

## ORIGINAL ARTICLE

## Agronomic Application of Genetic Resources

## Yield protection afforded by imidacloprid during Hessian fly infestation in six genotypes

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## Abstract

The Hessian fly (*Mayetiola destructor* Say) is a gall midge that infests and feeds upon wheat (*Triticum aestivum* L.). Recently, a new form of tolerance (*QHft.nc-7D*) was identified in the breeding line LA03136E71 (PI 700336). Partial resistance allows immature Hessian fly to thrive in small numbers and does not function like antibiosis. Little is known about the potential yield drag of using partial resistance. In this study, we evaluated six genotypes: one containing *QHft.nc-7D* (LA03136E71), one containing *H13*, and four potentially susceptible genotypes. All genotypes were evaluated with two different seed treatment regimens of imidacloprid: no treatment and a two times rate of imidacloprid. All tested genotypes were planted in six-to-eight replications of a full factorial design in four environments. Subsamples of yield trial plots were taken to measure percent infested tillers and a number of larvae/pupae per tiller. Plots were harvested and grain yield was recorded. There was a significant ( $p[>F] < 0.05$ ) reduction of percent infested tillers and a number of larvae/pupae per tiller related to the imidacloprid treatment. Imidacloprid treatment significantly ( $p[>T] < 0.05$ ) reduced the number of larvae/pupae per tiller for LA03136E71. There was no significant ( $p[>T] > 0.05$ ) grain yield increase associated with treatment for LA03136E71. This indicates that a two times application of imidacloprid on LA03136E71 (*QHft.nc-7D*) did not improve yield yet reduced infestation. Therefore, *QHft.nc-7D*, while allowing Hessian fly to thrive on the plant, does not significantly compromise yield.

## 1 | INTRODUCTION

The Hessian fly (*Mayetiola destructor* Say) is a gall midge whose larvae infest and feed upon wheat (*Triticum aestivum* L.). It has been demonstrated that Hessian flies will

lay and infest other small grains; yet wheat is the preferred host (Jones, 1939). In the initial stages of infestation, Hessian fly females will mate and then oviposit their eggs on the exterior grooves of wheat leaves. After a gestational period of around 3–5 days, maggots will hatch from the eggs and descend from the leaf to the whorl of the plant to establish a feeding site (Flanders et al., 2013). Hessian fly larvae induce several physiological changes to the cells at the established feeding site to extract substrates from the host for sustenance; it is these changes that are induced by Hessian fly larval feed-

**Abbreviations:** CAS22, Caswell Research Station in Kinston, North Carolina in 2022; CUN22, Cunningham Research Station in Kinston, North Carolina in 2022; HUG21, Hugo Research Station in Hugo, North Carolina in 2021; HUG22, Hugo Research Station in Hugo, North Carolina in 2022.

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ing, which are suspected to cause the symptoms of Hessian fly infestation (Harris et al., 2006). The physiological changes induced by Hessian fly larval feeding are irreversible and can lead to stunting, failure to flower and set seed, and, in profound infestations, premature death of the host (Shukle et al., 2016).

There are many different methods of cultural control for Hessian flies, most of which require action prior to planting (Schmid et al., 2018). These include planting after a “Hessian-fly-free date,” removal of volunteer wheat prior to planting, and tillage (Buntin & Chapin, 1990; Flanders et al., 2013; Schmid et al., 2018). Hessian-fly-free dates are supposed periods when growers may safely avoid Hessian fly infestation due to the temperature conditions. However, Hessian-fly-free dates are not available in latitudes that do not experience cold enough temperatures, and planting after the Hessian-fly-free date can increase the risk of winter kill (Buntin & Chapin, 1990; Campbell et al., 1991). Furthermore, there is evidence to suggest that current “Hessian-fly-free date” recommendations may require revision or reconsideration and that the practice may not be as broadly applicable as once thought (Schmid et al., 2018). There is a lack of literature for the removal of volunteer wheat prior to planting as a control measure for Hessian flies (Parks, 1917; Schmid et al., 2018).

Beyond cultural control, rescue foliar applications of insecticide may be applied early in the growing season when adult Hessian flies are present or when eggs have not yet hatched (Flanders et al., 2013). However, no economic threshold has been established for Hessian flies, making it difficult for growers to make decisions on when to apply this type of control (Shukle, 2008). In addition, Howell et al. (2017) found that lambda-cyhalothrin, an insecticide labeled for the control of Hessian fly in wheat, did not provide any benefit to the reduction of Hessian fly abundance when used as a foliar insecticide, although insecticidal seed treatment was effective when preventatively applied. Additionally, imidacloprid has been documented to control an initial infestation of Hessian fly and aphids (Hemiptera: Aphidoidea) in wheat (Howell et al., 2017; Wilde, 2001; Zhang et al., 2016).

Nevertheless, genetic resistance remains the most cost-effective and efficient form of Hessian fly control available (Howell et al., 2017). Hessian fly resistance in wheat follows the gene-for-gene hypothesis, wherein an interaction occurs between a resistance gene (*H*) in the host and a corresponding avirulence gene (*Avr*) in a pest. When both the resistance gene and the avirulence gene are present in the host–pest interaction, this results in an incompatible reaction leading to the failure of the pest to thrive on the host (Hatchett & Gallun, 1970).

