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A Comparison of Methods for Evaluating the Suppressive Ability of Winter Wheat Cultivars against Italian Ryegrass (*Lolium perenne*)

Margaret L. Worthington, S. Chris Reberg-Horton, David Jordan, and J. Paul Murphy*

Infestations of Italian ryegrass are problematic in both conventional and organic wheat production systems. The development of wheat cultivars with superior competitive ability against Italian ryegrass could play a role in maintaining acceptable yields and suppressing weed populations. Research was conducted in North Carolina to identify indirect methods of selection for Italian ryegrass suppressive ability (hereafter referred to as weed suppressive ability) of winter wheat cultivars that correlate well with Italian ryegrass-to-wheat biomass ratios. Two winter wheat cultivars (Dyna-Gro Baldwin and Dyna-Gro Dominion) and one experimental wheat line (NC05-19684) with differing morphological traits were overseeded with varying densities of Italian ryegrass. Wheat height measured throughout the growing season in weed-free plots was strongly associated with weed suppressive ability, but high wheat tillering capacity had no significant effect on weed suppressive ability in the lines tested in this study. Italian ryegrass seed head density during grain fill was strongly correlated ($r = 0.94$) with Italian ryegrass-to-wheat biomass ratio, the generally accepted measure of weed suppressive ability. Visual estimates of percent Italian ryegrass biomass relative to the plot with the highest level of Italian ryegrass infestation in each replicate were also strongly correlated with weed suppressive ability at all growth stages, especially during heading ($r = 0.87$) (Zadoks growth stage [GS] 55). Measurements from nonimaging spectrophotometers and overhead photographs taken from tillering (Zadoks 23 to 25) to early dough development (Zadoks 80) were unreliable estimates of end-of-season Italian ryegrass-to-wheat biomass ratios because they failed to account for wheat cultivar differences in biomass, color, and growth habit. Italian ryegrass seed head density and visual estimates of Italian ryegrass biomass during grain fill are appropriate indirect methods of selection for weed suppressive ability in breeding programs.

Nomenclature: Italian ryegrass, *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot LOLMU; wheat, *Triticum aestivum* L.
Key words: Indirect selection, organic wheat production, weed interference, wheat breeding.

Italian ryegrass, a winter annual, is a major weed in small-grain crops in the southeastern Unites States (Everman and Jordan 2011; Webster 2000). Italian ryegrass reduces grain yield by competing for nutrients and light, decreasing the number of productive tillers, and promoting lodging (Appleby et al. 1976). Infestations of Italian ryegrass are problematic in both conventional and organic wheat production systems, and with the efficacy of herbicidal mechanisms of action reduced due to the development of resistant biotypes and expanding market opportunities for organic grain, alternative or supplementary methods for control are worth exploring.

Significant variation in competitive ability against weeds has been identified in soybean [*Glycine max* (L.) Merr.] (Jannink et al. 2000; Place et al. 2011), corn (*Zea mays* L.) (So et al. 2009), grain sorghum [*Sorghum bicolor* (L.) Moench ssp. *bicolor*] (Guneyli et al. 1969), rice (*Oryza sativa* L.) (Saito et al. 2010; Zhao et al. 2006), and wheat (Lemerle et al. 2001b; Wicks et al. 1986). Competitive ability can be described by (1) ability to maintain acceptable grain yield in the presence of weeds (tolerance), or (2) ability to suppress the vegetative and reproductive growth of weeds (weed suppressive ability) (Goldberg 1990; Jannink et al. 2000). Weed suppressive ability is measured by cutting a quadrat from a weedy plot, separating the weed stems from the crop, and calculating the ratio of weed-to-crop biomass relative to the weed-free plot (Coleman et al. 2001; Paynter and Hills 2009). This method is effective in studies with small numbers of plots, but is impractical in large breeding programs.

Although many researchers have suggested breeding crops for increased competitive ability against weeds (Bertholdsson 2010; Hoad et al. 2008; Lemerle et al. 2001a; Wolfe et al. 2008), very few programs are actually breeding cultivars with improved weed-suppressive ability or tolerance (Worthington and Reberg-Horton 2013). The lack of simple, efficient, and reliable methods for quantifying weed suppressive ability is a factor undermining efforts to breed crops with superior competitive ability.

Weed scientists frequently use visual estimates of weed biomass to estimate weed cover or biomass and predict crop yield loss. These qualitative evaluations are rapid, inexpensive, and require no specialized equipment, making them suited for large breeding programs. But to be effective estimators of weed suppressive ability, these ratings must be: correlated with weed-to-crop biomass ratios, reliable across evaluators, and repeatable (Andujar et al. 2010; Donald 2006).

Tools developed for precision agriculture and site-specific weed management can be employed as indirect measures of weed suppressive ability. Many vegetation indices have been developed which use combinations of spectral signatures in the visible (VIS) and near infrared wavelengths (NIR) to infer information about plant health, biomass, and weed competition (Jackson and Huete 1991). The most common of these indices is the normalized difference vegetation index (NDVI), a ratio of the difference between near infrared and visible reflectance and the sum of the reflectance from both wavelengths (Rouse 1973). Although frequently used to describe plant health, NDVI readings obtained from aerial photographs have been used to differentiate weeds from crops (Lamb and Weedon 1998; Lamb et al. 1999). Image analysis of overhead photographs taken with a standard digital camera was used to estimate weed biomass in maize at a fine resolution appropriate for rating small plots (Brown et al. 1994). Nonimaging spectrophotometers were employed to discern between the spectral signatures of weeds and a number of crops, including wheat (Girma et al. 2005).

