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Evaluation of wheat stem sawfly-resistant solid stem *Qss.msub-3BL* alleles in hard red winter wheat

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Abstract

Host plant resistance provided by solid stems has been the most effective means for mitigating wheat stem sawfly (WSS) (*Cephus cinctus* Norton) damage in spring and winter wheat (*Triticum aestivum* L.). The solid stem trait originates from the spring wheat cultivar “Rescue” and is associated with a quantitative trait locus allele *Qss.msub-3BL.b* that explains the majority of the variation for stem solidness. Recently, a new *Qss.msub-3BL* solid stem allele, designated *Qss.msub-3BL.c*, was identified in the spring wheat cultivar “Conan”. It produces a solid stem phenotype early in plant development but dissipates during plant growth. The *Qss.msub-3BL.c* allele provides effective WSS resistance in spring wheat but has not been tested in winter wheat. To examine if the *Qss.msub-3BL.c* allele provides adequate WSS resistance in winter wheat, near-isogenic lines (NILs) were developed using marker-assisted backcrossing. This enabled comparisons between the hollow stem *Qss.msub-3BL.a*, solid stem *Qss.msub-3BL.b* and solid stem *Qss.msub-3BL.c* alleles for stem solidness, WSS resistance, and agronomic traits in Montana growing environments. Compared to the hollow stem allele, the NILs with the *Qss.msub-3BL.c* allele increased stem solidness and reduced WSS stem cutting. However, the *Qss.msub-3BL.c* allele resulted in lower solid stem scores and greater WSS stem cutting compared to the *Qss.msub-3BL.b* allele. Overall, these findings indicate that the *Qss.msub-3BL.c* allele failed to provide sufficient WSS resistance in the winter wheat backgrounds tested in this study.

1 | INTRODUCTION

Wheat stem sawfly (WSS), *Cephus cinctus* Norton, is a native grass-boring insect that has been one of the most destructive

Abbreviations: KASP, Kompetative allele-specific polymerase chain reaction; MAB, marker-assisted backcrossing; NIL, near-isogenic line; SNP, single-nucleotide polymorphism; WSS, wheat stem sawfly.

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spring wheat (*Triticum aestivum* L.) pests in the Northern Great Plains of North America since the early 1900s (Bekkerman, 2014; Beres et al., 2011; Lesieur et al., 2016; Wallace & McNeal, 1966). In the 1980s, WSS evolved in Montana to become a devastating winter wheat pest due to the life cycle of this opportunistic native species adapting to winter wheat phenology (Morrill & Kushnak, 1996). Adult

WSS emerges from wheat stubble between late spring and early summer (Morrill & Kushnak, 1996). Females lay eggs in elongating stems, resulting in larvae that feed inside the stem (parenchyma, pith, and vascular tissue), which reduces water and nutrient transport during grain fill (Delaney et al., 2010). At the end of the growing season, WSS larvae migrate to the base of the stem and girdle the stem interior to prepare for overwintering. The cut stem is weakened, which makes it highly vulnerable to lodging. Larval feeding and severe lodging lead to reduced yield, end-use quality, and profitability (Bekkerman & Weaver, 2018; Beres et al., 2007; Delaney et al., 2010; Knodel et al., 2010). WSS has caused an estimated \$350 million USD in annual losses in the Northern Great Plains (Beres et al., 2011).

The geographic range of WSS is expanding to other wheat-growing regions where wheat infestation has been historically low (Cockrell et al., 2021; McCullough et al., 2020). For example, substantial WSS infestations have recently occurred in eastern Colorado, the panhandle of Nebraska, and southeastern Wyoming (Cockrell et al., 2021; Irell & Peairs, 2014; Lesieur et al., 2016; McCullough et al., 2020). WSS populations have also been observed in wheat fields located in northwestern Kansas, the Red River Valley region of Minnesota, and eastern Idaho. These observations, coupled with climate warming, present a serious risk for additional economic loss in more regions (Bekkerman, 2014; Beres et al., 2011; Marshall et al., 2019; Michaud, 2013; Olfert et al., 2019).

Host plant resistance, conferred by a solid (pith-filled) stem trait, has been the most successful means of reducing WSS damage (Beres et al., 2011, 2017; Nilsen et al., 2017). Solid stems impede larval development and movement, resulting in larval death and reduced stem cutting (Buteler et al., 2015). The solid stem trait was first discovered in a Portuguese spring wheat landrace, “S-615” (Kemp, 1934), and was used to breed the first solid stem WSS-resistant spring wheat variety, “Rescue,” that was released in 1946 (Platt et al., 1948). The genetic architecture of the Rescue-derived solid stem trait was studied, and a single locus, *Qss.msub-3BL*, was found control 76% of the solid stem variation (Cook et al., 2004). Subsequent studies have identified several minor loci that further increase the expression of the solid stem trait including *Qss.msub-3DL* (Lanning et al., 2006). This locus controlled 31% of the variation in a spring wheat genetic mapping population that was fixed for the Rescue-derived *Qss.msub-3BL.b* solid stem allele (Lanning et al., 2006). Currently, most of the WSS-resistant solid stem spring and winter wheat varieties grown in the United States and Canada are derived from Rescue (Cook et al., 2017; Nilsen et al., 2017).

Wheat varieties with the solid stem trait are still infested and lodge due to variable expression of the solid stem trait in different environments (Beres et al., 2013, 2017; Subedi et al., 2021), although the use of the Rescue-derived solid stem trait significantly reduces WSS damage. Inconsistent expression

Core Ideas

- *Qss.msub-3BL* solid stem alleles for wheat stem sawfly (WSS) resistance in hard red winter wheat are compared.
- *Qss.msub-3BL.b* solid stem allele provides better WSS resistance than the *Qss.msub-3BL.c* solid stem allele.
- Winter wheat variety, Judee, has a unique temporal solid stem phenotype.

in stem solidness is affected by location, genotype, and their complex interactions (Beres et al., 2011, 2017; Cook et al., 2004; Hayat et al., 1995; Lanning et al., 2006). To enhance the WSS resistance provided by the solid stem trait inherited from Rescue, research was conducted to screen novel sources of resistance and identify new WSS resistance genes that augment the solid stem genes inherited from Rescue (Sherman et al., 2010; Talbert et al., 2014; Varella, Weaver, et al., 2019; Varella, Zhang, et al., 2019). Outcomes from this research include the identification of a novel source of solid stem WSS resistance in the spring wheat variety “Conan” (Sherman et al., 2010; Talbert et al., 2014). Stems in the Conan variety are solid early in plant development but become hollow as they elongate (Varella et al., 2016). This early solidness in spring wheat coincides with WSS oviposition and early larval growth, which hinders WSS infestation and survival (Varella et al., 2017).