In wheat, the use of *H* genes leads to the death of the Hessian fly larvae during larval feeding (antibiosis), which places strong selection pressure on the development of resistant Hessian fly biotypes. This phenomenon is illustrated by the loss of control efficacy afforded by *H* genes, like *H9*, over time (Cambron et al., 2010; Shukle et al., 2016). Currently, the resistance

### Core Ideas

- Application of a two-times regiment of imidacloprid provides yield protection for susceptible genotypes.
- Partial resistance and/or tolerance to Hessian fly (*QHft.nc-7D*) yields similarly regardless of seed treatment.
- Antibiosis-based Hessian fly resistance (*H13*) yields similarly regardless of seed treatment.
- Partial resistance and/or tolerance, while allowing Hessian fly to persist, does not compromise yield.

gene *H13* is effective against Hessian fly biotypes found in the southeast of the United States (Cambron et al., 2010; Shukle et al., 2016).

There are reports of Hessian fly partial resistance and/or tolerance, where in the host plant is successfully infested by the Hessian fly larvae, yet it still outperforms truly susceptible genotypes (Hao et al., 2013; Winn et al., 2021). This type of Hessian fly tolerance does not appear to function like traditional *H* genes, and the mode of action remains unknown. Furthermore, little has been reported about the potential yield loss associated with the deployment of these partial resistance and/or tolerance loci.

The objective of this study was to evaluate the yield loss associated with varying levels of resistance when lines are treated with imidacloprid seed treatment and when left untreated. We evaluated several known susceptible lines, a line anecdotally reported to exhibit tolerance, a line containing the *H13* locus, and the two parental lines from the QTL analysis performed by Winn et al. (2021) to assess their yield when treated with imidacloprid and when left untreated.

## 2 | METHODS

### 2.1 | Plant materials

Six soft red winter wheat lines of differing genetic backgrounds were selected for evaluation. The advanced breeding line, ‘LA03136E71’ (PI 700336), contains the recently identified Hessian fly field partial-resistance locus *QHft.nc-7D* that provides a significant reduction in the number of infesting larvae on the plant (Winn et al., 2021); its pedigree is ARLA97-1047-4-2 (P2684/3/N7840//PARULA/VEERY#6)/LA95125BUB73-1.

‘NC11546-14’ is an advanced breeding line developed by the North Carolina State University Small Grains Breeding Program; its pedigree is AGS 2027/NC09-20986. NC11546-

14 is noted for containing the Hessian fly resistance locus *H13* and producing incompatible reactions with Hessian fly biotypes B, C, D, O, and L (Murphy et al., 2019).

‘Jamestown’ (Reg. No. CV-1041, PI 653731) was produced by the Virginia Agricultural Experiment Station, was derived from the cross ‘Roane’/Pioneer Brand ‘2691’, and appeared, in the past, as resistant to Hessian fly (Griffey et al., 2010). However, Jamestown has appeared susceptible to biotypes C, D, O, and L over several years in Hessian fly screenings performed by the USDA-ARS Crop Protection and Pest Control Research Unit in West Lafayette, IN, USA (Murphy et al., 2017, 2018, 2019, 2020).

‘Shirley’ (Reg. No. CV-1039, PI 656753) was developed by the small grains breeding program at Virginia Polytechnic Institute and State University and has been shown to be highly susceptible to Hessian flies (Winn et al., 2021). Shirley was derived from a top cross of VA94-52-25/Coker9835//VA96-54-234.

‘USG3404’ (pedigree not publicly available) is a widely adapted Hessian fly susceptible variety released by UniSouth Genetics (Dickson, TN, USA). ‘SS8641’ was produced by the University of Georgia Wheat Breeding Program and derived from the cross GA-881130/2\*GA-881582 (Rahman et al., 2017). There have been antidotal reports of SS8641 exhibiting “field tolerance” or partial resistance akin to LA03136E71; however, greenhouse screening of SS8641 has shown susceptibility to biotypes C and D (Murphy et al., 2020).

## 2.2 | Experimental design and field management

In both the 2020–2021 and 2021–2022 growing seasons, plots were planted in a full factorial, randomized complete block design. All six genotypes were replicated within a block to contain an untreated plot and a plot treated with a rate of Gaucho 600 FS that was double the highest labeled rate (3.13 mL kg<sup>-1</sup> [4.8 fl oz hundredweight<sup>-1</sup>]; Bayer CropScience LP, Research Triangle Park, NC, USA). The weight of seed per genotype was taken in kilograms and was used to calculate the volume of pesticide to be applied to each genotype. The calculated volume of seed treatment was applied to seed by placing the liquid pesticide in a vessel of seed and mixing until thoroughly incorporated. Each block was replicated six-to-eight times in a single field, depending on seed availability.

The recommended seeding rate in North Carolina for winter wheat lies between 1.1 and 1.5 million seeds per acre (Weisz et al., 2013); therefore, plots were planted to remain within the bounds of this seeding rate in both growing seasons. To encourage Hessian fly infestation, plots were sown (early for the area) in late September to early October. Plots

planted in the 2020–2021 growing season were sown on October 5, 2020; plots planted in the 2021–2022 growing season were sown on September 28, 2021. Plots were scouted on a regular basis for the presence of immature Hessian fly eggs/larvae/pupae as well as other potential pests. No significant infestation of any other common pest in North Carolina, such as aphids, was noted in any location reported.

