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RESEARCH

Relative Contributions of Allelopathy and Competitive Traits to the Weed Suppressive Ability of Winter Wheat Lines Against Italian Ryegrass

Margaret Worthington,* S. Chris Reberg-Horton, Gina Brown-Guedira, David Jordan, Randy Weisz, and J. Paul Murphy

ABSTRACT

Allelopathy and competitive ability have been identified as independent factors contributing to the weed suppressive ability of crop cultivars; however, it is not clear whether these factors have equal influence on weed suppression outcomes of winter wheat (*Triticum aestivum* L.) lines in the field. Fifty-eight winter wheat lines adapted to the southeastern United States were screened for allelopathic activity against Italian ryegrass (*Lolium perenne* L. *ssp. multiflorum* [Lam.] Husnot) in an agar-based seedling bioassay. Eight strongly and weakly allelopathic lines were identified and evaluated for weed suppressive ability and grain yield tolerance in a replicated field experiment conducted in North Carolina. Significant genotypic differences in weed suppressive ability were found in three of four study environments, while genotypic differences in yield tolerance were identified in all environments. Although the allelopathic activity of genotypes varied in the seedling bioassay, no correlations between allelopathy and weed suppressive ability or grain yield tolerance were observed. Weed suppressive ability was correlated with competitive traits, including vigor and erect growth habit during tillering (Zadoks GS 29), high leaf area index (LAI) at stem extension (GS 31), plant height at tillering and stem extension (GS 29, 31), grain yield in weedy conditions, and grain yield tolerance. Therefore, breeders in the southeastern United States should focus their efforts on improving competitive traits within adapted germplasm rather than selecting for cultivars with high allelopathic activity to achieve maximum gains in weed suppressive ability against Italian ryegrass.

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Abbreviations: ECAM, Equal-compartment-agar method; GS, Zadoks growth stage; LAI, leaf area index; LS means, least square means; NC OVT, North Carolina official variety test; NDVI, normalized difference vegetation index.

Weed suppressive winter wheat (*Triticum aestivum* L.) cultivars could potentially supplement chemical and cultural practices for weed control (Worthington and Reberg-Horton, 2013). Breeders in the southeastern United States are increasingly interested in developing weed suppressive winter wheat cultivars due to the proliferation of herbicide-resistant Italian ryegrass (*Lolium perenne* L. *ssp. multiflorum* [Lam.] Husnot) populations (Kuk et al., 2008; Preston et al., 2009) and the rapid expansion of the organic wheat market (Wolfe et al., 2008; Hoad et al., 2012). Promising new cultivars should have the ability to suppress the vegetative and reproductive growth of weeds (weed suppressive ability) and sustain higher yields relative to other cultivars in the presence of weeds (grain yield tolerance) (Goldberg, 1990).

The weed suppressive ability of a crop cultivar is determined by the combined effects of its competitive and allelopathic activity (Harper, 1977). Competitive genotypes have the ability to access scarce light, nutrients, and water resources in a limited space,

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thus suppressing the growth and reproduction of nearby weed species. Allelopathic crop cultivars, on the other hand, suppress neighbors by exuding phytotoxins into the near environment (Muller, 1969). Although researchers have suggested that breeders should strive to improve a crop's allelopathic and competitive ability simultaneously to achieve maximum weed suppression (Lemerle et al., 2001; Olofsdotter et al., 2002; Belz, 2007), it is not clear whether these factors contribute equally to weed suppression outcomes in the field.

Allelopathy and competition function independently to suppress weeds and are virtually impossible to differentiate in field studies (Inderjit and del Moral, 1997). Therefore, controlled laboratory bioassays are considered useful initial screening tools to identify lines which possess superior allelopathic activity but may lack competitive traits (Wu et al., 2001). Unfortunately, highly allelopathic lines identified in laboratory bioassays are rarely screened for weed suppressive ability under field conditions in follow-up experiments. Significant variation in wheat seedling allelopathy has been established in laboratory bioassays (Wu et al., 2000a, 2003; Bertholdsson, 2005, 2010, 2011). However, the only field studies focused on confirming the weed suppressive ability of wheat lines identified as allelopathic in laboratory bioassays have been conducted with 20 or fewer wheat lines in Sweden (Bertholdsson, 2005, 2010, 2011).

Bertholdsson (2005) found that the influence of early vigor and allelopathy on weed suppressive ability in Swedish spring wheat cultivars were both far weaker than in barley (*Hordeum vulgare* L.) or rice (*Oryza sativa* L.) (Olofsdotter et al., 1999; Seal et al., 2008). Still, models predicted that a 20% improvement in wheat allelopathy would cause a corresponding decrease in weed biomass of 8 to 15% (Bertholdsson, 2005). In a subsequent study focused on winter wheat, least squares predictions indicated that weed biomass could be decreased by 60% if allelopathy and early vigor could be improved to the level of rye (*Secale cereale* L.) (Bertholdsson, 2011).

Despite promising results in Sweden, it is unclear if significant variation in allelopathic activity exists within winter wheat germplasm adapted to the southeastern United States. Temperature, solar irradiation, mineral deficiencies, water stress, and rhizosphere organisms can all impact the expression of allelopathy (Rice, 1984). Thus, allelopathy and competitive traits may vary in importance across different environmental and edaphic conditions. Therefore, the objectives of this study were to measure the allelopathic activity of winter wheat lines adapted to the southeastern United States in a laboratory seedling bioassay and to assess the relative contributions of allelopathy and competitive traits to weed suppression outcomes and yield tolerance of winter wheat lines in field experiments in North Carolina.