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The number of weed and crop stems or heads in a quadrat can be used to approximate weed suppressive ability. Wicks et al. (2004) determined the number of wheat stems m^{-2} to assess competitive ability of winter wheat cultivars against Italian ryegrass. The end-of-season weed-to-crop biomass ratio was not determined in this study, so it is unclear whether this method sufficiently approximated the commonly accepted biomass ratio measurement of weed suppressive ability.

Numerous studies have shown that morphological traits such as height at the end of the growing season (Lemerle et al. 1996, 2001b; Mason et al. 2007), high tillering capacity (Lemerle et al. 1996, 2001b; Mason et al. 2007), early biomass accumulation (Lemerle et al. 1996, 2001b), leaf morphology (Lemerle et al. 1996), and time to maturity (Mason et al. 2007) contribute to weed suppressive ability in spring wheat. Fewer studies have been conducted on weed suppressive ability in winter wheat, and it is unclear whether the same traits confer a competitive advantage to wheat characterized by a long period of slow growth during vernalization. The objective of this study was to compare indirect methods for estimating the ability of winter wheat lines to suppress Italian ryegrass, identify methods that correlate well with the more labor-intensive measurement of Italian ryegrass-to-wheat biomass ratios, and determine which morphological features contributed to the weed suppressive ability of winter wheat lines in North Carolina.

Materials and Methods

Experimental Design and Plant Material. Two locally adapted winter wheat cultivars and one experimental line (hereafter referred to as lines) were chosen for this study based on their dissimilar morphological characteristics. The lines selected for the study were Dyna-Gro Baldwin, Dyna-Gro Dominion, and NC05-19684, chosen respectively for their tall stature, short stature, and high tillering abilities. These three lines were overseeded with varying rates of Gulf Italian ryegrass, a commercial turf cultivar, in a factorial treatment structure. The study was established as a randomized complete block design with four replications at each location.

Wheat was planted in 6.1-m-long plots using a calibrated cone drill with seven rows at 20.3 cm spacing in 2011 and 17.1 cm spacing in 2012. All three wheat lines were seeded at a rate of 375 seeds m^{-2}, adjusted according to seed weight to achieve uniform plant density. This relatively high seeding rate is typical for organic wheat production in North Carolina. Italian ryegrass was broadcast at seeding rates of 0, 50, 150, and 300 seeds m^{-2} and incorporated via a cultipacker.

Growing Conditions. This experiment was conducted during the 2011 and 2012 growing seasons at Caswell Research Station in Kinston, NC (35.16°N, 77.36°W) and Piedmont Research Station in Salisbury, NC (35. 41°N, 80.37°W). Wheat and Italian ryegrass were planted on October 18, 2010 at the Piedmont Research Station on a Hiawassee Clay Loam (fine, kaolinitic, thermic Rhodic Kanhapludult) and on October 25, 2010 at the Caswell Research Station on a Pocalla loamy sand (loamy, siliceous, subactive, thermic Arenic Plinthic Paleudult) in the first year of the study. During the second year of the experiment, wheat and Italian ryegrass were planted on October 24, 2011 at the Piedmont Research Station on a Cecil Clay Loam (fine, kaolinitic, thermic Typic Kanhapludult) and October 25, 2011 at the Caswell Research Station on a Kenansville loamy sand (loamy, siliceous, subactive, thermic Arenic Hapludult).

Both sites were treated with 33.6 kg ha^{-1} of preplant nitrogen and top-dressed with potassium based on soil test recommendations. A second application of fall nitrogen was applied to Caswell during the 2011 growing season because of heavy rains within hours of planting. A total of 100.7 kg ha^{-1} of spring nitrogen was applied at Caswell in both growing seasons. In the 2012 growing season 22.5 kg ha^{-1} of nitrogen was applied on December 23 to compensate for patches of nutrient deficiency in Caswell. A total of 89.6 kg ha^{-1} of spring nitrogen was applied during March of 2011 and 2012 at the Piedmont location. Broadleaf weeds were controlled with thifensulfuron-methyl plus tribenuron-methyl (Harmony Extra®, Dupont, Wilmington, DE) in both locations as needed throughout the growing season.

Growing conditions during both seasons were favorable with 308 mm and 436 mm rainfall between sowing and maturity at Caswell and Piedmont locations in 2011, and 495 mm and 615 mm rainfall in Caswell and Piedmont locations in 2012. Twelve hundred and thirty-eight and 950 growing degree-days were accumulated in the Caswell and Piedmont locations between November and February of 2011 to 2012. In contrast, only 816 and 590 growing degree-days were accumulated in those locations during the same period in the 2011 growing season. Consequently, tiller development was generally higher and stem extension (Zadoks GS [growth stage] 31) was reached earlier during the second yr of the experiment. Total growing degree-days did not differ significantly between two seasons, because temperatures between March and May 2012 were cooler than the previous year.

Indirect Methods for Evaluating Weed Suppressive Ability. Visual estimates of percent Italian ryegrass suppression relative to the plot with the highest level of Italian ryegrass infestation in each replicate and spectral indices derived from digital photographs and a nonimaging spectrometer were collected throughout the growing season (at Zadoks GS 25, 29, 31, 55 to 60, 69 to 80) (Zadoks et al. 1974) and Italian ryegrass seed head density was recorded after anthesis (Zadoks GS 69 to 80) to identify what measurements taken at which growth stages correlated best with end-of-season hand-sorted Italian ryegrass-to-wheat biomass ratios. All measurements throughout the season were recorded in a selected 1 m^2 area of the plot to ensure that end-of-season measurements of Italian ryegrass-to-wheat biomass ratios were unbiased. The initial number of Italian ryegrass plants that established in the selected area of the plot was counted in a 1 m^2 quadrat area during early tillering (Zadoks 23 to 25) to test whether wheat lines varied in their ability to suppress weed seedling germination and establishment.