In the 2020–2021 growing season, due to seed limitations, plots were disk seeded into small 4-by-14-ft<sup>2</sup> plots using a seeding rate of approximately 1.13 million seeds per acre over six replications at a single location at the Hugo Agricultural Research Station in Hugo, NC, USA (HUG21). A separate increase location was planted in Wilson, NC, and seed was harvested in 2021 for use in the 2021–2022 growing season. In the 2021–2022 growing season, plots were disk seeded into larger 5-by-13-ft<sup>2</sup> plots using a seeding rate of approximately 1.27 million seeds per acre over eight replications at three locations: Cunningham Agricultural Research Station in Kinston, NC, USA (CUN22), Caswell Agricultural Research Station in Kinston, NC, USA (CAS22), and Hugo Agricultural Research Station in Hugo, NC, USA (HUG22).

In the 2020–2021 growing season, 336 kg ha<sup>-1</sup> of preplant 10-10-10 fertilizer was applied to HUG21 on September 22, 2020. To control ryegrass (*Lolium rigidum* L.), Zidua (BASF) herbicide was applied at 73 mL ha<sup>-1</sup> on October 9, 2020, followed by an application of Huskie (Bayer CropScience LP) herbicide at a rate of 949 mL ha<sup>-1</sup> on October 19, 2020. No other pesticidal substances were applied to the field for the rest of the growing season. Two subsequent applications of 24-0-0 nitrogen fertilizer were applied at 130 L ha<sup>-1</sup> on January 20, 2021, and at 168 L ha<sup>-1</sup> on March 3, 2021.

In the 2021–2022 growing season, 336 kg ha<sup>-1</sup> of preplant 10-10-10 fertilizer was applied to HUG22, CUN22, and CAS22 on September 20, 2021. To control rye grass, Zidua herbicide was applied at 73 mL ha<sup>-1</sup> on September 29, 2021, followed by an application of Huskie herbicide at a rate of 949 mL ha<sup>-1</sup> on October 13, 2021. No other pesticidal substances were applied to the field for the rest of the growing season. Two subsequent applications of 24-0-0 nitrogen fertilizer were applied at 112 L ha<sup>-1</sup> on January 27, 2022, and at 262 L ha<sup>-1</sup> on February 23, 2022.

## 2.3 | Data collection

Yield plot subsamples were removed from every plot in HUG21, CUN22, CAS22, and HUG22 using the methods described in Winn et al. (2021). A 0.5 m subsample was dug up from each plot within each location in their respective year from December 1 to 14. For each subsample, the number of tillers, the number of visually infested tillers, and the number of immature Hessian fly larvae/pupae were recorded. In the case of this study, any stem producing three or more leaves

was considered a tiller. A visually infested tiller was defined by stunted, darkened leaves and the presence of Hessian fly larvae/pupae.

The percent infested tillers in a subsample of a plot were calculated by taking the number of apparently infested tillers and dividing by the total number of tillers in a subsample. The number of larvae/pupae per tiller was calculated by taking the total number of immature Hessian flies identified in a subsample and dividing by the total number of tillers in the plot. Plots were harvested from mid-May to mid-June, bagged, weighed, and measured for percent moisture and weight in grams. Grain yield was recorded in metric tons per hectare and adjusted to 13% moisture content.

## 2.4 | Statistics and software

All statistical analysis was done in R statistical software version 4.1.2 (R Core Team, 2013). Visual examination of data distributions and QQ plots were conducted to confirm the normality of the data using the “base” package and “stats” package within R. Upon initial inspection of distributions, percent infested tillers and number of larvae/pupae per tiller exhibited a non-normal, zero-inflated, positive, non-integer distribution.

The popular Box-Cox transformation method (Box & Cox, 1964) is not defined for data containing zero; in lieu of this, we applied an inverse hyperbolic sine transformation as described by Burbidge et al. (1988):

$$f(y, \theta) = \frac{\sinh^{-1}(\theta y)}{\theta}$$

where  $y$  is a response of any distribution,  $\sinh^{-1}$  is the inverse hyperbolic sine, and  $\theta$  is a value that results in the best fit of  $y$  to a normal distribution. The parameter  $\theta$  was estimated empirically via log-likelihood:

$$-\frac{1}{2} \sum \log(1 + \theta^2 y_i^2)$$

where  $\theta$  is any value bound between zero and a positive integer, and  $y_i$  is the  $i$ th response. All values of  $\theta$  such that  $\{\theta: 0 \leq \theta \leq 200\}$  were entertained, and the value of  $\theta$  that maximized the log-likelihood was used as the  $\theta$  in the inverse hyperbolic sine transformation of that response.

The “lme4” package was used to run mixed linear models and estimate fixed effect significance via the function “lmer()” (Bates et al., 2015). Within environments, a mixed linear model was employed to assess the significance of genotype and treatment effects:

$$y_{ijk} = \mu + G_i + T_j + GT_{ij} + r_k + tr_{jk} + \varepsilon_{ijk}$$

where  $y$  is the response,  $\mu$  is the intercept,  $G$  is the fixed genotype effect,  $T$  is the fixed treatment effect,  $GT$  is the fixed genotype by treatment interaction,  $r$  is the random replication effect, which is identically and independently distributed across all levels,  $tr$  is the random replication by treatment interaction, which is identically and independently distributed across all levels, and  $\varepsilon$  is the residual error that is identically and independently distributed across all levels.