MATERIALS AND METHODS

Allelopathy Bioassay

Fifty-eight entries from the 2011 North Carolina Official Variety Test (NC OVT) were evaluated for seedling allelopathy using the equal-compartment-agar method (ECAM) (Wu et al., 2000b) (Table 1). Seeds of each wheat line and 'Gulf' Italian ryegrass, a commercial turf cultivar, were soaked in 70% ethanol for 2.5 min, and rinsed four times with sterilized distilled water for surface sterilization. Afterward, the seeds were soaked in 2.5% sodium hypochlorite for 15 min and rinsed four more times with sterilized distilled water. Wheat and Italian ryegrass seeds were then placed in individually labeled Petri dishes lined with autoclaved filter paper, doused with 1 mL of sterilized distilled water, and sealed with parafilm. Wheat and Italian ryegrass seeds were incubated at 25°C for 48 and 72 h, respectively.

Twelve pre-germinated seeds of each wheat line were placed on the surface of a 600 mL beaker filled with 30 mL of nutrient free 0.3% water agar. Wheat seeds were placed embryo up in three rows on one half of the beaker surface. Each beaker was then sealed with parafilm and placed in a growth chamber with fluorescent light intensity set to $3.56 \pm 0.16 \times 10^3$ lux. The daily light/dark cycle consisted of 13/11 h, and the temperature cycle was set to 25°C/13°C. Seven days later, 12 pre-germinated Italian ryegrass seeds were sown in three rows with embryos facing up on the half of the beaker surface not occupied by wheat seedlings. An autoclaved piece of paper board was then suspended 1 cm above the agar surface to separate the wheat and Italian ryegrass leaves and control for the effects of light competition. Each beaker was sealed with a new piece of parafilm and returned to the growth chamber. After 10 d of co-growth, the longest root of each of the 12 ryegrass seedlings was measured. The allelopathic activity of each wheat line was calculated as the percent reduction in the average root length of Italian ryegrass seedlings grown with wheat seedlings compared to the average root length of Italian ryegrass seedlings grown in a no-wheat control beaker. Each wheat line was evaluated in a randomized complete block design with four replicates over time.

Statistical analyses were conducted using the MIXED procedure in SAS 9.2 (SAS Institute Inc., Cary, NC). Genotype was treated as a fixed effect, and replicate was treated as a random effect. Plots of model-predicted values versus residual errors showed that allelopathic activity met the assumption of normal error distribution. Genotypic LS means were compared using the Fisher's protected least significant difference ($P \leq 0.05$).

Field Trial

Planting Material and Experimental Design

Eight lines with high (Coker 9553, Pioneer 25R32, Oakes, and SS 560) and low (NC05–19896, NC-Neuse, Pioneer 26R12, and Pioneer 26R22) allelopathic activity in the ECAM bioassay were chosen for inclusion in field trials. The selected genotypes had allelopathic activity that did not differ from the line with the highest or lowest least square (LS) mean for Italian ryegrass root length suppression according to Fisher's protected LSD ($P \leq 0.05$). Lines from both allelopathic classes were selected to include a wide range of final heights based on results from the NC OVT (2011) to obtain broad variation in competitive ability within both allelopathy classes.

Table 1. Percent reduction in the average root length of Italian ryegrass seedlings grown with wheat seedlings from each of the 58 experimental entries screened for allelopathic activity using the equal-compartment-agar method (ECAM) seedling bioassay compared to the nil wheat control.

Line†	Accession Number‡	Percent Italian ryegrass root length suppression
NC05–19896	NA	12
USG 3555	PI 654454	21
NC-Neuse	PI 633037	24
Pioneer 26R22	PI 638717	25
SS 8600	NA	25
Pioneer 26R12	PI 631475	29
Progeny PGX10–7	NA	32
NC-Yadkin	PI 663206	32
AGS 2056	NA	34
USG 3201	NA	34
SS 8308	PI 634979	35
TV 8861	PI 659787	35
USG 3452	NA	35
NC-Cape Fear	PI 659089	36
SS 8404	PI 638718	37
Roane	PI 612958	38
Jamestown	PI 653731	39
Coker 9804	PI 654420	40
DG Baldwin	PI 657988	40
NC05–19864	NA	40
NC07–25169	NA	41
DG Shirley	PI 656753	43
Pioneer 26R31	PI 634854	43
Progeny 117	NA	44
VA05W-139	NA	45
NC07–23880	NA	46
Pioneer 26R15	PI 633874	46
Progeny PGX10–5	NA	46
DG Dominion	PI 642937	47
AGS 2026	PI 658065	48
SY 9978	PI 659818	48
USG 3665	NA	48
DG 9171	PI 657988	49
NC06–20401	NA	49
TV 8525	NA	49
Oakes	PI 658040	51
TV 8535	NA	51
VA05W-251	NA	51
Progeny PGX10–2	NA	52
SS 8700	NA	52
USG 3120	NA	52
Featherstone VA	PI 664272	53
NC07–24445	NA	53
DG 9053	PI 657988	54
Progeny 185	NA	54
SS 560	GSTR 11101	55
USG 3592	PI 634600	55
Pioneer 25R32	PI 658151	56
Pioneer 26R20	PI 658150	56
Progeny 166	NA	56
DG 9012	NA	57
Progeny 125	NA	58
USG 3438	NA	58
SS 8641	PI 652450	60
Coker 9553	PI 643092	61
USG 3209	PI 617055	61
Merl	PI 658598	64
Mean		45
LSD (0.05)§		23
F genotype		2.05
P incl		< 0.001

† AGS, AgSouth Genetics; DG, DynaGro; SS, Southern States; SY, Syngenta; TV, Terral; USG, UniSouth Genetics.