Genetic studies determined that the early solid stem trait found in Conan is attributed to a second solid stem allele at the *Qss.msub-3BL* locus (Sherman et al., 2010; Talbert et al., 2014; Varella et al., 2016). The level of WSS resistance provided by the solid stem alleles were compared by testing hard red spring wheat near-isogenic lines (NILs) that varied for the hollow stem *Qss.msub-3BL.a* allele, Rescue-derived *Qss.msub-3BL.b* solid stem allele, and Conan-derived *Qss.msub-3BL.c* solid stem allele (Cook et al., 2019). The Conan-derived allele resulted in significantly greater reduction in WSS infestation and cutting compared with the hollow stem and the Rescue-derived alleles (Cook et al., 2019).

The *Qss.msub-3BL.c* solid stem allele provides sufficient WSS resistance in hard red spring wheat; however, we do not know if the allele provides sufficient WSS resistance in hard red winter wheat. The objective of this study is to use marker-assisted backcrossing (MAB) to develop multiple pairs of winter wheat NILs with contrasting alleles at the *Qss.msub-3BL* locus to evaluate the effectiveness of the *Qss.msub-3BL.c* allele in winter wheat. Results of this study will help determine if the *Qss.msub-3BL.c* allele is sufficient for providing WSS resistance in winter wheat.

2 | MATERIALS AND METHODS

2.1 | NIL development

Conan, a hard red spring wheat released by WestBred LLC (1997, PI 607549), possesses the *Qss.msub-3BL.c* allele that causes a solid stem phenotype early in plant development providing resistance to WSS (Varella et al., 2016, 2017). Conan was the *Qss.msub-3BL.c* donor parent for this study. Recurrent parents were the hard red winter wheat varieties Yellowstone and Judee. Yellowstone is a hollow stem, WSS-susceptible variety containing the hollow stem *Qss.msub-3BL.a* allele, and Judee is a solid stem, moderately WSS-resistant variety possessing the solid stem Rescuederived *Qss.msub-3BL.b* allele. As described by Cook et al. (2019), MAB was used to select heterozygous progeny in backcross generations BC₁, BC₂, and BC₃ using microsatellite marker *Xgwm340*, and a Kompetitive allele-specific polymerase chain reaction (KASP) marker, *Conan_3B_7*. Microsatellite marker *Xgwm340* is diagnostic for all three *Qss.msub-3BL* alleles, and the primers are *Xgwm340-F*: GCAATCTTTTTTCTGACCACG and *Xgwm340-R*: ACGAGGCAAGAACACACATG. The *Conan_3B_7* KASP marker is a new high throughput marker that cosegregates with *Xgwm340*. It was developed from an exome-capture SNP (G/A) (Jordan et al., 2015). Primers for *Conan_3B_7* are *Conan_3B_7_G*: tgatctgatcgccggttgG, *Conan_3B_7_A*: tgatctgatcgccggttgA and *Conan_3B_7_Common*: ccacgaa-gagcgcgaacg. The *Qss.msub-3BL.c* allele cosegregates with the “A” SNP genotype. Protocols for the preparation and running of KASP reactions are provided in the KASP manual (<http://www.kbioscience.co.uk/>). All KASP PCR reactions were performed on the Bio-Rad CFX96 Touch Real-Time polymerase chain reaction (PCR) Detection System (Bio-Rad, Hercules, CA), and biallelic calls were made using the Bio-Rad CFX Manager software version 3.1 (Bio-Rad, Hercules, CA). Multiple NIL pairs were developed from different BC₃ plants that are predicted to contain 87.5% of the recurrent parent genetic background. Heterozygous BC₃ individuals were allowed to self-pollinate, and BC₃F₂ plants homozygous for the alternative alleles were selected based on marker genotype. Ten NIL pairs were derived from each recurrent parent.

2.2 | Phenotyping

The NILs and check varieties were first planted in late September of 2017 at the Arthur Post Research Farm at Bozeman, MT (40° 33.6" N, 111° 9' 25.2" W), as a randomized complete block design to increase seed for future experiments and evaluate initial stem solidness. These NILs and check varieties were replicated three times and planted in single row

plots 1.8 m long with 60 cm row spacing. The seeding rate for the single row plots was 70 seeds m⁻². Check varieties included in this experiment were the recurrent parents, Judee and Yellowstone. During the 2018 growing season, the NILs were evaluated for stem solidness. Solid stem scores were collected from the main stem of three plants in each plot early in plant development (Zadoks 33) and at plant maturity (Zadoks et al., 1974). The main stem of plants collected at Zadoks 33 averaged three internodes, while the main stem of mature plants averaged five internodes. The main stem was split longitudinally, and a solid stem rating between 1 (hollow) and 5 (solid) was assigned to each internode. The average solid stem score across internodes was calculated for each main stem and then averaged across the three main stems from each plot. The average solid stem scores per internode collected from plants at Zadoks 33 were multiplied by five to give a solid stem score ranging between 5 (completely hollow) and 25 (completely solid), which allowed solid stem score comparisons between data acquired at Zadoks 33 and at maturity.