Across environments, a multi-environmental mixed linear model was employed to assess the significance of genotype and treatment effects:

$$ry_{ijkl} = \mu + G_i + T_j + E_k + GT_{ij} + GE_{ik} + TE_{jk} + GET_{ijk} + er_{kl} + ter_{jkl} + \varepsilon_{ijkl}$$

where  $y$  is the response,  $\mu$  is the intercept,  $G$  is the fixed genotype effect,  $T$  is the fixed treatment effect,  $E$  is the fixed environment effect,  $GT$  is the fixed genotype by treatment interaction,  $GE$  is the fixed genotype by environment interaction,  $TE$  is the fixed environment by treatment interaction,  $GET$  is the fixed genotype by environment by treatment three-way interaction,  $er$  is the replication by environment interaction random effect that is independently and identically distributed across levels,  $ter$  is the random treatment by environment by replication three-way interaction that is independently and identically distributed across levels, and  $\varepsilon$  is the residual error that is independently and identically distributed across levels.

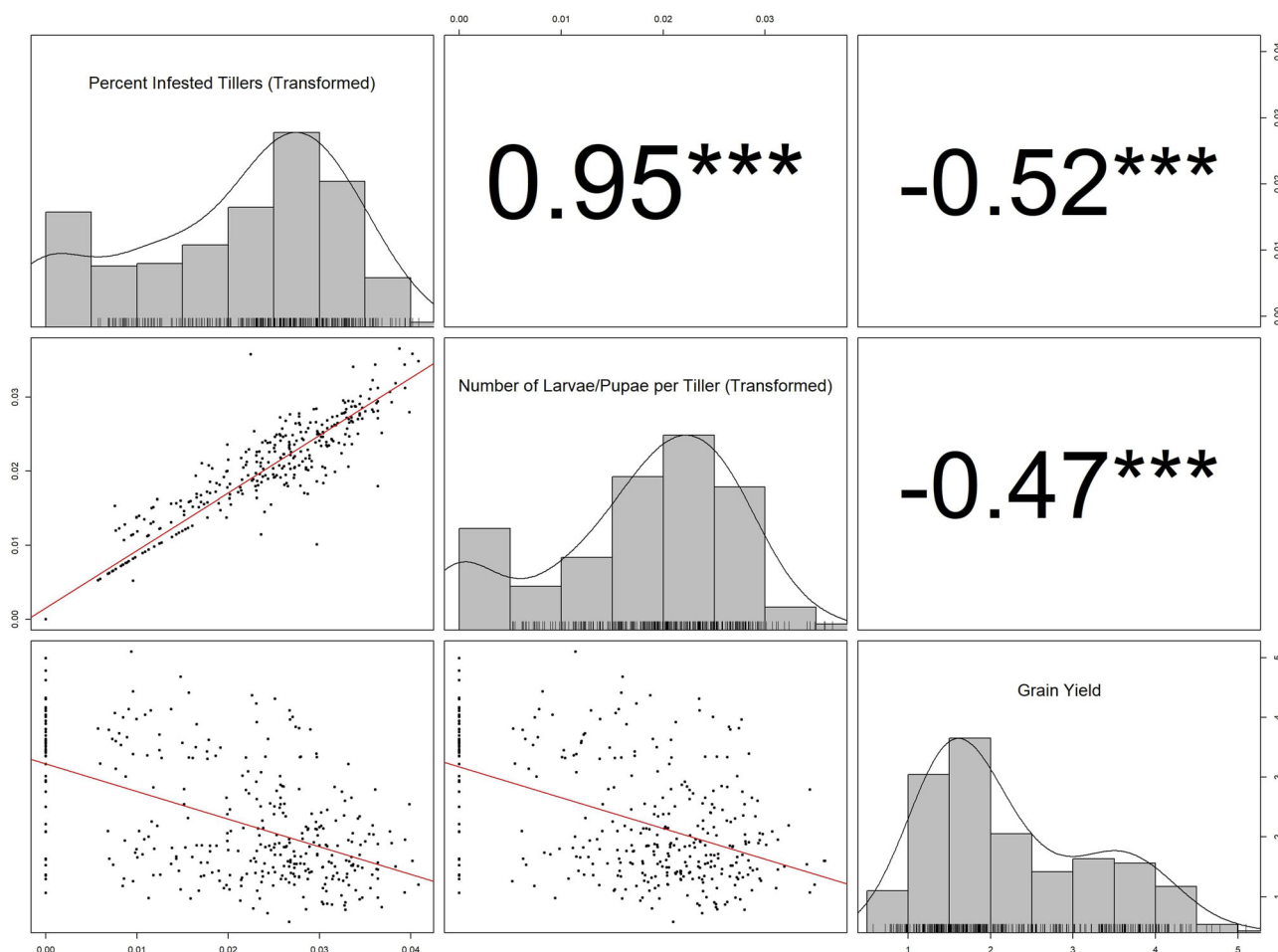
Adjusted group means, standard errors, confidence intervals, pairwise contrast, and Tukey’s honestly statistically different multiple comparisons were calculated for all models using the “emmeans” package (Lenth et al., 2018). Data were visualized using the “ggplot2” package (Wickham et al., 2016). Pair plots were visualized using the function “pairs.panels()” in the “psych” package (Revelle & Revelle, 2015).

## 3 | RESULTS

### 3.1 | Preliminary data analysis

Plot level observations were checked for normality by the visual assessment of distributions and QQ plots. Upon the visual examination of distributions, grain yield exhibited near normality; however, percent infested tillers and number of larvae/pupae per tiller exhibited a zero-inflated distribution. An inverse hyperbolic sine transformation was applied to percent infested tillers and number of larvae/pupae per tiller, where  $\theta \approx 132$  and  $\theta \approx 200$ , respectively. After transformation, percent infested tillers and the number of larvae/pupae per tiller approximated a normal distribution.





