NS	2.5
Falling number	***	**	**	5.4
DON	NS	**	**	7.4
Flour				
Patent	**	NS	NS	6.0
Clear	NS	*	NS	4.8
Extractable	*	NS	NS	6.6
Moisture	NS	NS	NS	1.1
Protein	NS	NS	NS	7. 9
Falling number	**	*	NS	6.0
DON	NS	NS	NS	19.8
Cookie spread	NS	NS	NS	5.6

Table 2. (continued)

Farinograph				
Flour absorption	NS	NS	NS	2.7
Development time	NS	NS	NS	6.4
Stability time	**	NS	NS	28.1
MTI	NS	NS	NS	22.0
Breakdown time	**	NS	†	20.8
Alveograph				
Overpressure	NS	NS	NS	26.3
Extensibility	***	NS	NS	11.2
Curve configuration	†	NS	NS	63. 5
Work	NS	NS	NS	10.8

†, *, **, ***, Significant at the 0.10, 0.05, 0.01 and 0.001 probability levels, respectively. NS, Not significant at $p < 0.10$.

Table 3. Least square mean estimates for grain yield and test weight of winter wheat for the timely and delayed harvests with percent change between harvests in six trials evaluated in 2002 and 2003.

Trial[†]	Days between harvest	Grain yield		Change in yield between harvests	Test weight		Change in test weight between harvests
		Timely harvest	Delayed harvest		Timely harvest	Delayed harvest	
		-------Mg ha^{-1}-------		-- % --	--------kg m^{-3}--------		-- % --
B-1	16	4.45a[‡]	4.29a	-3.6	777a	715b	-7.9
C-1	8	NA[§]	NA	NA	812a	777b	-4.3
T-1	8	5.14a	4.27b	-16.9	755a	730b	-3.3
C-2	19	4.46a	3.85b	-13.7	748a	640b	-14.4
P-2	15	5.11a	5.35a	+4.7	727a	676b	-6.9
T-2	8	4.26a	3.43b	-19.5	719a	711a	-1.0

[†] B-1 = Circle Grove Seed Farm 2002, C-1 = Cunningham Research Station 2002, T-1 = Tidewater Research Station 2002, C-2 = Cunningham Research Station 2003, P-2 = Piedmont Research Station, T-2 = Tidewater Research Station 2003.
[‡]Grain yield or test weight means within trials (rows) followed by the same letter are not significantly different at the 0.05 probability level.
[§] NA = Not applicable, yield data lost.

Table 4. Least square mean estimates for the main effect of trial for grain protein and selected milling and baking quality parameters of winter wheat in six trials evaluated in 2002 and 2003.

Trial[†]	Grain protein	Kernel weight	Patent flour	Extractable flour	Flour falling number	Farinograph stability	Alveograph extensibility	Alveograph curve configuration
	--g kg^{-1} --	--- mg---	-- % --	---- % ----	--- s ---	--- min ---	---- mm -----	
B-1	118c[‡]	43.9a[§]	50.9a	71.2a	390a	6.4ab	106c	0.36b
C-1	113c	NA[¶]	NA	NA	NA	NA	NA	NA
T-1	125b	38.8b	51.8a	71.3a	427a	7.4a	103c	0.34b
C-2	131a	44.7a	42.2b	60.4b	299c	8.0a	160a	0.23b
P-2	125b	35.1c	39.6b	57.9b	225d	5.1b	136b	0.29b
T-2	108d	30.3d	41.6b	60.1b	343b	3.2c	80d	0.73a

[†] B-1 = Circle Grove Seed Farm 2002, C-1 = Cunningham Research Station 2002, T-1 = Tidewater Research Station 2002, C-2 = Cunningham Research Station 2003, P-2 = Piedmont Research Station, T-2 = Tidewater Research Station 2003.
[‡]Grain protein means among trials followed by the same letter are not significantly different at the 0.05 probability level.
[§]Milling and baking quality parameters means among trials followed by the same letter are not significantly different at the 0.10 probability level.
[¶] NA = Not applicable, milling and baking quality data lost.

Table 5. Least square mean estimates of grain falling number, grain DON, and farinograph breakdown of winter wheat by harvest (timely and delayed) for each of five trials evaluated in 2002 and 2003.

Trial[†]	Days between harvests	Grain falling number		Grain DON		Farinograph breakdown time	
		Timely harvest	Delayed harvest	Timely harvest	Delayed harvest	Timely harvest	Delayed harvest
		---------- s ----------		---------- µg g^{-1} ----------		---------- min ----------	
B-1	16	400a[‡]	375a	0.59b	2.90a	7.0a	3.2b
T-1	8	447a	418a	0.88a	1.10a	8.3a	8.7a
C-2	19	288a	307a	1.45b	1.80a	8.0a	10.0a
P-2	15	318a	170b	NA[§]	NA	6.5a	6.0a
T-2	8	326a	341a	1.70b	2.6a	5.2a	3.0b

[†] B-1 = Circle Grove Seed Farm 2002, T-1 = Tidewater Research Station 2002, C-2 = Cunningham Research Station 2003, P-2 = Piedmont Research Station, T-2 = Tidewater Research Station 2003, C-1 = Cunningham Research Station 2002 not included because of insufficient sample for analysis.

[‡]Grain falling number, grain DON, or farinograph breakdown time means within trials (rows) followed by the same letter are not significantly different at the 0.10 probability level.

[§] NA = Not applicable, milling quality data lost.

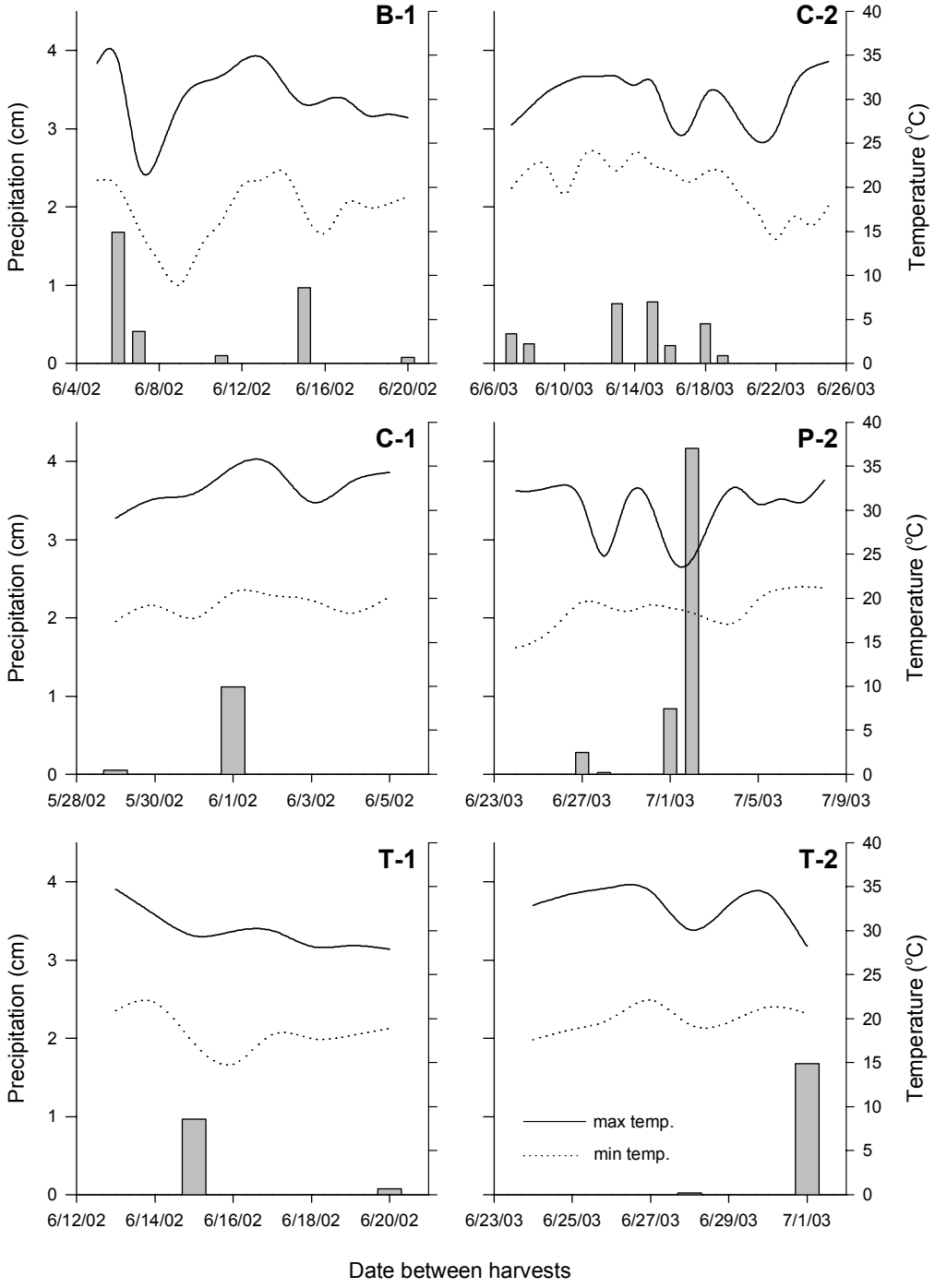


Fig. 1. Daily mean maximum and minimum temperature (ºC) and daily total precipitation (cm) measured between the timely and delayed harvests of winter wheat at each of six trial locations.

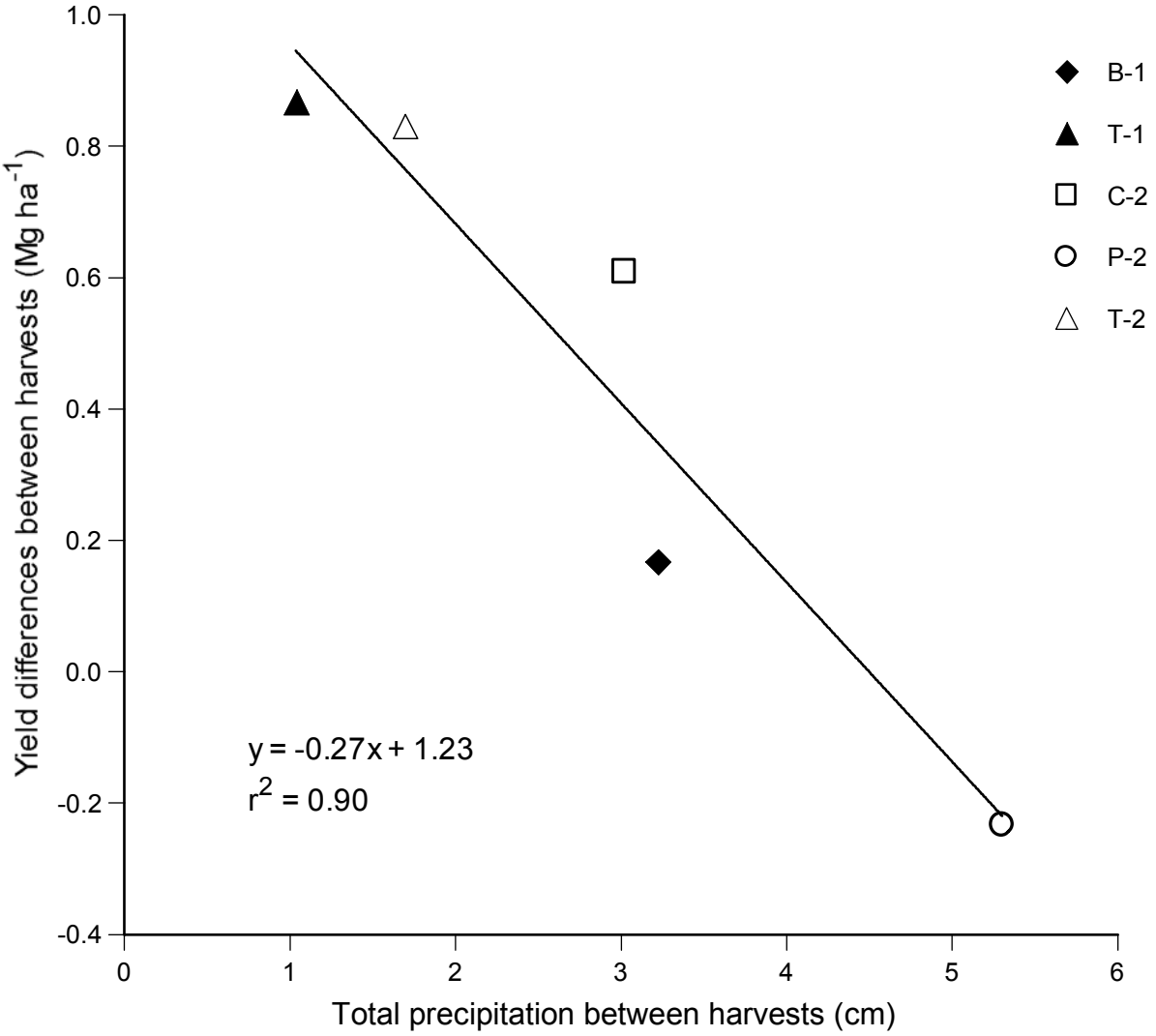


Fig. 2. Simple linear regression of winter wheat yield differences (Mg ha^{-1}) between the timely and delayed harvests versus total precipitation (cm) between harvests across five trials (trial C-1 yield data lost).

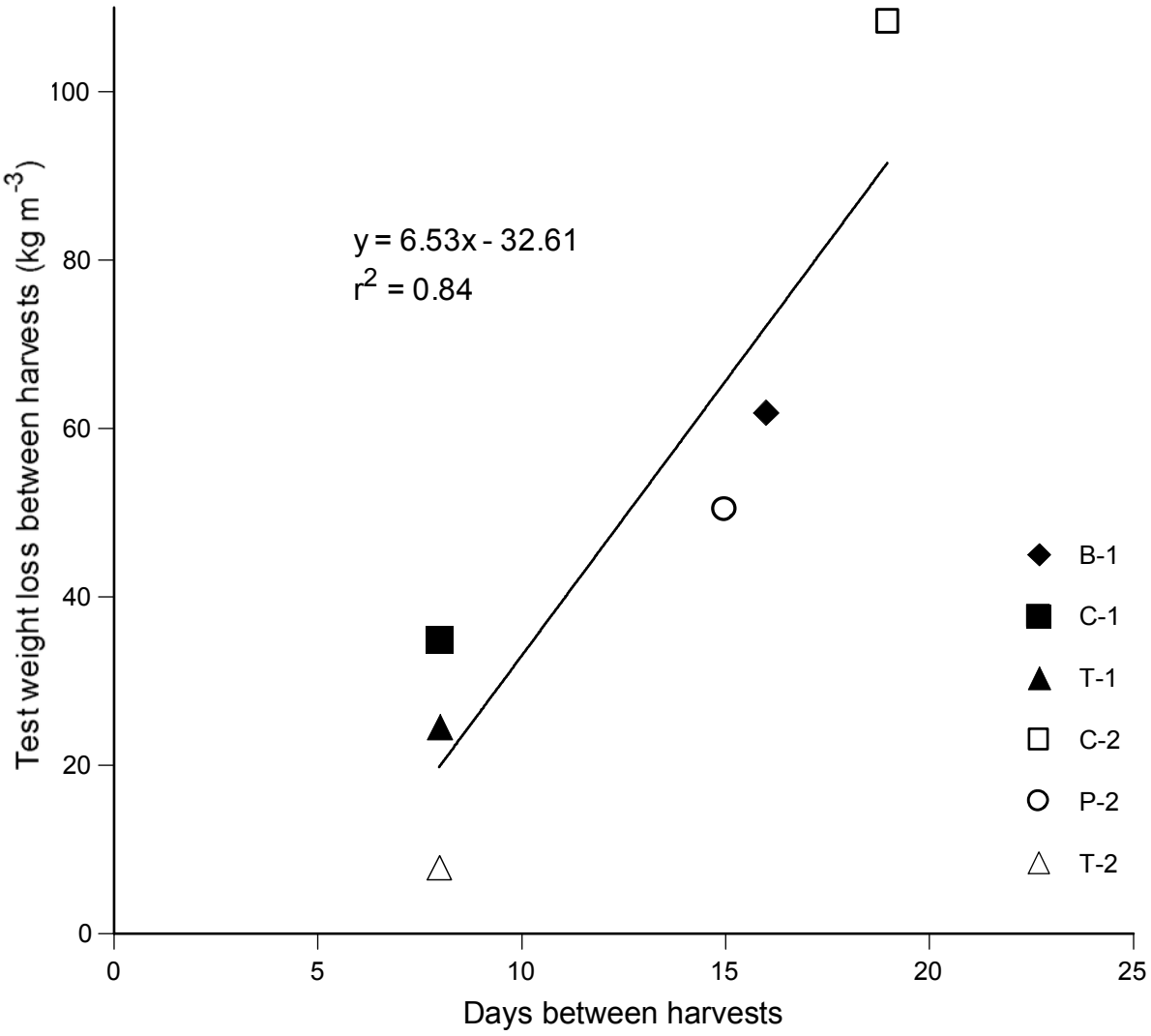


Fig. 3. Simple linear regression between reductions in winter wheat test weight (kg m^{-3}) and the number of days between the timely and delayed harvests across six trials.

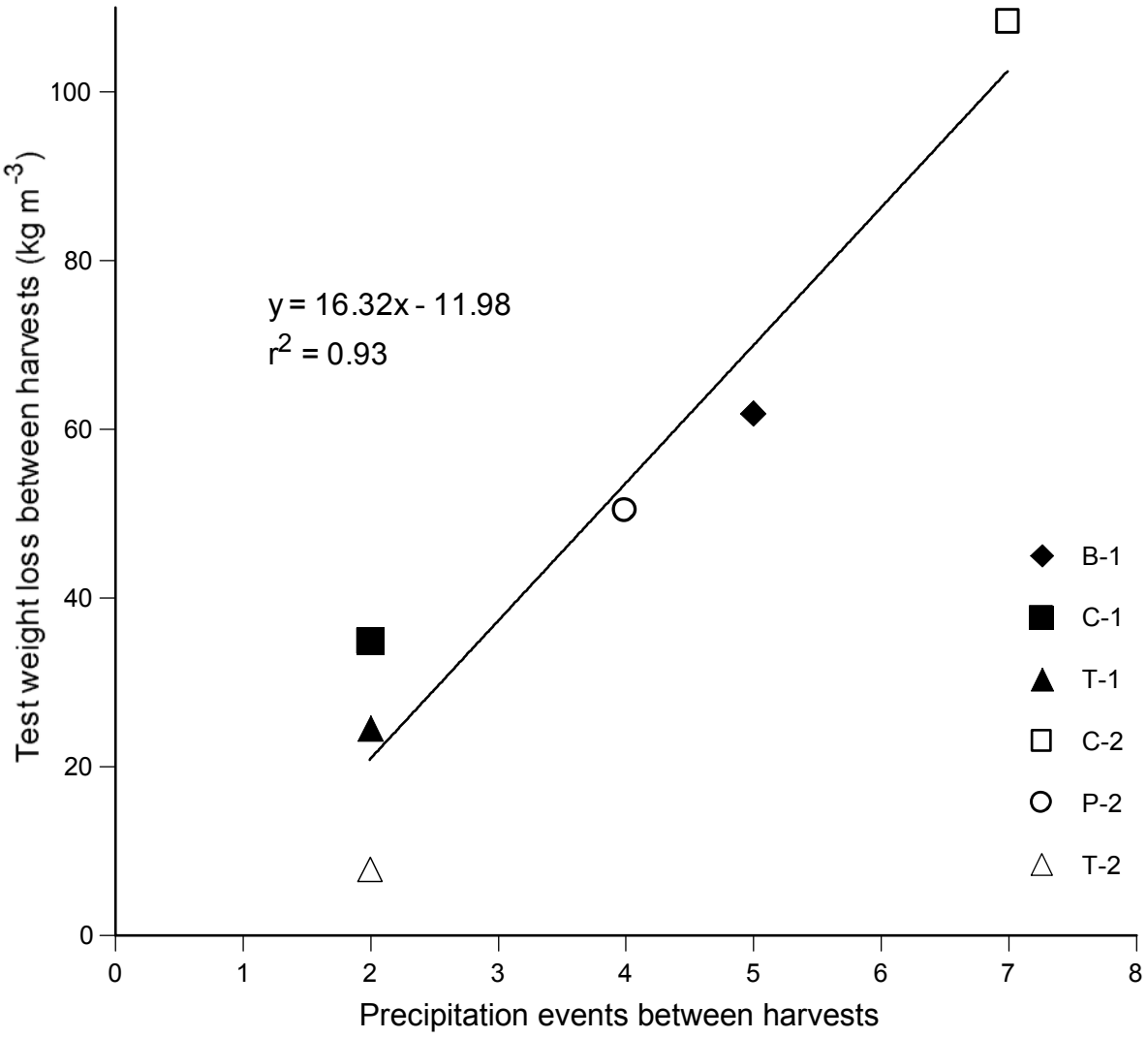


Fig. 4. Simple linear regression between reductions in winter wheat test weight (kg m^{-3}) and the number of precipitation events between the timely and delayed harvest across six trials.

CHAPTER TWO: Protein Variability in Southeastern Winter Wheat: Impact of Nitrogen Timing and Application Rates

Protein Variability in Southeastern Winter Wheat: Impact of Nitrogen Timing and Application Rates

ABSTRACT

Grain protein content in soft red winter wheat (*Triticum aestivum* L.) is highly variable across years and environments in the southeastern USA. This high variability makes southeastern wheat undesirable to millers and negatively impacts its value in the export market. The objectives of this study were to determine how different N fertilizer rates and times of application would affect overall grain protein variability and to determine if there were N fertilizer recommendations that would minimize regional protein variation. Experiments were conducted in the North Carolina Piedmont and Coastal Plain regions in 2002 and 2003 to capture the soil and environmental variability that exists annually. Partitioning of the total ANOVA model variability indicated that environment contributed the most variability to yield and test weight, 66.5% and 90.5%, respectively. Though environment contributed to grain protein variability (22.8%), the majority of that variability was attributed to timing and rate of N application (52.6%). As grain protein levels increased at higher N rates, so did overall protein variability as indicated by three stability indexes employed. In addition, applying the majority of total N at the later growth stage (GS) 30 decreased grain protein stability. The recommendations to reduce grain protein variability in the southeastern USA are: 1) to reduce the range in N fertilizer rates used across the southeast, 2) to avoid over application of N beyond what is required to optimize yield and economic return, and 3) to apply spring N at GS 25.

INTRODUCTION

The demand for soft red winter wheat (*Triticum aestivum* L.) by the milling and baking industry in the southeastern USA continues to grow, and the region's millers generally pay a premium for locally grown high quality grain. However, southeastern grain protein content is highly variable across years and environments. The milling and baking industry's desired range in grain protein content for soft wheat is 80 to 110 g kg^{-1} (Gooding and Davis, 1997). However, the desired grain protein ranges for specific uses such as cookies or pastry are much narrower. Grain protein content in North Carolina has been reported to range from 86 to 135 g kg^{-1} across cultivars and environments (Bowman, 2002, 2003, and 2004). This high variability makes regional wheat undesirable to the millers who currently import approximately 50% of their soft red winter wheat from the midwestern USA, where grain protein content is generally more consistent. High protein variability in southeastern soft red winter wheat not only makes this grain less desirable to regional millers, but also negatively impacts its value in the export market.

Variability in grain protein can be attributed to differing environments that exist across locations and years with respect to seasonal temperatures, precipitation amounts, and soil type and texture (Gooding and Davis, 1997; Gooding et al., 1997). Variability in grain protein can also be attributed to differences in cultivar genetic potential and to management decisions or cultural practices (Beuerlein et al., 1992; López-Bellido et al., 1998; Miezan et al., 1977; Smith and Gooding, 1996; Vaughan et al., 1990).

Among the most important management practices influencing grain protein content is N fertilizer rate application and timing. To a point, increasing the amount of N applied generally increases grain protein regardless of the type of wheat cultivar or climate (Fowler

et al., 1989; Johnson et al., 1973; Kelley, 1995; López-Bellido et al., 1998; Nelson et al., 1978; Smith and Gooding, 1996; Terman, 1979; Vaughan et al., 1990; Woodard and Bly, 1998). Nitrogen fertilizer rate recommendations in the southeastern USA generally call for N to be applied at growth stage (GS) 25 and/or 30 (Zadoks et al., 1974) with total amounts at these two growth stages not to exceed 136 kg N ha^{-1} (Alley et al., 1994; Scharf et al., 1993; Scharf and Alley, 1993; Weisz et al., 2001; Weisz, 2004). However, it is not unusual for soft red winter wheat producers to apply spring N fertilizer rates that range from as low as 45 kg N ha^{-1} when the price of N is high or the crop appears to have low yield potential, to rates as high as 202 kg N ha^{-1}.

Very little N is applied pre-plant in the southeastern USA, whereas producers from other regions often apply at least half of the total N as pre-plant. These different application strategies exist because of the warm temperatures and high precipitation typical of the southeastern region, which result in denitrification and leaching of pre-plant N fertilizer (Counce et al., 1984; Scharf et al., 1993; Scharf and Alley, 1993). In the southeastern region, the majority of N is typically applied at GS 30 if GS 25 tiller densities are high (above 550 tiller m^{-2}), or in split applications (the first split at GS 25 and the second at GS 30) if GS 25 tiller density is low (Scharf et al., 1993; Scharf and Alley, 1993, Weisz et al., 2001). While this practice optimizes yield (Weisz et al., 2001), it may have an impact on protein variability. Splitting N applications between GS 20 and later stages (e.g., GS 30) can result in an increase in grain protein compared to a single application (Beuerlein et al., 1992; Kelley, 1995; Nass et al., 1976; Stark and Tindall, 1992; Vaughan et al., 1990; Woodard and Bly, 1998). Kelley (1995) and Woodard and Bly (1998) reported that applications of N at GS 30 resulted in consistently higher grain protein concentrations compared to N applied at earlier

growth stages. Beuerlein et al. (1992) confirmed this by reporting that timing of N application had greater impact on grain protein then the amount applied. Clearly, differences in how producers time their N applications could also be contributing to regional grain protein variability.

Given the negative influence grain protein variability has on the marketability of soft red winter wheat in the southeastern USA, a determination of how N management influences grain protein variability is needed. In this light, our primary objectives were to determine how different N fertilizer rates and times of application would affect overall grain protein variability. Additionally, we wanted to compare the proportion of grain protein variability caused by different N treatments to that caused by associated environmental effects. Our secondary objective was to determine if there were N fertilizer recommendations that would minimize regional protein variation.

MATERIALS AND METHODS

Experimental Environments

Experiments were conducted in the North Carolina Piedmont and Coastal Plain regions in 2002 and 2003 to encompass the range of soil and environmental variability that exists annually in the state. Three site-years used in the Piedmont region were located near Salisbury, NC in 2002 and 2003 (P-1, P-2, and P-3, see Table 1) at the Piedmont Research Station. Site-years used in the Coastal Plain region were the Cunningham Research Station located near Kinston, NC in 2002 and 2003 (C-1 and C-2, see Table 1), the Lower Coastal Plain Tobacco Research Station located near Kinston, NC in 2002 (L-1), and the Tidewater Research Station located near Plymouth, NC in 2003 (T-2). The soils at these respective site-years were a Hiwassee clay loam (fine, kaolinitic, thermic Typic Rhodudults), a Lynchburg

sandy loam (fine, loamy, siliceous, thermic, Aeric Paleaquults), a Goldsboro loamy sand (fine, loamy, siliceous, thermic Aquic Paleudults), and a Cape Fear loam (clayey, mixed, thermic, Typic Umbraquults).

Experimental Design

At each site-year a split plot randomized complete block design with five replications was used. The main plot treatment ("N_{25}") consisted of five N rates (0, 34, 68, 102, and 136 kg N ha^{-1}) applied at GS 25. The sub-plot treatment consisted of the same five N rates applied at GS 30 ("N_{30}"). At P-3, the N_{25} rates of 34 and 136 kg N ha^{-1} were lost due to N application problems. The combination of main and sub-plots resulted in 25 different N treatments consisting of different N application rates and times of application. Site-years C-1 and C-2 received a pre-plant N application of approximately 30 kg ha^{-1} as N-P-K:10-10-30, with the source of N unknown. All N_{25} and N_{30} treatments were applied as urea ammonium nitrate (UAN: 30 % N), with the exception of N_{25} at P-1, P-2, and P-3, where ammonium nitrate (NH_4NO_3: 34 % N) was used.

Agronomics

In all site-years, 'Coker 9704' cultivar of soft red winter wheat was planted. Plots at P-1 and P-2 were approximately 1.2 by 3 m, and consisted of rows spaced 190 mm apart. At C-1 and L-1, plots were approximately 2 by 3.6 m, and consisted of rows spaced 178 mm apart. At C-2, plots were approximately 2 by 7.3 m, and consisted of rows spaced 178 mm apart. At P-3 plots were approximately 2 by 6.1 m, and consisted of rows spaced 190 mm apart. At T-2, plots were approximately 2 by 4.3m, and consisted of rows spaced 170 mm apart. Planting dates, seeding rates, lime, K, and P applications followed standard recommendations for soft red winter wheat in North Carolina based on soil tests (Hardy et

al., 2002; Weisz, 2004) with the exception of environments P-3 and T-2, which were planted approximately 28 d late due to poor weather conditions early in the season. All trials followed corn and were conventionally tilled except P-2, where a no-till system was used (Table 1). Weed management was excellent for all site-years except L-1, where weed populations at GS 25 were rated at approximately 22% cover in each plot, higher than average plot weed coverage (5%) elsewhere.

Data Collection

The number of tillers (with a minimum of three leaves) in a 1-m section of row was determined at two random locations in each main plot prior to GS 25 N application. Main plot tiller density was then estimated as the average of these two samples. Sub-plots were harvested with a small plot Massey-Ferguson MF-8 or Gleaner K2 combine (ADCO Corp., Duluth, GA) and yields measured with a Harvest-Master grain gauge (Juniper Systems, Inc., Logan, UT). Yields were adjusted to a moisture content of 135 g kg^{-1}. From each harvested sub-plot, samples of approximately 0.45 to 3.0 kg of grain were taken for grain protein and test weight analysis. Grain samples of approximately 85 g were taken from each sub-plot and were analyzed for grain N concentration using a CHN analyzer (McGeehan and Naylor, 1988) at Waters Agriculture Laboratories (Camilla, GA). Grain N concentrations were converted to grain protein by multiplying by a conversion factor of 5.83 (Kent and Evers, 1994). Test weight was determined on a volume weight basis with a DICKEY-john Grain Analysis computer (model number GA C2000, DICKEY-john Corp., Auburn, IL).

Statistical Analysis

For statistical analysis, environment was defined as a combination of site-year and tillage system. Replications within environments and the error term for main plots were

treated as random effects, while all other sources of variations and their interactions were treated as fixed effects. PROC MIXED was used to test for significant effects caused by environments, N treatments, and their interactions and for covariate analysis using SAS version 8 (SAS Institute Inc., Cary, NC). Least square mean separations were employed for testing differences between and among treatments. Additionally, estimates of the proportion of the total variation associated with each main effect and all interactions were determined by defining all sources of variations as random effects in PROC MIXED and then computing their estimated variance as a percentage of the total model variance (Milus, 1994).

The variance associated with each of the 25 combinations of the N_{25} and N_{30} treatments was further evaluated using three measures of stability. In the first approach we computed the grain protein standard deviation associated with each N treatment. Bartlett's chi-square test for homogeneity was use in PROC GLM with SAS version 8 (SAS Institute Inc., Cary, NC) to test for homogeneity among the 25 N treatment standard deviations across the seven environments.

The second measure of stability was based on Francis and Kannenberg (1978) who developed a stability index using the coefficient of variation (CV) for a genotype's yield across several differing environments, with a low CV and high yield indicating a desirable and stable genotype. This approach was modified in this study to measure the stability of grain protein (instead of yield) from 25 N treatment combinations (instead of genotypes) over seven environments using the CV of the 25 N treatments across seven environments. These were plotted against both total spring N applied and treatment mean grain protein.

A third stability index employed the methods describe by Eberhart and Russell (1966) for estimating a genotype's comparative yield stability across multiple environments. They

regressed an individual genotype's mean yield at an environment against the mean yield of all genotypes at that environment to obtain a regression coefficient. A genotype was considered to be desirable if it had a high yield and a regression coefficient close to one, meaning that it was responsive to favorable environments. This approach was modified using N treatments instead of genotypes, and grain protein instead of yield, with a goal of grain protein stability across environments. For this purpose, we assumed that regression coefficients closer to zero would indicate N treatments more stable across environments. An additional check of stability was a low deviation from the regression (Eberhart and Russell, 1966). To identify the most stable N treatments, we plotted the deviations from the regression against the regression coefficients. PROC MIXED was used to estimate regression coefficients for each N treatment, to determine an F-value to test for homogeneity of regression coefficients, to make a separation of regression coefficients by treatment, and to determine which regression coefficients were not significantly different from zero. For each N treatment, the deviations from the regression were computed using PROC REG in SAS version 8 (SAS Institute Inc., Cary, NC).

Weather Data

Daily mean air temperature and daily total precipitation from GS 30 to harvest at each environment (Table 2) were obtained from the State Climate Office of North Carolina website (http://www.nc-climate.ncsu.edu, verified March 2005). Thirty-year daily mean temperature and precipitation data from GS 30 to harvest were obtained from the same source.

RESULTS

Grain Yield

Environment, N_{25}, N_{30}, and their interactions were all significant for yield with the exception of the three-way interaction (Table 3). The $N_{25} \times N_{30}$ interaction indicated the yield response to N_{30} depended on the level of N_{25}. With higher N_{25}, there was little or no yield response to N_{30} compared to lower N_{25} (Fig. 1A). The two-way interactions with environment and either N_{25} or N_{30} indicated that yield responses to N_{25} or N_{30} depended on environment. At P-1 and P-2 there was little to no yield response to N_{25} (Fig. 1B). At C-2, yield increased and then decreased with increasing N_{25}, while at L-1 and T-2 yield continued to increase as N_{25} rates increased. Similarly, the yield response to N_{30} differed by environment (Fig. 1C) with some environments showing little to no response (e.g. P-3), a yield plateau at higher N_{30} (e.g. C-2), or yields that tended to increase even through the highest N rates (e.g. T-2).

Partitioning of the total yield variance among sources showed that 66.5% of the variability in yield was attributed to environment (Table 3). Only 13.4% of the variation could be attributed to N treatments and their interaction (N_{25}, N_{30}, $N_{25} \times N_{30}$) and less than 6% of the variation could be attributed to the interactions of environment and N treatments ($E \times N_{25}$, $E \times N_{30}$, and $E \times N_{25} \times N_{30}$). This indicated that environment had the strongest influence on yield variability. The highest mean yields were at P-1 and P-2. These two environments also had the highest mean GS 25 tiller densities and a relatively dry spring (Table 2). In contrast, P-3 had the lowest mean yield; the lowest mean GS 25 tiller density, and an extremely wet spring. Across all environments, mean grain yield was positively correlated with mean GS 25 tiller density ($r = 0.90$, $p = 0.01$), indicating that at any given

environment, mean grain yield was related to the number of tillers that had developed by GS 25. Also yield was negatively correlated to total spring precipitation ($r = -0.88$, $p = 0.01$), indicating that wetter environments had lower yields.

Test Weight

Environment, N_{25}, N_{30}, and their interactions were all significant for test weight with the exception of the environment × N_{25} interaction (Table 3 and Fig. 2). The three-way interaction indicated that test weight response to any treatment factor depended on the levels of the other treatment factors (Fig. 2). However, partitioning the sources of variation showed that only approximately 2.0% of the variation could be attributed to N treatments and their interactions with environment, whereas 90.5% of the variability in test weight was attributed to environment (Table 3). A test weight of 747 kg m^{-3} or above is considered representative of good soft wheat grain quality (USDA/ARS Soft Wheat Quality Lab., 2004), and producers can be financially penalized when test weights are below this standard. Mean test weights at C-2, P-3, and T-2 were below this standard; these sites had cool and extremely wet springs (total precipitation above the 30-yr mean, Table 2). Mean test weights were not correlated with mean yield or grain protein. The highest mean test weights were found at C-1 and L-1 where total spring precipitation levels were slightly below the 30-yr mean (Table 2).