In early October of 2018, the NILs and check varieties were planted in yield trials at the Arthur Post Research Farm, and Carter, MT (47° 48' 21.62" N, 110° 52' 47.93" W). The check varieties included in the yield trials were Yellowstone, Judee, and Warhorse (PI 670157; Berg et al., 2014), a solid stem Montana-adapted winter wheat variety that carries the *Qss.msub-3BL.b* allele. Yield trials were planted as randomized complete block experiments with three replications. The yield trial plots at Bozeman, MT, were three 5.5 m rows with 30 cm row spacing. The yield trial plots at Carter, MT, were six 6.0 m rows with 22 cm row spacing. In addition to the yield trials, the NILs and checks were planted as randomized complete block design with single row plots replicated three times near Big Sandy, MT (49° 11' 21.35" N, 110° 29' 19.27" W), and the Western Triangle Agricultural Research Center at Conrad, MT (48° 18' 25.872" N, 111° 55' 29.703" W), for WSS resistance evaluation. Check varieties included in these experiments were Yellowstone, Judee, and Warhorse. All yield trial and single row plots were planted at 70 seeds m⁻² seeding rate.

During the 2019 growing season, agronomic data collected from the yield trials in Bozeman and Carter included yield (kg ha⁻¹) and test weight (kg m⁻³). Test weight was measured from a sample of cleaned grain on a Seedburo test weight apparatus. Solid stem scores were also measured in the Bozeman yield trial as described above. WSS infestation was evaluated at Big Sandy, Carter, and Conrad. A visual evaluation of percentage cut stems was performed at maturity. Stems were collected from a 30 cm length of a row from each plot in all infested locations. They were then dissected in the laboratory to determine the percentage of WSS larvae presence (infestation), early larval mortality, parasitism, and overall larval mortality. Early larval mortality is defined as

when the immature WSS died within the internode where the egg was deposited, or before the larva could bore through a stem node. Parasitism is determined by the presence of parasitoid cocoons and emergence holes from adult parasitoids of two bivoltine specialist species [*Bracon cephi* (Gahan) and *Bracon lissogaster* Muesebeck]. Parasitism is a second form of mortality in larger larvae. Overall larval mortality includes parasitism and the mortality of immature larva that were able to breach one or more stem nodes but died before the larva was able to descend to the base of the ripened stem and girdle it for overwinter diapause.

2.3 | Statistical analyses

All analyses were performed using R software version 4.0.3 (R Core Team, 2021). Linear mixed-effects models were used to determine the significance of fixed effects on WSS resistance and agronomic traits, including variety, allele at the *Qss.msub-3BL* locus, and location. Replications were treated as a random effect (R package *lme4*; Bates et al., 2015). Separate mixed model analyses were used to examine WSS resistance and agronomic trait differences among checks and between contrasting *Qss.msub-3BL* alleles in NIL pairs from each recurrent parent. Each model tested interactions among fixed effects. Data from each location were analyzed separately if there was a significant location effect or a significant interaction between location and other fixed effects. Type III analysis of variance with Satterthwaite's method was used to assess if fixed effects accounted for variation in response variables (WSS resistance and agronomic traits). Diagnostic plots of models were examined to ensure that normality and homogeneity of variance assumptions were satisfied. Tukey's honestly significant difference method was used for the post hoc pairwise comparisons (R package *emmeans*; Lenth, 2020). Pearson correlation matrix for WSS resistance traits was performed using the *cor* function.

3 | RESULTS

Solid stem scores were collected during early plant development and at maturity. Among the check varieties, hollow stem Yellowstone did not have significantly different temporal solid stem scores; however, the temporal scores for stem solidness were significantly different in the solid stem check variety Judee (Figure 1; $p \leq 0.001$). The solid stem check variety, Warhorse, did not have significantly different temporal scores for stem solidness. Solid stem scores were also compared among the three check varieties at both developmental stages. Yellowstone had significantly lower solid stem scores versus Judee and Warhorse at both stages ($p \leq 0.001$), while

Judee and Warhorse solid stem scores were not significantly different at both stages (Figure 1).

Among the NILs, significant differences in temporal solid stem scores were observed between early plant development and maturity, as well as between contrasting alleles at each developmental stage (Figure 2). Comparing NILs derived from Yellowstone contrasting *Qss.msub-3BL.a* and *Qss.msub-3BL.c* alleles, the NILs with the Conan-derived allele had significantly higher solid stem scores than those containing the hollow stem allele at both the early plant development stage and maturity ($p \leq 0.001$). Additionally, the solid stem scores among the Yellowstone-derived NILs were significantly different between early plant development and maturity for both alleles ($p \leq 0.001$). Among the NILs derived from Judee with contrasting *Qss.msub-3BL.b* and *Qss.msub-3BL.c* alleles, the Conan-derived allele had significantly lower solid stem scores than the Rescue-derived allele at both temporal stages ($p \leq 0.001$). Solid stem scores among all Judee-derived NILs were significantly higher at maturity versus early plant development ($p \leq 0.01$).

Except for early larval mortality, WSS resistance traits were significantly different among the three locations infested with WSS (Big Sandy, Carter, and Conrad) when averaged across checks and NILs (Table 1). The mean value of WSS infestation was significantly greater at Carter compared with Big Sandy ($p \leq 0.05$), but neither location was significantly different from Conrad. We characterized all three locations as being severely infested with WSS. Overall larval mortality was significantly greater at Big Sandy, while Carter had significantly lower overall larval mortality ($p \leq 0.05$). Conrad had significantly higher WSS parasitism compared with Big Sandy and Carter. WSS stem cutting was significantly higher at Carter than at either Big Sandy or Conrad ($p \leq 0.05$).

Allele by location interactions for WSS resistance traits were examined for both the NILs and check varieties (Table 2). A significant allele by location interaction for WSS infestation was observed among NILs derived from Yellowstone that varied for the hollow stem and Conan-derived solid stem allele ($p \leq 0.05$). Significant allele by location interactions for larval mortality was observed among NILs derived from Judee that varied for the Conan-derived solid stem allele and the Rescue-derived solid stem allele ($p \leq 0.01$). The Judee-derived NILs also had a significant allele by location interaction for WSS stem cutting ($p \leq 0.001$).