**FIGURE 1** A pairwise comparison of observations for transformed values of percent infested tillers, number of larvae/pupae per tiller, and non-transformed grain yield. The bottom left triangle displays a linear regression line over a scatterplot for two corresponding traits on the diagonal. The diagonal displays the name of the trait and histogram of observations fitted with a density line. The upper right triangle displays Pearson's correlation coefficient and significance of the correlation. \*\*\* Indicates significance ( $p > T < 0.001$ ).

Plot level observations were visualized in a pair plot and subjected to a correlational analysis for further illustration (Figure 1). Correlational analysis of plot level observations of transformed percent infested tillers and number of larvae/pupae per tiller values indicated positive, high Pearson's correlation coefficient ( $r = 0.95$ ), which reflects previous studies with similar measurements (Winn et al., 2021). A correlational study of plot level observations of grain yield indicated highly significant ( $p > T < 0.001$ ) negative correlations with transformed values of percent infested tillers ( $r = -0.52$ ) and number of larvae/pupae per tiller ( $r = -0.47$ ), which implies that lines which experienced high Hessian fly infestation incidence tended to yield lower.

### 3.2 | Within environment analysis

After adjusting distributions and assessing plot level correlation across environments, each environment was analyzed separately for each trait recorded. For each environment by

trait combination, the significance of fixed effects was estimated (Table S1). For transformed percent infested tillers, the genotype and treatment effects were highly significant ( $p > F < 0.01$ ) within all environments assessed, yet the genotype by treatment interaction was insignificant ( $p > F \geq 0.05$ ) within each environment assessed. Similarly, analysis of transformed number of larvae/pupae per tiller values indicated highly significant ( $p > F < 0.01$ ) genotype and treatment effect, but a lack of significant genotype by treatment interaction ( $p > F \geq 0.05$ ).

For grain yield, analysis within environment indicated that the genotype and treatment effect were highly significant ( $p > F < 0.001$ ) in each environment assessed. Unlike number of larvae/pupae per tiller and percent infested tillers, the genotype by treatment effect was significant ( $p > F < 0.05$ ) in HUG21 and HUG22 for grain yield. Pairwise contrast was conducted for the genotype by treatment interaction for HUG21 and HUG22, as well as all other locations (Table S2). Pairwise contrast of the genotype by treatment interaction in HUG21 indicated a significant dif-

**TABLE 1** Table of *F* statistics, *p*-values, and significance indication for percent infested tillers, number of pupae/larvae per tiller, and grain yield.

	Percent infested tillers (transformed)				Number of pupae/larvae per tiller (transformed)				Grain yield			
	nDF	dDF	<i>F</i>	<i>p</i> (> <i>F</i> )	nDF	dDF	<i>F</i>	<i>p</i> (> <i>F</i> )	nDF	dDF	<i>F</i>	<i>p</i> (> <i>F</i> )
Genotype	5	283	70.3	<0.0001	5	282	71.8	<0.0001	5	284	315.5	<0.0001
Treatment	1	283	52.3	<0.0001	1	282	65.8	<0.0001	1	284	89.5	<0.0001
Environment	3	26	13.9	<0.0001	3	26	4.6	0.0101	3	26	137.4	<0.0001
Genotype:treatment	5	283	1.6	0.1595	5	282	2.3	0.0490	5	284	8.6	<0.0001
Genotype:environment	15	283	2.0	0.0174	15	282	2.2	0.0070	15	284	18.2	<0.0001
Treatment:environment	3	283	7.6	<0.0001	3	282	10.7	<0.0001	3	284	1.4	0.2390
Genotype:treatment:environment	15	283	0.8	0.6990	15	282	0.9	0.6091	15	284	1.1	0.3894

Abbreviations: dDF, denominator degrees of freedom; *F*, *F*-statistic derived from model; nDF, numerator degrees of freedom; *p*(>*F*), probability derived from *F* test.

ference ( $p[>T] < 0.05$ ) between treated and untreated plots for Jamestown, Shirley, and USG3404, where the treated line yielded significantly higher than the untreated line. In HUG22, pairwise contrast between treated and untreated plots indicated that Shirley plots, treated with imidacloprid, yielded significantly ( $p[>T] < 0.05$ ) higher than Shirley plots without treatment.