‡ Accession number from the USDA-ARS National Small Grains Collection. NA indicates that no USDA-ARS accession number for the line is available.

§ Fisher's protected LSD.

The eight lines chosen for inclusion in the field experiment were evaluated for weed suppressive ability in 2012 and 2013 at a total of four sites. The test was organized as a split plot experiment with weedy and weed-free main plots organized in a randomized complete block design with four replicates per site and wheat lines randomly assigned to subplots. Each site was conventionally tilled with two disk passes before planting. Wheat was planted with depth set at 2.5 cm in 6-m-long plots using a calibrated cone drill with seven rows at 17.1 cm spacing. Wheat lines were seeded at a rate of 375 seeds m^{-2}, adjusted by seed weight to achieve uniform plant density. This seeding rate is typical for organic wheat production in North Carolina. Gulf Italian ryegrass was then sown in the main plots randomly assigned to the weedy treatment using the same planter driving perpendicular to the direction in which wheat was planted with depth set at 1 to 5 mm. Based on a preliminary experiment conducted in 2011, 300 Italian ryegrass seeds m^{-2} was chosen as the optimal seeding rate for evaluation of differences in the weed suppressive ability of winter wheat lines (Worthington et al., 2013).

Growing Conditions

The experiment was planted on 24 Oct. 2011 at Piedmont Research Station in Salisbury, NC (35.41°N, 80.37°W), on a Cecil clay loam (fine, kaolinitic, thermic Typic Kanhapludults) and on 25 Oct. 2011 at Caswell Research Station in Kinston, NC (35.16°N, 77.36°W), on a Kenansville loamy sand (loamy, siliceous, subactive, thermic Arenic Hapludults) during the first year of the experiment. In the following year the experiment was planted at Caswell Research Station on 25 Oct. 2012 on a Stallings loamy sand (coarse-loamy, siliceous, semiactive, thermic Aeric Paleaquults) and at the Tidewater Research Station in Plymouth, NC (35.85°N, 76.67°W) on 15 Nov. 2012 on a Roanoke loam, (fine, mixed, semiactive, thermic Typic Endoaquults).

Based on recommendations from soil tests performed by the North Carolina Department of Agriculture and Consumer Services Agrononomic Division (Raleigh, NC), all sites were treated with 33.6 kg ha^{-1} of pre-plant N and top-dressed with 67.2 kg ha^{-1} K. In the 2012 growing season 22.5 kg ha^{-1} of N was applied at Caswell on 23 December to compensate for patches of nutrient deficiency, followed by an application of 100.7 kg ha^{-1} of spring N in March. A total of 89.6 kg ha^{-1} of spring N was applied during March 2012 at the Piedmont

Table 2. Dates at which sites were planted and wheat morphological traits were measured at each experimental site when Pioneer 26R12 reached early tillering (Zadoks growth stage [GS] 25), late tillering (GS 29), stem extension (GS 31), heading (GS 55), grain fill (GS 70–80), and maturity (GS 92).

	2012		2013	
	Caswell	Piedmont	Caswell	Tidewater
Planting	25 Oct.	24 Oct.	25 Oct.	15 Nov.
Zadoks GS 25	31 Dec.	26 Dec.	14 Jan.	10 Jan.
Zadoks GS 29	7 Feb.	11 Feb.	4 Mar.	11 Apr.
Zadoks GS 31	18 Mar.	17 Mar.	28 Mar.	23 Apr.
Zadoks GS 55	16 Apr.	6 Apr.	19 Apr.	8 May
Zadoks GS 70–80	8 May	24 Apr.	5 May	17 May
Zadoks GS 92	24 May	29 May	12 June	21 June

location. In the 2013 growing season, 100.7 kg ha^{-1} and 91.1 kg ha^{-1} of spring N were applied in March at the Caswell and Tidewater sites. Broadleaf weeds were controlled as needed in both locations with thifensulfuron methyl plus tribenuron methyl (Harmony Extra, Dupont, Wilmington, DE).

Precipitation was normal in both years with 495 and 615 mm rainfall in Caswell and Piedmont locations, respectively, in 2012 and 520 and 467 mm in the Caswell and Tidewater locations, respectively, in 2013. The Tidewater site was planted 20 d later than all other sites and experienced suppressed tiller development and delayed onset of stem extension compared to the other experimental sites (Table 2). While 2298, 1930, and 1918 growing degree-d ays (0°C minimum base temperature) were accumulated in the Caswell 2012, Piedmont 2012, and Caswell 2013 sites between the date of planting and 1 May, only 1554 growing degree-days were accumulated in Tidewater 2013 during the same period.

Wheat Morphological Traits Measured

Crop morphology data was collected in the weed-free plots when Pioneer 26R12, a weakly allelopathic cultivar with intermediate heading date, reached early tillering (Zadoks Growth Stage, GS 25), advanced tillering (GS 29), stem extension (GS 31), heading (GS 55), and grain fill (GS 70–80) (Zadoks et al., 1974). The dates when these growth stages were reached varied widely across sites because of differences in growing conditions (Table 2). The range of heading dates for the eight wheat lines was 13 d (Julian date 92–105) in 2012 and 11 d (Julian date 106–117) in 2013. Thus, not all genotypes had attained the same growth stage as Pioneer 26R12 on the dates when crop morphology measurements were made.