Grain Protein

Environment, N_{25}, and N_{30} and all their interactions were significant for grain protein (Table 3), indicating that grain protein response to a treatment factor depended on the levels of both the other treatment factors as seen in Fig. 3. At all environments there was a decrease in grain protein at low N_{25} and N_{30} combinations, except at P-1 and P-2. This is a predictable response seen in grain protein accumulation with low N environments (Gooding and Davis,

1997), while this response was not seen at P-1 and P-2, indicating a possibility of high soil residual N present to buffer the decrease in grain protein seen at low N environments. Only at T-2 was there a definite plateau in grain protein response to high N_{25} and N_{30} combinations, while at L-1, P-1, and P-2 there only appears to be a plateau in grain protein response to high total N rates (Fig. 3).

Unlike yield and test weight, only 22.8% of the variability in grain protein was attributed to environment (Table 3) and there was no direct relationship between grain protein and daily average temperature and daily total precipitation that occurred from GS 30 to grain fill (Table 2). Also grain protein was not related to yield, test weight, or tiller density. Instead, the majority of the variability, 52.6%, was attributed to N treatments (N_{25}, N_{30}, $N_{25} \times N_{30}$, Table 3) with an additional 7.3% attributed to the interactions of N treatments and environment ($E \times N_{25}$, $E \times N_{30}$, $E \times N_{25} \times N_{30}$). The contrast between grain protein and test weight regarding the proportion of response attributable to N and to environment is clearly apparent in Fig. 3 (grain protein) versus Fig.2 (test weight).

Total Spring Nitrogen Applied

Across all N_{25} and N_{30} treatment combinations the total amount of spring N applied ranged from 0 to 272 kg ha^{-1} and the mean grain protein associated with these treatments ranged from 104.3 to 138.8 g kg^{-1} (Table 4). The minimum and maximum grain protein values across all environments were 84.5 and 158 g kg^{-1} respectively. There was a strong quadratic relationship ($r^2 = 0.96$, Fig. 4) between the total amount of spring N applied and the treatment mean grain protein.

Higher spring N rates not only resulted in higher mean grain protein (Fig. 4), but also resulted in higher grain protein variability. For all N treatments, the grain protein standard

deviations and means were correlated ($r = 0.81$, $p < 0.05$, Fig. 5). Bartlett's test for homogeneity indicated that there were statistically significant differences among the treatment standard deviations ($\chi^2 = 12.23$, $p = 0.98$).

For all N treatments, the grain protein CV and means were correlated ($r = 0.57$, $p < 0.05$, Fig. 6A). Additionally, the CV was related to the total spring N applied ($r = 0.59$, $p < 0.05$, Fig. 6B). The relationships in Fig. 4, 5, and 6 demonstrated that as total spring N increased, grain protein also increased but at the cost of lower stability across environments as measured by either the protein standard deviation or CV.

Across the seven environments used in this study, the mean grain protein ranged from 103 to 138 g kg-1. However, in the ANOVA for grain protein, all interaction terms involving environment and N treatment were significant (Table 3). An approach outlined by Eberhart and Russell (1969) was followed to elucidate the environment and N treatment interactions and how they related to protein stability across the environments tested. Figure 7 is an example of how this approach was implemented using four of the 25 N treatments. For each N treatment, mean grain protein at a given environment (Fig. 7, Y-axis) was regressed against the grand protein mean of all 25 treatments at that environment (Fig. 7, X-axis). The regression coefficient and deviations from the regression were then calculated (Table 4). An F-test for differences among these regression coefficients was significant ($p = 0.0001$, Table 4). For the N25-N30 treatments of 0-0, 34-0, and 34-34 kg N ha-1, the regression coefficients (0.35, 0.24, and 0.50 respectively) were not significantly different from zero (Table 4). Consequently, for these low N treatments, grain protein response was unrelated to the environments tested resulting in a low regression coefficient, fitting our definition of stable N treatments. In contrast, the 204 kg N ha-1 treatment had a regression coefficient of 1.46,

indicating a treatment that was highly responsive to the environments used and considered unstable in this study. The ideal stable N treatment would have a low regression coefficient and low deviation from the regression. To identify which N treatments best met these criteria, treatment deviations from the regression were plotted against their associated regression coefficients (Fig. 8) and this graph was then divided into quadrants. Treatments with the lowest deviations from the regression and the lowest regression coefficients are by definition in the lower left quadrant of such a figure. Using this approach, five N25 - N30 treatment combinations were identified as being potentially ideal for protein stability; 34-0, 34-34, 0-68, 68-0, and 34-68 kg N ha-1.

The N treatment regression coefficients were positively correlated with treatment mean grain protein($r = 0.83$, $p < 0.05$, Fig. 9A) and with the total amount of N applied ($r = 0.87$, $p < 0.05$, Fig. 9B). As the total amount of spring N applied increased, the mean grain protein increased (Fig. 3), but so did the treatment regression coefficient indicating that grain protein stability across environments decreased. This is consistent with Fig. 5 and 6. In fact the treatment regression coefficients were correlated with both the treatment standard deviations ($r = 0.83$, $p < 0.05$) and CV's ($r = 0.77$, $p < 0.05$). Apparently, grain protein was unresponsive to the environment at low N rates, and therefore relatively stable. At high N rates, however, there was a large difference between the protein content produced at environments with high protein potential compared to that produced at low protein potential environments and this caused the high standard deviations and CV's associated with high N rates. These data indicate that when high spring N rates are widely used, high protein grain may be produced at some locations, but those N rates will also result in substantial protein variability across environments.

Nitrogen Timing

Some of the variability in the data shown in Fig. 9A could be attributed to the timing of spring N applications. To illustrate this timing effect, we analyzed two subsets of the data. The first subset consisted of the five "early" treatments that received at least 80% of the total spring N at GS 25. These data were contrasted with the second subset consisting of the five "late" treatments that received at least 80% of the total spring N at GS 30 (Fig. 10). Application timing ("early" or "late") was statistically significant as a class variable, and both linear and quadratic terms for treatment mean grain protein were statistically significant co-variables (Table 5). On average, at any given mean protein level, applying N at GS 25 resulted in a regression coefficient that was 0.36 lower compared to applying N at GS 30, indicating more agronomic stable N treatments (Table 5, Fig. 10).

DISCUSSION

Results from partitioning the total ANOVA model variability indicated that environment contributed the most variability to yield. Many environmental factors including timeliness of planting (Frederick and Marshall, 1985; Knapp and Knapp, 1978), reduced winter growth and/or tillering (Weisz et al., 2001), and weed competition (Gooding and Davis, 1997) can contribute to yield variability. Site-years P-3 and T-2 had the highest spring precipitation and the lowest yield. Wheat was planted late at P-3 and T-2, and late planting coupled with the cool temperatures resulted in low tiller densities that may explain the lower yields. High weed populations at L-1 may also have negatively affected the tiller density and yield at that location.

The majority of the variability in test weight was also attributed to environment. Environments P-3 and T-2 had the lowest mean test weights and the highest spring

precipitation. This was consistent with reduced test weights being associated with environments that had an increased chance of grain wetting during the formation or filling process. Schuler et al. (1994), Weisz and Bowman (1999), and Yamazaki and Briggle (1969) reported that environment contributed the most to variability in test weight of soft red winter wheat grown in temperate climates.

Our primary objective was to determine how different N fertilizer rates might affect grain protein variability. The majority of protein variability (52.6%) was attributed to N treatments. Increases in spring-applied N increased grain protein in our study (Fig. 4), and this same relationship has been found by many other researchers in differing environments regardless of the type of wheat or cultivar (Fowler et al. 1989; Johnson et al., 1973; Kelley 1995; López-Bellido et al., 1998; Nelson et al., 1978; Smith and Gooding, 1996; Terman, 1979; Vaughan et al., 1990; Woodard and Bly, 1998). Clearly, if producers within a region use different N rates, that fact alone will result in variability in soft red winter wheat grain protein content.

Both grain protein level and variability among treatments were increased with increased N applications (Figs. 4, 5, 6, and 7). Fowler et al. (1989) reported that the final grain protein content was determined by an interaction of environmental factors and N rate. For grain protein in our study, all the interaction terms that included environment and N treatment were significant (Table 3), and the nature of these interactions is illustrated in Fig. 3. Low N rates resulted in low grain protein levels that were relatively stable across environments (Figs. 6 and 9). At environments with low mean protein, higher N rates had a relatively small effect on protein. However, high N rates in responsive environments resulted

in large increases in protein. Consequently, if high N application rates are used throughout a region this could result in a wide range of grain protein levels.

Beuerlein et al. (1992), Kelley (1995), and Woodard and Bly (1998) observed that applying N late in the spring (approximately GS 30) could increase grain protein. We did not see a significant difference in mean grain protein between applications made at GS 25 and GS 30 (Table 4). However, there was a difference in protein stability between treatments that applied the majority of spring N at GS 25 instead of GS 30 (Fig. 10). There was a trend for the regression coefficients associated with the five early N treatments to be lower than those associated with the five late treatments (Table 5 and Fig. 10). When the early and late treatments were pooled into two groups, this trend was statistically significant (Table 5, Fig. 10) and this is perhaps the most interesting and important of our findings. At a given grain protein content (Fig. 10, X-axis), applying the majority of spring N at GS 25 resulted in a lower regression coefficient (Fig. 10, Y-axis), and consequently a protein content that was less sensitive to environmental differences and thus would be more regionally stable.

Our secondary objective was to determine if there were N fertilizer recommendations that might result in minimizing regional grain protein variation especially for soft red winter wheat intended for the baking industry. Some of the 25 treatments explored in this study did result in lower protein variability. Based on the criteria of low deviations from the regression and a low regression coefficient, five $N_{25} - N_{30}$ treatment combinations (34-0, 34-34, 0-68, 68-0, and 34-68 kg N ha^{-1}) were identified as the most stable (Fig. 8). These treatment combinations also had relatively low standard deviations and CV's (Table 4). While these N rates might be ideal for stabilizing regional grain protein, all but the 34-68 kg N ha^{-1} are too low to optimize crop yield and consequently producer profit in most cases.

The data herein point out some important generalized recommendations that might lead to lower regional variability. Based on Fig. 4, the first recommendation might be to reduce the range (45 to 202 kg N ha^{-1}) in N fertilizer rates used across the region. One of the biggest contributors to high protein variability found in this study was high N fertilizer rates. Consequently, the second recommendation would be to avoid over application of N beyond what is required to optimize yield and economic return. Current recommendations in North Carolina are that spring N rates not exceed 136 kg ha^{-1} (Weisz, 2004). Applying 102 to 136 kg N ha^{-1} resulted in greater grain protein biological and agronomic stability than 170 to 272 kg N ha^{-1} as indicated by all the stability indexes. Limiting N application rates to 102 to 136 kg N ha^{-1} would reduce the regional protein variability compared to the range in rates currently used. Scharf and Alley (1993) proposed using a GS 30 tissue test to optimize spring N fertilizer rates in the southeastern USA. This technique might be a good method for optimizing wheat yields and minimizing the use of excessively high or low N fertilizer rates. The third recommendation to reduce protein variability would be to apply spring N at GS 25 and avoid waiting until later in the season. Four of the five treatment combinations identified as grain protein stable in Fig 8 have at least 50% of the total spring N applied at GS 25, and "early" N applications increased stability compared to "late" ones (Fig. 8). In essence, regional interest would be served well by reducing the range of N rates applied, realistically applying N based on yield potentials or on an in-season tissue test, and avoiding later N applications.

REFERENCES

Alley, M.M., P.C. Scharf, D.E. Brann, W.E. Baethgen, and J.L. Hamons. 1994. Nitrogen management for winter wheat: principles and recommendations. Virginia Cooperative Extension. Crop & soil Environmental Sciences. Publication 424-026.

Beuerlein, J.E., E.S. Oplinger, and D. Reicosky. 1992. Mineral content of soft winter wheat as influenced by nitrogen fertilization and management. Commun. Soil Sci. Plant Anal. 23: 455-467.

Bowman, D.T. 2002. North Carolina measured crop performance: small grains 2002. Crop Science Research Report No. 200. North Carolina State University, Raleigh, NC.

Bowman, D.T. 2003. North Carolina measured crop performance: small grains 2003. Crop Science Research Report No. 207. North Carolina State University, Raleigh, NC.

Bowman, D.T. 2004. North Carolina measured crop performance: small grains 2004. Crop Science Research Report No. 211. North Carolina State University, Raleigh, NC.

Counce, P.A., B.R. Wells, J.R. Davis, and A.M. Simpson. 1984. Response of wheat to N-source, N-rate and nitrification inhibitors. Arkansas Farm Res. 33:2.

Eberhart, S.A., and W.A. Russell. 1966. Stability parameters for comparing varieties. Crop Sci. 6:36-40.

Fowler, D.B., J. Brydon, and R.J. Baker. 1989. Nitrogen Fertilization of no-till winter wheat and rye. II. Influence on grain protein. Agron. J. 81:72-77.

Francis, T.R., and L.W. Kannenberg. 1978. Yield stability studies in short-season maize. I. A descriptive method for grouping genotypes. Can. J. Plant Sci. 58: 1029-1034.

Frederick, J.R., and H.G. Marshall. 1985. Grain yield and yield components of soft red winter wheat as affected by management practices. Agron. J. 77:495-499.

Gooding, M.J., and W.P. Davis. 1997. Wheat production and utilization: systems quality and the environment. Cab International. Wallingford. UK.

Gooding, M.J., G. Smith, W.P. Davis, and P.S. Kettlewell. 1997. The use of residual maximum likelihood to model grain quality characters of wheat with variety, climate and nitrogen fertilizer effects. J. Agric. Sci. 128:135-142.

Hardy, D.H., D.L. Osmond, and A. Wossink. 2002. an overview of nutrient management with economic considerations. Publication No. AG-565. North Carolina Coop. Ext. Ser., North Carolina State Univ., Raleigh, NC.

Johnson, V.A., A.F. Dreier, and P.H. Grabouski. 1973. Yield and protein responses to nitrogen fertilizer of two winter wheat varieties differing in inherent protein content of their grain. Agron. J. 65:259-263.

Kelley, K.W. 1995. Rate and timing of nitrogen application for wheat following different crops. J. Prod. Agric. 8: 339-345.

Kent, N.L., and A.D. Evers. 1994. Kent's technology of cereals. Pergamon Press, Oxford.

Knapp, W.R., and J.S. Knapp. 1978. Response of winter wheat to date of planting and fall fertilization. Agron. J. 70:1048-1053

.

López-Bellido, L., M. Fuentes, J.E. Castillo, and F.J. López-Garrido. 1998. Effects of tillage, crop rotation and nitrogen fertilization on wheat grain quality grown under rain fed Mediterranean conditions. Field Crop Res. 57: 265-276.

McGeehan, S.L., and D.V. Naylor. 1988. Automated instrumental analysis of carbon and nitrogen in plant and soil samples. Comm. Soil Sci. Plant Anal. 19:493-505.

Miezan, K., E.G. Heyne, and K.F. Finney. 1977. Genetic and environmental effects on the grain protein content in wheat. Crop Sci. 17: 591-593.

Milus, E.A. 1994. Effect of foliar fungicides on disease control, yield and test weight of soft red winter wheat. Crop Prot. 13:291-295.

Nass, H.G., J.A. MacLeod, and M. Suzuki. 1976. Effects of nitrogen application on yield, plant characters, and N levels in grain of six spring wheat cultivars. Crop Sci. 16: 877-879.

Nelson, L.R., C.T. Young, and M.J. Bitzer. 1978. Yield and protein quality of five soft red winter wheat cultivars as affected by nitrogen fertilization rate. Commun. Soil Sci. Plant Anal. 9:415-428.

Scharf, P.C., and M.M. Alley. 1993. Spring nitrogen on winter wheat: II. A flexible multicomponent rate recommendation system. Agron. J. 85:1186-1192.

Scharf. P.C., M.M. Alley, and Y. Z. Lei. 1993. Spring nitrogen on winter wheat: I. Farmer-field validation of tissue test-based rate recommendations. Agron. J. 85:1181-1186.

Schuler, S.F., R.K. Bacon, and E.E. Gbur. 1994. Kernel and spike character influence on test weight of soft red winter wheat. Crop Sci. 34:1309-1313.

Smith, G.P., and M.J. Gooding. 1996. Relationship of wheat quality with climate and nitrogen application in regions of England (1974-1993). Ann. Appl. Biol. 129: 97-108.

Stark, J.C., and T.A. Tindall. 1992. Timing split applications of nitrogen for irrigated hard red winter wheat. J. Prod. Agric. 5:221-226.

State Climate Office of North Carolina. 2004. [Online]. Available at www.nc-climate.ncsu.edu. (accessed Aug. 2004; verified March 2005). Raleigh, NC.

Terman, G.L. 1979. Yields and protein content of wheat grain as affected by cultivar, N, and environmental growth factors. Agron. J. 71: 437-440.

USDA/ARS Soft Wheat Quality Laboratories. 2004. Soft wheat quality goals. Available at http://www.oardc.ohio-state.edu/swql. (accessed Aug. 2004; verified March 2005). Wooster, OH.

Vaughan, B., D.G. Westfall, and K.A. Barbarick. 1990. Nitrogen rate and timing effects on winter wheat grain yield, grain protein, and economics. J. Prod. Agric. 3:324-328.

Weisz, R. 2004. Small grain production guide 2004-2005. Pub. Num. 580. North Carolina Cooperative Extension Service, Raleigh, NC. AG-580.

Weisz, R., and D.T. Bowman. 1999. Influence of tillage systems on soft red winter wheat cultivar selection. J. Prod. Agric. 12:415-418.

Weisz, R., C.R. Crozier, and R.W. Heiniger. 2001. Optimizing nitrogen application timing in no-till soft red winter wheat. Agron. J. 93:435-442.

Woodard, H.J., and A. Bly. 1998. Relationship of nitrogen management to winter wheat yield and grain protein in South Dakota. J. Plant. Nutr. 21:217-233.

Yamazaki, W.T., and L.W. Briggle. 1969. Components of test weight in soft wheat. Crop Sci. 9:457-459.

Zadoks, J.C., T.T. Chang, and C.F. Zonzak. 1974. A decimal code for the growth stages of cereals. Weed. Res. 14:415-421.

Table 1. Location, year, tillage system, and environment identification for the seven environments used in the study of soft red winter wheat protein variability in North Carolina.

Location	Year	Tillage system	Environment identification
Cunningham Research Station (Kinston)	2001	Conventional	C-1
Lower Coastal Plains Research Station (Kinston)	2001	Conventional	L-1
Piedmont Research Station (Salisbury)	2001	Conventional	P-1
Piedmont Research Station (Salisbury)	2001	No-till	P-2
Cunningham Research Station (Kinston)	2002	Conventional	C-2
Piedmont Research Station (Salisbury, NC)	2002	Conventional	P-3
Tidewater Research Station (Plymouth, NC)	2002	Conventional	T-2

Table 2. Overall environmental means for yield, test weight, grain protein, tiller density, mean daily temperature and daily total precipitation from growth stage (GS) 30 to harvest (i.e., "Experiment" = "Exp."). The mean values of daily temperature and daily total precipitation for the preceding 30 yr are also shown.

Environment	Yield	Test weight	Grain protein	Tiller density	Mean daily temperature		Total precipitation	
					Exp.	30-yr avg	Exp.	30-yr avg
	----Mg ha^{-1} ----	--- kg m^{-3}---	-----g kg^{-1} ----	----- m^{-2} -----	---------- ºC ----------		---------- cm ---------	
C-1	4.90c†	794a	113d	527d	15.3	16.6	26.6	28.7
L-1	4.30d	785b	119c	475e	15.3	16.9	26.6	30.2
P-1	6.10a	753d	125b	701b	16.4	17.1	11.2	26.8
P-2	6.10a	766c	127a	882a	16.4	17.2	11.2	27.5
C-2	5.40b	740e	120c	605c	16.8	19.1	34.5	23.1
P-3	2.90e	709g	125b	166g	16.2	18.9	55.9	27.0
T-2	4.40d	733f	108e	202f	18.0	19.4	44.6	32.6

† Means within a column followed by the same letter are not significantly different at $p = 0.05$.

Table 3. ANOVA for soft red winter wheat grain yield, test weight, and protein across seven environments including N treatments applied at growth stages (GS) 25 (N_{25}) and 30 (N_{30}). Variability is the percentage of total model variability for each main effect and interaction.

Source of variation†	df	Yield	Variability‡	Test weight	Variability‡	Grain protein	Variability‡
			----- %-----		-----%-----		-----%-----
Environment (E)	6	***	66.5	***	90.5	***	22.8
N_{25}	4	***	1.9	**	0.1	***	33.6
N_{30}	4	***	4.5	**	< 0.1	***	18.2
$N_{25} \times N_{30}$	16	***	7.0	***	0.5	***	0.8
$E \times N_{25}$	24	***	3.4	NS	0.1	**	2.1
$E \times N_{30}$	24	***	1.7	***	1.0	***	4.1
$E \times N_{25} \times N_{30}$	88	NS	0.2	*	0.3	**	1.1
Residual	480		12.3		4.2		9.9

***, **, and * significance at $p = 0.001$, 0.01, and 0.05 and NS is not significant.

† Error terms are not listed.

‡ Calculated following Milus (1994).

Table 4. Nitrogen applied at growth stage 25 (N_{25}) and/or growth stage 30 (N_{30}) and the resulting mean treatment grain protein, standard deviation, Bartlett's χ^2, coefficient of variation (CV), the regression coefficients, plus deviations from the regression across seven environments for soft red winter wheat grown in North Carolina.

Total spring N applied	N_{25} applied	N_{30} applied	Treatment mean grain protein	Standard deviation	CV	Regression coefficient	Deviations from regression
--- kg ha^{-1}---	--- kg ha^{-1}---	--- kg ha^{-1}---	---- g kg^{-1} ----	--- g kg^{-1} --	---- % ---		
0	0	0	106.8ab‡	7.01	6.6	0.35†a	0.55
34	0	34	104.3a	8.63	8.2	0.58 abc	0.74
34	34	0	106.5ab	5.28	4.9	0.24†a	0.30
68	0	68	107.8ab	6.28	5.8	0.75 bcd	0.17
68	34	34	107.9ab	5.20	4.4	0.50†ab	0.17
68	68	0	110.1 bc	4.84	4.4	0.53 ab	0.12
102	0	102	114.0 cde	8.90	7.8	1.17 efghi	0.14
102	34	68	109.3abc	7.48	6.8	0.86 bcde	0.26
102	68	34	113.4 cd	8.10	7.1	1.23 efghi	0.04
102	102	0	116.3 def	7.92	6.8	1.02 defg	0.16

Table 4. (continued)

136	0	136	117.7	def	8.95	7.6	1.25 efghi	0.10
136	34	102	117.2	def	8.22	8.1	1.20 efghi	0.08
136	68	68	118.5	ef	7.92	6.7	1.13 defghi	0.01
136	102	34	119.9	fg	9.51	7.9	1.34 fghi	0.01
136	136	0	120.0	fg	7.66	6.4	1.02 cdefg	0.08
170	34	136	121.0	fgh	9.76	8.1	1.34 fghi	0.11
170	68	102	123.8	gh	9.33	7.6	1.18 efghi	0.16
170	102	68	125.3	hi	8.63	6.9	1.12 defgh	0.16
170	136	34	125.8	hij	7.61	6.0	0.97 bcdef	0.24
204	68	136	128.8	ijk	11.35	8.8	1.35 fghi	0.40
204	102	102	132.3	kl	11.59	8.8	1.46 ghi	0.43
204	136	68	130.5	jkl	9.35	7.2	1.21 efghi	0.20
238	102	136	133.3	kl	11.11	8.4	1.54 i	0.11
238	136	102	134.8	lm	11.06	8.2	1.47 hi	0.13

Table 4. (continued)

272	136	136	138.8	m	10.82	7.8	1.46	hi	0.18
					$\chi^2 = 12.23^{\S}$		F-value = 8.94***		

***, Significant at $p = 0.001$ level.
[†]Regression coefficients not significantly different from zero.
[‡]Treatment mean grain protein and regression coefficients within N treatment followed by the same letter are not significantly different at $p = 0.05$ and $p = 0.10$ level, respectively.
[§] Non-significance ($p = 0.98$).

Table 5. Analysis of covariance to determine the significance of N application timing (early or late) as a factor affecting the stability analysis regression coefficient using the treatment mean grain protein (g kg^{-1}) as a covariate in a soft red winter wheat study.

Sources of Variation	df	Model significance	Model	
			Early	Late
N application time	1	**		
Mean protein (quadratic)	6	**	$y = 0.63x - 0.002x^2 - 37.80$	$Y = 0.63x - 0.002x^2 - 37.44$

** Significant at $p = 0.01$.

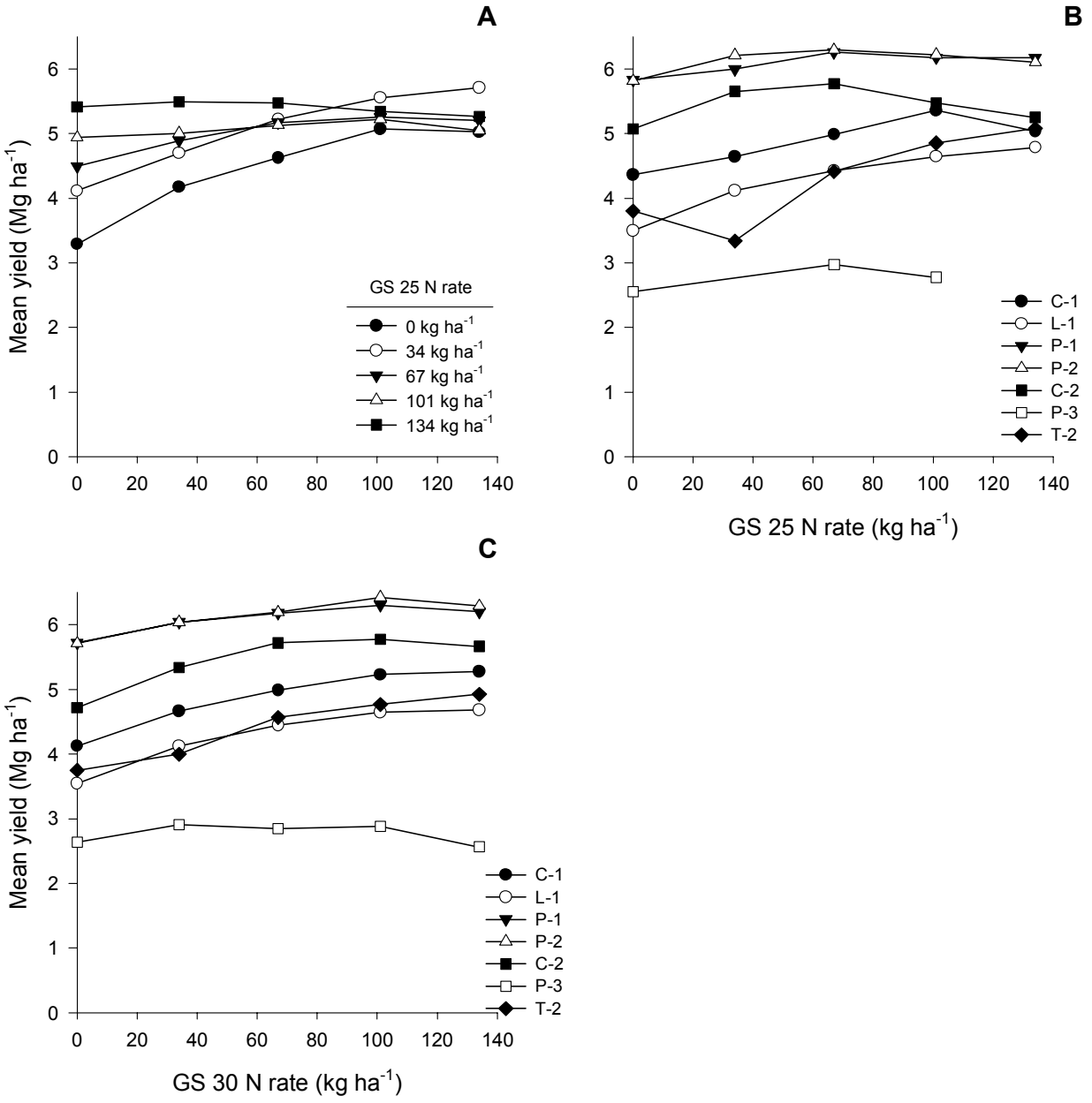


Fig. 1. Yield response of soft red winter wheat to N applications at seven environments in North Carolina displaying the two-way interactions from the ANOVA in Table 3: A) N treatments applied at GS 25 (N_{25}) × N treatments applied at GS 30 (N_{30}), B) environment (E) × N_{25}, and C) E × N_{30}.

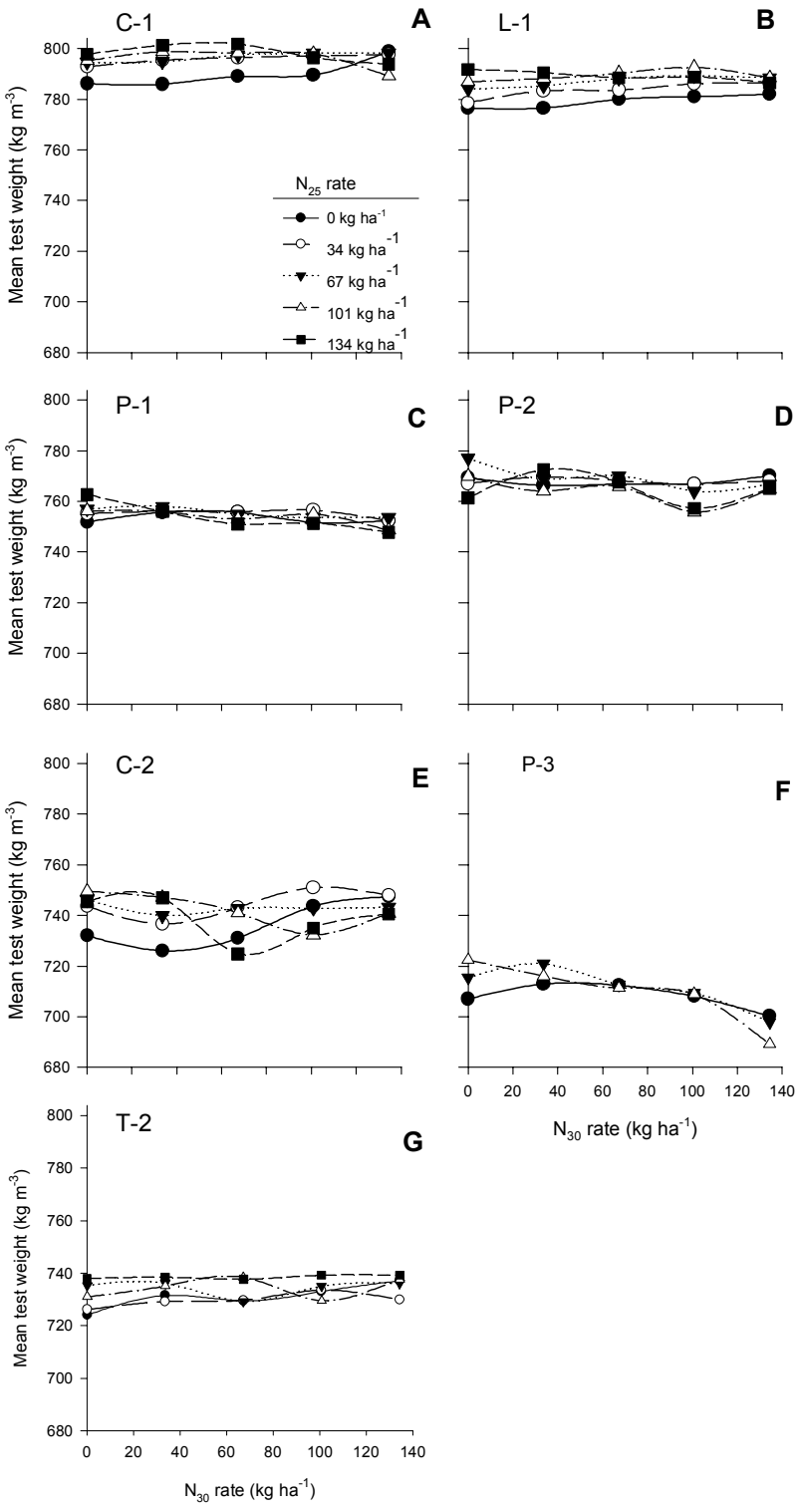


Fig. 2. Test weight response of soft red winter wheat to N applications at growth stage (GS) 25 (N_{25}) and GS 30 (N_{30}) at each of seven different environments in North Carolina: A) Cunningham Research Station 2002, C-1; B) Lower Coastal Plains Research Station 2002, L-1; C) Piedmont Research Station 2002, P-1; D) Piedmont Research Station, no-till 2002, P-2;

E) Cunningham Research Station 2003, C-2; F) Piedmont Research Station 2003, P-3; G) Tidewater Research Station 2003, T-2.

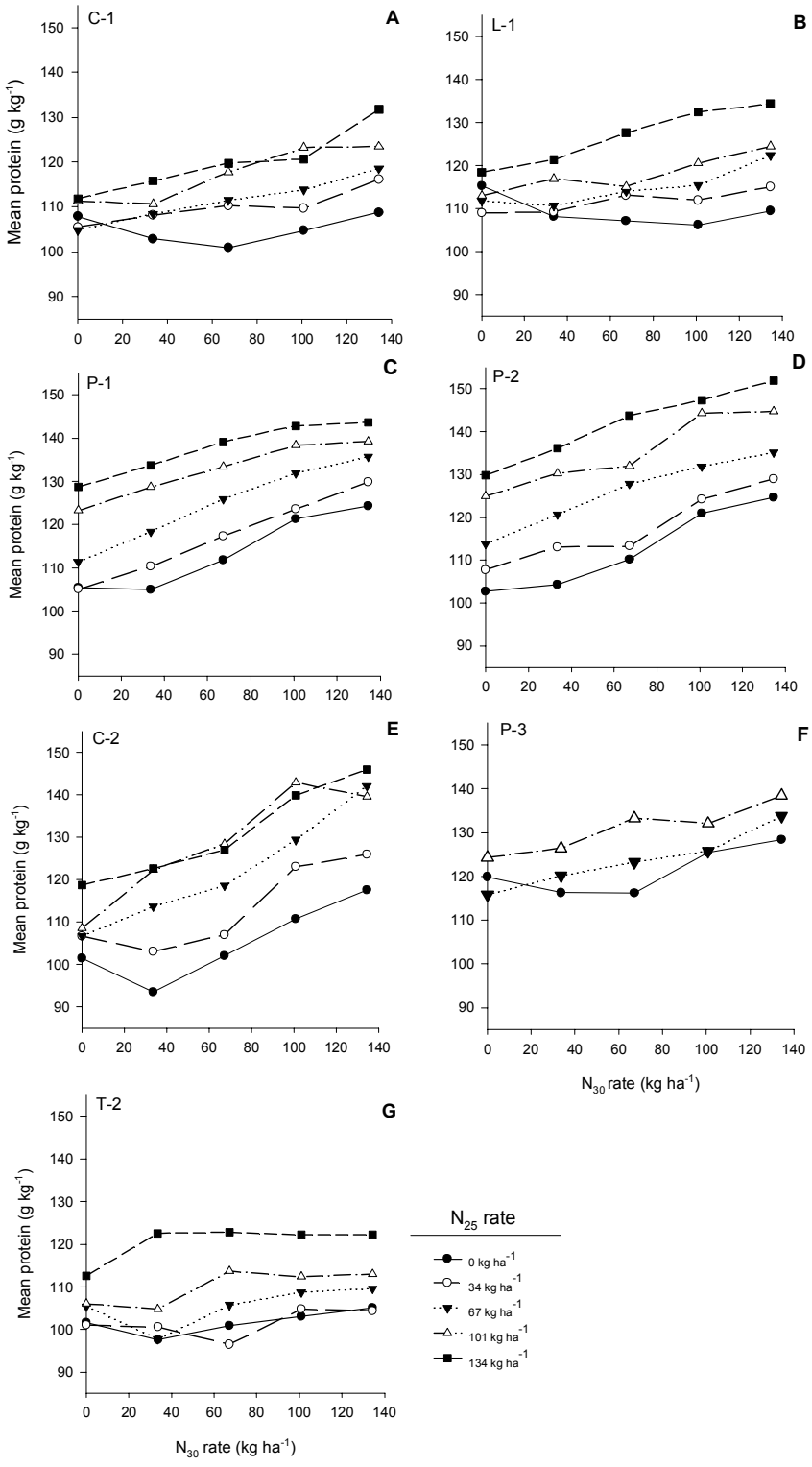


Fig. 3. Grain protein response of soft red winter wheat to N applications at growth stage (GS) 25 (N_{25}) and GS 30 (N_{30}) at each of seven different environments in North Carolina: A) Cunningham Research Station 2002, C-1; B) Lower Coastal Plains Research Station 2002, L-

1; C) Piedmont Research Station 2002, P-1; D) Piedmont Research Station, no-till 2002, P-2; E) Cunningham Research Station 2003, C-2; F) Piedmont Research Station 2003, P-3; G) Tidewater Research Station 2003, T-2.