### 3.3 | Across environment analysis

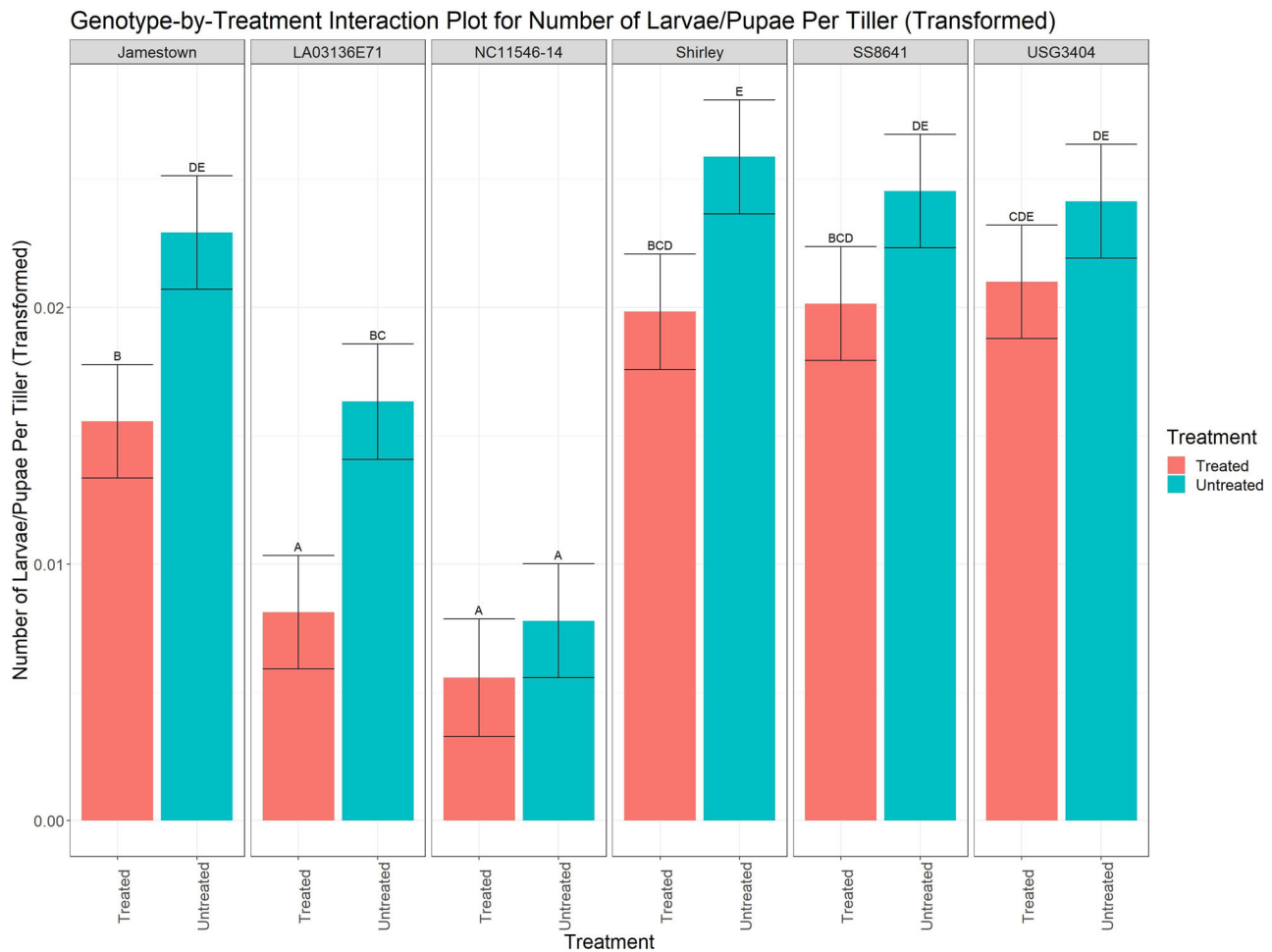
Analysis within environment indicated significant ( $p[>T] < 0.001$ ) effects for both genotype and treatment with minimal interaction for all traits assessed. A multi-environmental model was applied for each trait across assessed environments. Estimation of fixed effect significance was conducted for each trait assessed (Table 1). For transformed percent infested tillers, transformed number of larvae/pupae per tiller, and grain yield; the main effects for genotype, treatment, and environment were significant ( $p[>F] < 0.05$ ). The three-way genotype-by-treatment-by-environment interaction was insignificant ( $p[>F] > 0.05$ ) for percent infested tillers, number of larvae/pupae per tiller, and grain yield. Due to the insignificance of the three-way interaction, two-way interactions were investigated.

The two-way interaction for treatment-by-environment was significant ( $p[>F] < 0.05$ ) for transformed percent infested tillers and number of larvae/pupae per tiller. Adjusted means, standard errors, and 95% confidence intervals were calculated for the treatment-by-environment interaction for percent infested tillers and number of larvae/pupae per tiller and graphed (Figures S1 and S2). Pairwise comparison via Tukey's honestly significant difference of the treatment by environment interaction for percent infested tillers and number of larvae/pupae per tiller showed that the treated and untreated genotypes grouped together in CUN22.

The genotype-by-environment interaction was significant ( $p[>F] < 0.05$ ) for percent infested tillers, number of larvae/pupae per tiller, and grain yield. For transformed percent infested tillers, pairwise comparison by Tukey's honestly significant difference at 95% showed variability of groupings across environments and separation between CUN22 and HUG21 for Jamestown and HUG21 and HUG22 for Shirley (Figure S3). For transformed number of larvae/pupae per tiller, pairwise comparison by Tukey's honestly significant difference at 95% showed the variability of groupings across environments and separation between CUN22 and HUG21 for Jamestown (Figure S4). For grain yield, HUG21 appeared to be a significantly higher yielding environment across all genotypes, except for USG3404 (Figure S5).

The genotype-by-treatment interaction was significant ( $p[>F] < 0.05$ ) for the transformed number of larvae/pupae per tiller and grain yield. For transformed number of larvae/pupae per tiller, pairwise comparison by Tukey's honestly significant difference at 95% showed significant differences between treatments for LA03136E71, Jamestown, and Shirley where treated plots had significantly less severe infestation than untreated plots (Figure 2). For grain yield, pairwise comparison by Tukey's honestly significant difference at 95% revealed that there were significant differences between treatments for Jamestown, Shirley, and USG3404, where the treated plots had significantly higher yield than the untreated plots (Figure 3).