During early and late tillering (GS 25, 29), measurements of normalized difference vegetation index (NDVI) were taken using a Crop Circle ACS-210 Plant Canopy Reflectance Sensor (Holland Scientific, Inc., Lincoln, NE). Visual ratings of vigor, based on a combination of percent ground cover and height, were made on a one to nine scale with the most vigorous genotypes rated as one during early and late tillering (GS 25, 29) following Zhao et al. (2006). An additional visual rating of growth habit was made on a 1-to-9 scale with the most erect genotypes rated as 1 and the most prostrate genotypes rated as 9 at late tillering (GS 29). An LAI-2000 sensor (LI-COR Environmental, Lincoln, NE) was used to measure leaf area index (LAI) at stem extension (GS 31) and heading (GS 55) in overcast conditions. Visual estimates of vigor were also made on a 1-to-9 scale during heading (GS 55), with the fullest canopies rated as 1 and the sparsest canopies rated as 9. Plant height was estimated as the distance from ground level to the top of the canopy during tillering and stem extension (GS 29, 31) and as the distance from ground level to the tip of the average head, excluding awns during heading and grain fill (GS 55, 70–80).

The heading date of each experimental entry was evaluated in single 1.2-m row plots planted with 40–60 seeds at Lake Wheeler Road Field Laboratory in Raleigh, NC, during 2012 and 2013. Heading date for each line was recorded when 50% of the heads in the row had fully emerged from the sheath.

Measurements of Weed Suppression and Grain Yield

The initial numbers of Italian ryegrass seedlings in each weedy plot were counted in 1-m^{-2} quadrats during early tillering (GS 25) to determine whether wheat lines varied in their ability to suppress weed seedling germination and establishment. Counts of Italian ryegrass seed heads in 1-m^{-2} quadrats in each weedy plot were made during grain fill (GS 70). Italian ryegrass seed head density and Italian ryegrass to wheat biomass ratio were previously correlated in North Carolina (Worthington et al., 2013). Wheat grain yield (kg ha^{-1}) was harvested in weedy and weed-free plots with a combine at maturity (GS 92) and adjusted to 14% moisture. Grain yield tolerance to weed interference was calculated as the percent reduction of wheat grain yield in weedy plots compared to weed-free plots in each block.

Statistical Analyses

Statistical analyses were conducted using SAS 9.2 (SAS Institute Inc., Cary, NC). Plots of model-predicted values vs. residual errors showed that all measurements of yield, weed suppressive ability, and potentially correlated wheat morphological traits met the assumption of normal error distribution. The combined experiment was evaluated in the MIXED procedure with genotype treated as a fixed effect and site, block nested within site, and the interaction of genotype and site treated as random effects. The average pairwise Pearson correlations between genotype rankings for Italian ryegrass seed head density between Tidewater 2013 and other sites was nonsignificant ($r =$ 0.06), and the variance component for genotype by site interaction decreased from 792 to 169 when Tidewater 2013 was removed from the combined model. Thus, the results from Tidewater 2013 are presented separately from the pooled analysis of Caswell 2012, Piedmont 2013, and Caswell 2013 in this manuscript. Genotypic LS means were generated for initial Italian ryegrass seedling density, Italian ryegrass seed head density, wheat grain yield in weedy and weed-free conditions, wheat grain yield tolerance, and all wheat morphological traits potentially affecting competitive ability. Mean separation was performed using Fisher's protected LSD ($P \leq 0.05$).

Pearson's correlation coefficient was used to test the significance of correlations between the genotypic LS means for allelopathic potential and wheat morphological traits potentially affecting competitive ability with Italian ryegrass seed head density and wheat grain yield tolerance. Traits lacking significant genotypic effects were excluded from correlation analyses.

RESULTS AND DISCUSSION

ECAM Allelopathy Bioassay

Significant variation in allelopathic activity ($P \leq 0.05$) was found among the 58 genotypes evaluated with the ECAM seedling bioassay (Table 1). The average root length suppression of Italian ryegrass seedlings grown with various wheat lines included in this test was normally distributed and ranged from 12 to 63%. The highly allelopathic lines chosen for subsequent field evaluation (Coker 9553, Pioneer 25R32, SS 560, and Oakes) were among the 13 lines that did not differ from the genotype with the greatest root length suppression according to Fisher's protected LSD; whereas, the low allelopathy lines (NC05–19896, Pioneer 26R22, NC-Neuse, and Pioneer 26R12) were among the 37 lines that were not different from the least allelopathic genotype tested.

Weed Suppressive Ability and Grain Yield Tolerance

There were no significant genotypic differences in initial Italian ryegrass seedling density (GS 29) found in the pooled sites or Tidewater 2013, indicating that the tested lines did not differ in their ability to suppress Italian ryegrass germination or establishment (Table 3). Significant genotypic differences in Italian ryegrass seed head density (GS 70) were observed in the pooled sites, but not Tidewater 2013 (Table 3). Growth and tillering of Italian ryegrass was more extensive in Tidewater 2013 than the pooled sites. Least square means of Italian ryegrass seed heads m^{-2} were 303 in the pooled sites and 509 in Tidewater 2013. Thus, it is possible that genotypic difference in weed suppressive ability were obscured under very heavy weed interference in Tidewater 2013.

Table 3. Weed suppressive ability of the eight winter wheat lines and the means of the strongly allelopathic and weakly allelopathic groups in Tidewater 2013 and the pooled sites as measured by Italian ryegrass seedling density at tillering (Zadoks growth stage [GS] 25) and Italian ryegrass seed head density at grain fill (GS 70).