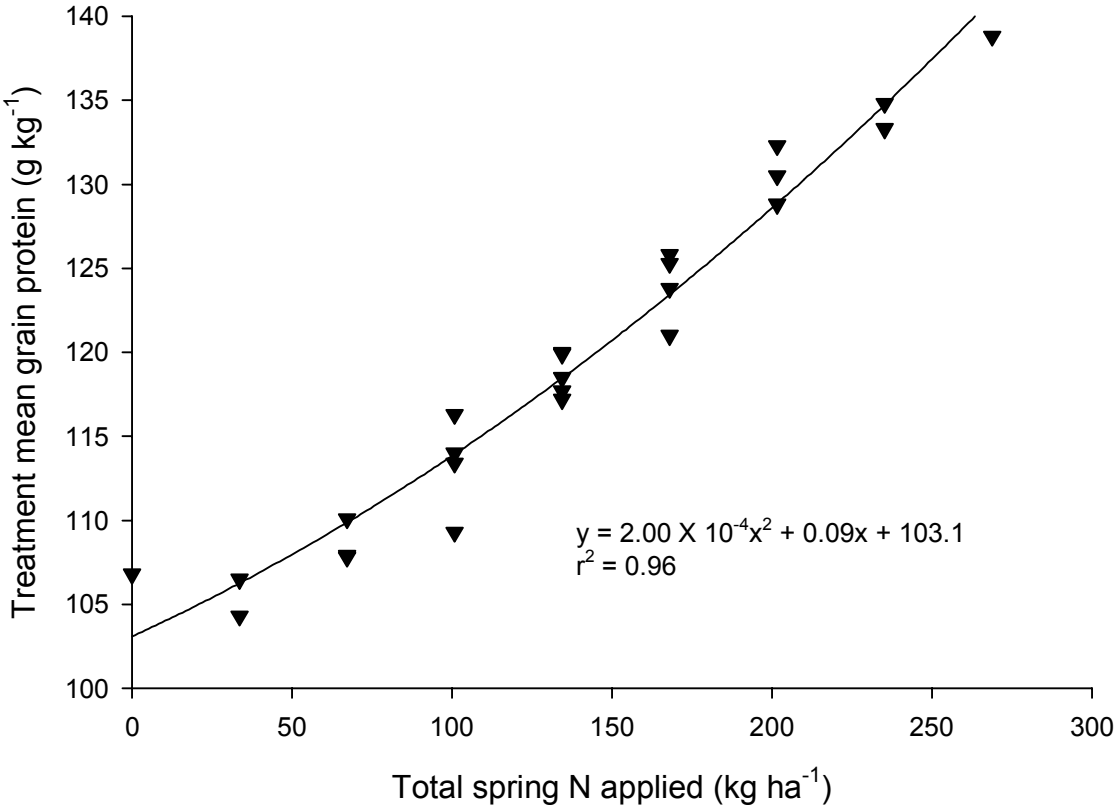


Fig. 4. The relationship of mean soft red winter wheat grain protein (g kg^{-1}) to total spring N applied (kg ha^{-1}) for 25 N treatments in North Carolina. Symbols represent N treatment mean values across environments (a combination of year, location, and tillage system).

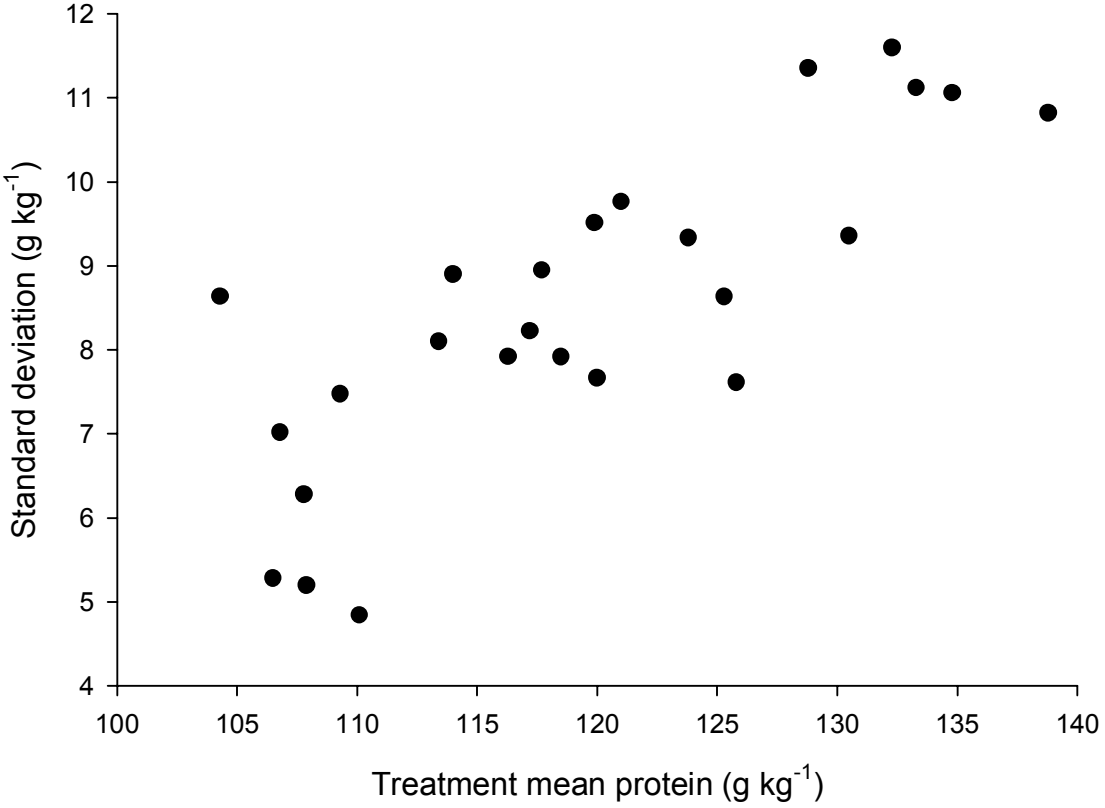


Fig. 5. The relationship of soft red winter wheat grain protein standard deviation (g kg^{-1}) to mean grain protein (g kg^{-1}) for 25 N treatments in North Carolina. Symbols represent N treatment mean values at each environment (a combination of year, location, and tillage system).

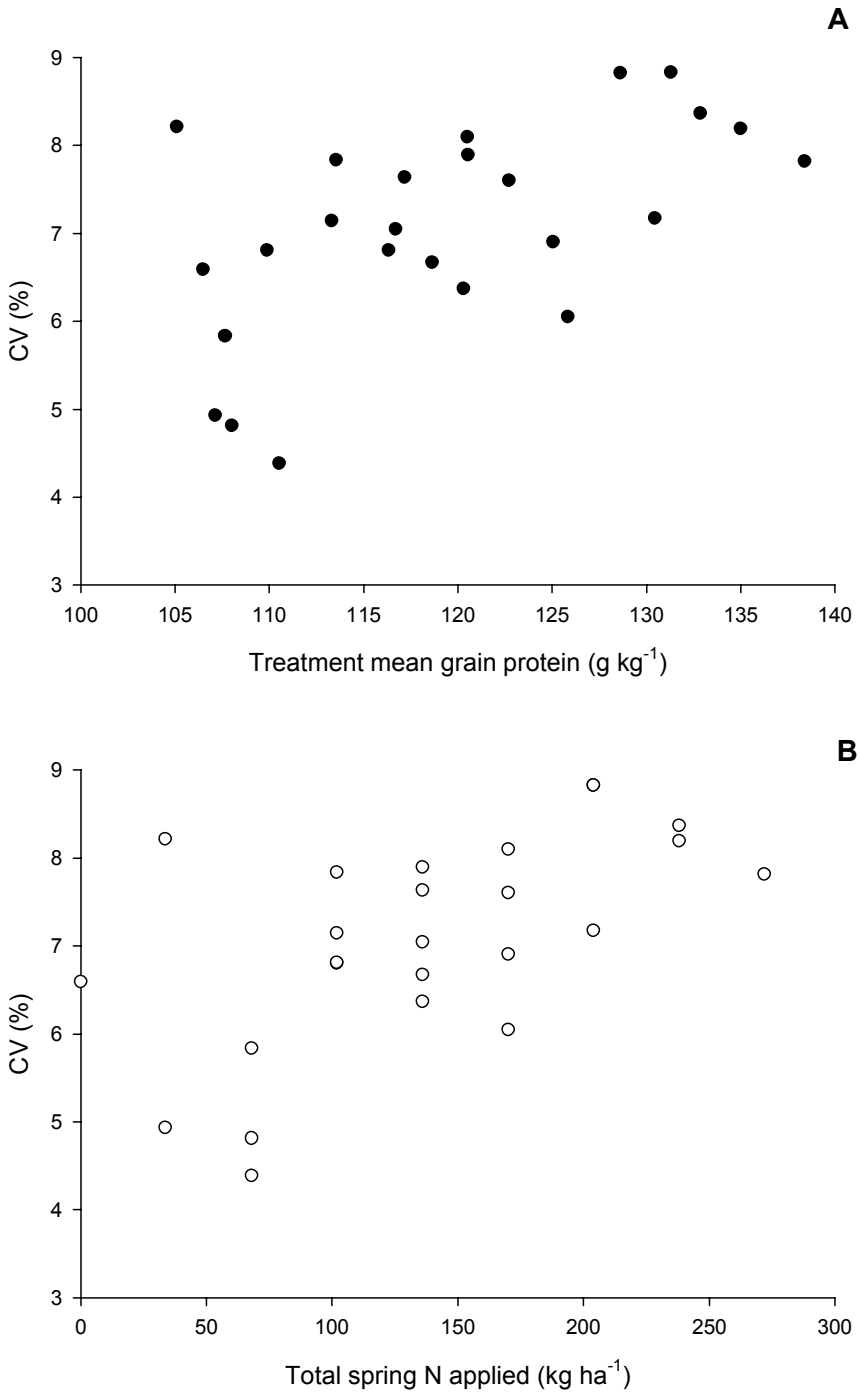


Fig. 6. The relationship of soft red winter wheat grain protein coefficients of variation (CV) of 25 N treatments to A) treatment mean grain protein (g kg^{-1}) and B) total spring N applied in North Carolina. Solid circles represent N treatment mean values across environments (a combination of year, location, and tillage system). Open circles represent treatment total N rates.

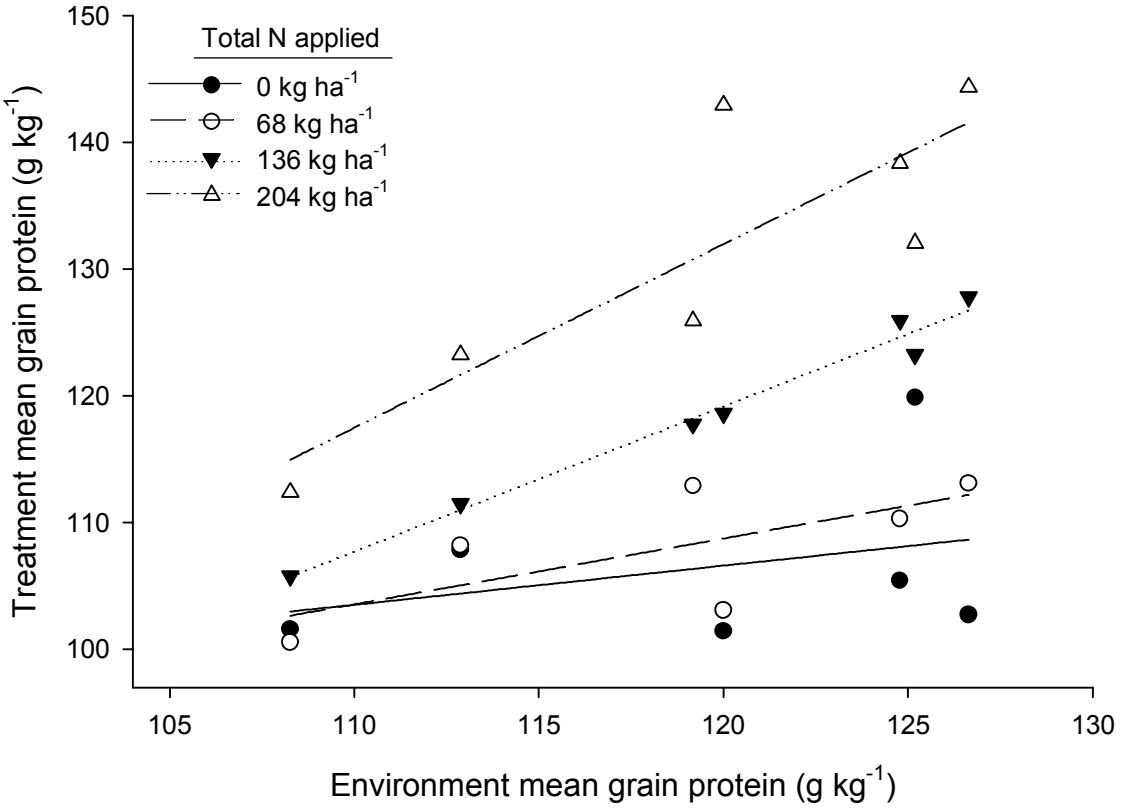


Fig. 7. Linear regressions of N treatment mean grain protein (g kg^{-1}) at a given environment against the overall mean grain protein (g kg^{-1}) for that environment in North Carolina. Four N treatments represented, with half the N applied at growth stage (GS) 25 and the second half at GS 30. Symbols represent each individual N treatment mean grain protein at one of seven environments.

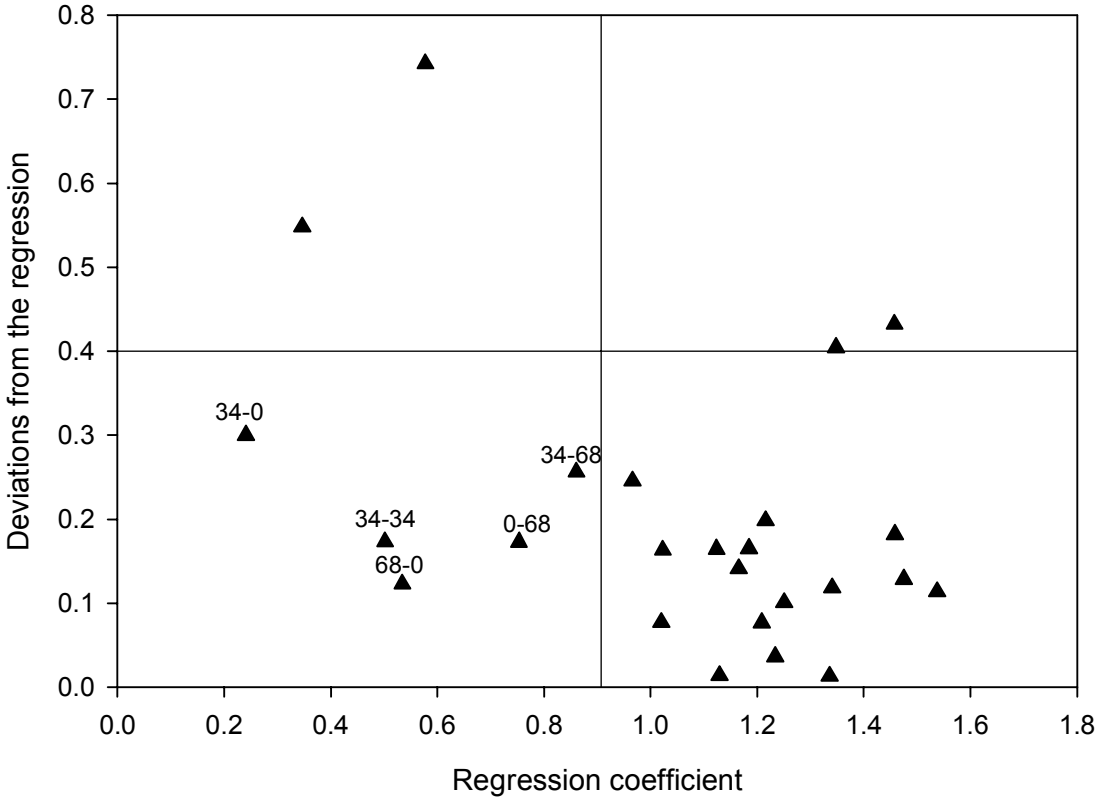


Fig. 8. The relationship of deviations from the regression coefficients to regression coefficients reported in Table 4, in a soft red winter wheat study in North Carolina. Numbers above symbols represent the combination of amount of N applied and time of application with the first number equaling the N amount applied (kg ha^{-1}) at growth stage (GS) 25 (N_{25}) and the second number is N amount applied (kg ha^{-1}) at GS 30 (N_{30}) for selected symbols.

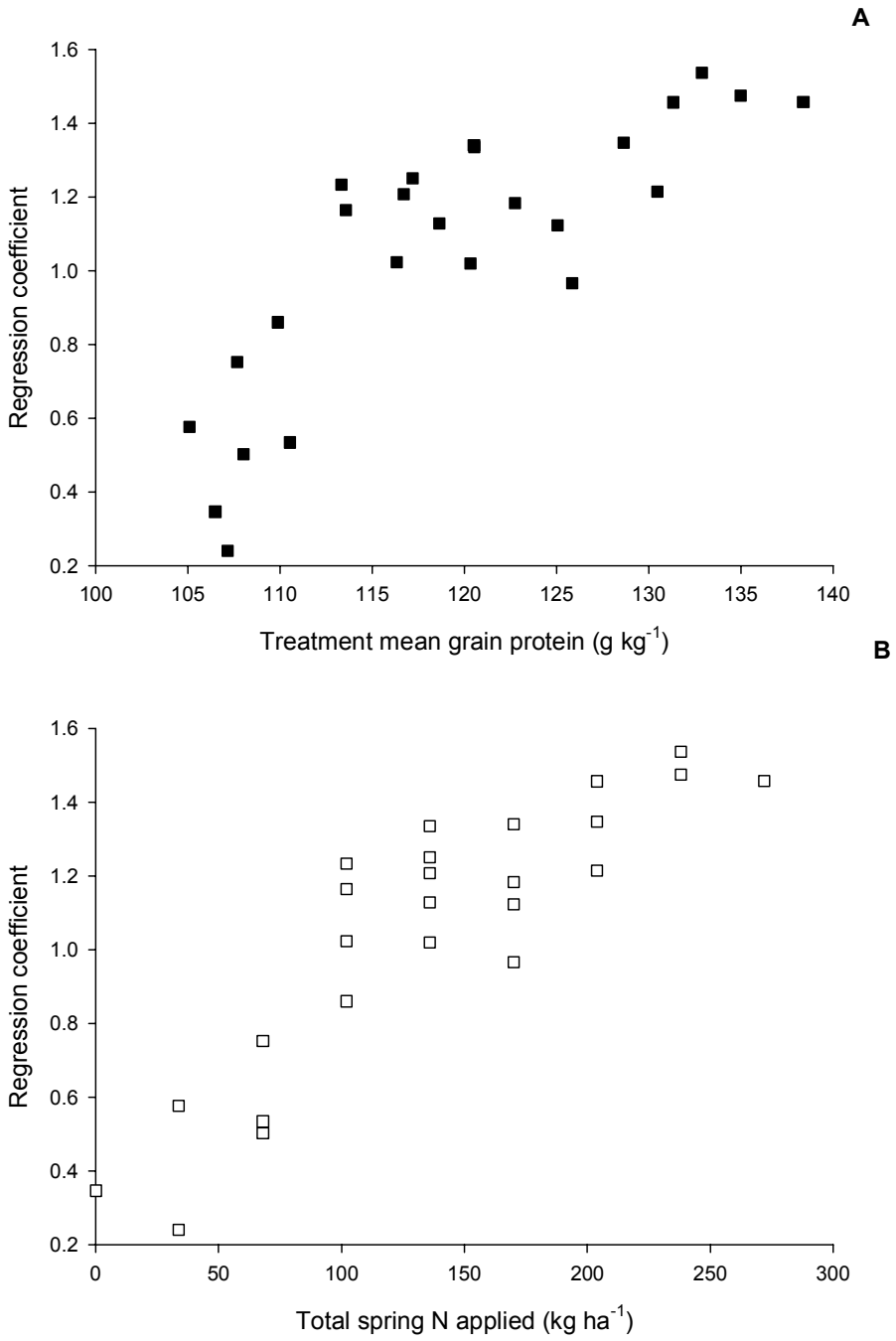


Fig. 9. The relationship of the regression coefficients reported in Table 4 to A) overall treatment mean grain protein of soft red winter wheat and B) total spring N applied in a soft red winter wheat study in North Carolina. Solid squares represent N treatment mean values across environments (a combination of year, location, and tillage system). Open squares represent treatment total N rates.

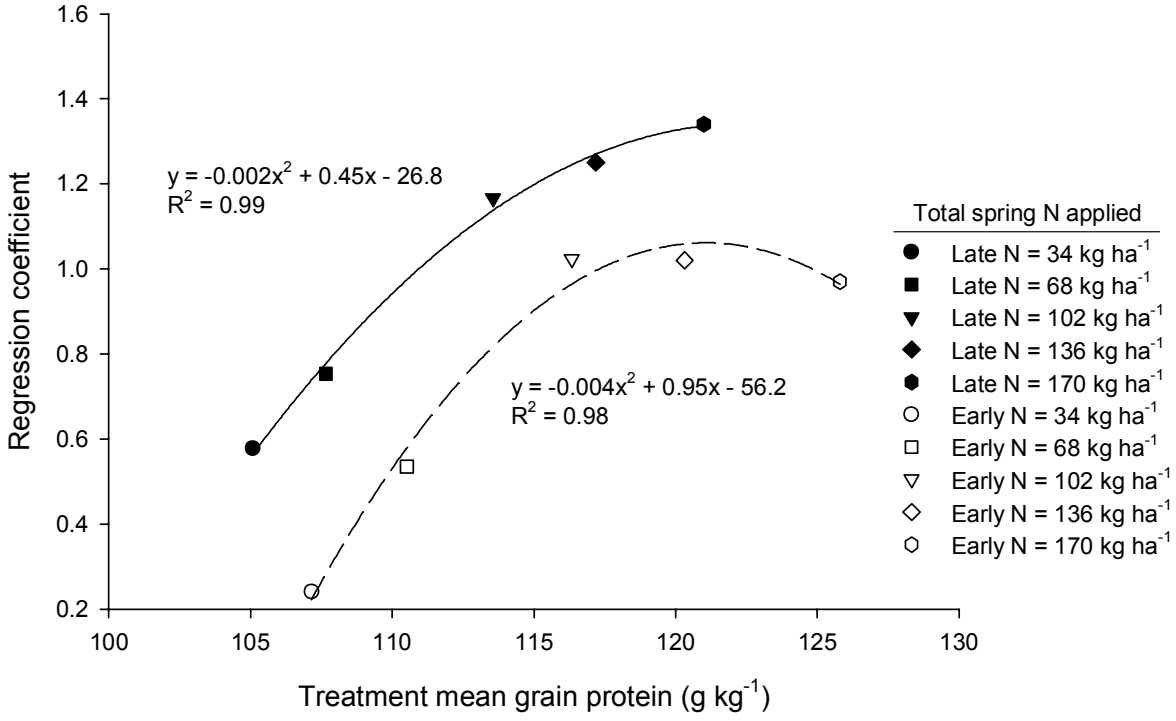


Fig. 10. The relationships of the regression coefficients from Table 4 to overall treatment mean soft red winter wheat grain protein (g kg^{-1}) for "Early" and "Late" N treatments, that is, those receiving 80 % or more of the spring N at growth stage (GS) 25 and GS 30, respectively, in a soft red winter wheat study in North Carolina. The solid (Late) and dashed (Early) lines represent regression equations used to fit the data.

CHAPTER THREE: Using Aerial Color Infrared Photography to Determine In-Season Nitrogen Recommendations in Winter Wheat

Using Aerial Color Infrared Photography to Determine In-Season Nitrogen Recommendations in Winter Wheat

ASTRACT

Finding an in-season nitrogen (N) management strategy that can accurately predict N requirement is an important step in increasing nitrogen use efficiency (NUE) in winter wheat (*Triticum aestivum* L.). The objectives of this study were to determine if in-season agronomic optimum N rate recommendations for soft red winter wheat at growth state (GS) 30 could be developed using spectral bands and indexes obtained from aerial color infrared (CIR) photography and to determine if and how biomass at GS 30 affects these relationships. Experiments were conducted in the Piedmont and Coastal Plain regions of North Carolina in 2002, 2003, and 2004. These regions represent the soil and environmental variability found in the southeastern USA. Relationships between optimum N and the three spectral bands and 39 indexes tested were weak, with approximately half the variability unexplained by the models. After separating the data into two biomass classifications (low, < 1000 kg ha^{-1} and high, > 1000 kg ha^{-1}), relationships between optimum N and the spectral bands and indexes improved substantially. Spectral indexes calculated from the Red or the Green band minus the Red or Green band of a high-N status reference plot (relative Red and relative Green, respectively) had the best relationships (quadratic) with optimum N rates (R^2 = 0.80 and 0.81, respectively) when biomass was > 1000 kg ha^{-1}. When biomass was < 1000 kg ha^{-1}, there was a significant relationship between optimum N and a spectral indicator, the relative ratio vegetation index (RVI), defined as: (near infrared [NIR]/Red) - ($NIR_{reference\ plot}$/$Red_{reference\ plot}$), but this was not as strong (R^2 = 0.45). These results indicate that

agronomic optimum N rates at GS 30 can be estimated using aerial CIR photographs if areas of low and high biomass can be distinguished.

INTRODUCTION

In the southeastern USA, soft red winter wheat (*Triticum aestivum* L.) is grown in regions that differ in soil types and climate. Both of these factors can affect nitrogen (N) management decisions (Baethgen and Alley, 1989; Counce et al., 1984). Though winter wheat requires less N for growth compared to corn and other grain crops, the N-use efficiency (NUE) of winter wheat is generally low; on average only about 33% of the N applied is utilized by the crop (Raun and Johnson, 1999). The variability in the factors influencing N management and low NUE make wheat production a major contributor to the increase of N in watersheds in the Southeast (Gilliam et al., 1997). Therefore, the development of a method for accurately predicting N requirement for soft red winter wheat could have a major impact in improving profitability and water quality.

While N can be applied to soft red winter wheat any time from just prior to planting to hard dough stage, Baethgen and Alley (1989) have shown that the best time to apply N is just before the rapid growth phase that occurs after stem elongation, that is at growth stage (GS) 30 (Zadoks et al., 1974). However, in some cases, N should be applied earlier than GS 30 to stimulate tillering when tiller densities are low, thus improving plant growth and development and yield potential (Scharf and Alley, 1993). Weisz et al. (2001) found that when wheat was planted during the recommended period when using no-till practices, up to 32 kg ha^{-1} of N applied at planting improved yield.

Weisz et al. (2001) also suggested an early or split application of N to no-till systems when GS 25 tiller densities were less than 550 tillers m^{-2} resulted in a yield increase. This result was confirmed in conventional tillage studies as well (Flowers et al., 2001). It is important to note that measurement of tiller density to determine N fertilizer

recommendations at GS 25 requires intensive monitoring of wheat fields (Flowers et al., 2001). Flowers et al. (2001) demonstrated that aerial photographs could be used to estimate tiller densities and thereby decrease the time and intensity of such monitoring. Flowers et al. (2003b) successfully validated this technique over a wide range of soil and climate conditions common in North Carolina.

While it appears that there are reasonable methods for determining when to apply N, accurate methods for determining how much N to apply have not been identified. With large amounts of N lost to dentrification and leaching in the humid Southeast, soil N tests are generally unreliable for estimating the amount of N that will be available to a wheat crop (Scharf and Alley, 1994). Baethgen and Alley (1989) found that winter wheat N uptake was affected by different N fertilizer treatments and climatic characteristics. Therefore, Scharf and Alley (1994) concluded that an in-season field-specific recommendation of N fertilizer in humid regions was the best method for increasing NUE.

Roth et al. (1989) found whole-plant N concentrations rather than leaf N concentration to be the best predictor of the N status in wheat. The period of maximum N uptake in winter wheat begins at GS 30 (stem elongation), with the N requirement of the crop indicated by the tissue N concentration at that time (Baethgen and Alley, 1989; Donohue and Brann, 1984; Scharf et al., 1993; Vaughan et al., 1990). Furthermore, Baethgen and Alley (1989) reported a good relationship between optimum N rates and tissue N concentrations measured at this time, and that this relationship provided a reliable N recommendation for growers in the southeastern USA. An N management system using tiller densities estimated at GS 25 to determine early N recommendations combined with the measurement of tissue N concentration at GS 30 to determine the amount of fertilizer to be applied at this stage

produced better management of N at a field-specific level (Alley et al. 1994; Flowers et al. 2004; Scharf and Alley, 1993). However, other researchers using spatial studies found that N-uptake as an estimator of N status at GS 30 was inconsistent across years (Roth et al. 1989, Raun and Westernman 1991). The process of collecting field samples and measuring tissue N concentrations to predict N need at GS 30 in winter wheat is time consuming and often creates unacceptable delays in the application of fertilizer. Therefore, remote sensing techniques could improve the efficiency of predicting the N status of winter wheat and help develop an appropriate N fertilizer recommendation, and thus improve NUE (Flowers et al, 2004; Raun and Johnson, 1999; Scharf et al. 1993, Scharf and Alley 1994,).

Raun et al. (2002) outlined seven steps for using a proximate active remote sensing technique to determine N recommendations in winter wheat at GS 30. This was accomplished by using an in-season estimate of yield derived from the normalize difference vegetation index (NDVI) divided by growing degree days, and estimates of N uptake derived from reference strips within a field where a high amount of N was applied and the NDVI. Flowers et al. (2003a) found that tissue N concentrations at GS 30 were related to the NDVI from aerial color infrared (CIR) photographs ($r^2 = 0.69$). They then applied a model developed by Scharf et al. (1993) that related optimum N rates to tissue N concentrations ($r^2 = 0.55$) to predict the final N recommendation (Flowers et al., 2003a). Both of the above-mentioned remote sensing techniques have the potential for introducing substantial variability into the final N recommendation since both the relationship between the spectral index and tissue N concentration and the relationship between tissue N concentration and an optimum N rate must be determined.

Gates et al. (1965) and Knipling (1970) reported that leaf chlorophyll and N concentration influence leaf reflectance in the blue, green, and red regions of the visible spectrum. Hinzman et al. (1986) reported that most differences between low (0 kg ha^{-1}) and high (120 kg ha^{-1}) N treatments could be detected by remote sensing techniques. Serrano et al. (2000) proposed that vegetative indexes are indicators of factors important in photosynthesis such as changes in leaf area and chlorophyll content. Unfortunately, there is evidence that factors other than N status and chlorophyll content may influence the relationship between reflectance from a crop canopy and chlorophyll content. Clark et al. (2000, 2001) reported that the canopy density of wheat and broccoli could influence the relationship between spectral reflectance and chlorophyll concentrations. This indicates that measures of chlorophyll concentrations alone may not accurately predict the N need of winter wheat unless other factors such as biomass are included (Reeves et al., 1993).

The primary objective of this study was to determine if an in-season N recommendation in soft red winter wheat at GS 30 could be developed directly from relationships between measured optimum N rates and spectral indicators derived from aerial CIR photography. The secondary objective was to determine if planting date-seeding rate combinations, tiller densities at GS 25, or biomass at GS 30 affected the relationships between agronomic optimum N rates and spectral indicators.

MATERIALS AND METHODS

Experimental Sites

Experiments were conducted in the North Carolina Piedmont and Coastal Plain in 2002, 2003, and 2004. These regions are representative of the soil and environmental variability that exists across two thirds of the state and much of the humid Southeast where

production of soft red winter wheat is concentrated. This environmental variability consists of fluctuating temperature and precipitation that occurs during the growing season, coupled with different soil types, color, moisture holding capacities, organic matter content, and nutrient availability. Locations and years (site-years) utilized were the Piedmont Research Station near Salisbury, NC in 2003 (P-1; Table 1), the Cunningham Research Station near Kinston, NC in 2002, 2003, and 2004 (C-1, C-2, and C-3), the Lower Coastal Plain Tobacco Research Station near Kinston, NC in 2002 (L-1), and the Tidewater Research Station near Plymouth, NC in 2004 (T-1). The taxonomic classifications of the soils at these sites are shown in Table 1. At most site-years 'Coker 9704' soft red winter wheat was planted with the exception of site-years C-3 and T-1, where 'Coker 9184' was planted due to a lack of high quality Coker 9704 seed in 2004 (Table 1).

Treatments and Experimental Design

Three treatment factors were employed in this study. A treatment factor consisting of three planting date-seeding rate (PDSR) combinations was created by: a timely planting date (Weisz, 2004) and high seeding rate (PDSR 1), a late planting date and high seeding rate (PDSR 2), and a late planting date and low seeding rate (PDSR 3) for all site-years except P-1 and T-1 (Table 1). At these site-years, poor weather conditions did not permit a timely planting date, so three different seeding rates were used to establish the desired treatment effects (Table 1). The PDSR were used to establish different tiller densities at GS 25 and amounts of biomass at GS 30 such that PDSR 1 was intended to produce the highest plant density and PDSR 3 the lowest. The second treatment factor consisted of five GS 25 N rates (N_{25}): 0, 34, 67, 101, and 134 kg N ha^{-1}, applied at all site-years except P-1. At site-year P-1, N_{25} rates were lowered to compensate for high N carry-over characteristic of this location,

and consisted of 0, 22, 45, 67, and 101 kg N ha^{-1}. The third treatment factor consisted of five GS 30 N rates (N_{30}): 0, 34, 67, 101, and 134 kg N ha^{-1}. At each site-year a randomized complete block design in a split-split plot arrangement with five replications was used. The main plot treatment factor was PDSR, the split-plot treatment factor was N_{25}, and the split-split-plot treatment factor was N_{30}. Site-years C-1, C-2, and C-3 received a pre-plant N application of approximately 30 kg N ha^{-1} as N-P-K: 10-13.2-24.9 % (N-P_2O_5-K_2O: 10-30-30%), N source unknown. All N treatments were applied as aqueous urea-ammonium nitrate (UAN: 30% N) with the exception of site-year P-1, where prilled ammonium nitrate (NH_4NO_3: 34% N) was applied at N_{25}.