Pairwise contrasts were calculated for the genotype-by-treatment interaction of grain yield (Table 2). A significant ( $p[>T] < 0.05$ ) difference between treatment levels was observed for Shirley, USG3404, and Jamestown where untreated plots were estimated to yield 0.74, 0.46, and 0.37 metric tons per hectare less than the treated plots. The *p*-values derived for LA03136E71 and NC11546-14 were far from significant ( $p[>T] > 0.90$ ), indicating that there was no significant effect of seed treatment for resistant or partially resistant genotypes.



**FIGURE 2** Genotype-by-treatment interaction plot for number of larvae/pupae per tiller with 95% confidence intervals and groupings via Tukey's honestly significant difference adjustment at 95%. Treatment levels are displayed on the X-axis, and genotype names are displayed in the heading of each subplot. Letters represent Tukey's derived groups. Black lines surrounding the end of bars represent a 95% confidence interval.

**TABLE 2** Pairwise contrast of genotype-by-treatment effect for grain yield in metric tons per hectare.

Genotype	Contrast	Grain yield			
		Estimate	DF	T ratio	p(>T)
Jamestown	Untreated – treated	0.37	266	4.29	0.0015
LA03136E71	Untreated – treated	0.12	265	1.44	0.9552
NC11546-14	Untreated – treated	0.04	265	0.44	1.0000
Shirley	Untreated – treated	0.74	265	8.60	<0.0001
SS8641	Untreated – treated	0.27	267	3.10	0.0882
USG3404	Untreated – treated	0.46	265	5.32	<0.0001

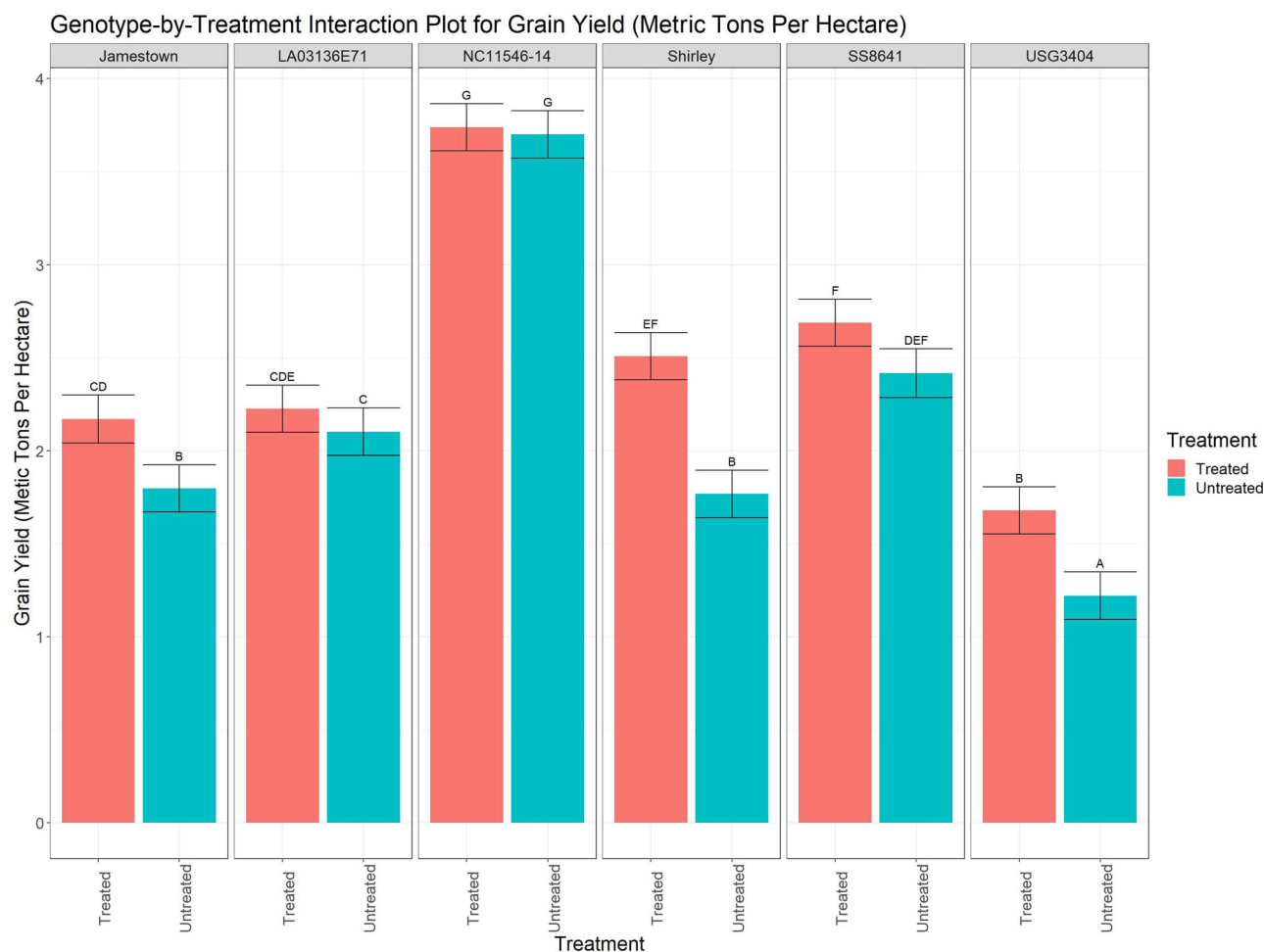
Note: Estimates are calculated as untreated minus treated.