The Italian ryegrass suppression in each plot was visually estimated during every field visit. The rater first observed all 12 plots in each replicate and selected the plot with the highest level of Italian ryegrass infestation. The weediest plot in each block was rated as 100 and Italian ryegrass-free plots were rated as 0. All other plots were assigned intermediate percentages based on the level of Italian ryegrass biomass and ground cover reduction in comparison to the weediest plot.

Spectrophotometer readings were taken on every plot during each field visit using a Crop Circle ACS-210 Plant Canopy Reflectance Sensor (Holland Scientific, Inc., Lincoln, NE), which used near-infrared reflectance and visible reflectance data to calculate NDVI.

Overhead digital photographs were taken throughout the growing season with a Nikon D5000 digital camera (Nikon, Inc., Melville, NY) mounted on an adjustable-height, custom-built camera stand, which was centered over the middle row of each plot. During each field visit the camera was positioned approximately 100 cm above the crop canopy and the AF-S Nikkor 18-55mm lens was adjusted to capture all seven rows of wheat in the plot and the soil between rows. No flash was employed in the photographs because excessive shadowing can interfere with the analysis of crop and weed spectral signatures. Digital image analysis of overhead photographs was conducted using ImageJ, a public domain Java image processing and analysis program (Abramoff et al. 2004). Hue, saturation, and brightness thresholds were manually selected to differentiate wheat pixels from soil and Italian ryegrass pixels. Different thresholds were optimal depending on the soil background and light conditions present when the images were collected, so photographs from each field visit were analyzed separately in ImageJ.

End-of-season counts of Italian ryegrass seed heads were made in a 1 m^2 quadrat centered in the selected region of each plot after anthesis (Zadoks GS 69 to 80). In 2011, a forage plot harvester (Haldrup Model 0978, Bredgade, Denmark) was used to cut an approximately 1.68 m^2 swath of wheat and Italian ryegrass stems from the previously selected area of each plot during grain development and ripening (Zadoks 80). In 2012, a sickle bar mower (Jari Monarch, Saint Peter, MN) was used to cut a slightly smaller (1.59 m^{-2}) swath of wheat and Italian ryegrass biomass at the same growth stage. Italian ryegrass and wheat stems were separated by hand and dried for 1 wk at 40 C during both years. Dried wheat and Italian ryegrass stems were then weighed and used to calculate end-of-season Italian ryegrass-to-wheat biomass ratios for each plot. Wheat grain yield was recorded with a combine in the plot area remaining after the destructive biomass sampling at maturity (after Zadoks 92) as an indicator of tolerance.

Morphological Traits Impacting Weed Suppressive Ability. Morphological traits were measured in all plots not overseeded with Italian ryegrass throughout the growing season to identify what factors might confer superior weed suppressive ability in winter wheat cultivars. Height notes for both wheat and Italian ryegrass plants were collected during grain fill on the same date as final counts of Italian ryegrass seed heads m^{-2} in the 2011 growing season. Plant height was estimated as the distance from ground level to the tip of the average head, excluding awns. Additional morphological data were collected in Italian ryegrass-free plots during the 2012 growing season, including height throughout the season (Zadoks 29, 31, and 69), visual estimates of early vigor during tillering (Zadoks 29), and diffuse noninterception (DIFN) (a measure of light penetration through the canopy) during stem extension and heading (Zadoks 31). Visual estimates of early vigor, a combination of percent ground cover and wheat height, were made on a 1 to 9 scale with the most vigorous plots rated as 1. DIFN was calculated using a LAI-2000 sensor (LI-COR Environmental, Lincoln, NE) in overcast conditions. Counts of reproductive wheat tillers per meter row were made in plots overseeded with 0 and 300 Italian ryegrass seeds m^{-2} at Caswell research station in 2011 (Zadoks 80) and at both locations in 2012 (Zadoks 69).

Statistical Analyses. Statistical analyses were conducted using SAS 9.2 (SAS Institute Inc., Cary, NC). Data collected from biomass harvest, visual estimates of Italian ryegrass-to-wheat biomass, measurements of Italian ryegrass seed head density, and spectral indices derived from digital photographs and a nonimaging spectrometer were first analyzed using analysis of variance (ANOVA) to test for the effect of treatment combination (twelve total combinations of wheat genotypes and Italian ryegrass seeding rate), block, environment, and their interaction. Least-square means were calculated for all measurements with significant treatment effects and used to calculate correlations between various estimates of weed suppressive ability. Pearson's correlation coefficient was used to test the significance of correlations between treatment least-square means for measurements with significant treatments effects.

Initial Italian ryegrass seedlings m^{-2}, and end-of-season wheat dry yield, wheat biomass, Italian ryegrass biomass, Italian ryegrass-to-wheat biomass ratio, and Italian ryegrass seed heads m^{-2}, and wheat grain yield adjusted to 14% moisture were tested for significant correlation with one another. Italian ryegrass-to-wheat biomass ratio was then tested for correlation with each of the visual estimates of percent Italian ryegrass biomass relative to the plot with the highest level of Italian ryegrass infestation in each replicate, overhead plot photographs, and nonimaging spectrometer data taken throughout the growing season. Measurements that were significantly correlated with end-of-season Italian ryegrass-to-wheat biomass ratios across all study environments were deemed acceptable substitutes for biomass harvesting and separation by hand.