Management

At site-years C-1 and L-1, plots were approximately 2 by 3.6 m, and consisted of rows spaced 178 mm apart. At site-years C-2 and C-3, plots were approximately 2 by 7.3 m, and consisted of rows spaced 178 mm apart. At site-years P-1, plots were approximately 2 by 6.1 m, and consisted of rows spaced 190 mm apart. At site-year T-1, plots were approximately 2 by 4.3 m, and consisted of rows spaced 170 mm apart. Lime and fertilizer rates other than N followed standard recommendations for small grain growers in North Carolina based on annual soil tests (Hardy et al., 2002; Weisz, 2004). All study sites followed corn and were conventionally tilled. Pre- and post-emergence herbicides were applied as recommended (Weisz, 2004), and weed management was excellent at all site-years except L-1, where weed cover between GS 25 and GS 30 was rated at approximately 10% in PDSR 1, 14% in PDSR 2, and 33% in PDSR 3, which were higher than the average plot weed coverage (5%) at the other site-years.

Data Collection

The number of tillers with a minimum of three leaves in a 1-m section of row was determined at GS 25 at two random locations in each main plot prior to the N_{25} application. Main-plot tiller density was then estimated as the average of these two samples. Aerial photographs and plant samples for biomass and tissue N concentration were taken at GS 30 prior to N_{30} applications. The plant samples were taken randomly from each split-split-plot after aerial photography by cutting whole plants just above the soil level. These samples were then pooled with the total area sampled equaling 3.0 m^2 from each split-plot. At site-year L-1 where weed populations were high, weed biomass was removed from the sample before biomass and tissue N concentrations measurements were taken. Plant samples were dried at 60°C for 48 hours for dry matter determination. Tissue N concentrations were determined on the dried plant samples by Waters Agriculture Laboratories (Camilla, GA) using a CHN analyzer (McGeehan and Naylor, 1988). Nitrogen uptake was calculated as the product of biomass and tissue N concentration.

Daily mean air temperature and daily total precipitation for the growing season at each site-year were obtained from the State Climate Office of North Carolina website (http://www.nc-climate.ncsu.edu, verified March 2005) for weather stations at or near each site-year location. The weather data was partitioned into three time periods corresponding to the time periods between applications of treatments; planting to GS 25, GS 25 to GS 30, and GS 30 to harvest (Table 2). Thirty-year growing-season mean daily temperature and precipitation data were obtained from the same source and averaged over these same time periods.

Split-split plots (approximately 9.24 m^2) were harvested with a Massey Ferguson MF-8 or Gleaner K2 plot combine (AGCO Corp., Duluth, GA) and yields measured with a HarvestMaster grain gauge (Juniper Systems, Inc., Logan, UT). Yields were adjusted to a moisture content of 135 g kg^{-1}. Analyses of variance were calculated using PROC GLM in SAS Version 8 (SAS Institute, Cary, NC) to determine the effects of treatments on: tiller densities at GS 25; biomass, tissue N concentration, and N uptake at GS 30; and yield for each individual site-year with replication as the random effect and all other sources of variation as fixed effects. Test statements were used to designate the appropriate error term for F-tests of the main and split-plot effects.

Determination of Optimum Nitrogen Rates at Growth Stage 30

The effects of the main-plot and split-plots treatments (PDSR and N_{25}, respectively) resulted in potentially different biomass and created a wide range of starting conditions for the N_{30} treatment at each of the six site-years utilized in this study. The five N_{30} treatments were used to establish the grain yield response to N_{30} and calculate an optimum N rate at GS 30. Optimum N_{30} rates were calculated for each combination of PDSR and N_{25} at each site-year, resulting in 90 estimates with 4 outliers removed (n = 86). Outliers consisted of data points that were more than two standard deviations from the mean. In trials where there was no statistically significant yield response to N_{30} as determined by the ANOVA in PROC GLM, the optimum N_{30} rate was designated as zero. Based on recommendations in the Small Grain Production Guide for North Carolina (Weisz, 2004), optimum N_{30} rates were not permitted to exceed 134 kg N ha^{-1}. In trials where there was a continual grain yield response to N_{30} without a yield plateau or decrease, the optimum N_{30} rate was determined as 134 kg N ha^{-1}. All other grain yield responses were modeled using a linear-plateau function, if

significant, or via least squares means separation using Fishers Protected LSD with $\alpha = 0.05$ using PROC NLIN (SAS Institute, Cary, NC) or PROC GLM, respectively. To avoid modeling negative yield responses when yield decreased when high rates of N were applied, plots with a significant yield decrease at high N_{30}, determined by least square means separation, were removed before optimum N_{30} rates were calculated.

Image Acquisition and Conversion to Spectral Radiation

Aerial CIR photographs were taken at each site-year at approximately GS 30 following the techniques described by Flowers et al. (2001). Aerial targets were placed at the four corners of each field and their geographic coordinates obtained with a differential global positioning system (DGPS) with 1-m accuracy (Trimble AG 132, Trimble Navigation, Sunnyvale, CA). The acquisition of CIR photographs, film processing, and the digitizing of the CIR photographs followed the methods described in Sripada et al. (2005). Digital images were georegistered using ERDAS Imagine version 8.7 (Leica Geosystems, Atlanta, GA). In all cases, the root mean square (RMS) errors after the georegistration were less than 1 m, indicating that the accuracy of targets in the photographs and DGPS coordinates were within 1 m of each other. The image ground resolution ranged from 0.45 to 0.68 m; differences in this range were due to the slightly different altitudes at which the images were obtained.

When used with a yellow filter to exclude blue light, the CIR film responds to light within the visible and near infrared (NIR) regions of the electromagnetic spectrum (490 to 900 nm), creating wide overlapping wavelengths for the three bands studied. This resulted in Band 1 wavelengths ranging from ~ 490 to 900 nm with maximum sensitivity at 730 nm (NIR), Band 2 wavelengths ranging from ~ 490 to 700 nm with maximum sensitivity at 650

nm (Red), and Band 3 wavelengths ranging from ~ 490 to 620 nm with maximum sensitivity at 550 nm (Green) (Eastman Kodak, 1996).

Individual split-plots, excluding the plot borders (approximately 0.6 m wide), were used as areas of interest (AOI) in the images obtained, resulting in approximately the same number of pixels for each split-plot. The mean digital number (DN) representing each spectral band for each individual split-plot was extracted from the AOI. A series of spectral indexes was calculated using the DN from the individual bands (Table 3). Relative bands and indexes were calculated in two ways: 1) by dividing the mean spectral band DN or index of a particular sub-plot N_{25} treatment by the mean spectral band or index for the sub-plot N_{25} treatment that received a high N rate at a particular site, and 2) by subtracting the mean spectral band DN or index for the sub-plot N_{25} treatment that received a high N rate at a particular site from the mean spectral band DN or index of a particular sub-plot N_{25} treatment. To avoid working with negative values resulting from the calculation of relative indexes, a constant value of 1 was added to NDVI, GNDVI, RVI, GRVI, SAVI, and GSAVI, a value of 1.5 was added to OSAVI and GOSAVI, and a value of 255 was added to DVI and GDVI (Sripada et at. 2005; Table 3).

The GS 30 tissue N concentrations and optimum N_{30} rates were regressed against the digital counts for the absolute and relative NIR, Red, and Green bands and all of the absolute and relative indexes using three different models. The linear and quadratic models were fit using PROC REG (SAS Institute, Cary, NC) and the exponential model was fit using PROC NLIN. Single covariate analyses were performed to test the influence of tiller densities at GS 25, biomass at GS 30, and the class variable PDSR on the relationships of GS 30 tissue N

concentrations or optimum N_{30} rates with the absolute and relative spectral bands and indexes using PROC GLM.

RESULTS AND DISCUSSION

Growing Conditions

Mean daily temperatures at C-1 and L-1 were predominantly near the 30-yr average (Table 2) across all time periods during the growing season. However, the mean daily total precipitation for C-1 and L-1 was lower than the 30-yr average across most time periods measured and was substantially lower during the period from planting to GS 25 (Table 2). At C-2 and P-1, the temperatures were approximately 2ºC cooler than the 30-yr average across all time periods (Table 2). In addition to being cooler at C-2 and P-1, the mean daily total precipitation after GS 25 was substantially greater than the 30-yr average. Site-years C-3 and T-1 experienced temperature and precipitation near the 30-yr average (Table 2) across all time periods.

Crop Responses

Due to the different PDSR and the different cultivars used at two site-years, we examined each site-year separately for response to PDSR, N_{25}, and N_{30}, and any interactions among them (Table 4). For tiller densities at GS 25, PDSR was the only treatment analyzed, while for biomass, tissue N concentration, and N uptake at GS 30, PDSR, N_{25}, and their interaction were analyzed for their effects (Table 4). The effects of PDSR, N_{25}, N_{30}, and their two- and three-way interactions were analyzed for their effects on grain yield (Table 4).

Tiller Densities at Growth Stage 25

Tiller densities at GS 25 responded to PDSR at all site-years (Table 4). Weisz (2004) stated that winter wheat fields in North Carolina with tiller densities below 540 m^{-2} will have

increased yields with an application of N at GS 25, while areas with higher tiller densities will not benefit. Across all site-years and PDSR treatments, the range of tiller density was 175 to 1300 tillers m^{-2}. However, PDSR 1 was above 540 tillers m^{-2} only at C-1 and C-2, while tiller densities were below this threshold for PDSR 2 and 3 at these site-years and for all PDSR treatments at all other site-years. This indicates that PDSR 2 and 3 at C-1 and C-2 and all the PDSR treatments at all other site-years would have potentially benefited from an application of N at GS 25 according to N management practices suggested in North Carolina (Wiesz, 2004).

Biomass at Growth Stage 30

The GS 30 biomass found in the study at the split-plot level ranged from 120 to 3060 kg ha^{-1} and was affected by PDSR and N_{25} at all site-years except C-3, where N_{25} was the only significant effect, and T-1, where only PDSR was significant (Table 4). There were no significant interactions between PDSR and N_{25} at any site-year. At site-years where PDSR was significant, the mean GS 30 biomass was statistically different among the various PDSR treatments (PDSR 1 > PDSR 2 > PSDR 3) with the exception of L-1 and T-1 where there were no significant differences in mean biomass between PDSR 2 and 3. Flowers et al. (2003a) found that locations with mean GS 30 biomass greater than 1000 kg ha^{-1} had relatively strong relationships with spectral bands or indexes. Only at C-1 and L-1 were mean biomass levels at all PDSR treatments above this value. At C-2 and P-1 only the mean biomass levels in the PDSR 1 treatments exceeded this value. At T-1, all PDSR treatment mean biomass values were below 1000 kg ha^{-1}.

The effect of N_{25} treatments on mean biomass at each site-year except T-1 resulted in an increase in biomass with increasing amounts of N applied. In all cases the lowest N_{25}

treatment had a significantly lower mean biomass when compared to the highest N_{25} treatment. All mean biomass values were greater than 1000 kg ha^{-1} for each of the five N_{25} treatments at C-1 and L-1, while at C-2 all but the lowest N_{25} treatment were greater than 1000 kg ha^{-1}. At P-3 only the 101 and 134 kg N ha^{-1} N_{25} treatments were greater than 1000 kg ha^{-1}, while at C-3, none of the N_{25} treatment mean biomass values were greater than 1000 kg ha^{-1}.

Tissue N Concentration at Growth Stage 30

The GS 30 tissue N concentrations at the split-plot level ranged from 15.4 to 60.6 g kg^{-1} and were affected by PDSR and N_{25} at all site-years. At L-1, the interaction between PDSR and N_{25} was also significant (Table 4). At L-1, the two-way interaction between PDSR and N_{25} (Fig. 1) resulted from increasing GS 30 tissue N concentrations with increasing N_{25} for all PDSR treatments but with a smaller increase in tissue N concentrations for PDSR 3. This was possibly due to the high weed pressure in PDSR 3 (33%), with the wheat competing for N with the weeds present, resulting in a lower tissue N accumulation at GS 30. The mean tissue N concentration increased from PDSR 1 to PDSR 3 at all site-years, but the mean tissue N concentrations between PDSR 2 and 3 did not differ significantly except at C-2 where there was a significant difference in the mean tissue N concentrations among all PDSR treatments. In all cases the lowest N_{25} treatment had a significantly lower mean tissue N concentration when compared to the highest N_{25} treatment.

Nitrogen Uptake at Growth Stage 30

Nitrogen uptake at GS 30 at the split-plot level ranged from 8.0 to 103.1 kg N ha^{-1} and was affected by N_{25} across all site-years (Table 4). At site-years C-2, P-1, and T-1, PDSR was significant, while at all other site-years PDSR had no effect on GS 30 N uptake (Table

4). There was no interaction between PDSR and N_{25} at any site-year (Table 4). At site-years where PDSR was significant, mean N uptake decreased (PDSR 1 > PDSR 2 > PDSR 3), with significant differences between PDSR 1 and PDSR 3. Increasing N_{25} increased N uptake at all site-years. In all cases, the lowest N_{25} treatment had a significantly lower mean N uptake when compared to the highest N_{25} treatment. Because N uptake is a function of biomass and tissue N concentration, lower biomass and low tissue N concentrations resulted in low N uptake and, likewise, more biomass and high tissue N concentrations resulted in the highest N uptake.

Grain Yield

Across all site-years, the range of yield at the split-split plot level was 1.40 to 10.10 Mg ha^{-1} with a mean of 4.60 Mg ha^{-1} (Table 5). Site-year P-1 had the lowest yields in the study possibly due to high disease pressure that resulted from increased precipitation during the grain filling period (Table 2) caused possibly by glume blotch [*Stagonospora nodorum* (Berk.)] which was observed at harvest. The yield at this site-year ranged from 2.00 to 3.90 Mg ha^{-1} with a mean of 3.00 Mg ha^{-1} (Table 5). Though site-year C-2 also had high precipitation amounts during grain fill (Table 2), the disease pressure of glume blotch was possibly not as high and it was not observed at harvest, and there was less yield loss compared to P-1. At T-1 yield ranged from 1.90 to 10.10 Mg ha^{-1} with a mean of 6.10 Mg ha^{-1}. This was the highest yielding site-year in this study and also the site-year with greatest range in yield (Table 5).

Site-year C-3 was the only site-year with a significant three-way interaction among PDSR, N_{25}, and N_{30} (Table 4) for grain yield. This indicated that yield response to any treatment factor depended on the levels of both of the other treatment factors. Grain yields

for each N_{25} and N_{30} combination were greater in PDSR 1 than they were in PDSR 2 or 3. There was minimal yield response to increasing N_{30} within each N_{25} in PDSR 3, while in PDSR 1 and 2 there was no yield response to N_{30} within the highest N_{25} treatments (101 and 134 kg N ha^{-1}). At this site-year, both GS 25 tiller densities and GS 30 biomass for PDSR 3 were statistically lower than those found in PDSR 1 and 2. This indicated that when tiller density and biomass were low, there was a limit to how much N the wheat could utilize at GS 30.

Grain yields were affected by the N treatments, N_{25} and N_{30}. Both the two-way interactions ($N_{25} \times N_{30}$) and main effects were significant at all site-years (Table 4). In general, as N_{25} increased, yield response to N_{30} decreased or was non-existent (Fig. 2). Overall, increasing N_{25} increased yield with the exception of the highest N_{25} treatment (134 kg ha^{-1}), which was frequently no different from the 101 kg ha^{-1} N rate (Y-axis of Fig. 2). Except as noted above for high N_{25} rates, yield tended to increase with increasing N_{30} rates. However, this was not always the case. Yield was less responsive to N_{30} at P-1, where at the 0 kg N ha^{-1} N_{25} rate there was a significant yield reduction at the 134 kg N ha^{-1} N_{30} rate compared to the 101 kg ha^{-1} N_{30} rate. This was possibly due to higher soil N carry-over at this site-year where lodging was observed in the higher N plots. It has been well documented that the yield of winter wheat will increase with increases in N applications at GS 25 and GS 30 in the southeast region (Baethgen and Alley, 1989; Flowers et al., 2004; Scharf and Alley, 1993; Scharf et al., 1993, Weisz, 2004). However, there is evidence that wheat can be over-fertilized resulting in a yield reduction (Weisz, 2004).

The two-way interaction between PDSR and N_{30} was significant at L-1, C-2, C-3, and T-1. Specifically, at C-2, C-3, and T-1, there was a decrease in yield when high rates of N_{30}

were applied within PDSR 1, while in PDSR 2 and 3 there were no yield reductions with high N_{30} rates. At L-1, the yield response to N_{30} was similar at both PDSR 1 and 2 with a plateau in the response at 101 kg N ha^{-1} N_{30} rate, while a plateau in the yield response to N_{30} within PDSR 3 occurred at the 67 kg N ha^{-1} N_{30} rate. The main effect of PDSR treatments on yield was also significant in three site-years: C-1, L-1, and C-2 (Table 4). Despite the significant two-way interaction at L-1 and C-2 for PDSR and N_{30}, there was a significant increase in grain yield with PDSR 3 < PDSR 2< PDSR1. At C-1 where PDSR was significant and there were no treatment interactions with PDSR, grain yield was significantly higher in PDSR 1 with no difference in yield between PDSR 2 and PDSR 3. In this study, there were significant increases in both tiller densities at GS 25 and biomass at GS 30 with PDSR 1 > PDSR 2 > PDSR 3 (Table 4). Weisz et al. (2001) reported that increases in tiller density at GS 25 resulted in yield increases, which was confirmed in this study at L-1, C-2, and C-1, where the highest GS 25 tiller densities and biomass at GS 30 in PDSR 1 were associated with higher yields compared to PDSR 2 and 3 which had lower GS 25 tiller densities and GS 30 biomass.

Relationships Between Tiller Density, Biomass, N Concentration, N Uptake and Yield

To better understand the influence of treatments on yield, the relationships between the various agronomic parameters were examined. Weisz et al. (2001) reported that across different planting dates and seeding rates in no-tillage systems there were positive relationships between soft red winter wheat yield and tiller densities at different site-years when N was not a confounding factor. In our study, because there was a significant interaction at each site-year between N_{25} and N_{30} for yield (Table 4), the data were separated by site-year and $N_{25} \times N_{30}$ to investigate whether there were relationships between tiller

density and yield similar to those reported by Weisz et al. (2001).. Overall, there were only a few weak positive relationships across PDSR treatments between yield and tiller density at C-1, L-1, C-3, and T-1 ($0.27 < r^2 < 0.44$, data not shown). Site-year P-1 had no significant relationship between yield and tiller densities. In contrast, at site-year C-2, all relationships were significant ($0.43 \leq r^2 \leq 0.87$, data not shown). Only seven out of 25 of the relationships between yield and tiller densities at C-2 had an r^2 below 0.60.

At C-1 and L-1, tiller density was higher than the other site-years, but did not result in the highest yield. This could be due to drier conditions that occurred at these site-years (Table 2). While C-3 and T-1 had lower tiller density than other site-years, they had the highest yields and the most conducive weather conditions for yield (Table 2). These inconsistencies between tiller density and yield might be responsible for the few and weak significant relationships found between yield and tiller density at these site-years (C-1, L-1, C-3, and T-1). At P-1, trials were planted late in the growing season, resulting in fewer tillers coupled with high disease pressure due to high precipitation during grain fill (Table 2), which likely resulted in no relationships between yield and tiller density. Conditions at C-2 were likely similar to those reported by Weisz et al. (2001), where there were highly significant relationships between yield and tiller density.

Across all site-years, PDSR, and N_{25}, there was a significant relationship between GS 30 biomass and GS 25 tiller density ($r^2 = 0.75$, data not shown), indicating that high GS 25 tiller density resulted in high GS 30 biomass. At all individual site-years, except P-1 and C-3, there were significant relationships between GS 30 biomass and GS 25 tiller density ($0.38 \leq r^2 \leq 0.84$, data not shown). Again, because there was a significant interaction at each site-year between N_{25} and N_{30} for yield (Table 4), the data was separated by site-year and $N_{25} \times N_{30}$ to

examine relationships between yield and GS 30 biomass. This resulted in an average of approximately five out of 25 significant positive relationships ($0.29 \leq r^2 \leq 0.81$, data not shown) at all site-years except C-2, where all relationships were significant ($0.31 \leq r^2 \leq 0.78$, data not shown).

The relationship of yield to GS 30 tissue N concentrations when separated by site-year and $N_{25} \times N_{30}$ had similar results to GS 30 biomass, with C-2 having the most significant positive relationships (21 out of 25) and all other site-years averaging approximately three out of 25 significant positive relationships per site-year. In general, high yields were associated with higher GS 30 tissue N concentration. Nitrogen uptake at GS 30 was a function of tissue N concentration and biomass and was not related to yield when pooled across all site-years and N treatments. However, this relationship was skewed by site-year T-1 where lower measured N uptake at GS 30 corresponded to higher mean yield. Separation by site-year revealed significant positive relationships between yield and N uptake at GS 30 ($0.18 \leq r^2 \leq 0.33$, data not shown) except at P-1 where there was no significant relationship. When the relationships between yield and N uptake were separated by site-year and $N_{25} \times N_{30}$, only about half of the 25 potential relationships were significant (positive correlations, $0.28 \leq r^2 \leq 0.74$, data not shown) at site-years C-1 and C-2. At all other site-years (L-1, P-1, C-3, T-1), on average only approximately two out of 25 relationships were significant (positive correlations, $0.27 \leq r^2 \leq 0.46$, data not shown).

Determination and Examination of Optimum Growth Stage 30 Nitrogen Rates

Examples of the yield responses used to calculate optimum N_{30} at two site-years, C-1 and C-3, are shown in Fig. 2. Optimum N_{30} at C-1 ranged from 0 to 123 kg N ha^{-1}, at site-year L-1 from 0 to 110 kg N ha^{-1}, and at C-2 from 0 to 100 kg N ha^{-1}. Site-years C-1, C-3,

and L-1 did not require as much N to reach their yield potentials compared to other site-years. At site-year P-1, optimum N_{30} rates tended to be lower, 0 to 67 kg N ha^{-1}. This was consistent with the fact that P-1 had the lowest mean yield. Though C-3 was not the highest yielding site-year, optimum N_{30} rates tended to be slightly higher than those found at T-1 (0 to 134 kg N ha^{-1}). This could be possibly due to sandier soils present at this site-year than at T-1 (Table 1), which was the highest yielding site-year with a slightly lower optimum N_{30} range (0 to 132 kg N ha^{-1}).

Physiology of Optimum Growth Stage 30 Nitrogen Rates

The optimum N_{30} rates derived in this study were the result of yield responses to N applications at GS 30 under varied starting conditions. The assumption was that the N_{30} treatment was responsible for the yield increases (or decreases). However, N application rates and timing are only one factor affecting yield potentials (Frederick and Bauer, 1999). Since other researchers have reported a relationship between tissue N and optimum N_{30} (Baethgen and Alley, 1989; Flowers et al., 2003a; Sharf and Alley, 1993; Scharf et al., 1993), the relationship between optimum N_{30} rates and tissue N concentrations was examined. The linear relationship between optimum N_{30} and tissue N concentrations at GS 30 in our study (Fig. 3A) was significant with a modest correlation ($r^2 = 0.43$). As tissue N concentration at GS 30 increased, the rate of N required to achieve optimal yield decreased.

To better understand the impact that biomass has on the relationship between GS 30 tissue N concentration and optimum N_{30}, the data in Figure 3B are shown by arbitrarily assigned GS 30 biomass classes. The different biomass classes explain much of the variability in the data (Fig. 3A). In a covariate analysis with biomass class as the covariate, the interaction between tissue N concentrations and biomass class were significant. This

indicates that biomass classes formed significantly different linear relationships (Fig. 3B). As tissue N concentrations increased, optimum N_{30} decreased more rapidly at the high biomass classes compared to the low biomass classes as seen with steeper slopes in the high biomass classes (Fig. 3B).

As stated earlier, past research has shown strong relationships between tissue N concentrations and spectral data (Flowers et al., 2003a; Raun et al. 2002). Our data indicated that the relationship of optimum N_{30} to GS 30 tissue N concentrations was influenced by biomass at GS 30. Since our primary objective was to determine a relationship between optimum N_{30} and spectral indicators, the relationships between GS 30 tissue N concentration and optimum N_{30} rate indicated that biomass at GS 30 has the potential to influence the final relationship we were pursuing. Other researchers have also speculated that biomass could affect relationships between spectral measurements of a crop and crop parameters (Clark et al., 2000 and 2001; Reeves et al., 1993; Serrano et al., 2000).

Spectral Indexes

Previous research indicated that using a high-N status reference plot to calculate a relative index can reduce differences caused when comparing data from different photographs taken at different times with differing exposures and ambient light levels (Blackmer and Schepers, 1994; Blackmer et al. 1996; Sripada et al., 2005). In addition, a high-N status plot provides an in-field reference that is likely to represent an extreme of spectral and agronomic response. In most cases, the N reference plot used in prior studies was the highest N rate used or a mean of the highest N rates (Blackmer and Schepers, 1994; Blackmer et al. 1996). In 78% of the site-year–PDSR treatment combinations in this study, there were no differences in yield between the 101 kg N ha^{-1} and 134 kg N ha^{-1} N_{25}

treatments when N_{30} was 0 kg N ha^{-1}. This indicated that N sufficiency for optimum yield was generally obtained with the 101 kg N ha^{-1} N_{25} treatment. Consistent with this finding, there were no spectral differences at GS 30 between the 101 kg N ha^{-1} and 134 kg N ha^{-1} N_{25} treatments in 83% of the site-year–PDSR treatment combinations. Based on this information, and with winter wheat having the potential to lodge due to an over application of N (Weisz, 2004), we used the 101 kg N ha^{-1} GS 25 treatment as the reference plot for the relative band and indexes calculated in this study rather than the highest N_{25} treatment.

Tissue Nitrogen and Optimum N_{30} Versus Spectral Bands and Indexes

As stated previously, research aimed at making accurate predictions of N rates for wheat have used the relationships of tissue N concentration or N uptake at GS 30 to spectral bands and indexes (Raun et al, 2002; Flowers et al. 2003a) in order to derive an N recommendation. The relationship between GS 30 tissue N concentrations and spectral indicators were examined in the present study, and the results indicated weaker relationships compared to those found by either Raun et al. (2002) or Flowers et al. (2003a) (Table 6). All linear relationships were significant for all spectral bands and indexes, but the coefficients of determination (r^2) were low ($0.01 \leq r^2 \leq 0.37$). The quadratic relationships had slightly higher coefficients of determination ($0.04 \leq R^2 \leq 0.40$) with approximately 48% of the spectral indicator being non-significant. The coefficients of determination for the exponential relationships were similarly low ($0.10 \leq R^2 \leq 0.37$), with approximately 79% of the spectral indicators non-significant. The strongest significant quadratic relationships between tissue N concentrations and spectral indexes were found with NDVI, SAVI, and NormRed ($R^2 = 0.40$, for all indexes). Calculating the spectral indicators relative to the high N reference did not

improve any of the relationships of spectral indicators with GS 30 tissue N concentrations. These results were consistent with the findings of Flowers et al. (2003a).

In general, optimum N_{30} showed stronger relationships with spectral bands and indexes (Table 6) than did GS 30 tissue N concentrations. All linear relationships were significant for all spectral bands and indexes with the exception of NIR. The coefficients of determination for the linear relationships ($0.06 \leq r^2 \leq 0.47$) were greater compared to linear relationships with tissue N concentrations, especially for the relative indicators. The quadratic relationships had higher coefficients of determination ($0.26 \leq R^2 \leq 0.52$), with approximately 38% of the spectral indicators being non-significant. It should be noted that the majority of the non-significant relationships were ones using absolute bands and indexes. The coefficients of determination for the exponential relationships ($0.33 \leq R^2 \leq 0.53$) were similar to those of the quadratic relationships, with approximately 45% of the spectral indicators non-significant. Again, the majority of the non-significant relationships were with the absolute bands and indexes. When comparing methods of calculating relative indexes (division versus subtraction), the subtraction method tended to result in more improvement in the relationships between the optimum N_{30} and spectral indicators. In all of the models tested, the only indexes where using subtraction did not improve the relationship between optimum N_{30} and spectral indicators more than using division were Rel_{div} RVI and Rel_{div} GRVI. Regardless of the method used to calculate relative indexes, the highest coefficient of determination for any of the relationships between optimum N_{30} and spectral bands and indexes was an $R^2 = 0.53$ (Table 6) which was found using Rel_{sub} NDVI, Rel_{sub} GNDVI, Rel_{sub} SAVI, and Rel_{sub} GSAVI (Fig. 4). Unfortunately, these relationships only explained about half of the variability in the model.

Covariate Analysis

Previous research into the influence of biomass on the relationship between optimum N and tissue N concentration showed that biomass has the potential to influence the relationships between spectral indicators and the N status of a crop (Clark et al, 2000 and 2001; Reeves et al., 1993). In our study, we purposefully created significant differences in GS 25 tiller density and GS 30 biomass via the PDSR and N_{25} treatments (Table 4), thus providing a test of the hypotheses that relationships of spectral indicators with crop N status and optimum N_{30} may be affected by GS 25 tiller density and GS 30 biomass. We tested GS 25 tiller density and GS 30 biomass as quantitative covariates and PDSR as a fixed factor to see if they affected and improved predictions of GS 30 tissue N concentrations and optimum N_{30} from spectral indicators.

In the covariate analysis, covariates were considered important when the spectral indicator and the covariate and/or the interaction of the covariate with the spectral indicator were significant. Relationships were considered improved when there was an increase (> 5%) in the coefficient of determination (R^2). With GS 30 biomass as a covariate, all relationships between GS 30 tissue N concentrations and spectral indicators improved when the covariate was included ($0.26 \leq R^2 \leq 0.51$) (Table 7), with the exceptions of the relationships with NIR, Red, Rel_{div} Red, Rel_{div} Green, and Rel_{div} DVI (Table 7). When using GS 25 tiller density as a covariate, 28 of 42 relationships between GS 30 tissue N concentrations and spectral indicators improved with resulting coefficients of determination $0.38 \leq R^2 \leq 0.59$ (Table 8). The fixed treatment factor covariate, PDSR, improved 30 out of 42 relationships between GS 30 tissue N concentrations and spectral indicators with resulting coefficients of determination $0.13 \leq R^2 \leq 0.52$ (Table 9).

While using GS 30 biomass as a covariate improved more of the relationships between the spectral indicators and GS 30 tissue N concentration, using GS 25 tiller density as a covariate resulted in the highest coefficient of determination ($R^2 = 0.59$), which was for the spectral indicator Rel_{sub} DVI (Table 8). On average, there was a 140% improvement in the coefficients of determination when using any one of the covariates tested here, but despite the drastic improvements with the covariates, the resulting simple linear models still only accounted for about half of the variability in tissue N concentration. These results indicated that biomass and/or tiller density influenced the relationship between GS 30 tissue N concentration and spectral indicators, but only accounted for part of the total variability found.

In contrast to the covariate models predicting GS 30 tissue N concentrations, the use of biomass as a covariate only improved 10 relationships between optimum N_{30} and the spectral indicators tested in this study (Table 7). Using GS 25 tiller density as a covariate improved five relationships between the spectral indictors and optimum N_{30} (Table 8), and the use of PDSR resulted in an improvement only between RVI and optimum N_{30} (Table 9). Using GS 25 tiller density or GS 30 biomass as a covariate improved the coefficients of determination by approximately 15%, with the models accounting for only half of the variability in the data. When using PDSR as a covariate, there was a 48% improvement in the one significant relationship (RVI), but the model still accounted for less than half of the variability in the model. These results indicated that while biomass and/or tiller density influenced some relationships between optimum N_{30} and spectral indicators, including them as covariates did not consistently improve the prediction of optimum N_{30}.

Segregation of Growth Stage 30 Biomass

It was suggested by Flowers et al. (2003a) that locations with GS 30 biomass below 1000 kg ha^{-1} had a weak spectral relationship with GS 30 tissue N concentrations; when such locations were removed from the analysis, relationships between these variables improved. The relationship between GS 30 tissue N concentrations and optimum N_{30} indicated that GS 30 biomass contributed to the variability in the data (Fig. 3). While the covariate analyses did not indicate that GS 25 tiller density, GS 30 biomass, and PDSR strongly influenced the relationship between optimum N_{30} and spectral indicators, we re-examined these relationships by separating the data into two biomass classes in two different ways.

First, we followed the approach of Flowers et al. (2003a) by separating the data by site-years into those with mean biomass > 1000 kg ha^{-1} and those with mean biomass < 1000 kg ha^{-1}. The site-years with high biomass were C-1, L-1, C-2, and P-1, while C-3 and T-1 had low biomass. Table 10 shows the coefficients of determination for the relationships of all of the relative spectral bands and indexes with optimum N_{30} separated based on mean site-year GS 30 biomass. We did not test the absolute bands and indexes because their relationships with optimum N_{30} were always weaker than those of the relative bands and indexes. The Rel_{sub} Red, Rel_{div} Red, and Rel_{sub} Green spectral indicators had the strongest relationships (quadratic) with optimum N_{30} for the high biomass site-years (Table 10; Fig. 5). There was a substantial increase in R^2 for these relationships when the high GS 30 biomass site-years were considered separately from the low GS biomass site-years (Rel_{sub} Red, $R^2 = 0.78$; Rel_{div} Red and Rel_{sub} Green, $R^2 = 0.75$). Figure 6 shows examples of the amount of variability in the data for the quadratic relationships of optimum N_{30} with the spectral indicators Rel_{sub} Red and Rel_{sub} Green when the data were not separated based on biomass ($R^2 = 0.49$ and 0.51,

respectively). In contrast to the increase in coefficients of determination for the high biomass site-years, there was a decrease in the linear r^2 values for the low biomass site-years for these same spectral indicators (Rel_{sub} Red, $R^2 = 0.32$; Rel_{sub} Green, $R^2 = 0.33$). In contrast, other spectral indicators showed improved performance for the low biomass site-years. The Rel_{sub} GNDVI and Rel_{sub} GSAVI indexes had the strongest relationships (exponential) with optimum N_{30} for the low biomass site-years ($R^2 = 0.63$ for both).

Flower et al. (2003a) plotted the significant linear coefficients of determination for relationships of GS 30 tissue N concentrations with NDVI for each location against the mean biomass of each location. The results further demonstrated that the low biomass locations had weaker relationships with NDVI, and justified their removal from the final model (Flowers et al., 2003a). We followed this approach to determine if it supported the segregation of the data into low and high biomass site-year. We examined the linear relationships of optimum N_{30} with Rel_{sub} Red and Rel_{sub} Green for each site-year since these two spectral indicators had the strongest relationships for the site-years with high mean biomass. The resulting r^2 of these relationships for each site year were plotted against the mean biomass for that site-year (Fig. 7). These figures confirmed the removal of site-years C-3 and T-1, which had relatively low biomass and low linear r^2 values. Unfortunately, confounding factors make it impossible to identify low site-year biomass as the only reason why site-years C-3 and T-1 differed from the others in this study. Site-years C-3 and T-1 were the two site-years where a different cultivar was grown, thus the "effect" of low biomass could be linked to genetic differences between the two cultivars used in this study, which might have influenced both spectral indicators and agronomic parameters. In addition, both of these low biomass site-years occurred in the same growing season, and the low biomass "effect" could be a result of a year

effect. It was our conclusion that removing a site-year without a clear understanding of the factors influencing the differences in optimum N_{30} and/or spectral indicators may lead to a bias in our results. Therefore, we examined a second method of accounting for the potential effect of biomass on the relationships between optimum N_{30} and spectral indicators. We examined each site-year by PSDR combination individually, as PDSR had a significant effect on GS 30 biomass (Table 4) for all site-years except one (C-3).