Correlation analysis was used to examine the relationship among WSS resistance traits measured from the NILs across all environments (Table 3). WSS infestation was negatively correlated with larval mortality and was positively correlated with stem cutting ($p \leq 0.001$). Moreover, stem cutting was negatively correlated with increased parasitism, early larval mortality, and overall larval mortality ($p \leq 0.05$). Altogether, greater infestation was associated with lower larval mortalities and greater sawfly cutting.

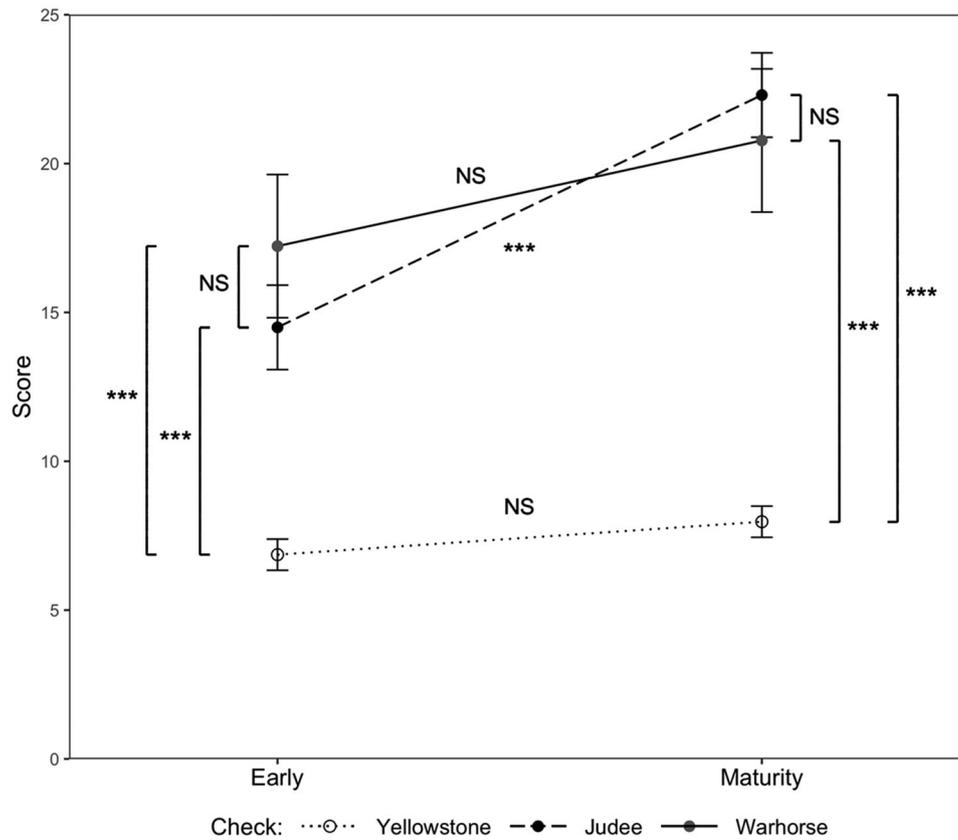


FIGURE 1 Temporal expression of stem solidness for check varieties, Yellowstone, Judee and Warhorse, grown at Bozeman, MT, and Carter, MT, during the 2018 and 2019 field seasons (Warhorse data were collected in 2019 only). Solid stem measurements were taken at Zadoks 33 (early) and plant maturity. Mean solid stem scores at early and maturity were compared among the three checks, and the temporal change (early vs. maturity) in mean solid stem scores was evaluated within each check. Mean solid stem scores are colored by check. The temporal change in means for each check is presented in different line types. Error bars represent standard errors. ***Significant at the 0.001 probability level. Abbreviation: NS, nonsignificant

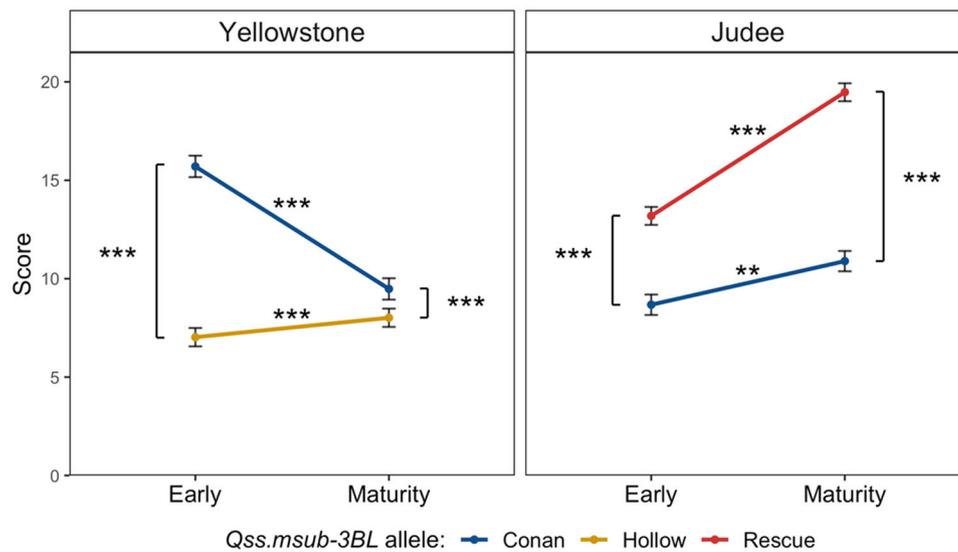


FIGURE 2 Temporal expression of stem solidness for near-isogenic line (NIL) pairs that differ for alleles at the *Qss.msub-3BL* locus in two recurrent parents, Yellowstone and Judee, grown at Bozeman, MT and Carter, MT, during the 2018 and 2019 field seasons. Solid stem measurements were taken at Zadoks 33 (early) and plant maturity. Mean solid stem scores at early and maturity were compared between NILs with contrasting alleles, and temporal change (early vs. maturity) in mean solid stem scores was evaluated within each allele of an NIL. Alleles are designated by a unique color. Error bars represent standard errors. *Significant at the 0.01 probability level; ***significant at the 0.001 probability level

TABLE 1 Wheat stem sawfly (WSS) resistance trait means in percent (%), all entries at Big Sandy, Carter, and Conrad in 2019

Location	WSS resistance trait (%)									
	Infestation		Larval mortality				Parasitism		Stem cutting	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Big Sandy	85.3 b	29.3–100	11.3 a	0–60	52.3 a	13.6–94.7	6.0 b	0–23.6	16.4 b	5–60
Carter	91.7 a	55.6–94.4	10.0 a	0–39.1	23.2 c	0–66.7	3.8 b	0–21.4	46.7 a	2–95
Conrad	88.5 ab	45.5–93.5	5.3 a	0–37.9	44.9 b	0–80.6	16.7 a	0–48.6	22.5 b	5–50

Note. Within a column, values not sharing a common letter indicate significant differences among locations for each trait value ($\alpha \leq 0.05$, Tukey-adjusted).