	Pooled sites†		Tidewater 2013	
Genotype	Italian ryegrass seedlings m^{-2}	Italian ryegrass seed heads m^{-2}	Italian ryegrass seedlings m^{-2}	Italian ryegrass seed heads m^{-2}
Coker 9553	119	295	102	496
NC05–19896	126	292	90	530
NC-Neuse	119	291	93	414
Oakes	115	304	111	542
Pioneer 25R32	141	366	114	513
Pioneer 26R12	126	268	110	534
Pioneer 26R22	119	314	89	453
SS 560	118	292	110	593
Mean	123	303	102	509
LSD (0.05)‡	ns§	42	ns	ns
F genotype	1.67	2.97	1.38	2.40
P incl	0.20	0.04	0.27	0.06
Strongly allelopathic lines¶	123	314	109	536
Weakly allelopathic lines#	122	291	95	483
Mean	123	303	102	509
LSD (0.05)	ns	ns	14	ns
F genotype	0.02	3.88	5.10	3.56
P incl	0.90	0.19	0.03	0.07

† Caswell 2012, Piedmont 2012, and Caswell 2013.

‡ Fisher's protected LSD.

§ No significant difference between genotypes.

¶ Coker 9553, Pioneer 25R32, Oakes, and SS 560

NC05–19896, NC-Neuse, Pioneer 26R12, Pioneer 26R22.

Wheat grain yields adjusted to 14% moisture in weedy and weed-free conditions were much lower in Tidewater 2013 than the pooled sites; average yields in weed-free and weedy conditions were 6190 and 3690 kg ha^{-1} in the pooled sites and 3400 and 1540 kg ha^{-1} in Tidewater 2013 (Table 4). The low grain yields recorded at the Tidewater site can be partially attributed to its late planting date and cool temperatures, which contributed to poor tiller development and reduced wheat biomass accumulation compared to other sites. The interaction between genotype and weed treatment (weedy vs. weed-free) for grain yield was significant in both the pooled sites and Tidewater 2013 (data not shown). Genotypic differences in wheat grain yield under weedy conditions were observed in both the pooled sites and Tidewater 2013 (Table 4). However, under weed-free conditions, no differences in grain yield were detected in the pooled sites.

Wheat grain yield was lower under weedy conditions than weed-free conditions in both the pooled sites and Tidewater 2013 (Table 4). Significant differences in grain yield tolerance to weed pressure among genotypes were found in both analyses (Table 4). However, genotypes yielded inconsistently across sites; the grain yield tolerance of genotypes in the pooled sites and Tidewater 2013 was not correlated ($r = -0.22$). While Pioneer 25R32 was the least tolerant genotype in the pooled sites, SS 560 was the least tolerant genotype in Tidewater 2013 (Table 4). Such genotype by environment interactions are common in studies of weed suppressive ability and tolerance (Coleman et al., 2001; Mokhtari et al., 2002); therefore, field screenings for weed suppressive ability should be conducted in multiple growing environments and years. Grain yield tolerance was correlated ($r = 0.81$) with weed suppressive ability in the pooled sites, indicating that weed suppressive winter wheat genotypes will likely also be tolerant of weed interference in North Carolina (Tables 5 and 6).

Allelopathy and Weed Suppressive Ability

Although significant variation in allelopathic activity was found among the 58 genotypes tested in this study, allelopathic activity measured using the ECAM bioassay

Table 4. Wheat grain yield of the eight winter wheat genotypes adjusted to 14% moisture in weedy and weed-free plots. Wheat grain yield tolerance, the ability to sustain high yields relative to other cultivars in the presence of weeds, was calculated as the percent yield reduction in weedy plots compared to weed-free plots in each block.

	Grain yield (kg ha^{-1})			Grain yield (kg ha^{-1})		
	No Italian ryegrass	Italian ryegrass	Tolerance %	No Italian ryegrass	Italian ryegrass	Tolerance %
		Pooled sites†			Tidewater 2013	
Coker 9553	6060	3590	55	3030	1350	55
NC05–19896	5610	3300	40	3640	1520	60
NC-Neuse	5930	3820	40	3650	1840	49
Oakes	6540	3980	44	3070	1820	37
Pioneer 25R32	5920	2720	44	3860	1640	57
Pioneer 26R12	6590	4020	39	3540	1470	58
Pioneer 26R22	6710	4130	40	3480	1670	51
SS 560	6170	4000	38	2930	970	67
Mean	6190	3690	43	3400	1540	54
LSD (0.05)‡	ns§	350	5	640	340	13
F genotype	2.05	5.08	6.44	2.69	10.62	3.94
P incl	0.12	< 0.01	< 0.01	0.04	< 0.01	0.01

† Caswell 2012, Piedmont 2012, and Caswell 2013.

‡ Fisher's protected LSD.

§ No significant difference between genotypes.

was not positively correlated with wheat grain yield tolerance or Italian ryegrass seed head density (Tables 5 and 6). Strongly allelopathic lines did not have better weed suppressive ability than weakly allelopathic lines in the pooled sites or Tidewater 2013 (Table 3). These findings are inconsistent with the important role of allelopathy in weed suppression trials conducted with spring and winter wheat lines in Sweden (Bertholdsson, 2005, 2010, 2011).