Figure 8 shows the coefficients of determination for the linear relationships of optimum N_{30} with Rel_{sub} Red and Rel_{sub} Green plotted versus GS 30 biomass for each PDSR at each site-year (n = 18). With some exceptions, the r^2 values tended to increase as GS 30 biomass increased, with little response beyond approximately 1000 kg ha^{-1} GS 30 biomass. Linear relationships between the linear coefficient of determination versus GS 30 biomass for the PDSR–site-year combinations in this figure were nearly significant for Rel_{sub} Green (r^2 = 0.18, p = 0.08) while with Rel_{sub} Red, only a weak positive trend was present. This suggests that biomass levels below 1000 kg ha^{-1} likely weakened the relationship between spectral indicators and optimum N_{30}.

Based on this result, the relationships of optimum N_{30} to the spectral indicators were re-examined after classifying each site-year by PDSR combination as either low (< 1000 kg ha^{-1}) or high (> 1000 kg ha^{-1}) GS 30 biomass (Table 11). The relationships were substantially stronger for the high biomass site-year by PDSR combinations compared to the low biomass site-year by PDSR combinations, and, more importantly, compared to data not separated by biomass. Figure 9 shows the best quadratic relationship between optimum N_{30} and relative spectral indicators when separated into low and high biomass classes. Compared to all data together, the Rel_{sub} Red and Rel_{sub} Green relationships were stronger in the high biomass

class (R^2 = 0.80 and 0.81, respectively) and weaker in the low biomass class (R^2 = 0.35 for both). For the low biomass class, the Rel_{sub} RVI had the strongest relationship (exponential) with optimum N_{30} (R^2 = 0.45).

The plot of optimum N_{30} versus the Rel_{sub} Green spectral indicator for the high biomass class (Fig. 9) indicated that one high Rel_{sub} Green data point appeared to unduly influence the shape of the quadratic model. This point had a spectral value that was almost twice as high as the majority of the data. The unusually high spectral value of this point might be due to low green absorption in this low N_{25} treatment, or the calculation of the optimum N_{30} might have underestimated the actual crop N requirement. Both of these possibilities were examined to determine whether this point might legitimately be eliminated as an outlier. Unfortunately, no cause could be identified that would justify the removal of this point. With this one data point removed the quadratic equation changed very little ($y = -0.007x^2 + 4.70x + 23.6$, R^2 = 0.81, data not shown) indicating that with or without this data point in the model, the relationship between optimum N rates and Rel_{sub} Green was largely unaffected.

In order to apply the high biomass models to predict optimum N_{30} using either the Rel_{sub} Red or Rel_{sub} Green spectral indicators, it would be necessary to first determine whether the target area had GS 30 biomass > 1000 kg ha^{-1}. Plant biomass can often be estimated spectrally using NDVI (Rouse et al., 1973) or RVI (Wanjura and Hatfield, 1987). We thus regressed GS 30 biomass against all the spectral bands and indexes in Table 3 in an effort to find a band or index to distinguish high from low GS 30 biomass. We found few significant relationships between biomass and the spectral bands and indexes (data not shown). The strongest relationship was with NIR (linear, r^2 = 0.32, data not shown). The data

was strongly influenced by site-year with a distinct separation of the data by site-year. The use of GS 25 tiller densities might be a potential means of distinguishing low from high biomass areas, as GS 25 tiller densities were related to GS 30 biomass ($r^2 = 0.74$).

The positive agronomic aspects of high GS 30 biomass (> 1000 kg ha^{-1}) in winter wheat, as defined by this study, would indicate a high amount of plant tissue and possibly a high number of tillers per plant, which would result in high yield potentials. With the bulk of N uptake occurring after GS 30, a higher biomass stand will potentially have a better root system and be able to utilize more to the available N compared to a low biomass stand with potentially smaller root systems (Weisz, 2004). This would indicate that there would be better NUE in high biomass stands. The negative agronomic aspects of high GS 30 biomass are that a late spring frost could potentially result in freeze damage to the tissue and drastically reduce yield potentials. Low GS 30 biomass (< 1000 kg ha^{-1}) does not automatically mean low yield potential, as indicated by site-years C-3 and T-1 where GS 30 biomass was lower than 1000 kg ha^{-1} but resulted in the highest yields. Nevertheless, there is still a possibility of lower yields with low GS 30 biomass as seen in P-1. Also with low GS 30 biomass, there is a reduced risk that freeze damage will lower yield potential.

The negative spectral aspects of low GS 30 biomass are less canopy coverage of the soil and more soil interference in the photographs. This was evident in this study when site-years with low mean GS 30 biomass had a better relationship between optimum N_{30} and a soil-adjusted spectral index, Rel_{sub} GSAVI ($R^2 = 0.63$) than with indexes that did not adjust for exposed soil. However, this did not hold true for the low GS 30 biomass site-year by PDSR combinations, for which the best relationship between optimum N_{30} and spectral indicators was found with Rel_{sub} RVI ($R^2 = 0.45$). It should be noted that this relationship did

not account for even half of the model variability, indicating that soil alone may not be the only source of variation in low GS 30 biomass areas.

SUMMARY

Our objectives were to determine if an in-season N recommendation for soft red winter wheat at GS 30 could be developed using aerial CIR photographs, and if crop biomass influenced the recommendation model. We used PDSR and N_{25} treatments to create different GS 25 tiller densities and GS 30 biomass, and the N_{30} treatments to determine optimum N rates at GS 30. In this study, the PDSR treatments influenced GS 25 tiller densities, GS 30 biomass, tissue N concentrations, and N uptake, as well as grain yield. Previous research pursued similar objectives but used GS 30 tissue N concentrations to derive in-season N recommendations (Flowers et al., 2003a; Raun et al., 2002). We took a different path by developing a direct link between optimum N_{30} and spectral indicators, thus eliminating the variability caused by first developing relationships between tissue N concentrations and spectral data, then translating the predicted tissue N concentration into an optimum GS 30 N recommendation.

When all data were considered together, the relationships of optimum N_{30} with spectral indicators were not strong. To discover the reasons behind this, the relationships between optimum N_{30} and GS 30 tissue N concentrations were examined and were found to be influenced by biomass, indicating that biomass might influence relationships between optimum N_{30} and spectral indicators. Analyses using GS 25 tiller density, GS 30 biomass, and PDSR as covariates showed that these covariates affected some of the relationships of optimum N_{30} with spectral indicators, but did not substantially improve prediction. However, when site-year–PDSR combinations were separated into high biomass (> 1000 kg ha^{-1}) and

low biomass (< 1000 kg ha^{-1}) classes, strong predictive relationships emerged for the high biomass combinations. Relationships between several spectral indicators and optimum N_{30} improved compared to the low biomass classes or all of the data considered together.

To facilitate the use of these improved relationships between spectral indicators and optimum N_{30}, the user must identify biomass levels. Unfortunately, we were not able to identify a method that used spectral data to determine GS 30 biomass. It is clear that additional research is necessary to determine if and how that might be achieved. There is some potential to pursue the relationship of optimum N_{30} with Rel_{sub} RVI with low biomass, though this was not investigated in this study.

To summarize, when only the PDSR plots with GS 30 biomass greater than 1000 kg ha^{-1} were considered, we found strong relationships of optimum N_{30} with the spectral indicators Rel_{sub} Red and Rel_{sub} Green (R^2 = 0.80 and 0.81, respectively). Additional research is needed to validate these indicators as predictors of optimum N_{30}. If an efficient means can be found to determine if a soft red winter wheat stand has GS30 biomass > 1000 kg ha^{-1}, these spectral indicators derived from aerial CIR photography show substantial potential to determine agronomic optimum N_{30}.

REFERENCES

Alley, M.M., P.C. Scharf, D.E. Brann, W.E. Baethgen, and J.L. Hamons. 1994. Nitrogen management for winter wheat: principles and recommendations. Virginia Cooperative Extension. Crop & Soil Environmental Sciences. Publication 424-026.

Baethgen, W.E., and M.M. Alley. 1989. Optimizing soil and fertilizer nitrogen use by intensively managed winter wheat. I. Crop nitrogen uptake. Agron. J. 81:116-120.

Blackmer, T.M., and J.S. Schepers. 1994. Techniques for monitoring crop nitrogen status in corn. Commun. Soil Sci. Plant Anal. 25:1791-1800.

Blackmer, T.M., J.S. Schepers, G.E. Varvel, and E.A. Walter-Shea. 1996. Nitrogen deficiency detection using reflected shortwave radiation from irrigated corn canopies. Agron. J. 88:1-5.

Clarke, T.R., E.M. Barnes, and P.J. Pinter. 2001. Remote sensing of chlorophyll content in broccoli using a 3-band radiometer. [CD-ROM]. *In* 2001 Agronomy abstracts. ASA, Charlotte, NC.

Clarke, T.R., E.M. Barnes, P.J. Pinter, B.A. Kimball, J.A. Haberland, E.A. Riley, and T.L. Thompson. 2000. A three-band, two dimensional reflectance index for wheat and cotton canopy chlorophyll content. p. 27. *In* 2000 Agronomy abstracts. ASA,Minneapolis, MN.

Counce, P.A., B.R. Wells, J.R. Davis, and A.M. Simpson. 1984. Response of wheat to N-source, N-rate and nitrification inhibitors. Arkansas Farm Res. 33:2.

Donohue, S.J., and D. E. Brann. 1984. Optimum N concentration in winter wheat grown in the coastal plain region of Virginia. Commun. Soil Sci. Plant Anal. 15:651-661.

Eastman Kodak. 1996. KODAK EKTACHROME Professional Infrared EIR Film Technical Information Data Sheet TI2323. Eastman Kodak Co., Rochester, NY.

Flowers, M., R. Weisz, and R. Heiniger. 2001. Remote sensing of winter wheat tiller density for early nitrogen application decisions. Agron. J. 93:783-789.

Flowers, M., R. Weisz, and R. Heiniger. 2003a. Quantitative approaches for using color infrared photography for assessing in-season nitrogen status in winter wheat. Agron. J. 95:1189-1200.

Flowers, M., R. Weisz, R. Heiniger, D. Osmond, and C. Crozier. 2004. In-season nitrogen optimization and site-specific management for soft red winter wheat. Agron. J. 96:124-134.

Flowers, M., R. Weisz, R. Heiniger, B. Tarleton, and A. Meijer. 2003b. Field validation of a remote sensing technique for early nitrogen application decisions in wheat. Agron. J. 95:167-176.

Frederick, J.R., and P.J. Bauer. 1999. Physiological and numerical components of wheat yield. *In* E.H. Satorre, and G.A. Slafer (ed.) Wheat ecology and physiology of yield determination. Food Products Press, New York.

Gates, D.M., H.J. Keegan, J.C. Schleter, and V.R. Weidner. 1965. Spectral properties of plants. Applied Optics. 4(1):11-20.

Gilliam, J.W., D.L. Osmond, and R.O. Evans. 1997. Selected agriculture best management practices to control nitrogen in the Neuse river basin. North Carolina Agriculture Research Service Technical Bulletin 311, North Carolina State Univ., Raleigh, NC.

Gitelson, A.A., Y.J. Kaufman, and M.N. Merzlyak. 1996. Use of a green channel in remote sensing of global vegetation from EOS-MODIS. Remote Sens. Environ. 58:289-298.

Hardy, D.H., D.L. Osmond, and A. Wossink. 2002. An overview of nutrient management with economic considerations. Publication No. AG-565. North Carolina Coop. Ext. Ser., North Carolina State Univ., Raleigh, NC.

Hinzman, L.D., M.E. Bauer, and C.S.T. Daughtry. 1986. Effects of nitrogen fertilizer on growth and reflectance characteristics of winter wheat. Remote Sens. Environ. 19: 47-61.

Huete, A.R. 1988. A soil adjusted vegetation index (SAVI). Int. J. Remote Sens. 9:295-309.

Jordan, C.F. 1969. Derivation of leaf area index from quality of light on the forest floor. Ecology 50:663-666.

Knipling, E.B. 1970. Physical and physiological basis for the reflectance of visible and near-infrared radiation from vegetation. Rem. Sens. Environ. 1:155-159.

McGeehan S.L., and D.V. Naylor. 1988. Automated instrumental analysis of carbon and nitrogen in plant and soil samples. Comm. Soil Sci. Plant Anal. 19:493-505.

Raun, W.R., and G.V. Johnson. 1999. Improving nitrogen use efficiency for cereal production. Agron. J. 91:357-363.

Raun. W.R., J.B. Solie, G.V. Johnson, M.L. Stone, R.W. Mullen, K.W. Freeman, W.E. Thomason, and E.V. Lukina. 2002. Improving nitrogen use efficiency in cereal grain production with optical sensing and variable rate application. Agron. J. 94:815-820.

Raun, W.R., and R.L. Westerman. 1991. Nitrate-N and phosphate-P concentration in winter wheat at varying growth stages. J. Plant Nutr. 14:267-281.

Reeves, D.W., P.L. Mask, C.W. Wood, and D.P. Delaney. 1993. Determination of wheat nitrogen status with a hand-held chlorophyll meter: influence of management practices. J. Plant Nutr. 16:781-796.

Rondeaux, G., M. Steven, and F. Baret. 1996. Optimization of soil-adjusted vegetation indices. Remote Sens. Environ. 55:95-107.

Roth, G.W., R.H. Fox, and H.G. Marshall. 1989. Plant tissue tests for predicting nitrogen fertilizer requirements of winter wheat. Agron. J. 81:502-507.

Rouse, Jr., J.W., R.H. Hass, J.A. Schell, and D.W. Deering. 1973. Monitoring vegetation systems in the Great Plains with ERTS. ERTS Symp. 3rd, Washington, D.C., 10-14 Dec. 1972. NASA SP-351. Vol. I:309-317.

Scharf, P.C., and M.M. Alley. 1993. Spring nitrogen on winter wheat: II. A flexible multicomponent rate recommendation system. Agron. J. 85:1186-1192.

Scharf, P.C., and M.M. Alley. 1994. Residual soil nitrogen in humid region wheat production. J. Prod. Agric. 7:81-85.

Scharf. P.C., M.M. Alley, and Y. Z. Lei. 1993. Spring nitrogen on winter wheat: I. Farmer-field validation of tissue test-based rate recommendations. Agron. J. 85:1181-1186.

Serrano, L., I. Filella, and J. Peñuelas. 2000. Remote sensing of biomass and yield of winter wheat under different nitrogen supplies. Crop Sci. 40:723-731.

State Climate Office of North Carolina. 2004. [Online]. Available at www.nc-climate.ncsu.edu. (accessed Aug. 2004; verified March 2005). Raleigh, NC.

Sripada, R.P., R.W. Heiniger, J.G. White, and R. Weisz. 2005. Aerial color infrared photography for determining late-season nitrogen requirements in corn. Agron. J. 97:1443-1451.

Tucker, C.J. 1979. Red and photographic infrared linear combinations for monitoring vegetation. Remote Sens. Environ. 8:127-150.

Vaughan, B., K.A. Barbarick, D.G. Westfall, and P.L. Chapman. 1990. Tissue nitrogen levels for dryland hard red winter wheat. Agron. J. 82:561-565.

Wanjura, D.F., and J.L. Hatfield. 1987. Sensitivity of spectral vegetative indices to crop biomass. Trans. ASAE. 30:810-816.

Weisz, R. 2004. Small grain production guide 2004-2005. Pub. Num. 580. North Carolina Cooperative Extension Service, Raleigh, NC. AG-580.

Weisz, R., C.R. Crozier, and R.W. Heiniger. 2001. Optimizing nitrogen application timing in no-till soft red winter wheat. 2001. Agron. J. 93:435-442.

Zadoks, J.C., T.T. Chang, and C.F. Zonzak. 1974. A decimal code for the growth stages of cereals. Weed. Res. 14:415-421.

Table 1. Site-year identification, locations and soil types, years, cultivars, planting date-seeding rate (PDSR) treatments, planting dates, and seeding rates for the remote sensing study of soft red winter wheat in North Carolina.

Site-year	Locations and soil type	Year	Cultivar	PDSR treatment§	Planting date	Seeding rate
						seeds m^{-2}
C-1	Cunningham Research Station	2002	C 9704†	1	17 Oct.	480
	Lynchburg sandy loam (fine, loamy,			2	6 Nov.	480
	siliceous, thermic, Aeric Paleaquults)			3	6 Nov.	185
C-2	Cunningham Research Station	2003	C 9704	1	22 Oct.	480
	Lynchburg sandy loam (fine, loamy,			2	5 Nov.	480
	siliceous, thermic, Aeric Paleaquults)			3	5 Nov.	185
C-3	Cunningham Research Station	2004	C 9184‡	1	23 Oct.	480
	Lynchburg sandy loam (fine, loamy,			2	10 Nov.	480
	siliceous, thermic, Aeric Paleaquults)			3	10 Nov.	185
L-1	Lower Coastal Plain Tobacco Research Station	2002	C 9704	1	17 Oct.	480
	Goldsboro loamy sand (fine, loamy,			2	6 Nov.	480
	siliceous, thermic Aquic Paludults)			3	6 Nov.	185

Table 1. (continued)

P-1	Piedmont Research Station	2003	C 9704	1	25 Nov.	689
	Hiwassee clay loam (fine, kaolinitic,			2	25 Nov.	517
	thermic Typic Rhodudults)			3	25 Nov.	258
T-1	Tidewater Research Station	2004	C 9184	1	10 Nov.	504
	Cape Fear loam (clayey, mixed,			2	10 Nov.	349
	thermic, Typic Umbraquults)			3	10 Nov.	194

† Cultivar 'Coker 9704'
‡ Cultivar 'Coker 9184'
§ PDSR treatments were: timely planting date and high seeding rate, PDSR 1; late planting date and high seeding rate, PDSR 2; late planting date and low seeding rate, PDSR 3, except at T-1 and P-1 where; PDSR 1 was late planting date and high seeding rate, PDSR2 was late planting date and medium seeding rate, and PDSR 3 was late planting date and low seeding rate.

Table 2. Summary weather data for mean daily temperature and mean daily total precipitation for the periods between planting and the N application at growth stage (GS) 25 (N_{25}), GS 25 to N applied at GS 30 (N_{30}), and GS 30 to harvest, with the 30-yr averages for the same time periods.

	Planting to GS 25				GS 25 to GS 30				GS 30 to Harvest			
Site-year†	Mean daily temperature		Mean daily total precipitation		Mean daily temperature		Mean daily total precipitation		Mean daily temperature		Mean daily total precipitation	
	Trial	30-yr avg	Trial	30-yr avg	Trial	30-yr avg	Trial	30-yr avg	Trial	30-yr avg	Trial	30-yr avg
	------- °C -------		------- cm -------		------- °C -------		------- cm -------		------- °C -------		------- cm -------	
C-1	10.5	10.7	11.4	28.6	9.8	8.3	8.1	10.4	15.3	16.6	26.6	28.7
L-1	10.5	10.7	11.4	28.6	9.8	8.3	8.1	10.4	15.3	16.9	26.6	30.2
C-2	7.5	10.0	22.4	30.2	8.9	10.4	20.0	16.0	16.8	19.1	34.5	23.1
P-1	4.3	6.4	25.0	26.7	8.4	9.3	21.7	14.7	16.2	18.9	55.9	27.0
C-3	10.8	10.4	28.5	25.7	8.2	8.9	13.2	16.2	18.9	18.1	32.3	27.6
T-1	7.3	8.2	33.8	31.1	10.6	9.6	4.8	7.1	18.3	17.6	31.9	32.2

† C-1, Cunningham Research Station in 2002; L-1, Lower Coastal Plain Tobacco Research Station in 2002; C-2, Cunningham Research Station in 2003; P-1, Piedmont Research Station in 2003; C-3, Cunningham Research Station in 2004; T-1, Tidewater Research Station in 2004.

Table 3. Spectral bands, band combinations, and indexes used in the remote sensing study of soft red winter wheat in North Carolina.

Spectral Index	Formula	Reference
	Absolute indexes	
Near infrared spectral band (NIR)	NA	-
Red spectral band	NA	-
Green spectral band	NA	-
Normalized Difference Vegetation Index (NDVI)	(NIR[†] – Red)/(NIR + Red)	Rouse et al., 1973
Green Normalized Difference Vegetation Index (GNDVI)	(NIR – Green)/(NIR + Green)	Gitelson et al., 1996
Difference Vegetation Index (DVI)	NIR – Red	Tucker, 1979
Green Difference Vegetation Index (GDVI)	NIR – Green	Tucker, 1979
Ratio Vegetation Index (RVI)	NIR/Red	Jordan, 1969
Green Ratio Vegetation Index (GRVI)	NIR/Green	Sripada et al., 2005
Soil Adjusted Vegetation Index (SAVI)	[(NIR – Red)/(NIR + Red + 0.5)] × 1.5	Huete, 1988
Green Soil Adjusted Vegetation Index (GSAVI)	[(NIR – Green)/(NIR + Green + 0.5)] × 1.5	Sripada et al., 2005
Optimized Soil Adjusted Vegetation Index (OSAVI)	(NIR - Red)/(NIR + Red + 0.16)	Rondeaux et al., 1996
Green Optimized Soil Adjusted Vegetation Index (GOSAVI)	(NIR - Green)/(NIR + Green+ 0.16)	Sripada et al., 2005
NormNIR	NIR/(NIR + Red + Green)	Sripada et al., 2005
NormRed	Red/(NIR + Red + Green)	Sripada et al., 2005

Table 3. (continued)

NormGreen	Green/(NIR + Red + Green)	Sripada et al., 2005
<u>Relative bands and indexes using division by reference plot spectral value</u>		
Relative NIR (Rel_{div} NIR)	$NIR_{plot}/NIR_{reference\ plot}$	Sripada et al., 2005
Relative Red (Rel_{div} Red)	$Red_{plot}/Red_{reference\ plot}$	Sripada et al., 2005
Relative Green (Rel_{div} Green)	$Green_{plot}/Green_{reference\ plot}$	Sripada et al., 2005
Relative Normalized Difference Vegetation Index (Rel_{div} NDVI)	$NDVI_{plot}/NDVI_{reference\ plot}$	Sripada et al., 2005
Relative Green Normalized Difference Vegetation Index (Rel_{div} GNDVI)	$GNDVI_{plot}/GNDVI_{reference\ plot}$	Sripada et al., 2005
Relative Difference Vegetation Index (Rel_{div} DVI)	$DVI_{plot}/DVI_{reference\ plot}$	Sripada et al., 2005
Relative Green Difference Vegetation Index (Rel_{div} GDVI)	$GDVI_{plot}/GDVI_{reference\ plot}$	Sripada et al., 2005
Relative Ratio Vegetation Index (Rel_{div} RVI)	$RVI_{plot}/RVI_{reference\ plot}$	Sripada et al., 2005
Relative Green Ratio Vegetation Index (Rel_{div} GRVI)	$GRVI_{plot}/GRVI_{reference\ plot}$	Sripada et al., 2005
Relative Soil Adjusted Vegetation Index (Rel_{div} SAVI)	$SAVI_{plot}/SAVI_{reference\ plot}$	Sripada et al., 2005
Relative Soil Adjusted Vegetation Index (Rel_{div} GSAVI)	$GSAVI_{plot}/GSAVI_{reference\ plot}$	Sripada et al., 2005
Relative Optimized Soil Adjusted Vegetation Index (Rel_{div} OSAVI)	$OSAVI_{plot}/OSAVI_{reference\ plot}$	Sripada et al., 2005
Relative Green Optimized Soil Adjusted Vegetation Index (Rel_{div} GOSAVI)	$GOSAVI_{plot}/GOSAVI_{reference\ plot}$	Sripada et al., 2005
<u>Relative bands and indexes using subtraction of reference plot spectral value</u>		
Relative NIR (Rel_{sub} NIR)	NIR_{plot}-$NIR_{reference\ plot}$	-

Table 3. (continued)

Relative Red (Rel_{sub} Red)	Red_{plot}-$Red_{reference\ plot}$	-
Relative Green (Rel_{sub} Green)	$Green_{plot}$-$Green_{reference\ plot}$	-
Relative Normalized Difference Vegetation Index (Rel_{sub} NDVI)	$NDVI_{plot}$-$NDVI_{reference\ plot}$	-
Relative Green Normalized Difference Vegetation Index (Rel_{sub} GNDVI)	$GNDVI_{plot}$-$GNDVI_{reference\ plot}$	-
Relative Difference Vegetation Index (Rel_{sub} DVI)	DVI_{plot}-$DVI_{reference\ plot}$	-
Relative Green Difference Vegetation Index (Rel_{sub} GDVI)	$GDVI_{plot}$-$GDVI_{reference\ plot}$	-
Relative Ratio Vegetation Index (Rel_{sub} RVI)	RVI_{plot}-$RVI_{reference\ plot}$	-
Relative Green Ratio Vegetation Index (Rel_{sub} GRVI)	$GRVI_{plot}$-$GRVI_{reference\ plot}$	-
Relative Soil Adjusted Vegetation Index (Rel_{sub} SAVI)	$SAVI_{plot}$-$SAVI_{reference\ plot}$	-
Relative Soil Adjusted Vegetation Index (Rel_{sub} GSAVI)	$GSAVI_{plot}$-$GSAVI_{reference\ plot}$	-
Relative Optimized Soil Adjusted Vegetation Index (Rel_{sub} OSAVI)	$OSAVI_{plot}$-$OSAVI_{reference\ plot}$	-
Relative Green Optimized Soil Adjusted Vegetation Index (Rel_{sub} GOSAVI)	$GOSAVI_{plot}$-$GOSAVI_{reference\ plot}$	-

[†]NIR is the near infrared spectral band

Table 4. ANOVA by site-year for the effects of planting date-seeding rate (PDSR) combinations, N applied at growth stage (GS) 25 (N_{25}), and N applied at GS 30 (N_{30}) on soft red winter wheat tiller densities at GS 25; biomass, tissue N concentration, and N uptake at GS 30; and grain yield.

	Site-year					
Source of Variation	C-1†	L-1	C-2	P-1	C-3	T-1
			GS 25 tiller densities			
PDSR	***	**	***	***	***	***
			GS 30 biomass			
PDSR	***	***	***	***	NS	***
N_{25}	**	**	***	***	***	NS
PDSR × N_{25}	NS	NS	NS	NS	NS	NS
			GS 30 tissue N concentration			
PDSR	***	***	***	**	***	***
N_{25}	***	***	***	***	***	***
PDSR × N_{25}	NS	*	NS	NS	NS	NS
			GS 30 N uptake			
PDSR	NS	NS	***	***	NS	***
N_{25}	***	***	***	***	***	***
PDSR × N_{25}	NS	NS	NS	NS	NS	NS
			Grain yield			
PDSR	*	*	***	NS	NS	NS
N_{25}	***	***	***	***	***	***
PDSR × N_{25}	NS	NS	*	NS	NS	NS

Table 4. (continued)

N_{30}	***	***	***	***	***	***
$N_{25} \times N_{30}$	***	***	***	***	***	***
PDSR × N_{30}	NS	***	*	NS	***	*
PDSR × N_{25} × N_{30}	NS	NS	*	NS	NS	NS

*, **, ***, and NS, Significant at p = 0.05, 0.01, 0.001, and not significant, respectively.
† C-1, Cunningham Research Station in 2002; L-1, Lower Coastal Plain Tobacco Research Station in 2002; C-2, Cunningham Research Station in 2003; P-1, Piedmont Research Station in 2003; C-3, Cunningham Research Station in 2004; T-1, Tidewater Research Station in 2004.

Table 5. Summary statistics for grain yield by site-year, and main effect treatment means (planting date-seeding rate, PDSR; N applied at growth stage (GS) 25, N_{25}; N applied at GS 30, N_{30}) for grain yield at each site-year (*n* = number of split-split plots for each site-year) for a soft red winter wheat study in North Carolina.

	Site-year†					
Variable	C-1	L-1	C-2	P-1	C-3	T-1
n	373	369	340	353	370	360
CV, %	16.0	18.6	20.9	12.7	18.6	20.2
	---------- Mg ha^{-1} ----------					
Mean	4.9	4.3	4.6	3.0	4.5	6.1
SD	0.8	0.8	1.0	0.4	0.8	1.2
Min.	2.3	1.8	2.7	2.0	1.4	1.9
Max.	6.6	5.9	7.1	3.9	6.2	10.1
	PDSR treatment mean					
PDSR 1	5.1	4.5	5.4	3.0	4.6	6.2
PDSR 2	4.8	4.3	4.4	3.0	4.5	6.1
PDSR 3	4.7	4.0	3.8	3.0	4.6	5.9
	N_{25} treatment mean					
0 kg ha^{-1}	4.3	3.6	4.0	2.7	3.7	4.9
34 kg ha^{-1}	4.8	4.0	4.5	3.1	4.3	5.9
67 kg ha^{-1}	5.0	4.4	4.8	3.2	4.7	6.2
101 kg ha^{-1}	5.3	4.6	4.6	3.1	5.0	6.6
134 kg ha^{-1}	5.1	4.8	4.6	2.9	5.1	6.8

Table 5. (continued)

	N_{30} treatment mean					
0 kg ha^{-1}	4.1	3.5	4.0	2.7	3.7	5.1
34 kg ha^{-1}	4.7	4.1	4.4	3.1	4.3	5.6
67 kg ha^{-1}	5.0	4.5	4.7	3.2	4.7	6.4
101 kg ha^{-1}	5.3	4.7	4.8	3.1	5.0	6.6
134 kg ha^{-1}	5.3	4.7	4.8	2.9	5.1	6.7

† C-1, Cunningham Research Station in 2002; L-1, Lower Coastal Plain Tobacco Research Station in 2002; C-2, Cunningham Research Station in 2003; P-1, Piedmont Research Station in 2003; C-3, Cunningham Research Station in 2004; T-1, Tidewater Research Station in 2004.

Table 6. Coefficients of determination for significant ($p \leq 0.05$) linear, quadratic, and exponential relationships of growth stage (GS) 30 tissue N concentration ($n = 450$) and optimum GS 30 N (N_{30}) rate ($n = 86$) with spectral bands and indexes in soft red winter wheat remote sensing experiments conducted in North Carolina.

	GS 30 tissue N concentration			Optimum N_{30}		
Spectral bands and indexes	Linear	Quadratic	Exponential	Linear	Quadratic	Exponential
	r^2	R^2	R^2	r^2	R^2	R^2
			Absolute bands and indexes			
NIR	0.16	NS†	NS	NS	NS	NS
Red	0.37	NS	NS	0.10	NS	NS
Green	0.36	NS	NS	0.06	NS	NS
NDVI	0.23	0.40	0.37	0.26	NS	NS
GNDVI	0.22	0.27	NS	0.26	NS	0.33
DVI	0.14	0.27	NS	0.26	0.30	NS
GDVI	0.24	0.31	NS	0.25	NS	NS
RVI	0.14	0.35	NS	0.21	0.26	NS
GRVI	0.19	0.24	NS	0.24	NS	NS
SAVI	0.23	0.40	0.36	0.26	NS	NS
GSAVI	0.22	0.27	0.28	0.26	NS	NS
OSAVI	0.27	0.39	0.36	0.29	NS	NS
GOSAVI	0.23	0.28	0.28	0.28	NS	NS
NormNIR	0.22	0.34	0.35	0.26	NS	NS
NormRed	0.21	0.40	NS	0.23	NS	NS

Table 6. (continued)

NormGreen	0.12	NS	NS	0.18	NS	NS
Relative bands and indexes using division by reference plot spectral value						
Rel_{div} NIR	0.01	NS	NS	0.08	NS	NS
Rel_{div} Red	0.14	0.23	NS	0.34	0.51	NS
Rel_{div} Green	0.06	0.12	NS	0.28	0.49	NS
Rel_{div} NDVI	0.25	NS	NS	0.43	0.50	0.50
Rel_{div} GNDVI	0.12	NS	NS	0.45	0.51	0.51
Rel_{div} DVI	0.33	NS	NS	0.35	0.50	NS
Rel_{div} GDVI	0.19	NS	NS	0.42	0.48	0.49
Rel_{div} RVI	0.21	NS	NS	0.46	0.51	0.52
Rel_{div} GRVI	0.10	NS	NS	0.47	0.52	0.52
Rel_{div} SAVI	0.25	NS	NS	0.43	0.50	0.50
Rel_{div} GSAVI	0.12	NS	NS	0.45	0.51	0.51
Rel_{div} OSAVI	0.30	NS	NS	0.38	0.49	0.47
Rel_{div} GOSAVI	0.16	NS	NS	0.43	0.47	0.48
Relative bands and indexes using subtraction of reference plot spectral value						
Rel_{sub} NIR	0.03	0.04	NS	0.14	NS	0.14
Rel_{sub} Red	0.26	NS	NS	0.41	0.49	0.49
Rel_{sub} Green	0.14	0.15	NS	0.39	0.51	0.49
Rel_{sub} NDVI	0.22	0.23	NS	0.44	0.51	0.53
Rel_{sub} GNDVI	0.10	0.11	0.10	0.45	0.52	0.53
Rel_{sub} DVI	0.32	NS	NS	0.37	0.52	0.50

Table 6. (continued)

Rel_{sub} GDVI	0.17	NS	NS	0.43	0.50	0.52
Rel_{sub} RVI	0.12	0.16	0.15	0.37	0.49	0.50
Rel_{sub} GRVI	0.05	0.08	NS	0.42	0.51	0.51
Rel_{sub} SAVI	0.22	0.23	0.22	0.44	0.51	053
Rel_{sub} GSAVI	0.10	0.11	NS	0.45	0.52	0.53
Rel_{sub} OSAVI	0.27	NS	NS	0.41	0.50	0.51
Rel_{sub} GOSAVI	0.13	NS	NS	0.44	0.50	0.52

† NS, not significant at $p = 0.05$.

Table 7. Results of covariate analyses for the dependent variables growth stage (GS) 30 tissue N concentration and optimum GS 30 N (N_{30}) rate with spectral indicators as the independent variable and biomass at GS 30 as a quantitative covariate with the associated interaction. Study conducted in soft red winter wheat in North Carolina.