Genotypic differences in weed suppressive ability were evaluated using PROC MIXED with genotype, Italian ryegrass seedling density (at Zadoks 23 to 35), and their interaction considered fixed effects, and block, environment, and associated interactions considered random effects. Italian ryegrass-to-wheat biomass ratio and Italian ryegrass seed head density were each tested separately as response variables. Only plots overseeded with 50, 150, and 300 Italian ryegrass seeds m^{-2} were included in the model when testing for the effects of genotype, Italian ryegrass seedlings density, and their interaction on Italian ryegrass-to-wheat biomass ratio and Italian ryegrass seed heads density because there was no variation in the response variable in weed-free plots. Genotypic differences in tolerance were tested using the same PROC MIXED model with wheat grain yield substituted as the response variable. Significant Italian ryegrass seedling density-by-genotype interactions would indicate differences in tolerance.

Least-square means of each wheat line were also calculated for each of the morphological traits recorded throughout the growing season in Italian ryegrass-free plots, and for counts of reproductive wheat tillers per meter row in Italian ryegrass free plots and plots overseeded at the highest rate (300 Italian ryegrass seeds m^{-2}). Fisher's Protected LSD was then used to test for significant differences between genotypes for each trait.

Table 1. Least-square means of the 12 treatment combinations for traits related to Italian ryegrass competition, biomass, and grain yield in the four test environments.

Ryegrass seeds m^{-2}	Line	Ryegrass seedlings m^{-2} GS 23–25[a]	Visual Esimate GS 55–60	Ryegrass seed heads m^{-2} GS 69–80	Ryegrass biomass kg m^{-2} GS 80	Wheat biomass kg m^{-2} GS 80	Ryegrass-to-wheat biomass ratio GS 80	Grain yield kg ha^{-1} GS 92
0	Baldwin	0.0	0.0	0.0	0.00	1.67	0.00	5,780
50	Baldwin	12.8	7.5	38.6	0.05	1.63	0.03	5,500
150	Baldwin	32.8	36.4	97.2	0.13	1.55	0.09	4,830
300	Baldwin	52.9	79.0	153.0	0.18	1.39	0.14	4,580
0	Dominion	0.0	0.0	0.0	0.00	1.46	0.00	5,670
50	Dominion	12.4	15.1	60.4	0.06	1.48	0.04	5,650
150	Dominion	32.2	54.4	156.3	0.17	1.26	0.14	4,928
300	Dominion	51.8	94.0	254.9	0.26	1.18	0.23	4,480
0	NC05-19684	0.0	0.0	0.0	0.00	1.66	0.00	5,930
50	NC05-19684	12.3	12.1	51.2	0.05	1.46	0.04	5,360
150	NC05-19684	38.2	60.0	147.9	0.16	1.34	0.13	4,910
300	NC05-19684	57.2	94.56	241.5	0.23	1.24	0.19	4,590
	R^2 [b]	0.84	0.94	0.87	0.85	0.77	0.84	0.64
	CV	45.2	26.9	40.0	44.0	10.8	52.4	11.7
	Mean	25.2	37.8	100.2	0.11	1.44	0.09	5,160
	LSD (0.05)[c]	8.0	7.1	28.0	0.03	0.11	0.03	420

[a] Zadoks growth stage (GS) when data were collected.
[b] R^2 and coefficient of variation (CV) for each trait are based on a general linear model testing for the effect of treatment, environment, treatment by environment interaction, and replicate.
[c] Fisher-protected LSD.

Results and Discussion

Range of Weed Suppressive Ability. Significant variation between factorial treatment groups ($P < 0.01$) was detected for Italian ryegrass-to-wheat biomass ratio (Table 1). Least-square means of Italian ryegrass-to-wheat biomass ratios for treatments ranged from 0 in plots not overseeded with Italian ryegrass to 0.23 in Dyna-Gro Dominion plots overseeded with 300 Italian ryegrass seeds m^{-2}. Overall mean Italian ryegrass-to-wheat biomass ratios in the four study environments ranged from 0.06 at the Piedmont research station in both years to 0.14 at the Caswell station in 2011. Significant treatment differences in all environments were observed for other traits related to weed suppressive ability, including Italian ryegrass biomass m^{-2} at Zadoks 80, wheat biomass m^{-2} at Zadoks 80, Italian ryegrass seedlings m^{-2} at Zadoks 23 to 25, Italian ryegrass seed heads m^{-2} at Zadoks 69, and wheat dry yield at Zadoks 92 (Table 1). Correlations between traits related to weed competition were significant with the exceptions of final wheat biomass m^{-2} (Zadoks 80) with Italian ryegrass seedling density (Zadoks 23 to 25) (Table 2). As expected, wheat biomass m^{-2} and grain yield decreased in all environments as Italian ryegrass seeding rate increased.

Variation in Weed Suppressive Ability among Genotypes. The three lines differed in their ability to suppress Italian ryegrass biomass accumulation, tillering, and reproduction (Figure 1). Observed differences in weed suppressive ability were not attributed to genotypic effects on weed establishment, because Italian ryegrass seedling density (Zadoks 23 to 25) did not differ among the lines ($P \geq 0.05$; Table 1). Genotype and Italian ryegrass seedling density main effects were significant ($P < 0.05$) and their interaction was nonsignificant. Dyna-Gro Baldwin was the most suppressive line in all study environments, whereas Dyna-Gro Dominion was consistently the poorest suppressor of Italian ryegrass tillering and biomass accumulation. NC05-19684 performed intermediately across environments (Figure 1). The consistent weed suppression rankings of these three lines across experimental locations and years suggests that weed suppressive ability is a heritable trait and that significant variation for this trait exists within adapted southeastern winter wheat germplasm.