The average root length suppression of Italian ryegrass seedlings grown with the wheat lines tested in this study ranged from 12 to 63%. In a more extensive bioassay of 453 wheat cultivars from around the world, Wu et al. (2000a) found a normal distribution of allelopathic activity across the tested genotypes, with the average root length suppression of rigid ryegrass (*Lolium rigidum* Guadin) seedlings grown with wheat lines ranging from 10 to 91%. Thus, it is possible that none of the lines screened in this study had allelopathic potential on the highest end of the spectrum. However, Wu et al. (2000a) used a different species as a receiver, and it is plausible that rigid ryegrass is more or less responsive to wheat seedling allelopathy than Italian ryegrass.

Soil or climate conditions in North Carolina may also have affected the expression of allelopathy in this environment. Bertholdsson (2010) found that highly allelopathic spring wheat lines derived from a cross between allelopathic and non-allelopathic parents suppressed weed biomass 24% more than the non-allelopathic parent in a dry year and only 12% more in a wet year. Dilday et al. (1998) also found that year to year variation, soil type, weed density, crop density, and root density all affected the expression of allelopathic activity in rice lines tested in the field.

Competitive Traits and Weed Suppressive Ability

While allelopathic activity was not associated with weed suppressive ability in this study, wheat morphological traits commonly associated with competitive ability were positively correlated with weed suppressive ability and grain yield tolerance (Tables 5 and 6). Several wheat morphological traits including early vigor and erect growth habit during tillering (GS 29), high leaf area index (LAI) at stem extension (GS 31), and plant height at tillering and stem extension (GS 29, 31) were correlated with Italian ryegrass seed head density and grain yield tolerance in the pooled sites (Tables 5 and 6). Meanwhile, grain yield tolerance at Tidewater 2013 was only correlated with vigor at heading (GS 55) and final plant height (GS 70–80) (Table 6).

Some wheat morphological traits that have been implicated in weed suppressive ability in other studies were not associated with improved weed suppression in North Carolina. Though prostrate growth habit was correlated with high weed suppressive ability in spring wheat grown in Saskatchewan and Australia (Huel and Hucl, 1996; Lemerle et al., 1996), erect growth habit during tillering was strongly associated with weed suppressive ability in this study. Final cultivar height was also an important determinant of competitive ability in many studies (Huel and Hucl, 1996; Lemerle et al., 1996; Coleman et al., 2001; Vandeleur and Gill, 2004; Mason et al., 2007; Murphy et al., 2008) and was associated with weed suppressive ability in a preliminary study conducted in North Carolina (Worthington et al., 2013). However, final height was only associated with grain yield tolerance at Tidewater 2013 (Table 4). The competitive advantage gained by rapid early growth from tillering to stem extension (GS 29–55) was

Table 5. Correlations of weed suppressive ability with wheat morphological traits potentially conferring competitive ability and allelopathic activity measured in the equal-compartment-agar method (ECAM) seedling bioassay in the pooled sites†.

Trait	Italian ryegrass seed heads m^{-2}
	Zadoks GS 25
Early Vigor	0.50
NDVI‡	ns§
	Zadoks GS 29
Growth habit	0.81*
Height	−0.77*
Early vigor	0.82*
NDVI	0.06
	Zadoks GS 31
LAI¶	−0.83*
Height	−0.76*
	Zadoks GS 55
LAI	ns
Height	−0.67
Vigor	0.58
	Zadoks GS 70
Height	0.56
	Zadoks GS 92
Weed free yield	ns
Weedy yield	−0.70*
Grain yield tolerance	0.81*
	Not GS Specific
Heading date	−0.36
Allelopathic activity	0.48

* Significant at $P \leq 0.05$.

** Significant at $P \leq 0.01$.

† Caswell 2012, Piedmont 2012, and Caswell 2013Traits lacking significant genotypic effects were excluded from correlation testing.

‡ NDVI = Normalized difference vegetation index.

§ No significant difference between genotypes ($P > 0.05$).

¶ LAI = Leaf area index.

far more important than final cultivar height in determining weed suppressive ability in the pooled sites.

CONCLUSIONS

Researchers have suggested that elite allelopathic wheat lines from exotic sources could be crossed with locally adapted lines to achieve gains in weed suppressive ability (Belz, 2007; Bertholdsson, 2010). Bertholdsson (2010) crossed Mohan 73, a highly allelopathic Tunisian cultivar, to an adapted but weakly allelopathic Swedish cultivar and evaluated the agronomic performance and weed suppressive ability of highly and weakly allelopathic $F_{2:3}$ lines derived from the cross. Although early weed biomass was significantly lower in the highly allelopathic lines, the highly allelopathic lines were also significantly lower yielding (Bertholdsson, 2010). This yield loss was likely a result of linkage drag from the poorly adapted allelopathic parent used in the cross. Allelopathy is controlled by the action of multiple small effect QTLs (Niemeyer and Jerez, 1997; Wu et al., 2003), so it is unlikely that allelopathy could be recovered without some loss of adaptation in wide crosses.

Table 6. Correlations of grain yield tolerance, the ability to sustain high yields relative to other cultivars in the presence of weeds, with wheat morphological traits potentially conferring competitive ability and allelopathic activity measured in the equal-compartment-agar method (ECAM) seedling bioassay†.