	GS 30 tissue N concentrations				Optimum N_{30}			
Spectral bands and indexes	Spectral†	Biomass‡	Spectral × biomass	R^2	Spectral	Biomass	Spectral × biomass	R^2
	F-value	F-value	F-value		F-value	F-value	F-value	
				Absolute bands and indexes				
NIR	0.23	3.42	9.54**	0.24	1.43	3.34	2.69	NS
Red	28.59***	1.57	0.49	0.39	0.04	10.23**	4.47*	0.24
Green	21.92***	0.55	5.32*	0.39	0.04	12.11***	6.92*	0.22
NDVI	53.87***	19.24***	11.47***	0.40	10.66**	5.77*	2.40	0.31
GNDVI	46.42***	139.87***	6.49*	0.40	8.18**	1.82	1.31	0.30
DVI	7.18**	80.41***	1.39	0.34	4.25*	1.19	0.04	0.27
GDVI	41.56***	114.34***	5.35*	0.40	7.25**	3.07	0.89	0.30
RVI	46.55***	0.02	14.18***	0.35	11.46**	5.57*	4.33*	0.27
GRVI	44.84***	0.11	8.28**	0.39	7.38**	2.25	1.58	0.27

Table 7. (continued)

SAVI	53.58***	19.40***	11.36***	0.40	10.64**	5.75*	2.39	0.31
GSAVI	46.42***	139.8***	6.49*	0.40	8.18**	1.82	1.31	0.30
OSAVI	54.63***	14.75***	9.26**	0.42	9.96**	5.40*	1.56	0.33
GOSAVI	46.18***	133.16***	5.01*	0.41	8.48**	2.08	1.05	0.31
NormNIR	57.44***	3.86	12.48***	0.41	11.09**	3.45	2.77	0.31
NormRed	37.21***	13.99***	6.32*	0.37	6.97**	0.67	1.08	0.27
NormGreen	23.56***	4.34*	1.89	0.34	2.88	0.09	0.14	0.19
			Relative bands and indexes using division by reference plot spectral value					
Rel_{div} NIR	12.77***	28.49***	33.71***	0.26	2.52	19.46***	18.87***	0.28
Rel_{div} Red	1.52	6.09*	26.36***	0.41	1.16	8.28**	6.90*	0.41
Rel_{div} Green	0.46	4.03*	13.60***	0.33	0.47	6.29*	5.73*	0.34
Rel_{div} NDVI	33.72***	12.82***	7.08**	0.50	8.68**	0.63	0.80	0.46
Rel_{div} GNDVI	18.20***	9.02**	5.49*	0.41	13.84***	0.04	0.09	0.49
Rel_{div} DVI	48.83***	1.70	0.40	0.51	2.93	2.65	2.82	0.38

Table 7. (continued)

Rel_{div} GDVI	19.09***	16.48***	11.55***	0.45	6.72*	1.21	1.37	0.45
Rel_{div} RVI	21.98***	24.28***	8.70**	0.47	12.75***	0.01	0.05	0.50
Rel_{div} GRVI	14.24***	9.42**	3.28	0.38	18.95***	0.41	0.13	0.51
Rel_{div} SAVI	33.72***	12.80***	7.08**	0.50	8.67**	0.63	0.81	0.46
Rel_{div} GSAVI	18.17***	9.03**	5.51*	0.41	13.83***	0.04	0.09	0.49
Rel_{div} OSAVI	48.08***	6.21*	2.54	0.51	4.31*	2.37	2.68	0.41
Rel_{div} GOSAVI	24.66***	12.62***	7.62**	0.45	6.86*	1.90	2.25	0.46
		Relative bands and indexes using subtraction by reference plot spectral value						
Rel_{sub} NIR	4.12*	80.25***	23.03***	0.26	0.72	8.93**	12.52***	0.28
Rel_{sub} Red	14.75***	79.18***	12.93***	0.46	4.50*	10.10**	3.60	0.47
Rel_{sub} Green	4.14*	90.32***	21.46***	0.40	2.28	8.56**	8.21**	0.46
Rel_{sub} NDVI	22.85***	109.64***	10.69**	0.48	11.7**	3.87	0.04	0.47
Rel_{sub} GNDVI	14.06***	133.74***	3.45	0.38	18.04***	4.87*	0.21	0.50
Rel_{sub} DVI	42.96***	104.40***	1.85	0.51	4.03*	3.66	2.10	0.40

Table 7. (continued)

Rel_{sub} GDVI	16.16***	116.82***	11.58***	0.44	9.56**	4.14*	0.37	0.46
Rel_{sub} RVI	10.99**	112.18***	3.30	0.37	13.79***	3.87	1.64	0.45
Rel_{sub} GRVI	7.68**	123.67***	0.35	0.30	26.93***	5.33*	3.74	0.51
Rel_{sub} SAVI	22.86***	109.59***	10.69**	0.48	11.69**	3.87	0.05	0.47
Rel_{sub} GSAVI	14.04***	133.69***	3.47	0.38	18.03***	4.86*	0.21	0.50
Rel_{sub} OSAVI	39.45***	117.36***	5.49*	0.51	7.31**	3.82	1.15	0.44
Rel_{sub} GOSAVI	20.33***	139.81***	6.35*	0.43	12.06***	4.72*	0.28	0.48

*, **, and *** Significant at $p = 0.05$, 0.01, and 0.001, respectively.
† Spectral bands and indexes as independent variable, referring to the 42 listed in the first column of this table.
‡ Biomass at GS 30 as a quantitative covariate.

Table 8. Results of covariate analyses for the dependent variables growth stage (GS) 30 tissue N concentration and optimum GS 30 N (N_{30}) rate with spectral indicators as the independent variable and GS 25 tiller densities as a quantitative covariate with the associated interaction. Study conducted in soft red winter wheat in North Carolina.

	GS 30 tissue N concentrations				Optimum N_{30}			
Spectral bands and indexes	Spectral†	Tiller densities‡	Spectral × tiller densities	R^2	Spectral	Tiller densities	Spectral × tiller densities	R^2
	F-value	F-value	F-value		F-value	F-value	F-value	
				Absolute bands and indexes				
NIR	0.13	0.02	1.92	0.40	2.35	1.53	1.86	NS
Red	32.54***	26.20***	0.99	0.52	0.01	1.13	0.98	0.11
Green	19.45***	7.76**	0.06	0.51	0.30	2.21	2.21	0.09
NDVI	41.12***	68.35***	5.70*	0.50	14.55***	2.74	4.24*	0.30
GNDVI	24.90***	218.87***	0.56	0.48	9.14**	0.10	1.06	0.27
DVI	4.71*	123.33***	1.43	0.43	10.77**	0.92	1.94	0.27
GDVI	27.49***	199.06***	1.06	0.48	10.25**	0.38	1.58	0.27
RVI	34.33***	8.63**	6.85**	0.46	12.91***	3.34	4.51*	0.26

Table 8. (continued)

GRVI	22.00***	9.54**	0.51	0.47	7.37**	0.69	0.77	0.25
SAVI	40.88***	68.54***	5.63*	0.50	14.55***	2.73	4.24*	0.30
GSAVI	24.91***	218.81***	0.56	0.48	9.14**	0.11	1.06	0.27
OSAVI	44.23***	57.40***	5.29*	0.51	15.20***	3.56	4.13*	0.31
GOSAVI	26.04***	216.35***	0.48	0.49	10.08**	0.19	1.16	0.29
NormNIR	36.54***	0.35	3.68	0.49	12.92***	2.92	2.96	0.29
NormRed	32.94***	18.13***	3.83	0.48	10.53**	2.80	2.83	0.25
NormGreen	31.10***	0.94	0.01	0.42	3.14	0.04	0.03	0.18
		Relative bands and indexes using division by reference plot spectral value						
Rel_{div} NIR	6.51*	10.06**	14.90***	0.38	1.74	7.03**	7.14**	0.16
Rel_{div} Red	7.69**	3.34	3.62	0.49	3.77	0.57	0.67	0.35
Rel_{div} Green	3.62	1.56	2.00	0.44	2.80	0.50	0.70	0.30
Rel_{div} NDVI	36.06***	2.83	0.19	0.58	9.89**	0.01	0.01	0.43
Rel_{div} GNDVI	20.42***	1.08	0.01	0.51	13.06***	0.27	0.27	0.46

Table 8. (continued)

Rel_{div} DVI	64.63***	0.36	2.53	0.60	5.09*	0.51	0.46	0.36
Rel_{div} GDVI	20.48***	2.99	0.65	0.53	6.97**	0.13	0.11	0.42
Rel_{div} RVI	26.99***	11.67***	0.34	0.55	13.61***	0.30	0.27	0.46
Rel_{div} GRVI	17.45***	3.46	0.00	0.48	17.15***	1.17	1.11	0.48
Rel_{div} SAVI	36.10***	2.81	0.18	0.58	9.89**	0.01	0.01	0.43
Rel_{div} GSAVI	20.52***	1.08	0.01	0.51	13.05***	0.27	0.27	0.46
Rel_{div} OSAVI	51.17***	0.62	0.20	0.60	5.42*	0.76	0.68	0.39
Rel_{div} GOSAVI	11.20***	5.11*	1.58	0.50	6.16*	0.48	0.43	0.43
		Relative bands and indexes using subtraction by reference plot spectral value						
Rel_{sub} NIR	1.83	163.69***	14.41***	0.39	0.10	0.34	5.34*	0.20
Rel_{sub} Red	23.76***	164.38***	1.72	0.55	6.94*	0.48	0.24	0.41
Rel_{sub} Green	9.97**	170.33***	3.17	0.49	4.11*	0.14	1.58	0.40
Rel_{sub} NDVI	27.65***	192.58***	0.45	0.56	13.44***	0.03	0.39	0.44
Rel_{sub} GNDVI	18.83***	129.38***	19.22***	0.38	17.21***	0.09	1.43	0.46

Table 8. (continued)

Rel_{sub} DVI	58.08***	206.34***	1.11	0.59	6.46*	0.02	0.18	0.37
Rel_{sub} GDVI	25.30***	204.35***	0.02	0.52	9.93**	0.08	0.06	0.43
Rel_{sub} RVI	24.93***	217.53***	1.41	0.47	13.88***	0.08	2.63	0.40
Rel_{sub} GRVI	11.26***	218.18***	0.36	0.42	23.25***	0.07	5.05*	0.46
Rel_{sub} SAVI	27.69***	192.55***	0.45	0.56	13.42***	0.03	0.38	0.44
Rel_{sub} GSAVI	18.64***	223.35***	0.05	0.48	17.19***	0.09	1.42	0.46
Rel_{sub} OSAVI	41.74***	205.13***	0.02	0.59	8.59**	0.01	0.06	0.41
Rel_{sub} GOSAVI	27.54***	112.89***	27.33***	0.39	11.63**	0.06	0.11	0.45

*, **, and *** Significant at $p = 0.05$, 0.01, and 0.001, respectively.
† Spectral bands and indexes as independent variable, referring to the 42 listed in the first column of this table.
‡ Tiller density at GS 25 as a quantitative covariate.

Table 9. Results of covariate analyses for the dependent variables growth stage (GS) 30 tissue N concentration and optimum GS 30 N (N_{30}) rate with spectral indicators as the independent variable and planting date-seeding rate (PDSR) treatment combination as a fixed factor covariate with the associated interaction. Study in soft red winter wheat conducted in North Carolina.

	GS 30 tissue N concentrations				Optimum N_{30}			
Spectral bands and indexes	Spectral†	PDSR‡	Spectral × PSDR	R^2	Spectral	PDSR	Spectral × PDSR	R^2
	F-value	F-value	F-value		F-value	F-value	F-value	
				Absolute bands and indexes				
NIR	103.15***	0.91	4.00*	0.26	0.09	0.55	0.69	NS
Red	370.94***	10.55***	0.28	0.51	8.56**	0.23	0.47	NS
Green	384.89***	3.86*	2.16	0.52	6.13*	0.63	1.01	NS
NDVI	252.61***	8.04***	12.31***	0.42	37.12***	0.35	2.46	0.33
GNDVI	230.29***	65.11***	5.09**	0.41	32.99***	1.76	0.77	0.30
DVI	117.75***	8.70***	4.16*	0.28	35.63***	0.42	2.41	0.31
GDVI	255.81***	64.16***	5.74**	0.42	31.00***	1.37	0.51	0.28
RVI	177.35***	8.55***	21.50***	0.36	32.00***	2.74	3.83*	0.31
GRVI	196.85***	2.08	7.79***	0.38	28.94***	0.65	1.11	0.29

Table 9. (continued)

SAVI	251.81***	8.04***	12.27***	0.42	37.14***	0.36	2.46	0.33
GSAVI	230.38***.	65.11***	5.10**	0.41	32.98***	1.76	0.77	0.30
OSAVI	290.51***	9.43***	8.52***	0.45	38.80***	0.31	1.96	0.33
GOSAVI	250.2***	68.27***	3.68*	0.42	35.53***	1.88	0.62	0.32
NormNIR	250.61***	6.14**	12.18***	0.43	36.88***	1.61	2.03	0.33
NormRed	204.12***	14.79***	9.14***	0.38	29.89***	2.25	2.00	0.28
NormGreen	106.42***	0.24	0.01	0.28	17.52***	0.00	0.01	0.20
			Relative bands and indexes using division by reference plot spectral value					
Rel_{div} NIR	6.14*	0.77	1.34	0.11	7.42**	0.26	0.27	NS
Rel_{div} Red	76.96***	4.82**	1.20	0.23	45.80***	1.29	1.37	0.37
Rel_{div} Green	30.57***	3.04*	0.79	0.15	33.78***	0.55	0.56	0.30
Rel_{div} NDVI	178.42***	0.37	0.98	0.35	61.26***	0.15	0.16	0.43
Rel_{div} GNDVI	71.06***	0.65	1.22	0.22	67.83***	0.16	0.16	0.46
Rel_{div} DVI	272.9***	0.06	0.29	0.44	42.43***	0.04	0.04	0.35

Table 9. (continued)

Rel_{div} GDVI	116.4***	0.49	0.57	0.28	58.76***	0.05	0.06	0.42
Rel_{div} RVI	137.49***	0.46	2.10	0.30	70.03***	0.39	0.38	0.47
Rel_{div} GRVI	55.10***	0.75	2.10	0.19	72.21***	0.47	0.45	0.48
Rel_{div} SAVI	178.57***	0.37	0.98	0.35	61.24***	0.15	0.16	0.43
Rel_{div} GSAVI	71.26***	0.65	1.21	0.22	67.83***	0.16	0.16	0.46
Rel_{div} OSAVI	225.58***	0.09	0.46	0.40	49.23***	0.01	0.02	0.38
Rel_{div} GOSAVI	81.60***	0.74	0.96	0.23	61.08***	0.03	0.03	0.44
		Relative bands and indexes using subtraction by reference plot spectral value						
Rel_{sub} NIR	14.55***	17.73***	2.29	0.13	13.98***	0.04	0.25	0.15
Rel_{sub} Red	179.46***	25.25***	0.47	0.35	56.52***	0.38	0.23	0.42
Rel_{sub} Green	82.37***	20.9***	0.40	0.23	51.60***	0.26	0.07	0.40
Rel_{sub} NDVI	141.08***	25.32***	1.72	0.31	63.18***	0.13	0.41	0.44
Rel_{sub} GNDVI	31.45***	20.85***	15.48***	0.15	66.80***	0.26	0.52	0.46
Rel_{sub} DVI	253.49***	28.34***	0.25	0.42	47.01***	0.06	0.08	0.37

Table 9. (continued)

Rel_{sub} GDVI	103.89***	22.91***	0.36	0.26	61.51***	0.18	0.06	0.44
Rel_{sub} RVI	65.89***	24.77***	5.29**	0.21	52.79***	0.14	2.39	0.42
Rel_{sub} GRVI	23.92***	22.35***	3.27*	0.14	61.90***	0.2	1.91	0.45
Rel_{sub} SAVI	141.29***	25.32***	1.71	0.31	63.17***	0.13	0.41	0.44
Rel_{sub} GSAVI	53.09***	22.31***	1.88	0.19	66.80***	0.26	0.52	0.46
Rel_{sub} OSAVI	194.25***	26.42***	0.63	0.37	56.22***	0.09	0.10	0.41
Rel_{sub} GOSAVI	43.68***	20.66***	22.09***	0.17	64.73***	0.28	0.07	0.45

*, **, and *** Significant at $p = 0.05$, 0.01, and 0.001, respectively.
† Spectral bands and indexes as independent variable, referring to the 42 listed in the first column of this table.
‡ PDSR is the planting date-seeding rate treatment combination as a fixed factor in the covariate analysis.

Table 10. Coefficients of determination for significant ($p \leq 0.05$) linear, quadratic, and exponential relationships of optimum growth stage (GS) 30 N (N_{30}) rate with spectral bands and indexes separated into two site-year mean-biomass classes, high (> 1000 kg ha^{-1}) (*n* = 58) and low (< 1000 kg ha^{-1}) (*n* = 28) in a remote sensing experiment in soft red winter wheat in North Carolina.

	High biomass site-years			Low biomass site-years		
Spectral bands and indexes	Linear	Quadratic	Exponential	Linear	Quadratic	Exponential
	r^2	R^2	R^2	r^2	R^2	R^2
Relative bands and indexes using division by reference plot spectral value						
Rel_{div} NIR	0.30	0.37	0.34	NS†	0.21	NS
Rel_{div} Red	0.52	0.75	0.72	0.36	NS	0.43
Rel_{div} Green	0.35	0.63	NS	0.37	NS	0.45
Rel_{div} NDVI	0.59	0.64	0.64	0.46	NS	0.54
Rel_{div} GNDVI	0.56	0.62	0.63	0.46	0.56	NS
Rel_{div} DVI	0.46	0.60	NS	0.49	NS	NS
Rel_{div} GDVI	0.51	0.60	0.60	0.45	0.56	NS
Rel_{div} RVI	0.64	0.67	0.67	0.48	NS	0.54
Rel_{div} GRVI	0.58	0.63	0.63	0.49	NS	0.59
Rel_{div} SAVI	0.59	0.64	0.64	0.46	NS	0.54
Rel_{div} GSAVI	0.56	0.62	0.63	0.46	0.56	NS
Rel_{div} OSAVI	0.50	0.61	0.58	0.44	NS	0.53
Rel_{div} GOSAVI	0.53	0.60	0.60	0.43	NS	0.53
Relative bands and indexes using subtraction of reference plot spectral value						
Rel_{sub} NIR	0.32	0.37	0.35	NS	NS	NS

Table 10. (continued)

Rel_{sub} Red	0.70	0.78	0.77	0.32	NS	0.37
Rel_{sub} Green	0.56	0.75	0.72	0.33	NS	0.38
Rel_{sub} NDVI	0.60	0.66	0.66	0.45	0.55	0.58
Rel_{sub} GNDVI	0.55	0.63	0.63	0.47	0.59	0.63
Rel_{sub} DVI	0.50	0.62	059	0.49	0.56	0.58
Rel_{sub} GDVI	0.53	0.61	0.62	0.47	0.58	0.36
Rel_{sub} RVI	0.55	0.67	0.65	0.47	0.57	0.59
Rel_{sub} GRVI	0.52	0.61	0.60	0.52	0.61	0.61
Rel_{sub} SAVI	0.60	0.66	0.66	0.45	0.55	0.59
Rel_{sub} GSAVI	0.55	0.63	0.63	0.47	0.60	0.63
Rel_{sub} OSAVI	0.55	0.63	0.62	0.44	0.54	0.58
Rel_{sub} GOSAVI	0.55	0.62	0.63	0.44	0.56	0.62

† NS, not significant at $p = 0.05$.

Table 11. Coefficients of determination for significant ($p \leq 0.05$) linear, quadratic, and exponential relationships of optimum growth stage (GS) 30 N (N_{30}) rate to spectral bands and indexes separated by site-year and planting date-seeding rate (PDSR) combination into two GS 30 biomass classes: high biomass (> 1000 kg ha^{-1}, n = 39) and low biomass (< 1000 kg ha^{-1}, n = 47) in a remote sensing experiment in soft red winter wheat in North Carolina.

	GS 30 biomass > 1000 kg ha^{-1}			GS 30 biomass < 1000 kg ha^{-1}		
Spectral bands and indexes	Linear	Quadratic	Exponential	Linear	Quadratic	Exponential
	r^2	R^2	R^2	r^2	R^2	R^2
Relative bands and indexes using division by reference plot spectral value						
Rel_{div} NIR	0.45	0.52	0.50	NS†	NS	NS
Rel_{div} Red	0.57	0.78	NS	0.23	0.37	0.39
Rel_{div} Green	0.37	0.68	NS	0.25	0.39	0.39
Rel_{div} NDVI	0.59	0.67	0.68	0.33	0.40	0.40
Rel_{div} GNDVI	0.62	0.71	0.71	0.37	NS	0.40
Rel_{div} DVI	0.46	0.67	NS	0.28	0.41	NS
Rel_{div} GDVI	0.55	0.68	NS	0.34	NS	0.38
Rel_{div} RVI	0.64	NS	0.70	0.36	NS	0.41
Rel_{div} GRVI	0.64	0.71	0.70	0.39	NS	0.41
Rel_{div} SAVI	0.59	0.67	0.68	0.33	0.40	0.40
Rel_{div} GSAVI	0.62	0.71	0.71	0.37	NS	0.40
Rel_{div} OSAVI	0.52	0.66	0.65	0.29	0.40	0.39
Rel_{div} GOSAVI	0.59	0.69	0.70	0.33	NS	0.37
Relative bands and indexes using subtraction of reference plot spectral value						
Rel_{sub} NIR	0.46	NS	0.50	NS	NS	0.06

Table 11. (continued)

Rel_{sub} Red	0.73	0.80	0.80	0.24	0.35	0.33
Rel_{sub} Green	0.63	0.81	0.76	0.27	0.35	0.35
Rel_{sub} NDVI	0.60	0.69	0.69	0.35	0.42	0.43
Rel_{sub} GNDVI	0.61	0.70	0.71	0.38	NS	0.43
Rel_{sub} DVI	0.50	0.69	0.67	0.29	0.43	0.41
Rel_{sub} GDVI	0.56	0.68	0.69	0.36	NS	0.41
Rel_{sub} RVI	0.57	0.68	0.68	0.40	NS	0.45
Rel_{sub} GRVI	0.59	0.68	0.67	0.42	NS	0.44
Rel_{sub} SAVI	0.60	0.69	0.69	0.35	0.42	0.43
Rel_{sub} GSAVI	0.61	0.70	0.71	0.38	NS	0.43
Rel_{sub} OSAVI	0.56	0.66	0.67	0.31	0.41	0.42
Rel_{sub} GOSAVI	0.60	0.70	0.71	0.36	NS	0.41

† NS, not significant at $p = 0.05$.

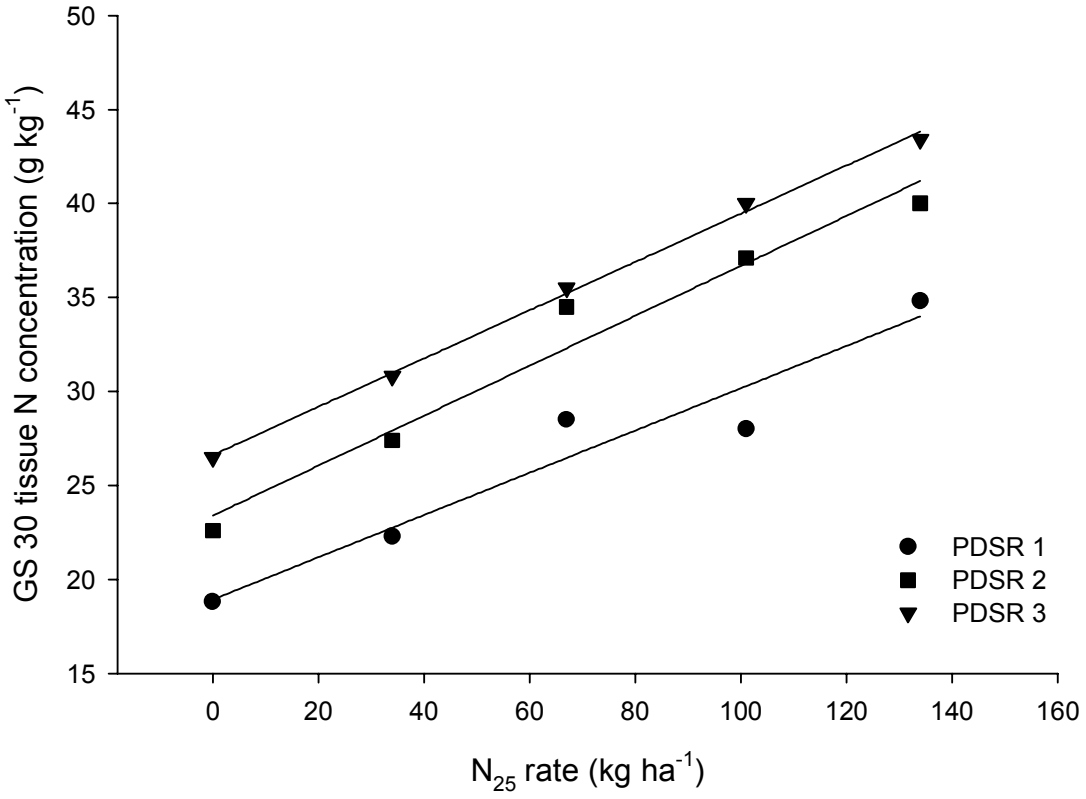


Fig. 1. Response of soft red winter wheat tissue N concentration at growth stage (GS) 30 to N applied at GS 25 (N_{25}) at each planting date-seeding rate combination (PDSR) at the Lower Coastal Plain Tobacco Research Station in 2002 (Site-year L-1) in North Carolina.

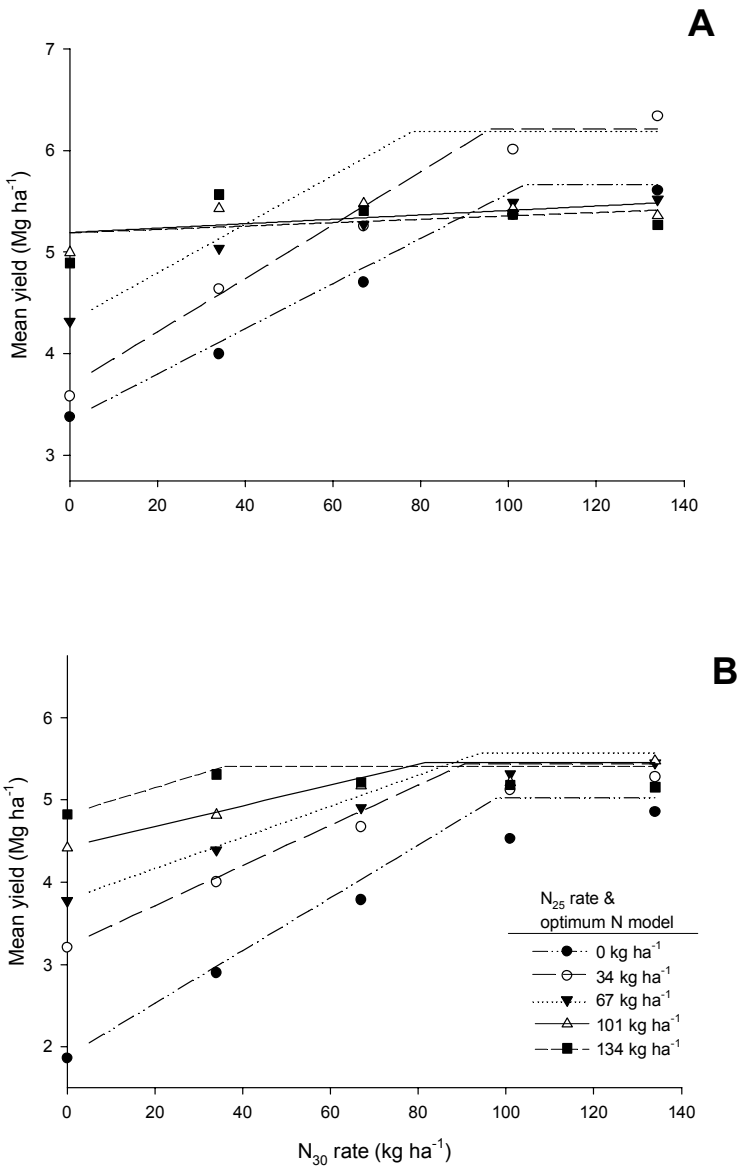


Fig. 2. Yield response of soft red winter wheat to N applied at growth stage (GS) 30 (N_{30}) for the first planting date and high seeding rate (PDSR 1) combination for each GS 25 N rate (N_{25}) for two site-years: A) Cunningham Research Station in 2002 (C-1) and B) Cunningham Research Station in 2004 (C-3). Optimum GS 30 N rates were determined from these responses using a linear plateau model, if significant, or Fisher's Protected LSD.

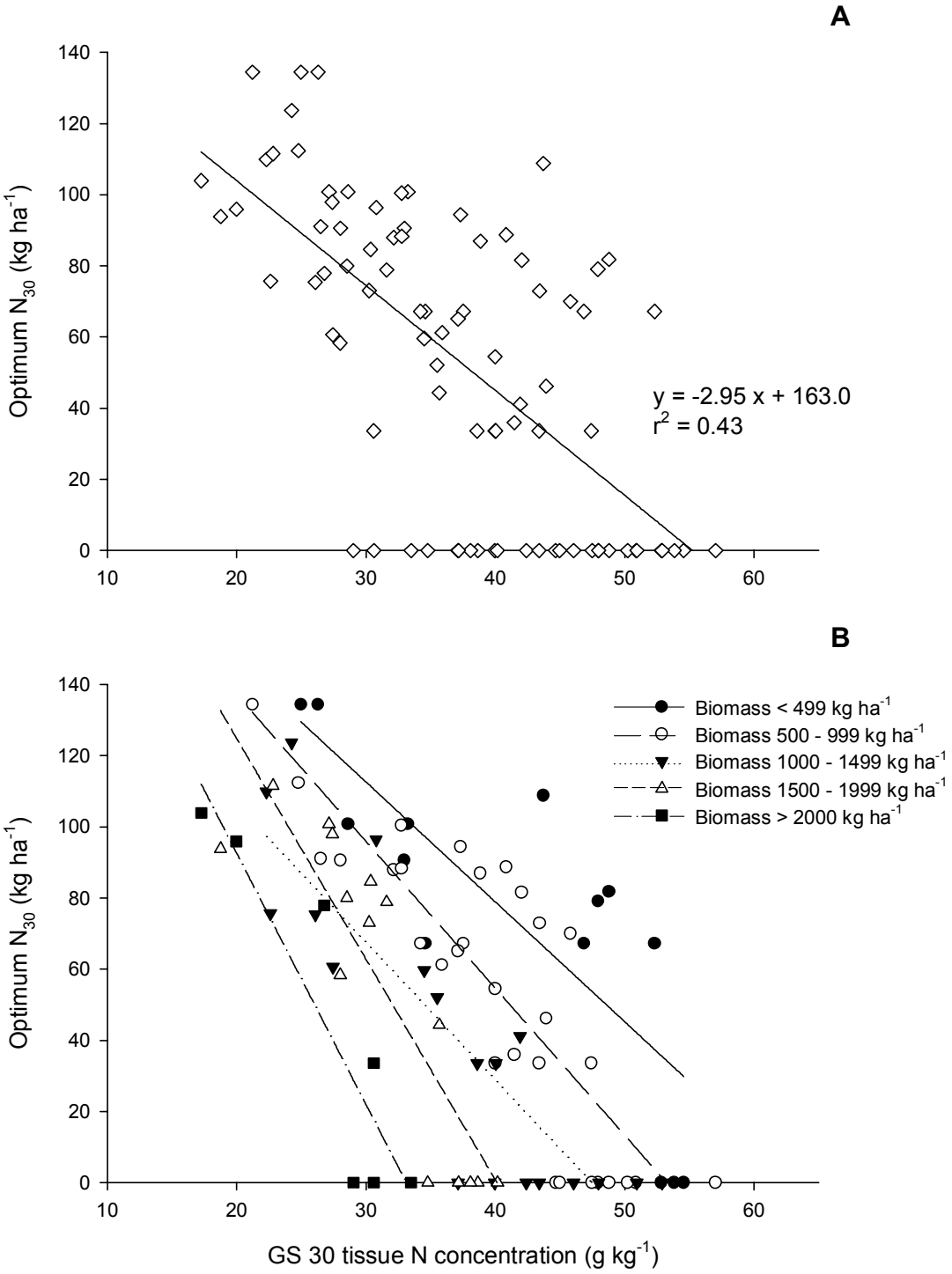


Fig. 3. The relationships of optimum N rate at growth stage (GS) 30 (N_{30}) to tissue N concentration at GS 30 in soft red winter wheat across six site-years in North Carolina, with A) simple linear relationship and B) separation into five biomass classes and their individual linear relationships.

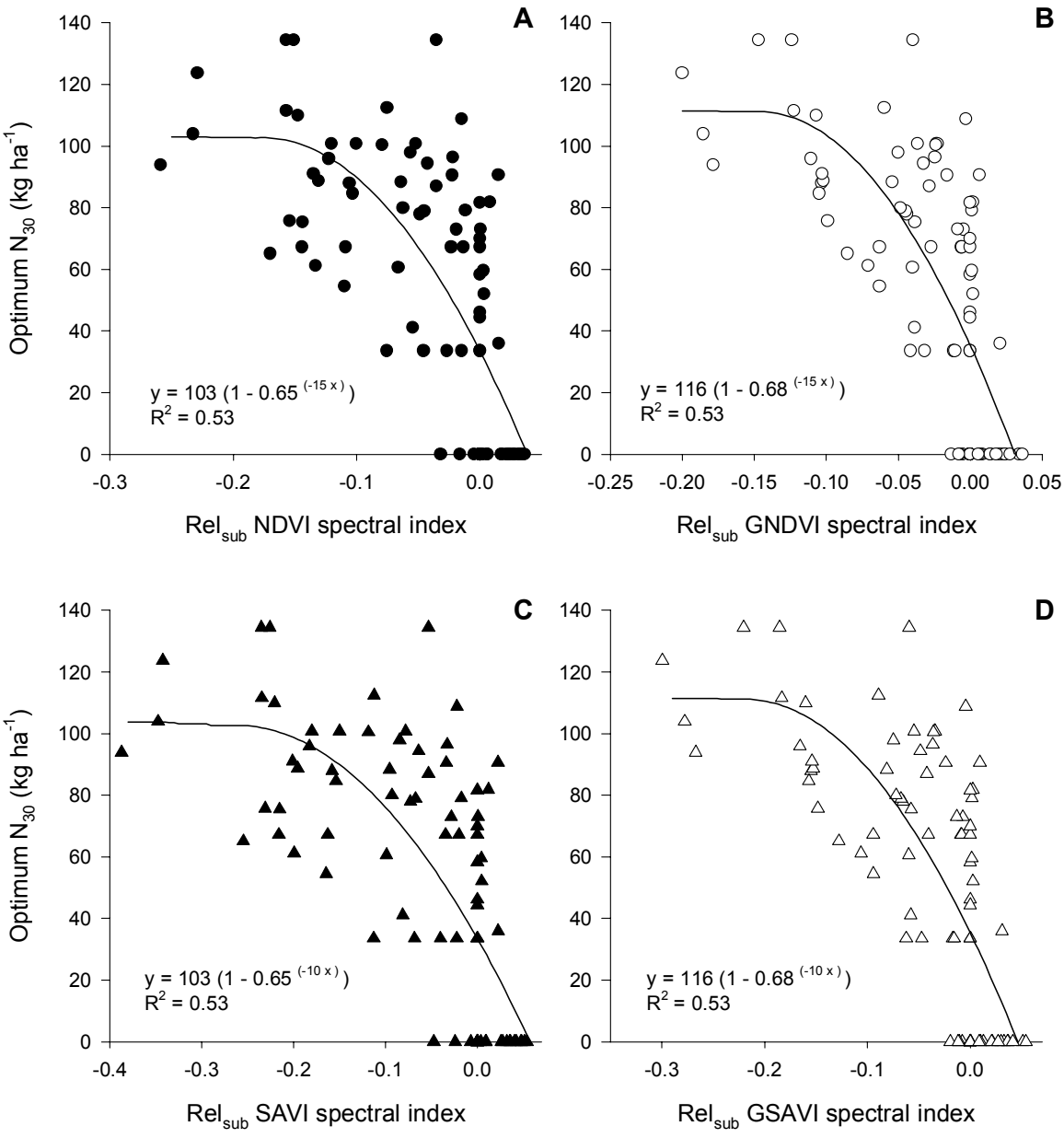


Fig. 4. The relationship of optimum N rates at growth stage (GS) 30 in soft red winter wheat to: A) Rel_{sub} NDVI, B) Rel_{sub} GNDVI, C) Rel_{sub} SAVI, and D) Rel_{sub} GSAVI spectral indicators fit with exponential models (n = 86).