Differences in weed suppressive ability between lines were most evident at the highest Italian ryegrass-seeding rate tested (300 seeds m^{-2}; Figure 1). Italian ryegrass germination and establishment varied across environments, ranging from a mean of 37 to 78 seedlings m^{-2} at the highest seeding rate. Seedling establishment rates can increase or at least become more consistent if Italian ryegrass seeds are drilled into the soil rather than broadcast, but our results suggested that breeders can accentuate genotypic differences in weed suppressive ability by overseeding plots with at least 300 Italian ryegrass seeds m^{-2}.

No significant differences in tolerance (ability to maintain acceptable grain yield in the presence of weeds) were detected

Table 2. Correlations (r) of traits related to Italian ryegrass competition, biomass, and grain yield in the four test environments.

	Ryegrass seedlings m^{-2}	Ryegrass seed heads m^{-2}	Ryegrass biomass kg m^{-2}	Wheat biomass kg m^{-2}	Ryegrass-to-wheat biomass	Wheat grain yield kg ha^{-1}
Ryegrass seedlings m^{-2}						
Ryegrass seed heads m^{-2}	0.83**					
Ryegrass biomass kg m^{-2}	0.82**	0.94**				
Wheat biomass kg m^{-2}	ns	−0.48**	−0.41**			
Ryegrass-to-wheat biomass	0.68**	0.93**	0.94**	−0.59**		
Wheat grain yield kg ha^{-1}	−0.62**	−0.67**	−0.77**	0.37*	−0.75**	

[a] Abbreviation: ns, no significant correlation ($P > 0.05$).
*, ** r significantly different from zero at $P \leq 0.05$ and $P \leq 0.01$, respectively.

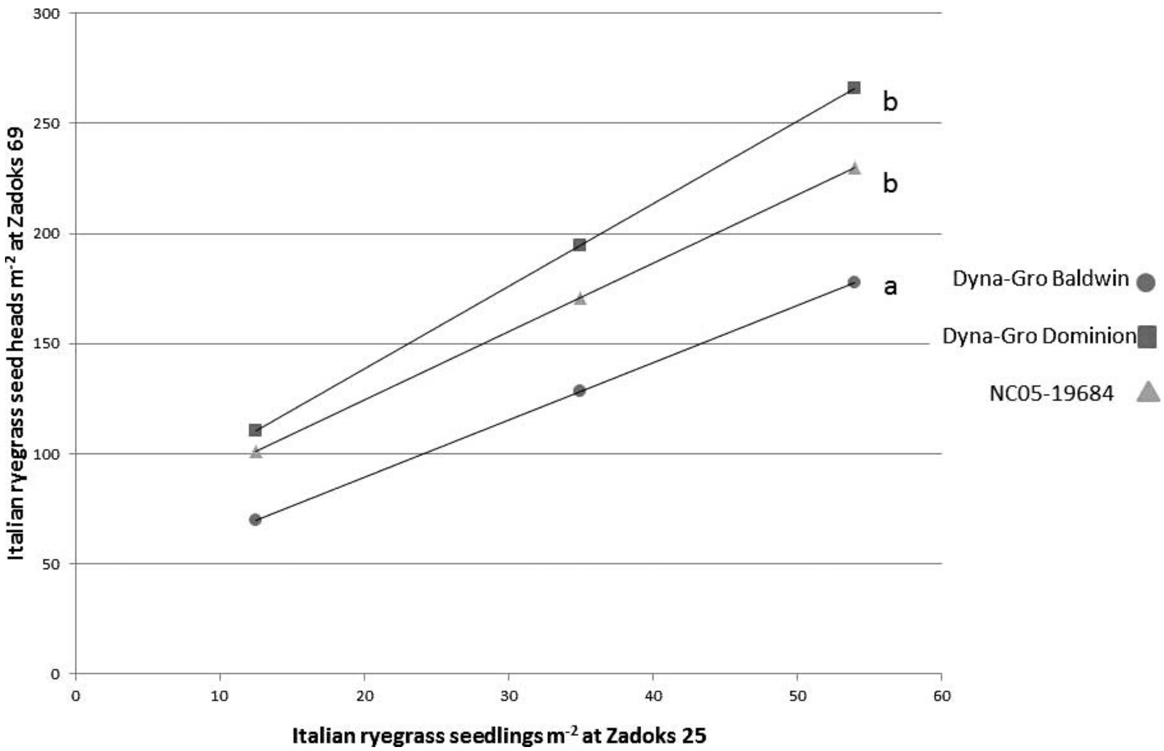

Figure 1. Relationship between Italian ryegrass seedling density during tillering (Zadoks 23 to 25) and Italian ryegrass seed heads density after anthesis (Zadoks 69 to 80) for Dyna-Gro Baldwin, Dyna-Gro Dominion, and NC05-19684. The predicted Italian ryegrass seed head density for each experimental line is plotted at the mean Italian ryegrass seedling density for all plots overseeded at 50, 150, and 300 Italian ryegrass seedlings m^{-2}. Lowercase letters indicate significant differences in the weed suppressive ability of lines ($P \leq 0.05$).