Trait	Tolerance‡ Pooled sites§	Tolerance‡ Tidewater 2013
	Zadoks GS 25	
Early Vigor	0.55	ns
NDVI¶	ns	0.13
	Zadoks GS 29	
Growth habit	0.73*	−0.08
Height	−0.73*	−0.08
Early vigor	0.79*	0.13
NDVI	−0.21	−0.09
	Zadoks GS 31	
LAI††	−0.86**	−0.56
Height	−0.72*	−0.26
	Zadoks GS 55	
LAI	ns	−0.61
Height	−0.61	−0.46
Vigor	0.46	0.71*
	Zadoks GS 70	
Height	0.50	−0.72*
Ryegrass seed heads m^{-2}	0.81*	ns
	Zadoks GS 92	
Weed free yield	ns	0.05
Weedy yield	−0.93**	−0.81*
	Not GS Specific	
Heading date	−0.36	−0.05
Allelopathic activity	0.56	< 0.01

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$.

† Traits lacking significant genotypic effects were excluded from correlation testing.

‡ Wheat grain yield tolerance was calculated as the percent yield reduction in weed free plots compared to weed-free plots in each block.

§ Caswell 2012, Piedmont 2012, and Caswell 2013.

¶ Normalized difference vegetation index.

\# No significant difference between genotypes ($P > 0.05$).

†† LAI = Leaf area index.

Significant variation in weed suppressive ability was found among the small group of lines tested in this study in the pooled sites and variation in grain yield tolerance was found in all sites. The lack of correlation between the grain yield tolerance of genotypes in Tidewater 2013 and the pooled sites and lack of significant genotypic differences in weed suppressive ability observed in Tidewater 2013 indicate that selection for weed suppressive ability may not be equally efficient in all environments and that the performance genotypes identified as highly weed suppressive may be affected by planting date and environmental conditions. Still, wheat breeders in the southeastern United States should be able to improve weed suppressive ability and grain yield tolerance within locally adapted material by selecting for yield in weed-free environments as well as competitive traits, including early vigor (GS 29), erect growth habit (GS 29), and height at tillering, stem extension, and heading (GS 29, 31, 55). The effects of competitive morphological traits on weed suppressive ability will likely vary from year to year, but yields should not be compromised by selection for improved competitive ability. Researchers have suggested that breeders should strive to improve allelopathic and competitive ability simultaneously to achieve maximum weed suppression (Lemerle et al., 2001; Olofsdotter et al., 2002; Belz, 2007). However, the results of this study suggest that wheat breeders in the southeastern United States would make greater gains in weed suppressive ability against Italian ryegrass and grain yield tolerance by focusing their time and resources on improving competitive traits within adapted germplasm.

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References

Belz, R.G. 2007. Allelopathy in crop/weed interactions — an update. Pest Manag. Sci. 63:308–326. doi:10.1002/ps.1320

Bertholdsson, N.O. 2005. Early vigour and allelopathy — two useful traits for enhanced barley and wheat competitiveness against weeds. Weed Res. 45:94–102. doi:10.1111/j.1365-3180.2004.00442.x

Bertholdsson, N.O. 2010. Breeding spring wheat for improved allelopathic potential. Weed Res. 50:49–57. doi:10.1111/j.1365-3180.2009.00754.x

Bertholdsson, N.O. 2011. Use of multivariate statistics to separate allelopathic and competitive factors influencing weed suppression ability in winter wheat. Weed Res. 51:273–283. doi:10.1111/j.1365-3180.2011.00844.x

Coleman, R.D., G.S. Gill, and G.J. Rebetzke. 2001. Identification of quantitative trait loci for traits conferring weed competitiveness in wheat (*Triticum aestivum* L.). Aust. J. Agric. Res. 52:1235–1246. doi:10.1071/AR01055

Dilday, R.H., W.G. Yan, A.K. Moldenhauer, and K.A. Gravois. 1998. Allelopathic activity in rice for controlling major aquatic weeds. In: M. Olofsdotter, editor, Proceedings of the Workshop on Allelopathy in Rice. International Rice Research Institute Publishing, Los Banos, The Philippines. p. 7–26.

Goldberg, D. 1990. Components of resource competition in plant communities. In: J. Grace and D. Tilman, editors, Perspectives in Plant Competition. Academic Press, San Diego, California. p 27-49.

Harper, J.L. 1977. Population Biology of Plants. Academic Press, New York.

Hoad, S.P., N.O. Bertholdsson, D. Neuhoff, and U. Kopke. 2012. Approaches to breed for improved weed suppression in organically grown cereals. In: E. Lammerts van Bueren and J.R. Myers, editors, Organic crop breeding. Wiley-Blackwell, John Wiley & Sons, West Sussex, UK. p. 3–13.

Huel, D.G., and P. Hucl. 1996. Genotypic variation for competitive ability in spring wheat. Plant Breeding. 115:325–329. doi:10.1111/j.1439-0523.1996.tb00927.x

Inderjit, and R. del Moral. 1997. Is separating resource competition from allelopathy realistic? Bot. Rev. 63:221–230.

Kuk, Y.I., N.R. Burgos, and R.C. Scott. 2008. Resistance profile of diclofop-resistant Italian ryegrass (*Lolium multiflorum*) to ACCase- and ALS-inhibiting herbicides in Arkansas, USA. Weed Sci. 56:614–623. doi:10.1614/WS-08-003.1

Lemerle, D., G.S. Gill, C.E. Murphy, S.R. Walker, R.D. Cousens, S. Mokhtari, S.J. Peltzer, R. Coleman, and D.J. Luckett. 2001. Genetic improvement and agronomy for enhanced wheat competitiveness with weeds. Aust. J. Agric. Res. 52:527–548. doi:10.1071/AR00056

Lemerle, D., B. Verbeek, R.D. Cousens, and N.E. Coombes. 1996. The potential for selecting wheat varieties strongly competitive against weeds. Weed Res. 36:505–513. doi:10.1111/j.1365-3180.1996.tb01679.x