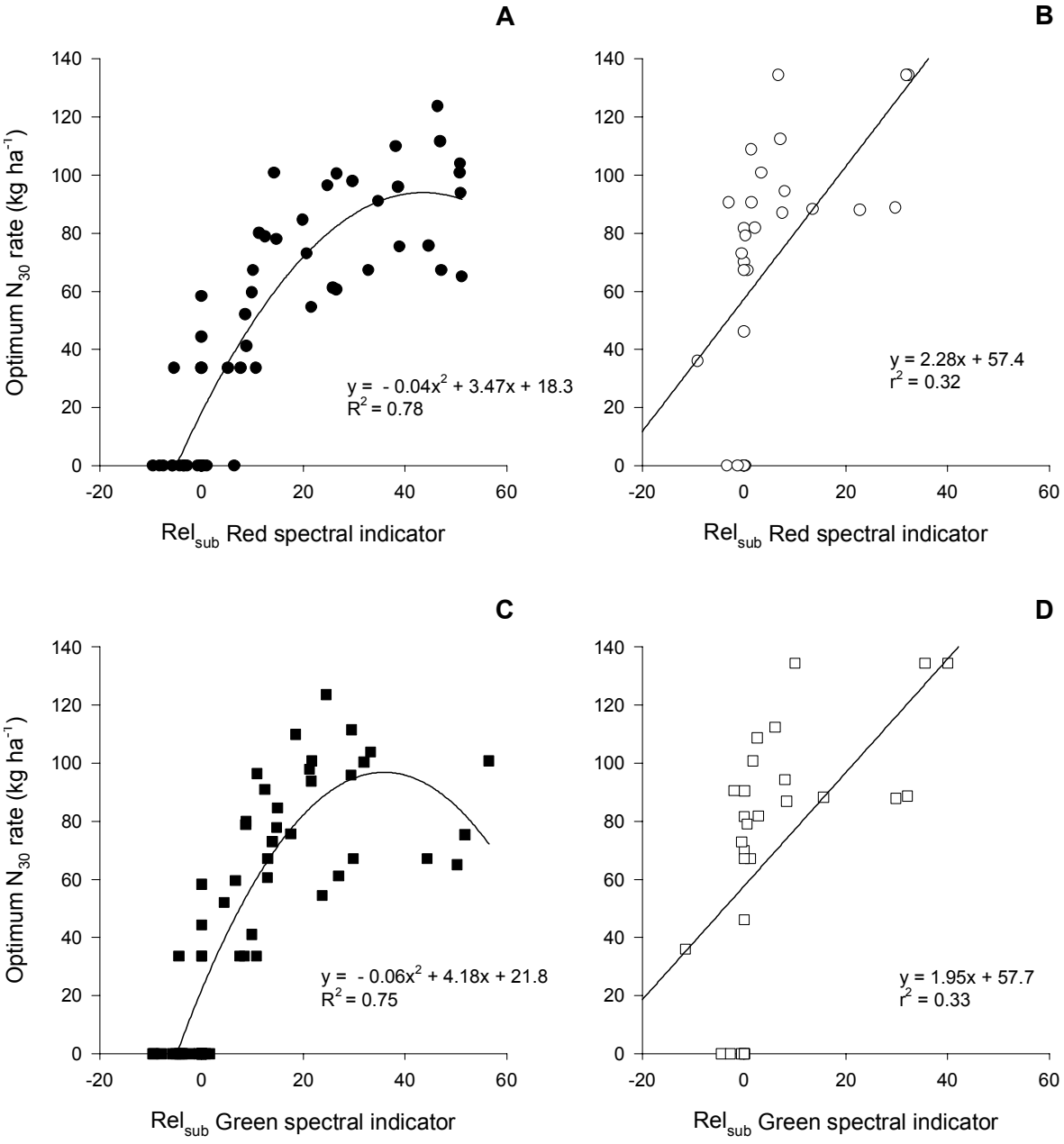


Fig. 5. The relationships of optimum N rates at growth stage (GS) 30 (N_{30}) in soft red winter wheat to A) Rel_{sub} Red for high biomass site-years, B) Rel_{sub} Red for low biomass site-years, C) Rel_{sub} Green for high biomass site-years, and D) Rel_{sub} Green for low biomass site-years in North Carolina.

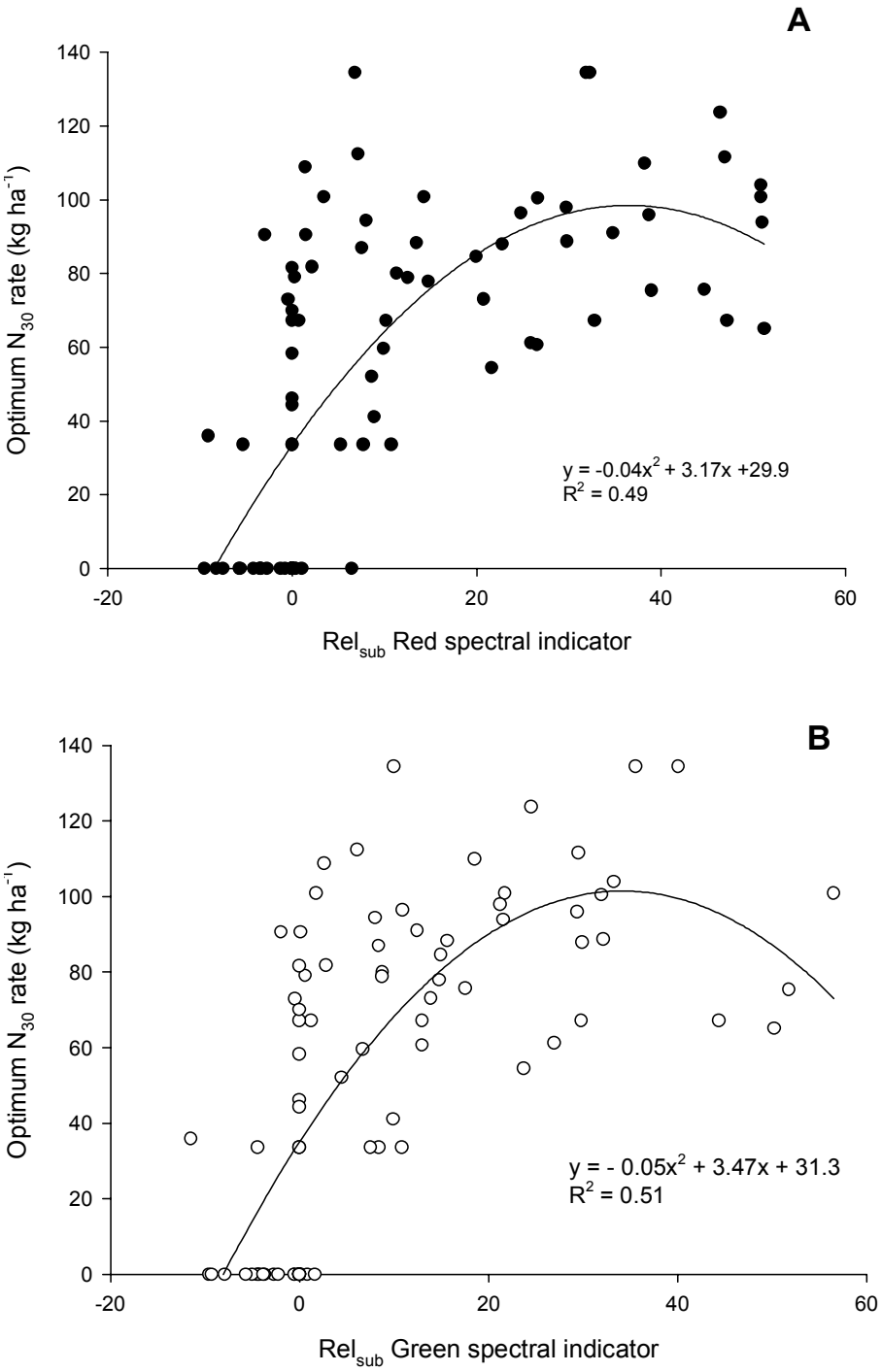


Fig. 6. The relationship of optimum N rate at growth stage (GS) 30 (N_{30}) in soft red winter wheat to: A) Rel_{sub} Red, and B) Rel_{sub} Green spectral indicators fit with quadratic models (*n* = 86).

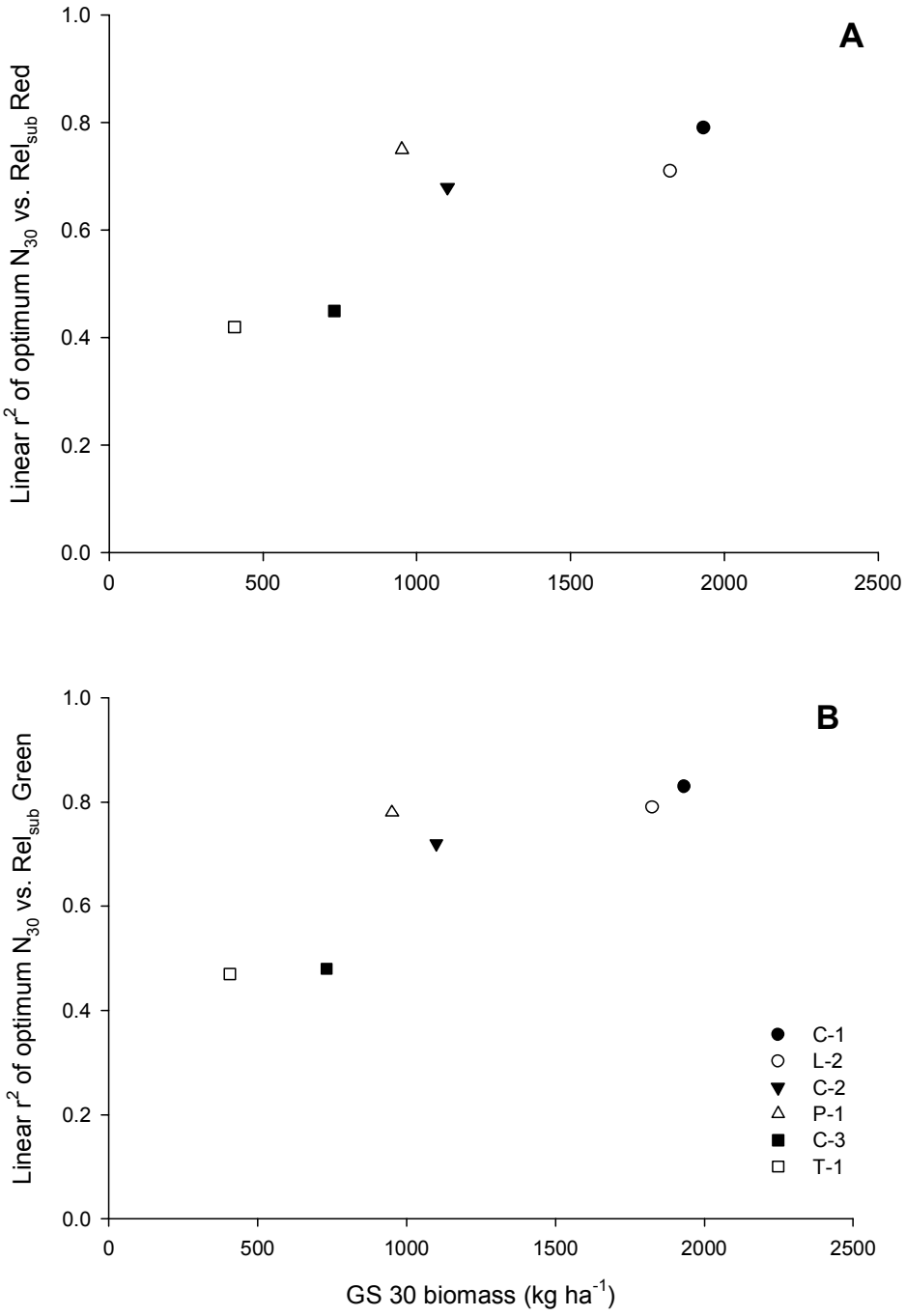


Fig. 7. The coefficients of determination (r^2) for the linear relationships of optimum N rates at growth stage (GS) 30 to: A) Rel_{sub} Red and B) Rel_{sub} Green, both plotted against site-year mean biomass measured at GS 30.

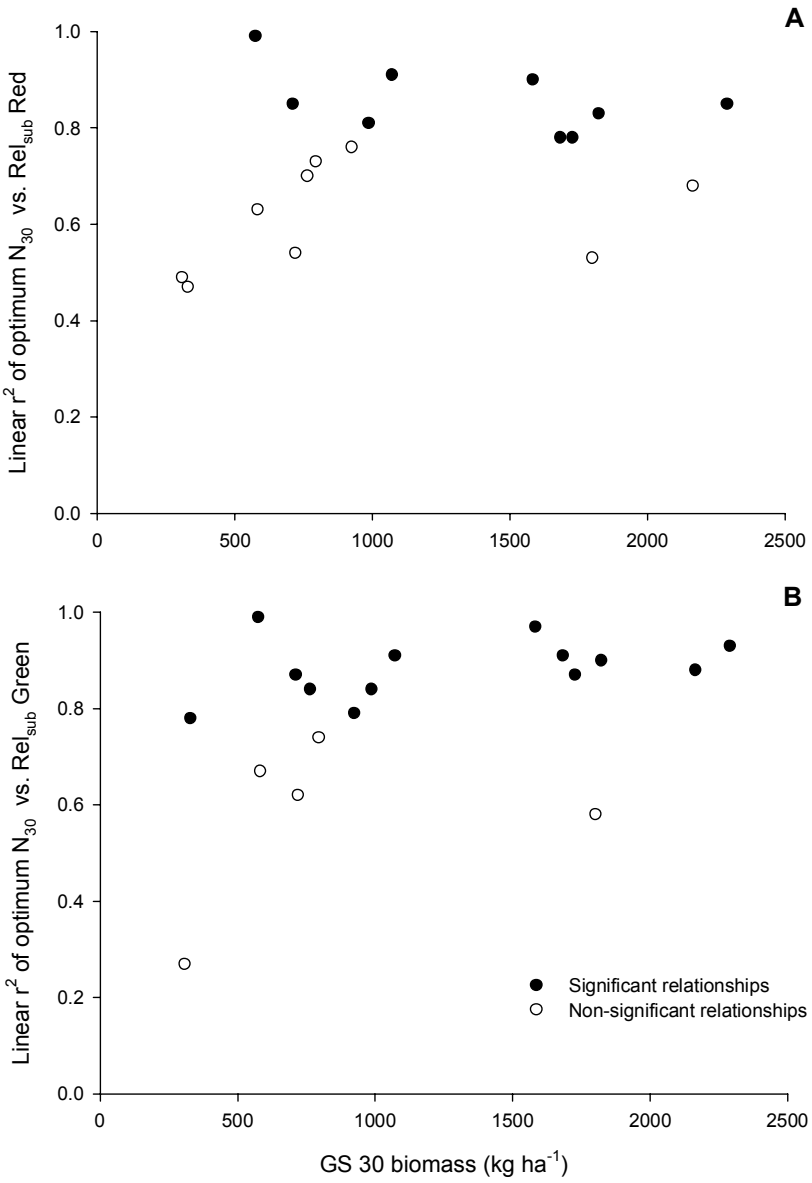


Fig. 8. The coefficients of determination (r^2) for the linear relationships of derived optimum N rates at growth stage (GS) 30 (N_{30}) in soft red winter wheat to: A) Rel_{sub} Red and B) Rel_{sub} Green, both plotted against biomass measured at GS 30 for each location by planting date-seeding rate (PDSR) combination. Open symbol represent non-significant relationships and closed symbols significant relationships ($p = 0.05$).

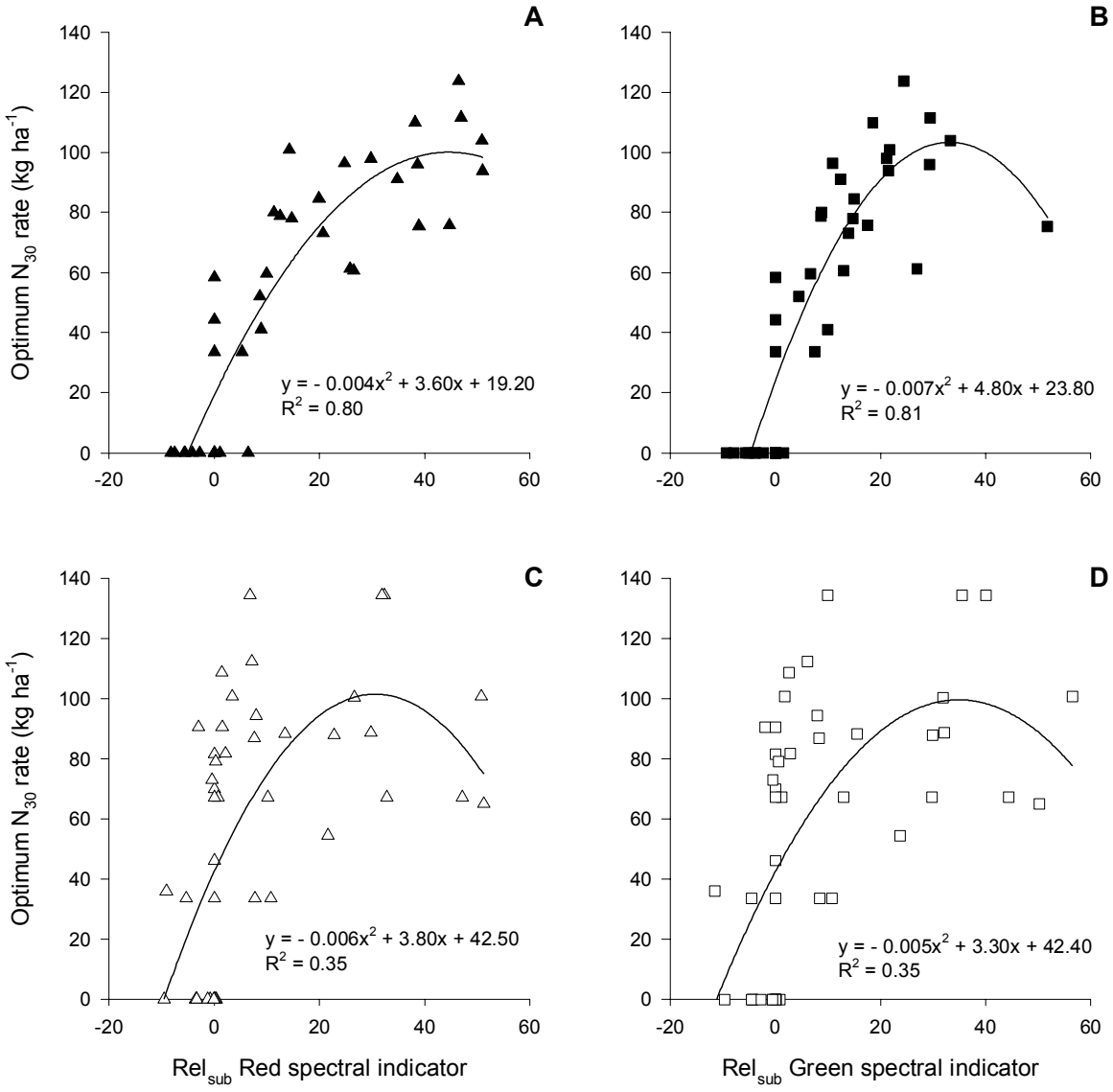


Fig. 9. The relationships of optimum N rates at growth stage (GS) 30 (N_{30}) in soft red winter wheat to spectral indicators separated by site-year and planting date-seeding rate (PDSR) into high and low biomass classes: A) Rel_{sub} Red for high (> 1000 kg ha^{-1}) GS 30 biomass, B) Rel_{sub} Green with high biomass (> 1000 kg ha^{-1}), C) Rel_{sub} Red with low biomass (< 1000 kg ha^{-1}), and D) Rel_{sub} Green with low biomass (< 1000 kg ha^{-1}) in North Carolina.

APPENDIX A

Table 1. Days between harvests, number of precipitation events, and select environmental conditions measured between 1st (timely) and 2nd (delayed) harvest dates of winter wheat at each of six trial locations.

Trial†	Days between harvests	Precipitation events	Total precipitation	Mean daily maximum temperature	Mean daily minimum temperature	Mean daily wind speed	Mean daily relative humidity
		n	---cm---	---ºC---	---ºC---	-km hr^{-1}-	---%---
B-1	16	5	3.22	30.3	17.1	8.4	69.5
C-1	8	2	1.17	32.4	18.6	6.1	68.5
T-1	8	2	1.04	30.5	18.6	7.1	72.1
C-2	19	7	3.02	30.1	20.0	8.7	76.3
P-2	15	4	5.31	30.0	18.4	4.5	77.3
T-2	8	2	1.70	32.2	19.5	4.2	72.0

† B-1 is Circle Grove Seed Farm near Belhaven, NC in 2002; C-1 is Cunningham Research Station near Kinston, NC in 2002; T-1 is Tidewater Research Station near Plymouth, NC, in 2002; C-1 is Cunningham Research Station near Kinston, NC in 2003; P-2 is Piedmont Research Station near Salisbury, NC, in 2003; and T-1 is Tidewater Research Station near Plymouth, NC in 2003.

Table 2. Correlation of yield losses caused by delayed harvest to environmental conditions that occurred between harvest dates for five trials in North Carolina.

	Variables							
Variables	Yield loss	Days between harvest	Number of precipitation events	Daily total precipitation	Daily maximum temperature	Daily minimum temperature	Daily average wind speed	Daily average Relative humidity
	r	r	r	r	r	r	r	r
Yield loss	1.00	-0.56	-0.35	-0.95*	0.55	0.55	0.14	-0.35
Days between harvests	-0.56	1.00	0.97**	0.67	-0.70	-0.07	0.55	0.42
Number of precipitation events	-0.35	0.97**	1.00	0.49	-0.63	0.08	0.66	0.37
Daily total precipitation	-0.95*	0.67	0.49	1.00	-0.54	-0.26	-0.16	0.59
Daily maximum temperature	0.55	-0.70	-0.63	-0.54	1.00	0.32	-0.56	-0.41
Daily minimum temperature	0.55	-0.07	0.08	-0.27	0.32	1.00	-0.16	0.53
Daily average wind speed	0.14	0.55	0.66	-0.15	-0.56	-0.16	1.00	-0.23
Daily average relative humidity	-0.35	0.42	0.37	0.59	-0.41	0.53	-0.23	1.00

* and ** are significant at $p = 0.05$ and 0.01, respectively.

Table 3. Correlation of test weight reductions caused by delayed harvest to environmental conditions that occurred between harvest dates for six trials in North Carolina.

	Variables							
Variables	Test weight reductions	Days between harvest	Number of precipitation events	Daily total precipitation	Daily maximum temperature	Daily minimum temperature	Daily average wind speed	Daily average Relative humidity
	r	r	r	r	r	r	r	r
Test weight reductions	1.00	0.91**	0.96**	0.47	-0.66	0.14	0.71	0.45
Days between harvests	0.92**	1.00	0.98***	0.73	-0.76	-0.03	0.54	0.55
Number of precipitation events	0.96**	0.98***	1.00	0.57	-0.70	0.10	0.64	0.50
Daily total precipitation	0.47	0.73	0.57	1.00	-0.65	-0.21	-0.10	0.68
Daily maximum temperature	-0.66	-0.76	-0.70	-0.65	1.00	0.21	-0.51	-0.60
Daily minimum temperature	0.15	-0.03	0.10	-0.21	0.21	1.00	-0.15	0.47
Daily average wind speed	0.71	0.54	0.64	-0.10	-0.51	-0.15	1.00	-0.13
Daily average relative humidity	0.45	0.55	0.50	0.68	-0.60	0.47	-0.13	1.00

** and *** are significant at $p = 0.01$ and 0.001, respectively.

Table 4. Correlation of grain falling number reductions caused by delayed harvest to environmental conditions that occurred between harvest dates for five trials in North Carolina.

	Variables							
Variables	Falling number reductions	Days between harvest	Number of precipitation events	Daily total precipitation	Daily maximum temperature	Daily minimum temperature	Daily average wind speed	Daily average Relative humidity
	r	r	r	r	r	r	r	r
Falling number reductions	1.00	0.12	-0.10	0.75	-0.47	-0.43	-0.44	0.46
Days between harvests	0.12	1.00	0.97**	0.67	-0.70	-0.07	0.55	0.42
Number of precipitation events	-0.10	0.97**	1.00	0.49	-0.63	0.08	0.66	0.37
Daily total precipitation	0.75	0.67	0.49	1.00	-0.54	-0.27	-0.16	0.59
Daily maximum temperature	-0.47	-0.70	-0.63	-0.54	1.00	0.32	-0.56	-0.41
Daily minimum temperature	-0.43	-0.07	0.08	-0.27	0.32	1.00	-0.16	0.53
Daily average wind speed	-0.44	0.55	0.66	-0.16	-0.56	-0.16	1.00	-0.23
Daily average relative humidity	0.46	0.42	0.37	0.59	-0.41	0.53	-0.23	1.00

** is significant at $p = 0.01$.

Table 5. Correlation of increased grain DON (deoxynivalenol) levels caused by delayed harvest to environmental conditions that occurred between harvest dates for four trials in North Carolina.

	Variables							
Variables	Increased grain DON	Days between harvest	Number of precipitation events	Daily total precipitation	Daily maximum temperature	Daily minimum temperature	Daily average wind speed	Daily average Relative humidity
	r	r	r	r	r	r	r	r
Increased grain DON	1.00	0.27	-0.88	0.59	-0.02	-0.80	0.16	-0.75
Days between harvests	0.27	1.00	0.21	0.92	-0.68	-0.03	0.81	0.38
Number of precipitation events	-0.88	0.21	1.00	-0.13	-0.25	0.85	0.17	0.97*
Daily total precipitation	0.59	0.92	-0.13	1.00	-0.46	-0.23	0.63	0.09
Daily maximum temperature	-0.02	-0.68	0.25	-0.46	1.00	0.28	-0.98*	-0.23
Daily minimum temperature	-0.80	-0.03	0.85	-0.23	0.28	1.00	-0.31	0.86
Daily average wind speed	0.16	0.81	0.17	0.63	-0.98*	-0.31	1.00	0.21
Daily average relative humidity	-0.75	0.38	0.97*	0.09	-0.23	0.86	0.21	1.00

* is significant at $p = 0.05$.

Table 6. Correlation of farinograph breakdown time reductions caused by delayed harvest to environmental conditions that occurred between harvest dates for five trials in North Carolina.

	Variables							
Variables	Farinograph breakdown time reductions	Days between harvest	Number of precipitation events	Daily total precipitation	Daily maximum temperature	Daily minimum temperature	Daily average wind speed	Daily average Relative humidity
	r	r	r	r	r	r	r	r
Farinograph breakdown time reductions	1.00	-0.22	-0.31	0.03	0.40	-0.69	-0.21	-0.73
Days between harvests	-0.22	1.00	0.97**	0.67	-0.70	-0.07	0.55	0.42
Number of precipitation events	-0.31	0.97**	1.00	0.49	-0.63	0.08	0.66	0.37
Daily total precipitation	0.03	0.67	0.49	1.00	-0.54	0.27	-0.16	0.59
Daily maximum temperature	0.40	-0.70	-0.63	-0.54	1.00	0.32	-0.56	-0.41
Daily minimum temperature	-0.69	-0.07	0.08	-0.27	0.32	1.00	-0.16	0.53
Daily average wind speed	-0.21	0.55	0.66	-0.16	-0.56	-0.16	1.00	-0.23
Daily average relative humidity	-0.73	0.42	0.37	0.59	-0.41	0.53	-0.23	1.00

** is significant at $p = 0.01$.

APPENDIX B

Table 1. Correlation of yield, test weight, grain protein, tiller density of soft red winter wheat and daily average temperature from growth stage (GS) 30 to harvest and daily total precipitation (from GS 30 to harvest) in seven different environments in North Carolina.

	Variables					
Variables	Yield	Test weight	Grain protein	Tiller density	Daily average temperature	Daily total precipitation
	r	r	r	r	r	r
Yield	1.00	0.43	0.29	0.90**	0.08	-0.88**
Test weight	0.43	1.00	-0.12	0.53	-0.65	-0.67†
Grain protein	0.29	-0.12	1.00	0.56	-0.30	-0.41
Tiller density	0.90**	0.53	0.56	1.00	-0.26	-0.92**
Daily average temperature	0.08	-0.65	-0.30	-0.26	1.00	0.30
Daily total precipitation	-0.88**	-0.67†	-0.41	0.92**	0.30	1.00

† and **, Significant at p = 0.10 and 0.01

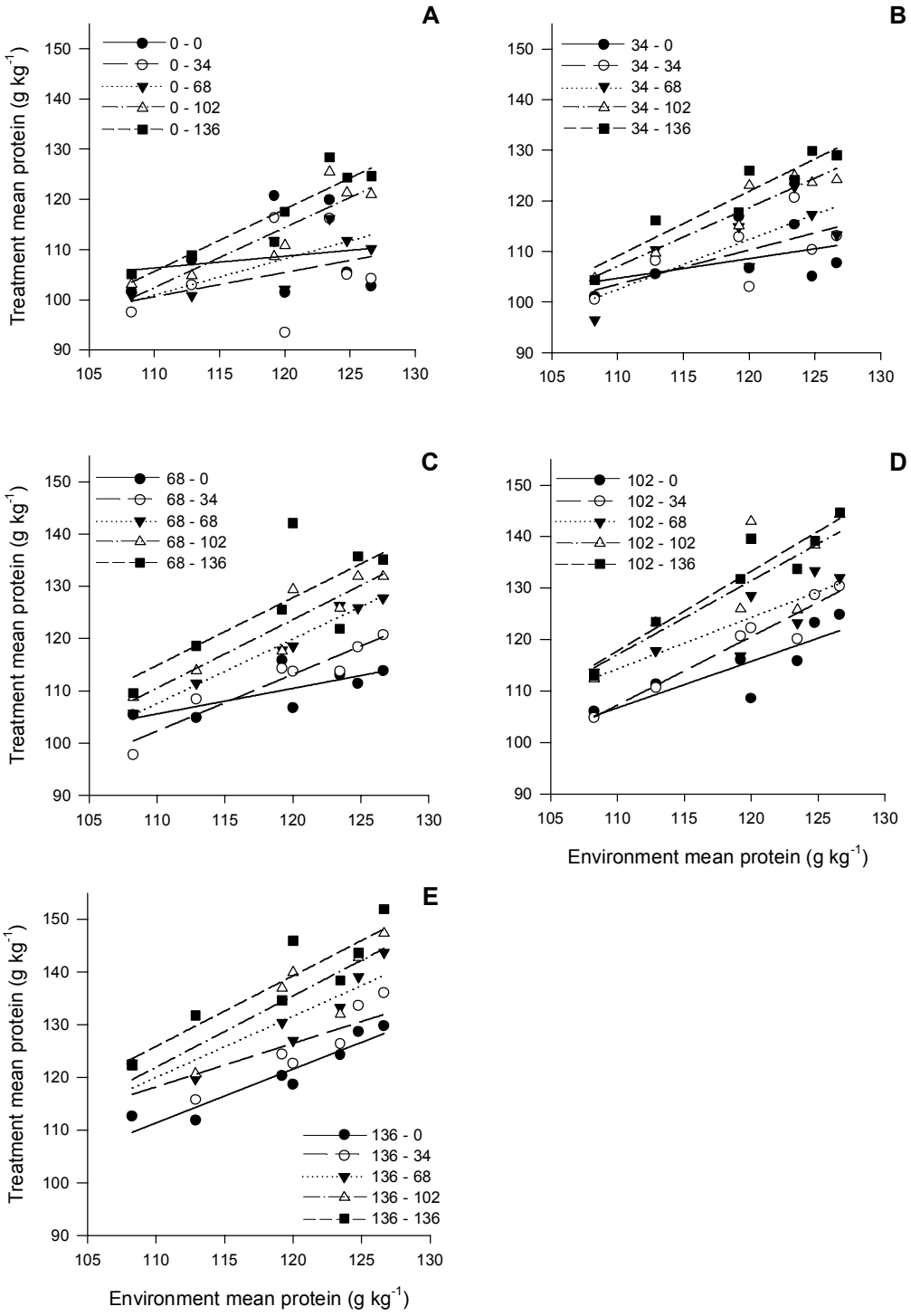


Fig. 1. Linear relationships of mean grain protein for each of 25 N treatment combinations of N applied at growth stage (GS) 25 and GS 30 across seven different environments regressed against each environment mean grain protein across all 25 N treatments with A) all GS 25 N treatments of 0 kg N ha^{-1} combined with five incremental N rates at GS 30 (kg ha^{-1}), B) all

GS 25 N treatments of 34 kg N ha^{-1} combined with five incremental N rates at GS 30 (kg ha^{-1}), C) all GS 25 N treatments of 68 kg N ha^{-1} combined with five incremental N rates at GS 30 (kg ha^{-1}), D) all GS 25 N treatments of 102 kg N ha^{-1} combined with five incremental N rates at GS 30 (kg ha^{-1}), and E) all GS 25 N treatments of 136 kg N ha^{-1} combined with five incremental N rates at GS 30 (kg ha^{-1}).

APPENDIX C

Table 1. Response of soft red winter wheat growth stage (GS) 25 tiller density to a planting date-seeding rate (PDSR) treatments at six site-years in North Carolina.

	Site-year†					
Treatment	C-1	L-1	C-2	P-1	C-3	T-1
	tiller m^{-2}					
PDSR 1	696a‡	563a	605a	314a	342a	283a
PDSR 2	529b	474b	291b	245b	293b	267b
PDSR 3	420c	365c	126c	139c	236c	215c

† C-1 Cunningham Research Station 2002; L-1, Lower Coastal Plain Tobacco Research Station 2002; C-2, Cunningham Research Station 2003; P-1, Piedmont Research Station 2003; C-3, Cunningham Research Station 2004; T-1, Tidewater Research Station 2004.
‡ Different letters denote significant differences ($p = 0.05$) in GS 25 tiller density between PDSR treatments at each site-year.

Table 2. Response of soft red winter wheat growth stage (GS) 30 biomass to a planting date-seeding rate (PDSR) treatments and N applied at GS 25 (N_{25}) treatment at six site-years in North Carolina.

	Site-year†					
Treatments	C-1	L-1	C-2	P-1	C-3	T-1
	kg ha^{-1}					
PDSR						
1	2290a‡	2166a	1801a	1072a	NA§	583a
2	1823b	1727b	925b	987b	NA	330b
3	1683c	1583b	575c	796c	NA	308b
N_{25} rate						
0 kg ha^{-1}	1739c	1645c	769c	624d	449c	NA
34 kg ha^{-1}	1855bc	1790bc	1047b	832c	706b	NA
67 kg ha^{-1}	2006ab	1871ab	1196ab	943b	834a	NA
101 kg ha^{-1}	2090a	1881ab	1310a	1129a	828a	NA
134 kg ha^{-1}	1972ab	1940a	1179ab	1230a	841a	NA

† C-1, Cunningham Research Station 2002; L-1, Lower Coastal Plain Tobacco Research Station 2002; C-2, Cunningham Research Station 2003; P-1, Piedmont Research Station 2003; C-3, Cunningham Research Station 2004; T-1, Tidewater Research Station 2004.
‡ Different letters denote significant differences ($p = 0.05$) in GS 30 biomass between PDSR treatments and N_{25} treatments within each site-year.
§ NA is not applicable, as effects in the ANOVA were not significant.

Table 3. Response of soft red winter wheat growth stage (GS) 30 tissue N concentrations to a planting date-seeding rate (PDSR) treatments and N applied at GS 25 (N_{25}) treatment at six site-years in North Carolina.