Abbreviation: DF, degrees of freedom.

## 4 | DISCUSSION

In the present study, genotypes of varying genetic backgrounds were evaluated with and without a rate of imidacloprid seed treatment two times the highest labeled rate in fields predisposed to infestation by Hessian fly and

planted early to maximize infestation pressure. The purpose of the high rate of insecticide was to compare genotypes with varying degrees of susceptibility to Hessian fly at differing levels of infestation. We showed that the application of a two times rate of imidacloprid: (1) reduces infestation broadly but does not eliminate Hessian fly infestation; (2) is often



**FIGURE 3** Genotype-by-treatment interaction plot for grain yield with 95% confidence intervals and groupings via Tukey's honestly significant difference adjustment at 95%. Treatment levels are displayed on the X-axis, and genotype names are displayed in the heading of each subplot. Letters represent Tukey's derived groups. Black lines surrounding the end of bars represent a 95% confidence interval.

more effective in susceptible backgrounds; and (3) provides a sizable amount of yield protection to genotypes with a susceptible background.

There was a significant genotype by treatment interaction for grain yield across environments. The  $p$ -value derived from the pairwise contrast for SS8641 indicated a marginally insignificant value ( $p[>T] \approx 0.09$ ). If we consider a critical value of  $\alpha = 0.10$  in lieu of the traditional  $\alpha = 0.05$ , there is a significant ( $p[>T] < 0.10$ ) difference between the treated and untreated plots with an estimated loss of 0.27 metric tons per hectare for SS8641 when left untreated. This may imply that an anecdotal evidence of SS8641's "field tolerance" may be misleading, and that SS8641 is indeed truly susceptible to Hessian fly infestation.

When pairwise comparisons were made at an  $\alpha = 0.05$  between the treatment levels for each genotype, it was found that all lines except NC11546-14 and LA03136E71 had a significant increase in yield associated with imidacloprid treatment. Both NC11546-14 and LA03136E71 contain resistance loci, and these results may indicate that there is no

increased protection against Hessian fly infestation when applying a high rate of imidacloprid seed treatment on resistant or tolerant lines.

NC11546-14 contains the resistance locus *H13* that is a well characterized and heavily utilized Hessian fly resistance locus, which functions on the gene-for-gene principal and produces incompatible reactions with Hessian flies of a specific biotype (Gill et al., 1987; Joshi, 2018; Liu et al., 2005). There was an exceptionally low level of infestation on NC11546-14 in comparison to all other lines, and there was no significant reduction in infestation from applying a two times rate of imidacloprid. This is expected of a line containing a resistance locus like *H13* that produces an incompatible reaction with specific biotypes. These results support that *H13* is still durable in North Carolina, and that applying insecticidal seed treatment to lines, which contain resistance loci, like *H13*, does not provide any level of extra protection.

LA03136E71 contains a partial resistance and/or tolerance locus (*QHft.nc-7D*), which has been observed to have



a large effect on reduction in infestation (Winn et al., 2021). The evidence presented in the current work supports that LA03136E71 did not experience a significant yield boost from seed treatment application. However, we showed that when treated with a two-times rate of imidacloprid, that infestation severity (in terms of number of larvae/pupae per tiller) is significantly reduced for LA03136E71, but not NC11546-14. This further supports that the partial resistance and/or tolerance granted by *QHft.nc-7D* is not akin to the antibiosis incompatible reactions of resistance loci like *H13*.

There was a significant reduction in the number of immature Hessian fly on the treated plots of LA03136E71, yet there was no significant increase in yield for the treated plots. Therefore, even though there were significantly more immature Hessian fly surviving on LA03136E71 when left untreated, they did not reduce the yield potential of the line. This phenomenon of a line being infested yet still managing to thrive is often classified as “tolerance,” and because we observed a reduction in infestation for LA03136E71, yet no reduction in yield, this may imply that the partial resistance imparted by *QHft.nc-7D* may be akin to tolerance (Gallun, 1972).

Thus, the cost of infestation for lines, which contain loci like *QHft.nc-7D*, is marginal at best. Through these results, we suggest that the use of partial-resistance loci, like *QHft.nc-7D*, may be sufficient to adequately protect wheat from yield loss related to Hessian fly infestation. In addition, using a locus like *QHft.nc-7D*, which does not result in the death of the pest, may reduce the risk for the “boom-and-bust” cycle previously seen with other antibiosis-based resistance loci (Cambron et al., 2010; Shukle et al., 2016; Thompson & Burdon, 1992).

## AUTHOR CONTRIBUTIONS

**Zachary J. Winn:** Conceptualization; data curation; formal analysis; investigation; methodology; project administration; software; writing—original draft. **Dominic Reisig:** Conceptualization; supervision; writing—review and editing. **Joseph P. Murphy:** Conceptualization; data curation; funding acquisition; methodology; project administration; supervision; writing—review and editing.

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## CONFLICT OF INTEREST STATEMENT

The authors claim no conflicts of interests.

## DATA AVAILABILITY STATEMENT

All data and associated code may be found at <https://github.com/zjwinn/Yield-Protection-Afforded-By-Imidacloprid-During-HF-Infestation-In-Six-Backgrounds>.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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