among the three wheat lines (data not shown). The main effect of Italian ryegrass seedling density was highly significant ($P < 0.01$) in all environments, indicating that Italian ryegrass infestation reduced wheat yield, but genotypic main effects and Italian ryegrass seedling density by genotype interactions were nonsignificant ($P \geq 0.05$). All three lines suffered yield losses at approximately equal rates in plots with high Italian ryegrass infestations and no significant differences were observed in the grain yield of the three tested lines in weed-free conditions (Table 3). Many studies have found that weed biomass suppression and yield tolerance are broadly correlated (Balyan et al. 1991; Challaiah et al. 1986; Huel and Hucl 1996; Lemerle et al. 1996, 2001b; Wicks et al. 1986). Still others have found no relationship between tolerance and weed suppressive ability (Coleman et al. 2001; Cousens and Mokhtari 1998). It should be noted that only three wheat lines were tested in this study; a larger sampling of adapted genotypes should be tested to discern the relationship between yield tolerance and weed suppressive ability in winter wheat.

Morphological Traits Impacting Weed Suppressive Ability. No significant differences between lines were detected for heading date, grain yield, vigor rating during tillering, or DIFN (at Zadoks 31) in any of the environments. Dyna-Gro Baldwin, the most weed-suppressive line, was significantly taller than Dyna-Gro Dominion and NC05-19684 at all growth stages and in all environments (Table 3). Dyna-Gro Baldwin also had a more erect growth habit during tillering

Table 3. Least-square means of various morphological measurements collected throughout the growing season in Italian ryegrass free plots on the three wheat lines in the four test environments.

	Vigor Rating[a]	DIFN[a]	Wheat height[a] cm	Wheat height[a] cm	Wheat height[a] cm	Wheat height cm	Wheat biomass kg m^{-2}	Wheat grain yield kg ha^{-1}
Line	GS 29[b]	GS 31	GS 29	GS 31	GS 69	GS 80	GS 80	GS 92
Dyna-Gro Baldwin	3.8	0.17	27	50	93	102	1.80	5,780
Dyna-Gro Dominion	4.5	0.28	22	37	75	81	1.58	5,670
NC05-19684	4.1	0.25	16	34	76	82	1.79	5,950
RSquare[c]	0.40	0.78	0.82	0.95	0.97	0.95	0.90	0.47
CV[c]	27.6	36.4	13.4	7.6	3.2	3.5	14.2	10.5
Mean	4.1	0.24	22	40	82	88	1.72	5,800
LSD (0.05)[d]	ns[e]	0.09	3	3	3	2	0.18	ns

[a] These data were collected only in 2012.
[b] Zadoks growth stage (GS) when data were collected.
[c] R^2 and coefficient of variation (CV) for each trait are based on a general linear model testing for the effect of genotype, environment, replicate, and genotype by environment interaction.
[d] Fisher-protected LSD.
[e] Abbreviations: DIFN, diffuse noninterception; ns = no significant difference between lines.

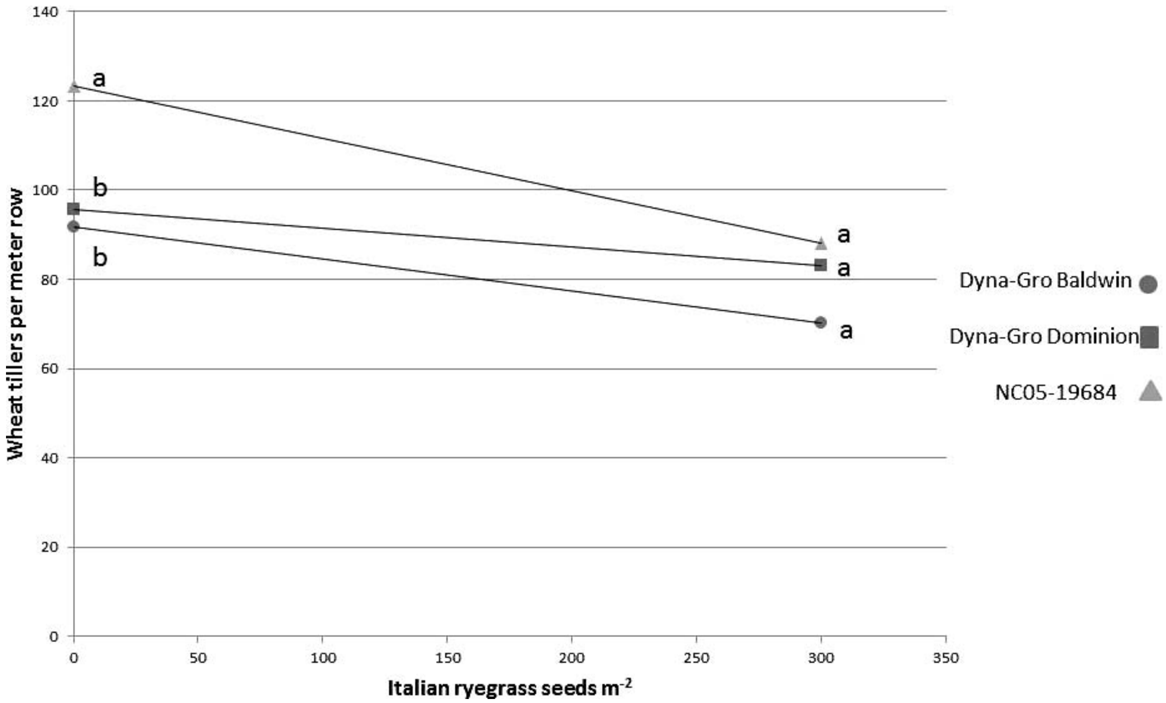

Figure 2. The number of wheat tillers per meter row in plots overseeded with 0 and 300 Italian ryegrass seeds m^{-2} for Dyna-Gro Baldwin, Dyna-Gro Dominion, and NC05-19684. Lowercase letters indicate significant differences in the number of wheat tillers per meter row among the three lines at the highest and lowest Italian ryegrass seeding rates.