Mason, H.E., A. Navabi, B.L. Frick, J.T. O'Donovan, and D.M. Spaner. 2007. The weed-competitive ability of Canada western red spring wheat cultivars grown under organic management. Crop Sci. 47:1167–1176. doi:10.2135/cropsci2006.09.0566

Mokhtari, S., N.W. Galwey, R.D. Cousens, and N. Thurling. 2002. The genetic basis of variation among wheat F_3 lines in tolerance to competition by ryegrass (*Lolium rigidum*). Euphytica 124:355–364. doi:10.1023/A:1015752021568

Muller, C.H. 1969. Allelopathy as a factor in ecological process. Vegetatio 18:348–357. doi:10.1007/BF00332847

Murphy, K.M., J.C. Dawson, and S.S. Jones. 2008. Relationship among phenotypic growth traits, yield, and weed suppression in spring wheat landraces and modern cultivars. Field Crops Res. 105:107–115. doi:10.1016/j.fcr.2007.08.004

North Carolina Official Varietal Trials. 2011. Characteristics of North Carolina Wheat Varieties. NC OVT. North Carolina State University. www.ncovt.com/files/SmallGrains2011_Table3_2011.pdf (accessed 9 Sept. 2011).

Niemeyer, H.M., and J.M. Jerez. 1997. Chromosomal location of genes for hydroxamic acid accumulation in *Triticum aestivum* L (wheat) using wheat aneuploids and wheat substitution lines. Heredity 79:10–14. doi:10.1038/hdy.1997.117

Olofsdotter, M., L.B. Jensen, and B. Courtois. 2002. Improving crop competitive ability using allelopathy — an example from rice. Plant Breeding. 121:1–9. doi:10.1046/j.1439-0523.2002.00662.x

Olofsdotter, M., D. Navarez, M. Rebulanan, and J.C. Streibig. 1999. Weed suppressing rice cultivars — does allelopathy play a role? Weed Res. 39:441–454. doi:10.1046/j.1365-3180.1999.00159.x

Preston, C., A.M. Wakelin, F.C. Dolman, Y. Bostamam, and P. Boutsalis. 2009. A decade of glyphosate-resistant *Lolium* around the world: Mechanisms, genes, fitness, and agronomic management. Weed Sci. 57:435–441. doi:10.1614/WS-08-181.1

Rice, E. 1984. Allelopathy, second edition. Academic Press, London.

Seal, A.N., J.E. Pratley, and T. Haig. 2008. Can results from a laboratory bioassay be used as an indicator of field performance of rice cultivars with allelopathic potential against *Damasonium minus* (starfruit)? Aust. J. Agric. Res. 59:183–188. doi:10.1071/AR06333

Vandeleur, R.K., and G.S. Gill. 2004. The impact of plant breeding on the grain yield and competitive ability of wheat in Australia. Aust. J. Agric. Res. 55:855–861. doi:10.1071/AR03136

Wolfe, M.S., J.P. Baresel, D. Desclaux, I. Goldringer, S. Hoad, G. Kovacs, F. Löschenberger, T. Miedaner, H. Østergård, and E.T.L. van Bueren. 2008. Developments in breeding cereals for organic agriculture. Euphytica 163:323–346. doi:10.1007/s10681-008-9690-9

Worthington, M., and C. Reberg-Horton. 2013. Breeding cereal crops for enhanced weed suppression: Optimizing allelopathy and competitive ability. J. Chem. Ecol. 39:213–231. doi:10.1007/s10886-013-0247-6

Worthington, M.L., S.C. Reberg-Horton, D. Jordan, and J.P. Murphy. 2013. A comparison of methods for evaluating the suppressive ability of winter wheat cultivars against Italian ryegrass (*Lolium perenne*). Weed Sci. 61:491–499. doi:10.1614/WS-D-12-00167.1

Wu, H., J. Pratley, D. Lemerle, and T. Haig. 2000a. Evaluation of seedling allelopathy in 453 wheat (*Triticum aestivum*) accessions against annual ryegrass (*Lolium rigidum*) by the equal-compartment-agar method. Aust. J. Agric. Res. 51:937–944. doi:10.1071/AR00017

Wu, H., J. Pratley, D. Lemerle, and T. Haig. 2000b. Laboratory screening for allelopathic potential of wheat (*Triticum aestivum*) accessions against annual ryegrass (*Lolium rigidum*). Aust. J. Agric. Res. 51:259–266. doi:10.1071/AR98183

Wu, H., J. Pratley, D. Lemerle, T. Haig, and M. An. 2001. Screening methods for the evaluation of crop allelopathic potential. Bot. Rev. 67:403–415. doi:10.1007/BF02858100

Wu, H., J. Pratley, W. Ma, and T. Haig. 2003. Quantitative trait loci and molecular markers associated with wheat allelopathy. Theor. Appl. Genet. 107:1477–1481. doi:10.1007/s00122-003-1394-x

Zadoks, J.C., T.T. Chang, and C.F. Konzak. 1974. Decimal code for growth stages of cereals. Weed Res. 14:415–421. doi: 10.1111/j.1365-3180.1974.tb01084.x

Zhao, D.L., G.N. Atlin, L. Bastiaans, and J.H.J. Spiertz. 2006. Cultivar weed-competitiveness in aerobic rice: Heritability, correlated traits, and the potential for indirect selection in weed-free environments. Crop Sci. 46:372–380. doi:10.2135/cropsci2005.0192