	Site-year†					
Treatments	C-1	L-1	C-2	P-1	C-3	T-1
	---------- g kg^{-1} ----------					
PDSR						
1	24.7b‡	NA§	31.5c	41.8b	34.0b	41.3b
2	30.6a	NA	35.3b	44.3a	37.6a	46.3a
3	32.6a	NA	42.8a	46.5a	38.5a	47.1a
N_{25} rate						
0 kg ha^{-1}	21.4d	NA	29.8e	36.3d	24.2e	30.3c
34 kg ha^{-1}	25.9c	NA	33.2d	38.8d	31.0d	44.0b
67 kg ha^{-1}	29.5b	NA	36.5c	44.2c	39.0c	46.7b
101 kg ha^{-1}	34.3a	NA	39.0b	48.1b	43.9b	51.0a
134 kg ha^{-1}	35.5a	NA	44.2a	53.5a	45.5a	52.4a

† C-1, Cunningham Research Station 2002; L-1, Lower Coastal Plain Tobacco Research Station 2002; C-2, Cunningham Research Station 2003; P-1, Piedmont Research Station 2003; C-3, Cunningham Research Station 2004; T-1, Tidewater Research Station 2004.
‡ Different letters denote significant differences ($p = 0.05$) in GS 30 tissue N concentrations between PDSR treatments and N_{25} treatments within each site-year.
§ NA is not applicable, as effects in the ANOVA were not significant.

Table 4. Response in soft red winter wheat growth stage (GS) 30 tissue N concentration at the Lower Coastal Plain Tobacco Research Station in 2002 to N applied at GS 25 (N_{25}) for each of three planting date-seeding rate (PDSR) treatments in North Carolina.

	Treatment		
N_{25} rate	PDSR 1	PDSR 2	PDSR 3
	---------- g kg^{-1} ----------		
0 kg ha^{-1}	18.8d†	22.6e	26.5e
34 kg ha^{-1}	22.3c	27.4d	30.8d
67 kg ha^{-1}	28.5b	34.5c	35.5c
101 kg ha^{-1}	28.0b	37.1b	40.0b
134 kg ha^{-1}	34.8a	40.0a	43.4a

† Different letters denote significant differences ($p = 0.05$) in GS 30 tissue N concentrations between N_{25} treatments with in PDSR treatment.

Table 5. Nitrogen uptake response to a planting date-seeding rate (PDSR) treatment and N applied at growth stage (GS) 25 (N_{25}) for each of six site-years in North Carolina.

	Site-year†					
Treatments	C-1	L-1	C-2	P-1	C-3	T-1
	kg ha^{-1}					
PDSR						
1	NA‡	NA	57.3a§	46.3a	NA	24.2a
2	NA	NA	33.4b	44.9a	NA	15.6b
3	NA	NA	24.8b	38.0b	NA	14.6b
N_{25} rate						
0 kg ha^{-1}	39.1d	36.3d	20.9d	22.6e	10.6d	9.5c
34 kg ha^{-1}	50.4c	47.6c	32.3c	32.1d	21.6c	17.1b
67 kg ha^{-1}	58.8b	60.7b	40.8b	41.3c	32.4b	20.5ab
101 kg ha^{-1}	70.1a	64.3b	48.8a	54.0b	36.3a	21.9a
134 kg ha^{-1}	71.4a	75.4a	50.1a	65.2a	38.2a	21.7a

† C-1, Cunningham Research Station 2002; L-1, Lower Coastal Plain Tobacco Research Station 2002; C-2, Cunningham Research Station 2003; P-1, Piedmont Research Station 2003; C-3, Cunningham Research Station 2004; T-1, Tidewater Research Station 2004.
‡ NA is not applicable, as the effect was not significant in the ANOVA.
§ Different letters denote significant differences ($p = 0.05$) in N uptake between PDSR treatments and N_{25} treatments within each site-year.

Table 6. Grain yield response of soft red winter wheat to planting date-seeding rate (PDSR) treatment by N treatment applied at growth stage (GS) 30 (N_{30}) in three site-years in North Carolina.

	Site-years†								
	L-1			C-3			T-1		
N_{30} rate	PDSR 1	PDSR 2	PDSR 3	PDSR 1	PDSR 2	PDSR 3	PDSR 1	PDSR 2	PDSR 3
	bu acre^{-1}								
0 kg ha^{-1}	53.4d‡	53.7d	50.5c	53.8e	53.5d	57.2d	79.8c	76.7d	72.5c
34 kg ha^{-1}	63.3c	61.7c	58.0b	64.2d	62.5c	64.9c	86.8b	84.0c	79.9b
67 kg ha^{-1}	69.8b	66.3b	62.7a	70.7c	69.0b	70.3b	97.1a	91.6b	96.2a
101 kg ha^{-1}	74.0a	69.1a	64.7a	75.5b	73.1a	73.7a	98.1a	100.6a	96.0a
134 kg ha^{-1}	75.5a	70.3a	63.7a	78.3a	74.6a	74.7a	98.1a	101.9a	97.0a

† L-1, Lower Coastal Plain Tobacco Research Station 2002; C-3, Cunningham Research Station 2004; T-1, Tidewater Research Station 2004.
‡ Different letters denote significant differences ($p = 0.05$) in grain yield between N_{30} treatments within a PDSR at each site-year.

Table 7. Grain yield response of soft red winter wheat to N treatments applied at growth stage (GS) 30 (N_{30}) for each level of N applied at GS 25 (N_{25}) in five site-years in North Carolina.

	Site-year†														
	C-1					L-1					P-1				
	N_{25} rate, kg ha^{-1}														
N_{30} rate	0	34	67	101	134	0	34	67	101	134	0	22	45	67	101
	bu acre^{-1}														
0 kg ha^{-1}	45.2d‡	52.8e	61.4d	72.3c	70.3c	36.0d	44.0d	52.0d	59.9c	70.7bc	30.8c	38.2c	41.7c	43.9c	47.9a
34 kg ha^{-1}	56.7c	65.7d	72.1c	78.1b	76.8ab	48.0c	54.8c	62.5c	66.7b	73.1ab	39.6b	44.3b	48.6ab	49.4a	47.6a
67 kg ha^{-1}	64.4b	73.3c	77.2b	80.9ab	78.9a	56.6b	61.4b	68.4b	70.8a	74.2a	45.5a	49.8a	49.6a	48.3a	43.9b
101 kg ha^{-1}	76.9a	79.1b	81.4a	82.2a	76.6ab	63.2a	68.9a	70.3ab	71.5a	72.6ab	44.7a	49.5a	49.0a	45.4b	41.0c
134 kg ha^{-1}	80.1a	84.7a	79.1ab	79.6ab	73.8bc	64.2a	71.7a	72.9a	71.1a	69.3c	41.7b	46.6b	46.5b	40.9d	37.8d

† C-1, Cunningham Research Station 2002; L-1, Lower Coastal Plain Tobacco Research Station 2002; P-1, Piedmont Research Station 2003.
‡ Different letters denote significant differences ($p = 0.05$) in grain yield between N_{30} treatments within a N_{25} treatment at each site-year.

Table 7. (continued)

	Site-year†									
	C-3					T-1				
	N_{25} rate, kg ha^{-1}									
N_{30} rate	0	34	67	101	134	0	34	67	101	134
	bu acre^{-1}									
0 kg ha^{-1}	31.8e‡	47.0d	57.4d	66.4d	71.7b	45.4d	70.0d	75.5c	92.1b	98.7b
34 kg ha^{-1}	46.2d	58.6c	65.6c	72.2c	76.8a	56.1c	80.9c	86.6b	95.7ab	98.8b
67 kg ha^{-1}	58.7c	67.8b	72.4b	75.3b	75.8a	76.6b	90.9b	98.2a	101.7a	107.3a
101 kg ha^{-1}	66.5b	73.6a	77.0a	77.1ab	76.2a	91.0a	96.0ab	101.4a	100.6a	102.2ab
134 kg ha^{-1}	71.2a	75.3a	79.1a	78.2a	75.5a	94.2a	100.4a	99.3a	100.5a	100.5b

† C-3, Cunningham Research Station 2004; T-1, Tidewater Research Station 2004

‡ Different letters denote significant differences ($p = 0.05$) in grain yield between N_{30} treatments within a N_{25} treatment at each site-year.

Table 8. Agronomic optimum N rates at growth stage (GS) 30 (N_{30}) calculated at each treatment of N applied at GS 25 (N_{25}) and planting date-seeding rate (PDSR) treatment at each of six site-years for a grain yield response to N_{30} in soft red winter wheat in North Carolina.

	Site-year†								
	C-1			C-2			C-3		
N_{25} rate	PSDR 1	PDSR 2	PDSR 3	PSDR 1	PDSR 2	PDSR 3	PSDR 1	PDSR 2	PDSR 3
	kg ha^{-1}								
0 kg ha^{-1}	104	112	124	75	101‡	67‡	134‡	134‡	134‡
34 kg ha^{-1}	96	98	85	101‡	100	0‡§	91	88	88
67 kg ha^{-1}	78	73	79	34‡	67‡	0‡	94	87	89
101 kg ha^{-1}	0‡	44	0‡	0‡	34‡	0‡	82	46	70
134 kg ha^{-1}	0‡	0‡	0‡	0‡	0‡	0‡	36	0‡	0‡§

† C-1, Cunningham Research Station 2002; C-2, Cunningham Research Station 2003; C-3, Cunningham Research Station 2004.
‡ Denoted that a linear plateau model was not used, that either mean separations or no yield response defined the optimum N_{30} rate.
§ Data removed from final model as outlier.

Table 8. (continued)

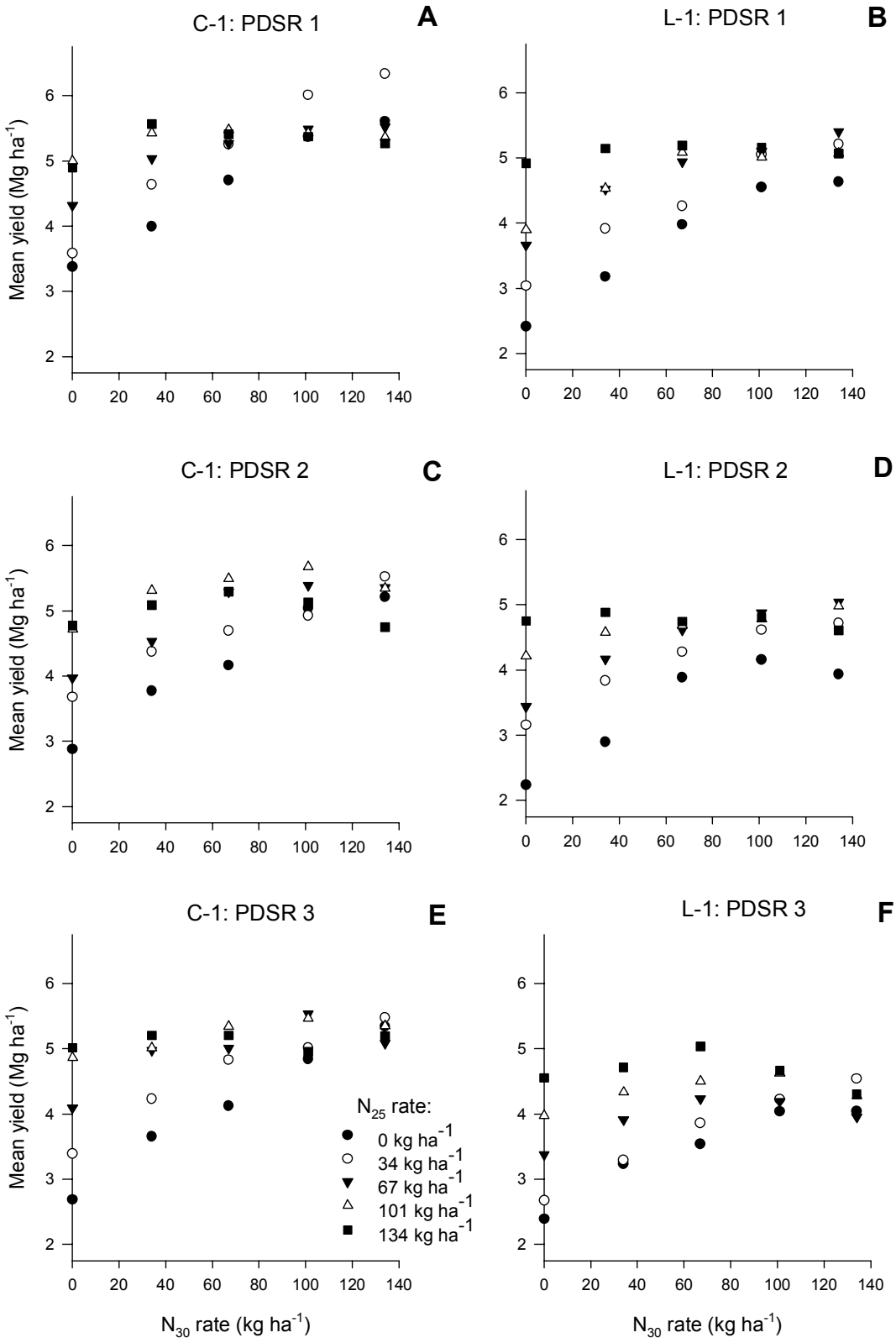
	Site-year†								
	L-1			P-1‡			T-1		
N_{25} rate	PSDR 1	PDSR 2	PDSR 3	PSDR 1	PDSR 2	PDSR 3	PSDR 1	PDSR 2	PDSR 3
	kg ha^{-1}								
0 kg ha^{-1}	94	76	91	47¶	65	67§	112	101§	91
34 kg ha^{-1}	110	61	96	61	54	34§	132¶	109	67§
67 kg ha^{-1}	80	60	52	41	34§	34§	73	79	82
101 kg ha^{-1}	58	0§	34§	0§	0§	0§	0§	0§	67§
134 kg ha^{-1}	0§	0§	0§	0§	0§	0§	0§	0§	0§

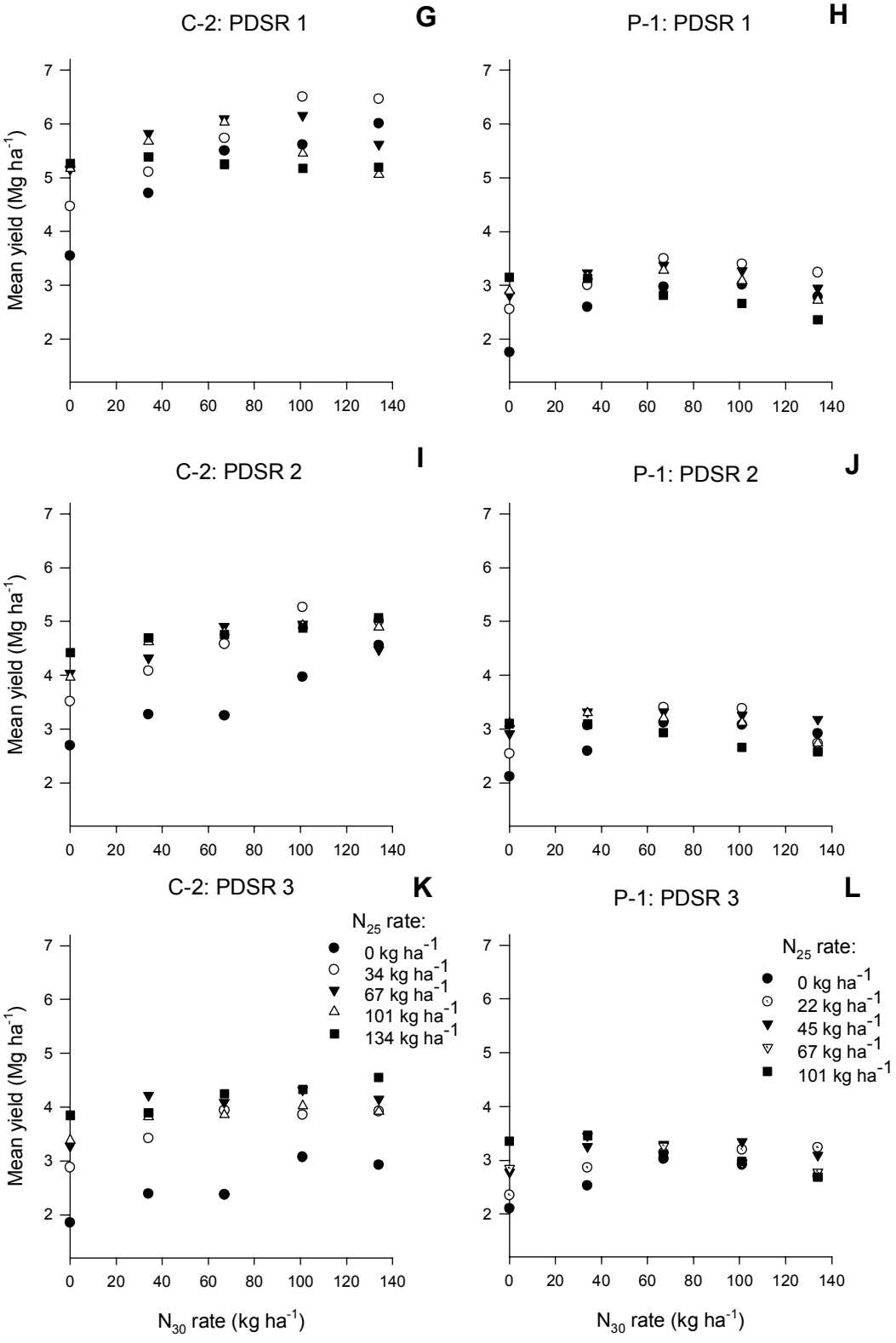
† L-1 Lower, Coastal Plain Tobacco Research Station 2002; P-1, Piedmont Research Station 2003; T-1, Tidewater Research Station 2004.
‡ At this site-year the N_{25} rates were as follows: 0, 22, 45, 67, and 101 kg N ha^{-1}.
§ Denoted that a linear plateau model was not used, that either mean separations or no yield response defined the optimum N_{30} rate.
¶ Data removed from final model as outlier.

Table 9. Yield and spectral response of soft red winter wheat between two high N rates where no yield or spectral response between the two high N rates is denoted as "0" and a yield or spectral response between the two high N rates denoted as "+" at each site-year and planting date-seeding rate (PDSR) treatment combination.

	Site-year†																	
	C-1			L-1			C-2			P-1			C-3			T-1		
Response variables	PDSR 1	PDSR 2	PDSR 3	PDSR 1	PDSR 2	PDSR 3	PDSR 1	PDSR 2	PDSR 3	PDSR 1	PDSR 2	PDSR 3	PDSR 1	PDSR 2	PDSR 3	PDSR 1	PDSR 2	PDSR 3
Yield	0	0	0	+	+	+	0	0	0	0	0	0	0	+	0	0	0	+
Spectral	0	0	0	+	+	0	0	0	0	0	0	0	0	0	0	0	0	+

†C-1, Cunningham Research Station 2002; L-1, Lower Coastal Plain Tobacco Research Station 2002; C-2, Cunningham Research Station 2003; P-1, Piedmont Research Station 2003; C-3, Cunningham Research Station 2004; T-1, Tidewater Research Station 2004.


A
C-1: PDSR 1
B
L-1: PDSR 1
C
C-1: PDSR 2
D
L-1: PDSR 2
E
C-1: PDSR 3
F
L-1: PDSR 3
Mean yield (Mg ha-1)
N30 rate (kg ha-1)
N25 rate:
0 kg ha-1
34 kg ha-1
67 kg ha-1
101 kg ha-1
134 kg ha-1


C-2: PDSR 1
G
P-1: PDSR 1
H
C-2: PDSR 2
I
P-1: PDSR 2
J
C-2: PDSR 3
K
P-1: PDSR 3
L
Mean yield (Mg ha-1)
N30 rate (kg ha-1)
N25 rate:
0 kg ha-1
34 kg ha-1
67 kg ha-1
101 kg ha-1
134 kg ha-1
22 kg ha-1
45 kg ha-1

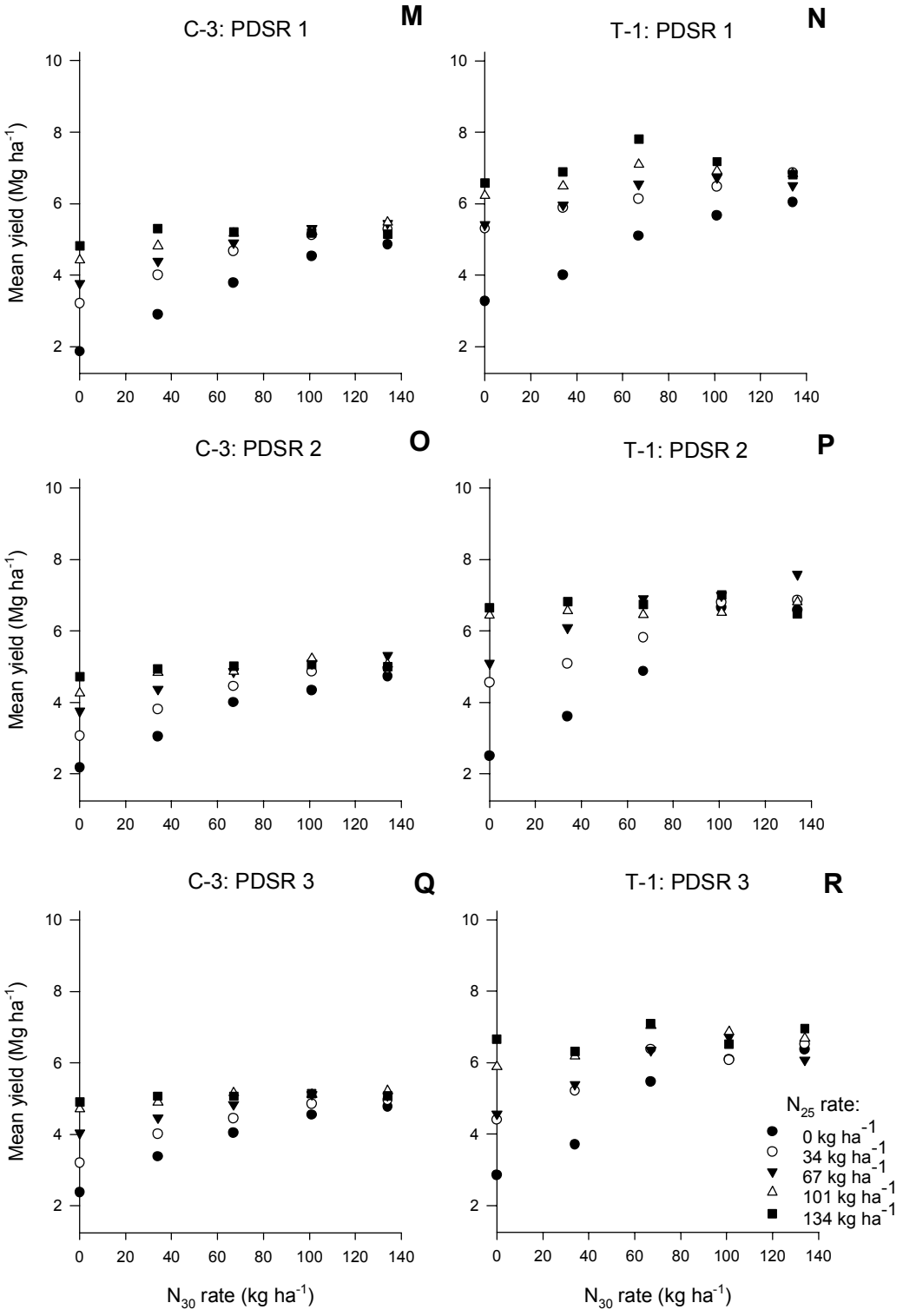


Fig 1. Grain yield response of soft red winter wheat to N applied at growth stage (GS) 30 (N_{30}) at each N_{25} treatment rate (N applied at GS 25) by planting date-seeding rate (PDSR) treatment at each of six site-years in North Carolina with: A) C-1 = Cunningham

Research station 2002, PDSR 1; B) L-1 = Lower Coast Plain Tobacco Research Station 2002, PDSR 1; C) C-1 = Cunningham Research station 2002, PDSR 2; D) L-1 = Lower Coast Plain Tobacco Research Station 2002, PDSR 2; E) C-1 = Cunningham Research station 2002, PDSR 3; F) L-1 = Lower Coast Plain Tobacco Research Station 2002, PDSR 3; G) C-2 = Cunningham Research station 2003, PDSR 1; H) P-1 = Piedmont Research Station 2003, PDSR 1; I) C-2 = Cunningham Research station 2003, PDSR 2; J) P-1 = Piedmont Research Station 2003, PDSR 2; K) C-2 = Cunningham Research station 2003, PDSR 3; L) P-1 = Piedmont Research Station 2003, PDSR 3; M) C-3 = Cunningham Research station 2004, PDSR 1; N) T-1 = Tidewater Research Station 2004, PDSR 1; O) C-3 = Cunningham Research station 2004, PDSR 2; P) T-1 = Tidewater Research Station 2004, PDSR 2; Q) C-3 = Cunningham Research station 2004, PDSR 3; and R) T-1 = Tidewater Research Station 2004, PDSR 3.

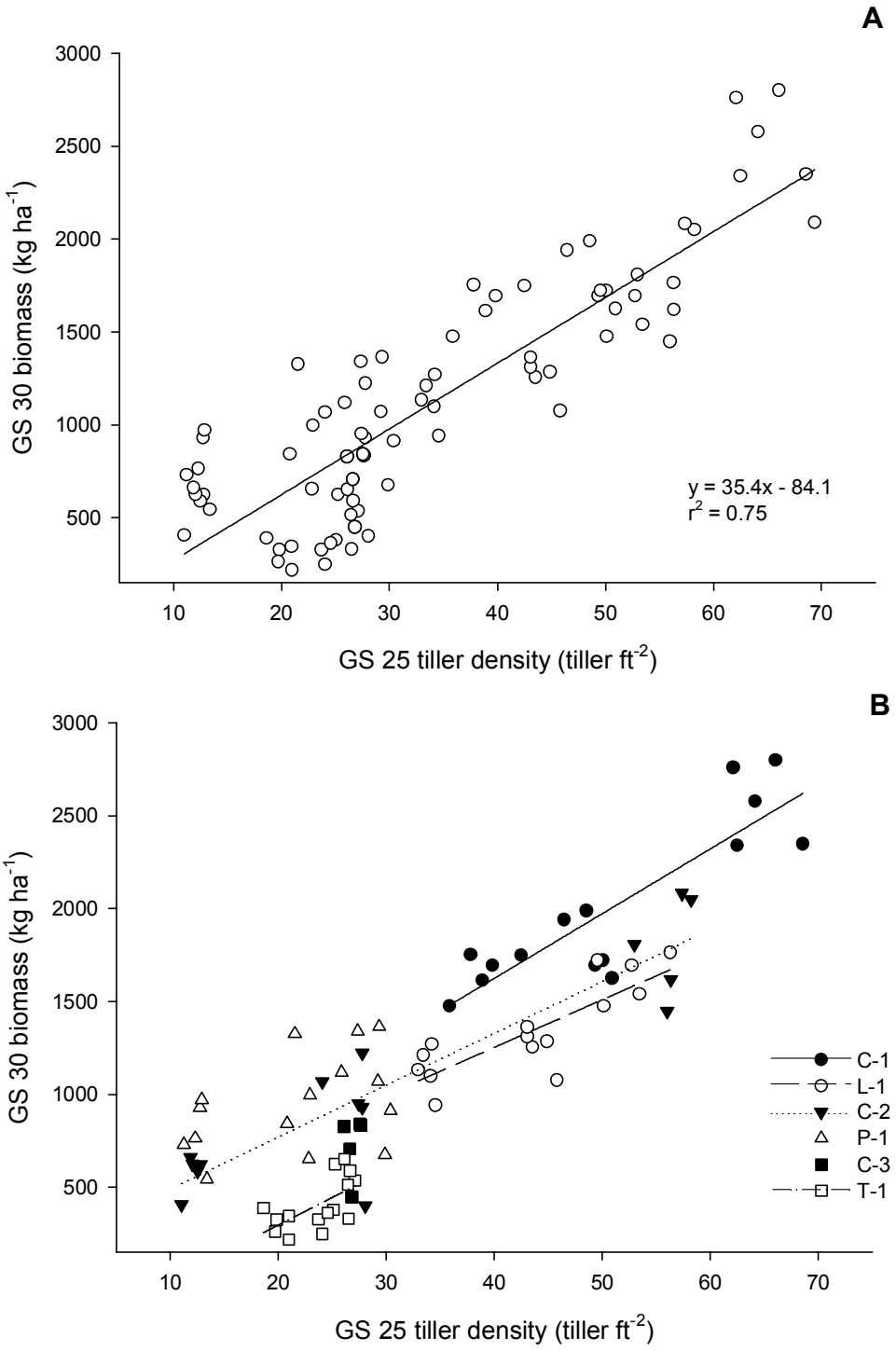


Fig. 2. The relationship of biomass at growth stage (GS) 30 to GS 25 tiller density in soft red winter wheat A) across all six site-years and B) for each individual site-year in North Carolina. Symbols represent a N_{25} (N applied at GS 25) treatment by planting date-seeding rate by site-year mean (n = 90).

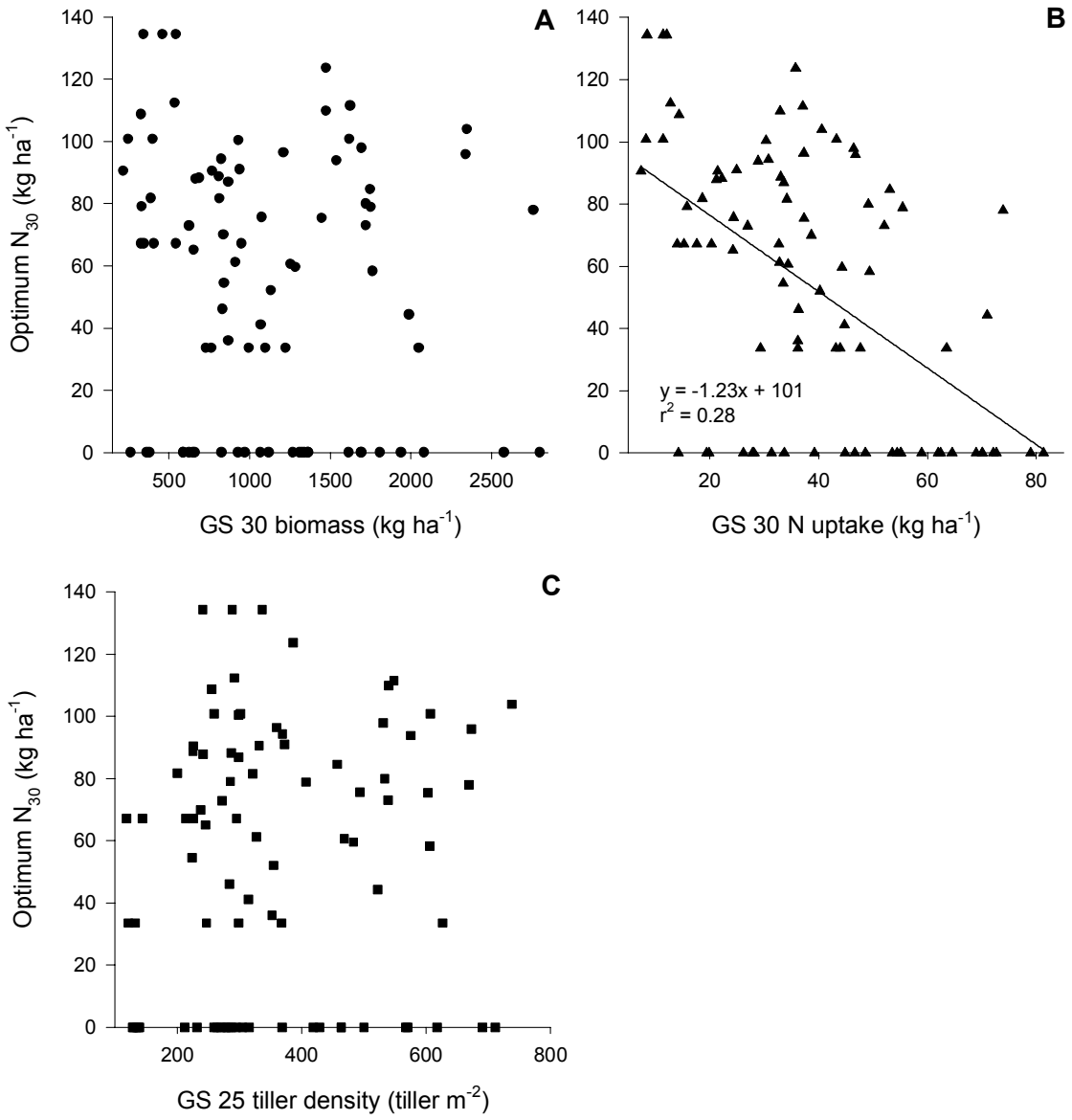


Fig 3. Agronomic optimum N rates at growth stage (GS) 30 (N_{30}) for soft red winter wheat plotted versus A) GS 30 biomass, B) GS 30 N uptake, and C) GS 25 tiller density across six site-years in North Carolina.

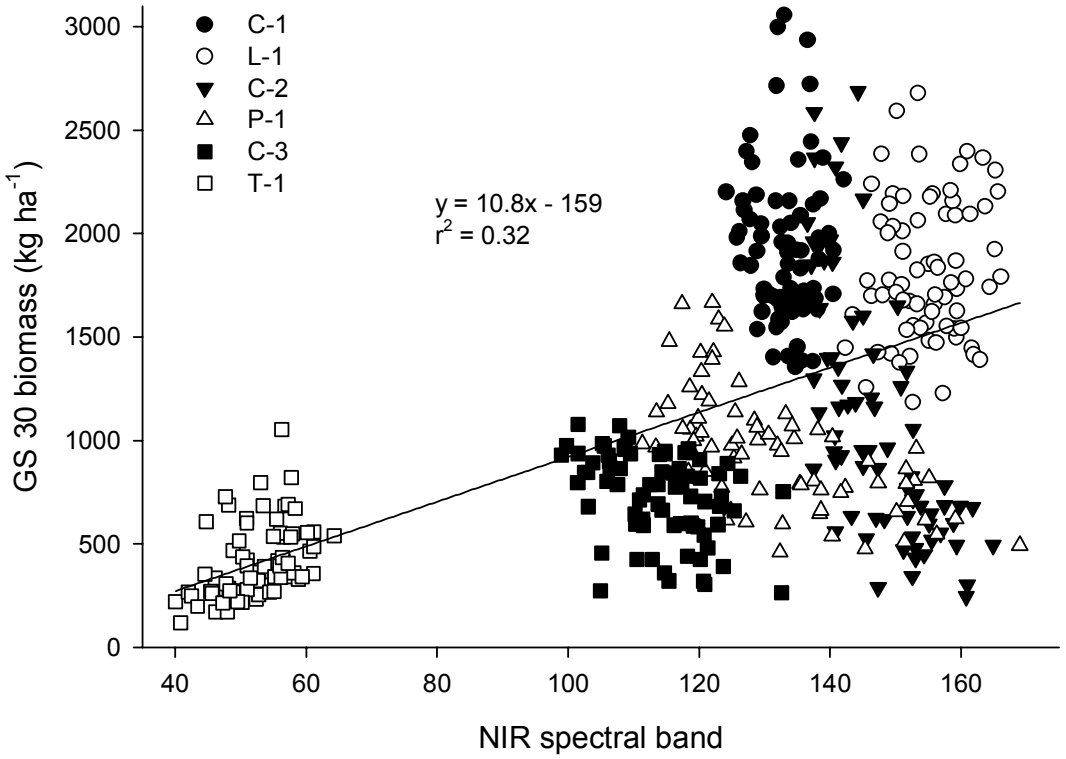


Fig 4. Relationship between growth stage (GS) 30 biomass in soft red winter wheat to near infrared (NIR) spectral band across six site-years (each symbol is a different site-year) in North Carolina with C-1 Cunningham Research Station 2002, L-1 Lower Coastal Plain Tobacco Research Station 2002, C-2 Cunningham Research Station 2003, P-1 Piedmont Research Station 2003, C-3 Cunningham Research Station 2004, and T-1 Tidewater Research Station 2004.