(data not shown), and allowed the least amount of light penetration through the canopy (Zadoks 55 to 60) at Caswell in 2012, according to DIFN measurements (Table 3).

Previous studies have found significant positive associations between wheat height and suppressive ability against rigid ryegrass (*Lolium rigidum* Gaudin) (Lemerle et al. 1996, 2001b), sterile oat (*Avena ludoviciana* Durieu) (Balyan et al. 1991), and a variety of summer annual weeds (Mason et al. 2007; Wicks et al. 2004). Italian ryegrass in Dyna-Gro Baldwin plots grew significantly taller (mean of 116 cm at GS 80) than Italian ryegrass grown with shorter varieties, Dominion and NC05-19684 (108 and 105 cm, respectively) in 2011. Balyan et al. (1991) also reported that the height of wheat lines remained the same regardless of weed pressure, but wild oats grow grew taller when grown with taller wheat lines. Weeds with strong phenotypic plasticity might suffer a fitness penalty as they allocate more of their resources to vegetative growth in order to surpass the canopy of taller wheat lines at the expense of tillering and reproductive growth. This effect might complement the increased shading ability of tall lines that deplete light resources available to weeds. Plant breeders usually select against taller lines because of their lower harvest index and propensity for lodging (Lemerle et al. 1996). Our results showed that Dyna-Gro Baldwin yielded as well as the shorter lines, regardless of whether the plots were overseeded with Italian ryegrass or not, and was taller during all growth stages. Fewer studies have investigated the effect of plant height during tillering, stem extension, and heading on weed suppression, particularly during the long period of slow growth and competition during mild southeastern winters. It is possible that increased early-season wheat height could induce a reproductive fitness penalty on Italian ryegrass similar to end-of-season height without the associated loss of wheat harvest index and threat of lodging. A larger set of lines should be screened to determine which morphological traits contribute to weed suppressive ability in winter wheat systems.

Although previous research on spring wheat cultivars has suggested that tillering capacity plays an important role in weed suppressive ability (Lemerle et al. 1996, 2001b; Mason et al. 2007), our results indicated that tillering had little effect on weed suppressive ability North Carolina. NC05-19684 had a significantly higher capacity for tillering in weed free plots than Dyna-Gro Baldwin and Dyna-Gro Dominion (Figure 2). But no significant differences were detected between lines in the counts of wheat tillers per meter row in plots overseeded with 300 Italian ryegrass seeds m^{-2} in any of the three environments where tillering was measured (Figure 2). Italian ryegrass depresses wheat yields in North Carolina primarily by reducing the density of reproductive tillers (Liebl and Worsham 1987). These results indicated that not all winter wheat lines experience the same rate of tillering suppression when infested with the same amount of Italian ryegrass, and that tillering capacity in Italian ryegrass free conditions is a poor predictor of tiller maintenance in weedy conditions.

Comparison of Screening Methods. *Visual Estimates*. Visual estimates of percent Italian ryegrass suppression relative to the plot with the highest level of Italian ryegrass infestation in each replicate, taken during tillering (Zadoks GS 23, 25, and 29), stem extension (Zadoks GS 31), heading (Zadoks GS 55 to 60), and ripening (Zadoks GS 69 to 80) differed significantly among the 12 treatment combinations. Correlations between visual estimates taken at all growth stages and Italian ryegrass-to-wheat biomass ratios were highly significant ($P < 0.01$), especially during and after heading ($r = 0.87$; Zadoks 55 to 60), when Italian ryegrass stems emerged from above the wheat canopy (Table 4). A single evaluator made

Table 4. Correlation coefficients (r) of potential screening methods with Italian ryegrass-to-wheat biomass ratio.[a]

Screening method	Correlation with Italian ryegrass-to-wheat biomass ratio
Zadoks 23–25	
Ryegrass seedlings m^{-2}	0.68**
NDVI	−0.14
Overhead photograph	ntd
Visual estimate	0.73**
Zadoks 29	
NDVI	−0.10
Overhead photograph	ntd
Visual estimate	0.81**
Zadoks 31	
NDVI	0.19
Overhead photograph	0.23
Visual estimate	0.80**
Zadoks 55–60	
NDVI	0.31*
Overhead photograph	0.24
Visual estimate	0.87**
Zadoks 69–80	
NDVI[b]	−0.02
Overhead photograph[b]	−0.14
Visual estimate	0.86**
Ryegrass seed heads m^{-2}	0.93**

[a] Abbreviations: NDVI, normalized difference vegetation index; ntd, no significant difference between treatments.

[b] Data collected only in 2011.

*,** r significantly different from zero at $P \leq 0.05$ and $P \leq 0.01$, respectively.

these estimates over a very large range of Italian ryegrass densities. Follow-up studies should therefore test the consistency of estimates across evaluators, especially in plots all overseeded with the same density of Italian ryegrass. Over 30 person-min of sorting is required to determine Italian ryegrass-to-wheat biomass ratios for each plot. In contrast, a trained evaluator can objectively estimate a plot's percent Italian ryegrass biomass relative to the plot with the highest level of Italian ryegrass infestation in each replicate in less than 1 min. Thus, visual estimates appear to be an efficient and repeatable substitute for Italian ryegrass-to-wheat biomass ratios. Visual ratings have also been used to estimate the weed suppressive ability of rice accessions against ducksalad [*Heteranthera limosa* (Sw.) Willd.] (Dilday et al. 1994), suggesting that this screening method could be adapted for use in other crop and weed species.