TABLE 2 Type III test of fixed effect interactions between location and near-isogenic lines (NILs), and location and checks for the wheat stem sawfly (WSS) resistance trait in percent (%) during the 2019 field season

Factor		WSS resistance trait (%)									
		Infestation		Larval mortality				Parasitism		Stem cutting	
		df	F value	df	F value	df	F value	df	F value	df	F value
NILs by recurrent parent											
Yellowstone	Allele (A)	1, 165	7.9 **	1, 176	0.2	1, 167	0.07	1, 173	1.4	1, 167	9.9**
	Location (L)	2, 17	9.4 **	2, 6	6.0*	2, 6	32.0***	2, 173	41.4 ***	2, 6	46.4***
	A × L	2, 165	3.3 *	2, 165	1.8	2, 165	0.4	2, 171	0.8	2, 165	2.8
Judee	Allele (A)	1, 166	2.6	2, 164	24.4***	1, 170	18.0***	1, 172	0.6	1, 164	0.8
	Location (L)	2, 6	3.3	2, 11	0.5	2, 170	33.0***	2, 172	45.7 ***	2, 9	53.0***
	A × L	2, 164	0.8	2, 164	6.9**	2, 170	5.1**	2, 170	2.3	2, 164	49.0***
Checks											
	Variety (V)	2, 16	0.8	2, 15	3.8*	2, 11	4.7*	2, 15	0.9	2, 11	26.5***
	Location (L)	2, 6	0.02	2, 6	5.0*	2, 13	6.4*	2, 6	3.1	2, 17	9.0**
	V × L	4, 12	2.8	4, 11	0.1	4, 11	5.4**	4, 12	1.2	4, 11	20.1***

Note. F values with degree of freedom (numerator, denominator) are shown.

* $p = 0.05$; ** $p = 0.01$; *** $p = 0.001$.

TABLE 3 Pearson correlation coefficients of wheat stem sawfly (WSS) resistance trait based on means from combined infested locations in 2019

Trait	Infestation	Early larval mortality	Parasitism	Overall larval mortality
Early larval mortality	−0.40***	–	–	–
Parasitism	0.09 NS	−0.09 NS	–	–
Overall larval mortality	−0.33***	0.47***	0.13*	–
Stem cutting	0.22***	−0.30***	−0.23***	−0.53***

Abbreviation: NS, nonsignificant.

* $p = 0.05$; ** $p = 0.01$; *** $p = 0.001$.

NIL pairs with contrasting *Qss.msub-3BL* alleles were compared to evaluate the effectiveness of these alleles for WSS resistance (Table 4). NIL developed from Yellowstone that carry the Conan-derived allele resulted in significantly lower infestation compared to the hollow stem allele at Big Sandy ($p \leq 0.05$), but infestation did not differ between the two alleles at Carter and Conrad. Stem cutting was signifi-

cantly lower among the NILs with the Conan-derived allele versus the hollow stem allele at Big Sandy ($p \leq 0.001$), whereas stem cutting was not significantly different at Carter or Conrad.

Comparing the NILs derived from Judee that carry the Rescue-derived solid stem allele versus the Conan-derived allele, the Rescue-derived allele had significantly greater early

TABLE 4 Near-isogenic line (NIL) and check means in percent (%) for wheat stem sawfly (WSS) infestation, larval mortalities, parasitism, and stem cutting from three locations grown in 2019 and the combined environments

	Variety	Allele	Infestation	Larval mortality		Parasitism	Stem cutting
				Early	Overall		
Big Sandy							
NILs by recurrent parent	Yellowstone	Hollow	92.0	4.4	40.9	5.1	33.2
		Conan	85.2	7.4	43.2	6.1	19.2
	<i>P</i> value		*	*	NS	NS	***
	Judee	Rescue	80.4	17.2	69.0	6.8	11.2
		Conan	86.0	6.3	53.2	4.9	13.5
	<i>P</i> value		NS	***	***	NS	NS
Checks	Yellowstone	Hollow	91.7	11.4	36.1	9.4	36.7 a
	Judee	Rescue	87.6	15.1	61.5	5.3	6.7 b
	Warhorse	Rescue	82.7	34.9	69.5	8.8	5.0 b
	<i>P</i> value		NS	NS	NS	NS	***
Carter							
NILs by recurrent parent	Yellowstone	Hollow	94.4	3.7	22.4	4.2	63.7
		Conan	96.4	1.9	20.3	2.8	60.3
	<i>P</i> value		NS	NS	NS	NS	NS
	Judee	Rescue	89.9	4.9	25.7	3.2	22.2
		Conan	90.3	4.1	23.6	5.1	54.5
	<i>P</i> value		NS	NS	NS	NS	***
Checks	Yellowstone	Hollow	97.9	0.0	36.3	2.2	78.3 a
	Judee	Rescue	86.9	3.6	12.2	2.8	16.7 b
	Warhorse	Rescue	78.5	3.0	3.0	1.8	4.2 b
	<i>P</i> value		NS	NS	NS	NS	***
Conrad							
NILs by recurrent parent	Yellowstone	Hollow	87.5	9.3	43.7	16.0	27.3
		Conan	90.6	9.5	45.3	13.9	24.2
	<i>P</i> value		NS	NS	NS	NS	NS
	Judee	Rescue	87.8	7.0	44.6	15.6	25.8
		Conan	91.6	7.8	44.2	20.6	23.2
	<i>P</i> value		NS	NS	NS	NS	NS
Checks	Yellowstone	Hollow	74.4	8.0	27.5	19.4	36.7 a
	Judee	Rescue	100.0	7.0	50.1	24.9	28.3 ab
	Warhorse	Rescue	87.3	7.7	56.3	7.0	11.7 b
	<i>P</i> value		NS	NS	NS	NS	***
Combined environments							
NILs by recurrent parent	Yellowstone	Hollow	92.4	5.8	35.7	4.8	41.4
		Conan	89.7	6.3	36.2	3.9	34.5
	<i>P</i> value		NS	NS	NS	NS	**
	Judee	Rescue	86.1	10.0	46.5	8.5	20.1
		Conan	89.2	5.8	40.3	10.2	30.2
	<i>P</i> value		NS	**	**	NS	***

(Continues)

TABLE 4 (Continued)

	Variety	Allele	Infestation	Larval mortality		Parasitism	Stem cutting
				Early	Overall		
Checks	Yellowstone	Hollow	88.0	2.2 b	33.3	6.2	50.6 a
	Judee	Rescue	91.5	4.1 ab	41.3	5.4	17.2 b
	Warhorse	Rescue	83.5	8.1 a	45.4	2.5	8.0 b
	<i>P</i> value		NS	*	NS	NS	***

Note. Values not sharing a common letter indicate significant differences between alternative alleles or among cultivars ($\alpha \leq 0.05$, Tukey-adjusted).

Abbreviation: NS, not significant.

* $p = 0.05$; ** $p = 0.01$; *** $p = 0.001$.

and overall larval mortality at Big Sandy ($p \leq 0.001$) but was not significantly different at Carter or Conrad. The Rescue-derived allele resulted in significantly lower stem cutting at Carter ($p \leq 0.001$); however, stem cutting was not significantly different between the two alleles at Big Sandy or Conrad.

Agronomic traits were measured at the Bozeman location, which had a miniscule WSS population, and the Carter location which was heavily infested by WSS. Among the checks at the Bozeman location, test weight was not significantly different, but Yellowstone had significantly greater grain yield than Judee and Warhorse (Figure 3; $p \leq 0.05$). At the Carter location, Yellowstone had a significantly lower test weight than the other two varieties ($p \leq 0.05$), whereas grain yield was not significantly different among the three checks.

Among the NILs in the Yellowstone genetic background, no differences between alleles were observed for yield and test weight at Bozeman or Carter (Figure 4). In the Judee genetic background, the NILs with the Conan-derived allele had significantly greater test weight than the Rescue-derived allele in Bozeman ($p \leq 0.05$); however, yield was not significantly different. At Carter, test weight was not significantly different among these NILs, but the NILs with the Rescue-derived allele had significantly greater yield ($p \leq 0.001$).

4 | DISCUSSION

In Montana, hard red spring and winter wheat are grown in many of the same areas where high levels of WSS infestation occur. However, winter wheat experiences greater exposure to peak WSS emergence than spring wheat. WSS emergence and oviposition activity can begin as early as mid-May and continue until mid-July (Buteler et al., 2015; Morrill & Kushnak, 1996; Subedi et al., 2021). In winter wheat, the peak WSS activity is concurrent with stem elongation to anthesis period; however, a substantial number of WSS continue to emerge post winter wheat anthesis (Varella et al., 2016). Conversely, spring wheat is planted from early April to mid-May, and peak WSS activity occurs when spring wheat starts stem elongation but substantially decreases after anthesis.

This study sought to examine whether the novel early solid stem Conan-derived *Qss.msub-3BL.c* allele provides adequate WSS resistance in winter wheat, despite high WSS activity after anthesis when stem solidness is significantly reduced.

To test the efficacy of the Conan-derived *Qss.msub-3BL.c* allele for WSS resistance in winter wheat, NILs were developed using MAB to introgress the *Qss.msub-3BL.c* allele into two Montana-adapted winter wheat varieties, Yellowstone (*Qss.msub-3BL.a*) and Judee (*Qss.msub-3BL.b*). Temporal expression of the *Qss.msub-3BL.c* solid stem phenotype in the Yellowstone NILs was significantly higher than NILs with the hollow stem *Qss.msub-3BL.a* allele at early plant development and maturity growth stages (Figure 2). This is similar to what was observed in the hollow stem spring wheat genetic backgrounds (Cook et al., 2019; Varella et al., 2016). Additionally, stem solidness expressed by the *Qss.msub-3BL.c* allele significantly declined between early and mature temporal solid stem measurements, which is also what was observed in spring wheat (Cook et al., 2019; Varella et al., 2016).

Conversely, NILs derived from Judee contrasting for the *Qss.msub-3BL.b* and *Qss.msub-3BL.c* solid stem alleles produced a different temporal solid stem phenotype than what was previously reported. First, the *Qss.msub-3BL.c* allele had a significantly lower solid stem score than the *Qss.msub-3BL.b* allele at both early and mature developmental stages (Figure 2). These results are contrary to findings in spring wheat where the *Qss.msub-3BL.c* allele expressed a similar solid stem score to the *Qss.msub-3BL.b* allele at early plant development, but the *Qss.msub-3BL.c* solid stem score decreased significantly as the plant approached anthesis (Cook et al., 2019; Talbert et al., 2014; Varella et al., 2016). Second, both alleles had significantly greater solid stem scores at maturity versus the early plant development stage. Comparison of the check varieties included in the experiment also showed that Judee had a significant increase in stem solidness from early to mature plant growth stages, whereas Yellowstone (hollow stem variety) and Warhorse (solid stem variety) did not have significantly different temporal solid stem scores (Figure 1). It is possible Judee has an unknown allele causing a previously uncharacterized temporal phenotype of late developing stem solidness.