Overhead Photographs. Spectral signatures derived from overhead photographs were unreliable substitutes for Italian ryegrass-to-wheat biomass ratios. Overhead photographs taken throughout the growing season were analyzed with ImageJ software, with thresholds set for hue, saturation, and brightness to separate wheat pixels from soil and Italian ryegrass pixels. The intended use was to calculate the percent plot area covered by Italian ryegrass pixels, but it was impossible to consistently separate wheat and Italian ryegrass pixels from one another. Variations in light conditions, soil background, and wheat line color and growth habit were confounding factors. Instead, we were only able to use ImageJ to estimate the percent of ground covered in vegetation. Vegetative ground cover estimates derived from overhead photographs were not significantly correlated with Italian ryegrass-to-wheat biomass ratios at any growth stage (Table 4). Although aerial photographs have successfully discerned between weeds and a number of crops, including natural Italian ryegrass infestations in winter wheat fields (Lopez-Granados et al. 2006), these techniques were generally ineffective in predicting Italian ryegrass-to-wheat biomass ratios across a diverse set of wheat lines.

NDVI. Measurements of NDVI obtained from a nonimaging spectrophotometer were slightly better correlated with Italian ryegrass-to-wheat biomass ratios than image analysis of overhead photographs, particularly during heading (Zadoks GS 55 to 60; Table 4). However, the utility of this measure was limited by many of the same factors as overhead photography, including variation in soil background, wheat line color, and growth habit. NDVI and Italian ryegrass-to-wheat biomass ratio were significantly correlated at three out of four study environments during heading (Zadoks 55 to 60; Table 4), but correlations were nonsignificant during tillering (Zadoks 23 to 25, 29) and grain ripening (Zadoks 69 to 80). Italian ryegrass seeding rate and genotypic main effects were both significant for NDVI measurements made between Zadoks 31 and 69 (data not shown), but genotypic differences in wheat color and growth habit obscured the effects of Italian ryegrass density and rendered NDVI an inadequate method for evaluating weed suppressive ability of diverse wheat lines.

Italian Ryegrass Seed Head Density. Italian ryegrass seed head density after anthesis (Zadoks GS 69 to 80) were highly correlated ($P < 0.01$) with Italian ryegrass-to-wheat biomass ratios in all environments (Table 4). Pearson's correlation coefficients between Italian ryegrass seed head density and Italian ryegrass-to-wheat biomass ratios ranged from $r = 0.97$ at Caswell during 2011 to $r = 0.99$ at both locations in 2012. The overall correlation between Italian ryegrass seed head density and Italian ryegrass-to-wheat biomass ratios was 0.93 when all environments were pooled. Wilson et al. (1988) and Korres and Froud-Williams (2002) also found a positive linear relationship between weed biomass and the density of reproductive structures in a number of important broadleaf and annual grass weeds of winter wheat, including annual bluegrass [*Poa annua* L.] and common chickweed [*Stellaria media* (L.) Vill.], indicating that this method could be transferable to weed species other than Italian ryegrass. Italian ryegrass seed head counts from 0.25 m^{-2} quadrats were also correlated strongly with Italian ryegrass-to-wheat biomass ratios (data not shown), suggesting that breeders could make effective seed head counts in a smaller area to save time and resources. Evaluators can objectively count Italian ryegrass seed heads in a 1 m^{-2} area in approximately 5 min, whereas over 30 person-min of sorting were required to determine Italian ryegrass-to-wheat biomass ratios in each plot. Furthermore, specialized equipment and dryer space are required to calculate Italian ryegrass-to-wheat biomass ratios across a large number of plots. Italian ryegrass seed head density approximated Italian ryegrass-to-wheat biomass ratios effectively and provided significant savings in time and expense. Reduced Italian ryegrass seed head density is a particularly appropriate measure of the weed-suppressive ability of wheat lines because the number of seed heads produced each season will directly impact the weed seed bank in that field during subsequent years.

Conclusion. Variation in weed suppressive ability was observed within the very small set of lines tested in this study. Dyna-Gro Baldwin, a tall line with relatively low tillering capacity in weed-free environments suppressed the vegetative and reproductive growth of Italian ryegrass more effectively than the shorter lines Dyna-Gro Dominion and NC05-19684. Italian ryegrass seed head density and visual estimates of Italian ryegrass suppression have been identified as simple, efficient, and reliable methods for quantifying weed suppressive ability. Both methods were rapid (1 min plot^{-1} for visual estimates of Italian ryegrass suppression and 5 min plot^{-1} for Italian ryegrass seed head density) and highly correlated with the more labor-intensive measurement of Italian ryegrass-to-wheat biomass ratios (visual estimates of Italian ryegrass suppression $r = 0.87$; Italian ryegrass seed head density $r = 0.93$). Although this study was focused solely on identifying appropriate methods for evaluating the weed suppressive ability of winter wheat lines against Italian ryegrass, related research indicates that these methods could be adapted for use in other crop and weed systems (Dilday et al. 1994; Korres and Froud-Williams 2002; Wilson et al 1988). The use of these efficient screening methods can enable breeders to screen a larger number of lines for weed-suppressive ability and facilitate the development of cultivars with improved weed suppressive ability

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