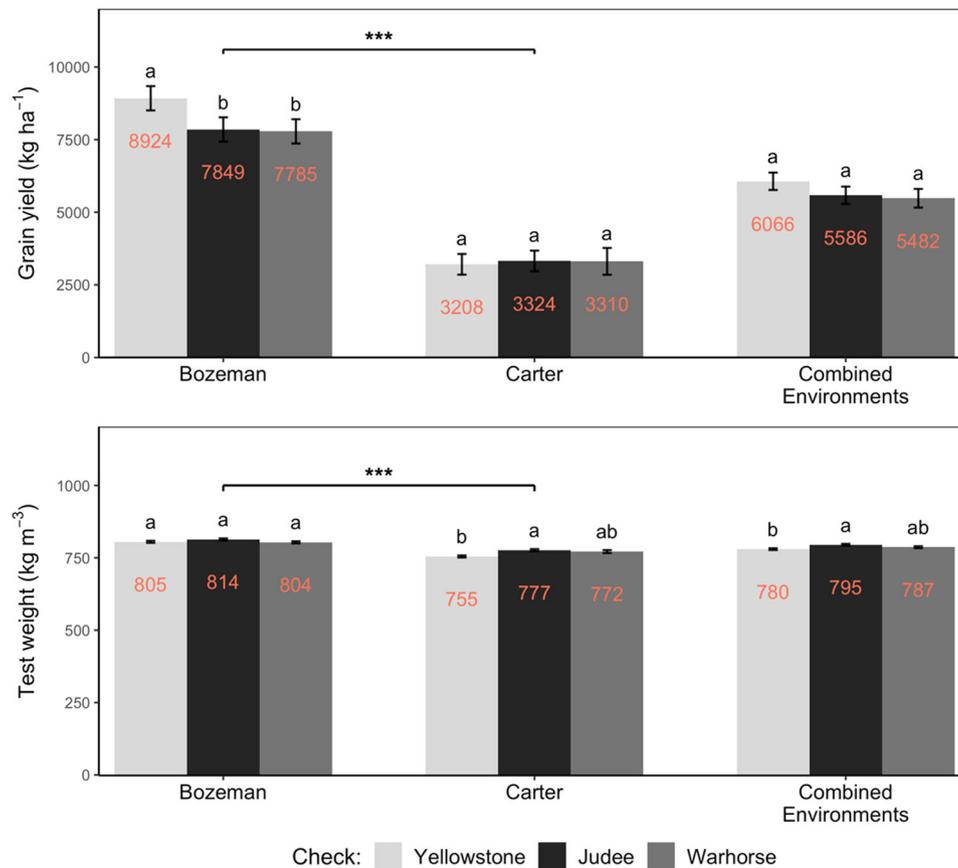


FIGURE 3 Mean grain yield (kg ha⁻¹) and test weight (kg m⁻³) for three check varieties: Yellowstone (hollow stem), Judee (solid stem), and Warhorse (solid stem). Means were derived from yield trials grown during the 2019 field season at Bozeman, MT (no wheat stem sawfly [WSS] infestation), Carter, MT (wheat stem sawfly [WSS]-infested site). Varieties are designated by a unique color. Error bars represent standard errors. ***Significant at the 0.001 probability level. Within a location, bars not sharing a common letter indicate significant differences among checks ($\alpha \leq 0.05$, Tukey-adjusted)

Despite the significant reduction of stem cutting provided by the *Qss.msub-3BL.c* allele compared with the *Qss.msub-3BL.a* allele in the Yellowstone genetic background, we still rate the NILs with *Qss.msub-3BL.c* allele as susceptible to WSS. Comparing WSS stem cutting scores among the Judee-derived NILs with contrasting *Qss.msub-3BL.b* and *Qss.msub-3BL.c* alleles found that the NILs with the *Qss.msub-3BL.c* allele had significantly higher WSS stem cutting at Carter and across combined locations than NILs with the *Qss.msub-3BL.b* allele. Overall, our study indicates that the *Qss.msub-3BL.c* does not provide sufficient improvement in WSS resistance when integrated into the hollow stem Yellowstone winter wheat, and it reduced WSS resistance when integrated into the solid stem Judee winter wheat originally containing the *Qss.msub-3BL.b* solid stem allele.

WSS stem cutting scores were also compared among the check varieties to determine if the unique temporal solid stem phenotype found in Judee affects WSS resistance. Judee had significantly lower WSS stem cutting scores than Yellowstone at two out of the three locations (Table 4). Compared

to Warhorse, Judee WSS stem cutting scores were higher at all locations but were not significantly different (Table 4). In another study published by Bruckner et al. (2020), Judee and Warhorse were evaluated for WSS stem cutting in 16 WSS infested environments where Judee had significantly higher cutting scores, but the solid stem scores measured at maturity were not significantly different. Although the Judee WSS stem cutting scores were not significantly higher than Warhorse in our study, Bruckner et al. (2020) indicate Judee is more vulnerable to WSS. Further research needs to be done to confirm the Judee temporal solid stem phenotype and how it affects WSS resistance.

Lastly, yield and test weight were measured among the NILs contrasting for the *Qss.msub-3BL* alleles to determine if *Qss.msub-3BL.c* negatively impacts these traits in winter wheat. At Bozeman (non-WSS infested) or Carter (WSS infested), no significant yield or test weight differences were observed among the NILs in the Yellowstone background. Grain yield among NILs derived from the Judee background was not significantly different at Bozeman (non-WSS infested); however, at Carter (WSS infested), NILs with the

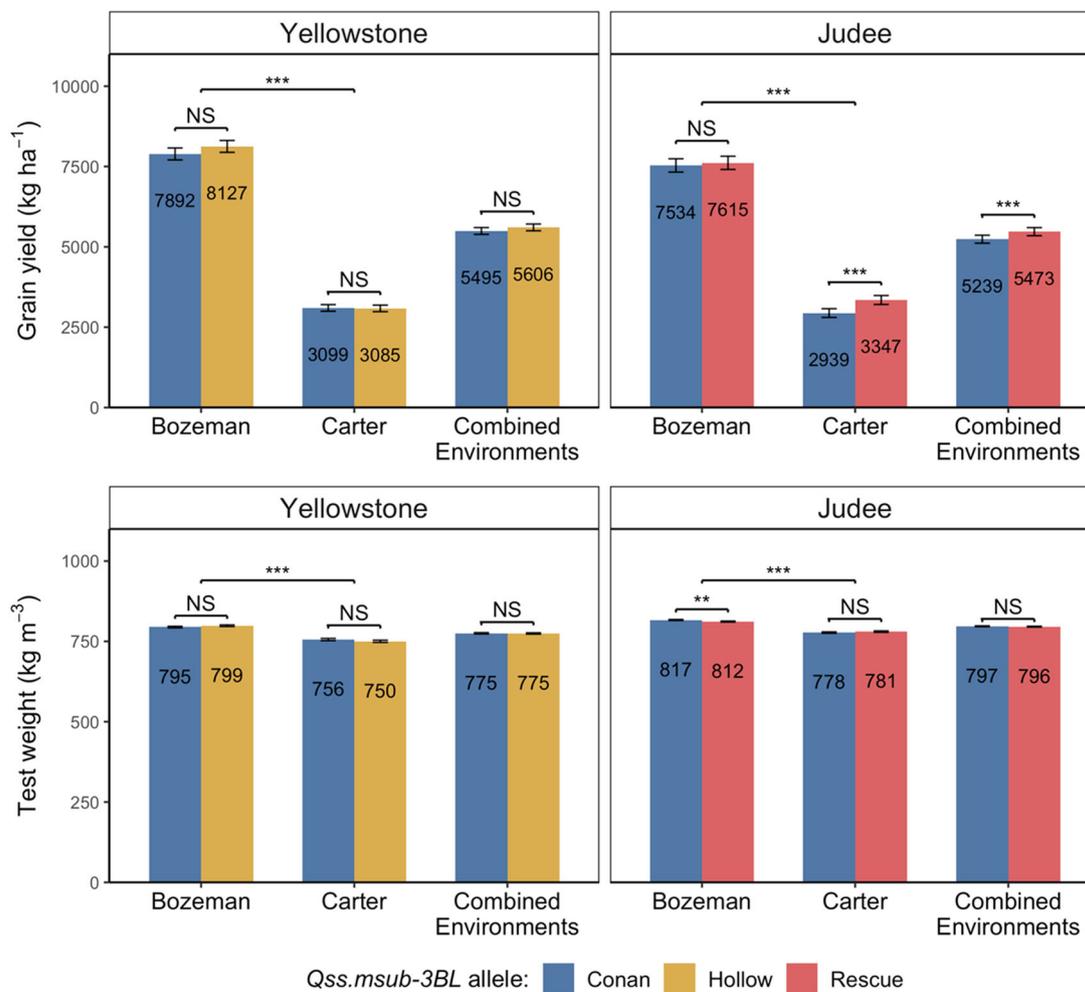


FIGURE 4 Mean grain yield (kg ha⁻¹) and test weight (kg m⁻³) for near-isogenic line (NIL) pairs derived from two recurrent parents: Yellowstone (hollow stem) and Judee (solid stem). Data were collected from Bozeman, MT (no wheat stem sawfly [WSS] infestation) and Carter, MT (wheat stem sawfly [WSS]-infested site), during the 2019 field season. Alleles are designated by a unique color. Error bars represent standard errors. **Significant at the 0.01 probability level; ***significant at the 0.001 probability level. Abbreviation: NS, nonsignificant

Qss.msub-3BL.b did have significantly higher grain yield than NILs with the *Qss.msub-3BL.c* allele. In non-WSS infested growing environments, it does not appear the *Qss.msub-3BL.c* allele negatively impacts grain yield and test weight in winter wheat, which is similar to what we observed in spring wheat (Cook et al., 2019). This also corresponds with no significant yield drag being associated with the *Qss.msub-3BL.b* allele in spring and winter wheat (Cook et al., 2004; Hayat et al., 1995; Sherman et al., 2010). The significant difference in grain yield at Carter is likely due to the *Qss.msub-3BL.c* NILs suffering significantly higher WSS stem cutting than the *Qss.msub-3BL.b* NILs, thus demonstrating the value of growing WSS-resistant varieties in WSS infested growing environments.

The focus of this study was to evaluate the use of the Conan-derived *Qss.msub-3BL.c* solid stem allele for WSS resistance in hard red winter wheat grown in Montana environments with high WSS infestation. Our results indicate that the use

of the Conan-derived allele for WSS resistance in winter wheat was not as effective as the Rescue-derived *Qss.msub-3BL.b* allele. These results contrast with the study of hard red spring wheat where the Conan-derived allele provided similar levels of WSS resistance as the Rescue-derived allele (Cook et al., 2019). We believe this is due to a greater number of adult WSS remaining active after the pith produced by the Conan-derived allele is degraded in winter wheat. Successful use of the *Qss.msub-3BL.c* allele is dependent on the synchronization of wheat phenology with WSS emergence in which the peak WSS oviposition period needs to precede early solid stem degradation. It is possible the unique temporal solid stem phenotype found in Judee may have negatively impacted the effectiveness of the *Qss.msub-3BL.c* allele in the Judee genetic background. Evaluating the *Qss.msub-3BL.c* allele in a WSS-resistant variety with a stable temporal solid stem phenotype, such as Warhorse, may improve *Qss.msub-3BL.c* WSS resistance.

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AUTHOR CONTRIBUTIONS

Mei Ling Wong: Data curation; Formal analysis; Methodology; Visualization; Writing – original draft; Writing – review & editing. **Philip Bruckner:** Investigation; Resources; Supervision. **Jim Berg:** Data curation; Investigation; Resources; Writing – original draft. **Peggy Lamb:** Investigation; Resources. **Megan Hofland:** Data curation; Investigation; Resources. **Christopher Caron:** Data curation; Investigation; Resources. **Hwa-Young Heo:** Investigation; Resources; Writing – review & editing. **Nancy Blake:** Resources; Writing – review & editing. **David K. Weaver:** Data curation; Investigation; Supervision; Writing – review & editing. **Jason Cook:** Conceptualization; Data curation; Funding acquisition; Project administration; Supervision; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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