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ORIGINAL ARTICLE

Mapping of a novel major effect Hessian fly field partial-resistance locus in southern soft red winter wheat line LA03136E71

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Abstract

Key message **Hessian fly resistance has centralized around resistance loci that are biotype specific. We show that field resistance is evident and controlled by a single locus on chromosome 7D.**

Abstract Hessian flies (*Mayetiola destructor* Say) infest and feed upon wheat (*Triticum aestivum* L) resulting in significant yield loss. Genetically resistant cultivars are the most effective method of Hessian fly management. Wheat breeders in the southern USA have observed cultivars exhibiting a "field resistance" to Hessian fly that is not detectable by greenhouse assay. The resistant breeding line "LA03136E71" and susceptible cultivar "Shirley" were crossed to develop a population of 200 random $F_{4:5}$ lines using single seed descent. The population was evaluated in a total of five locations in North Carolina during the 2019, 2020, and 2021 seasons. A subsample of each plot was evaluated for the total number of tillers, number of infested tillers, and total number of larvae/pupae. From these data, the percent infested tillers, number of larvae/pupae per tiller, and the number of larvae/pupae per infested tiller were estimated. In all within and across environment combinations for all traits recorded, the genotype effect was significant ($p < 0.05$). Interval mapping identified a single large effect QTL distally on the short arm of chromosome 7D for all environment-trait combinations. This locus was identified on a chromosome where no other Hessian fly resistance/tolerance QTL has been previously identified. This novel Hessian fly partial-resistance QTL is termed *QHft.nc-7D*. Fine mapping must be conducted in this region to narrow down the causal agents responsible for this trait, and investigation into the mode of action is highly suggested.

Introduction

Hessian fly (*Mayetiola destructor* Say) is an insect pest which feeds upon wheat (*Triticum aestivum* L) causing significant reduction in yield and financial loss. Hessian flies are presumed to have originated in the Fertile Crescent and prefer wheat as their host (Stuart et al. 2012). Once the first-instar larvae of the Hessian fly hatch from their eggs, they migrate down the leaf toward the central whorl where they settle to feed. Plants that are fed upon by larvae are permanently stunted, and primary shoots and tillers that are infested fail to flower (Shukle et al. 2016). Hessian fly infestation in the southern USA can be severe due to the Hessian fly's short life cycle (approximately 28 days) and the ability of Hessian fly to go through multiple life cycles per year in a conducive climate (Schmid et al. 2018).

Hessian flies pose a possible economic disaster for regions with amiable climates. Economic damage to susceptible spring wheat cultivars planted in Oregon during the 2001 and 2002 seasons ranged from 112 to 252 USD per

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hectare, while damage to resistant cultivars did not exceed 15 USD per hectare (Smiley et al. 2004). In Georgia during the 1988–1989 season, Hessian fly caused approximately 20 million USD worth of damage (Buntin et al. 1992). There are several ways to avoid damage from Hessian fly such as late planting (McColloch 1923), use of seed treatment and spray application insecticides (Howell et al. 2017), as well as crop rotation and removal of volunteer wheat prior to planting; however, the most efficient of these integrated pest management strategies is host genetic resistance.

Genetic resistance to Hessian fly mainly follows the gene-for-gene interaction model, where a single resistance (H) gene in the wheat plant corresponds to a single avirulence gene in the Hessian fly (Hatchett and Gallun 1970). The interaction between wheat R genes and Hessian flies is hypothesized to relate to effector proteins secreted by Hessian fly first-instar larvae into the plant cells; it is this interaction that produces a resistance response (Aljbory et al. 2020). This single gene resistance is to the advantage of plant breeders due to the ease with which single gene resistance may be introgressed into elite lines.

There are currently 36 identified loci containing H genes for Hessian fly on wheat chromosomes 1A, 1D, 2B, 3A, 3D, 4D, 5A, 5B, 6A, 6B, and 6D (McIntosh et al. 1998, 2014, 2016). Many of these loci are clustered on the distal, gene rich end of the 1A short arm as well as on 6D and 3D (Stuart et al. 2012). Of the total identified Hessian fly resistance genes, only 12 loci have shown reliable efficacy in the southern USA (Cambron et al. 2010; Shukle et al. 2016), which further punctuates the need for the identification of effective Hessian fly resistance loci for this region.

The "Marquillo" resistance, which has been cited as a form of Hessian fly tolerance with recessive expression, has been hypothesized to reside on either the A or B subgenomes because it originated in "Iumillo" durum (*Triticum durum* L) wheat (Maas III et al. 1987; Painter et al. 1940). Unlike the antibiosis incompatible resistance reactions produced by the named H genes, the Marquillo tolerance does not induce such a reaction. This tolerance phenotype is assumed to be related to the speed of tillering post-infestation (Painter et al. 1940). Other forms of tolerance to Hessian fly infestation, defined by the ability of the plant to prosper post-infestation, have also been identified on 1AL, 1AS, and 3DL, which may indicate that there are more loci in the wheat genome that contribute to Hessian fly tolerance (Hao et al. 2013).

The elite germplasm line "LA03136E71" from the Louisiana State University Small Grains Breeding Program was found to be susceptible to Hessian fly biotypes C, O, and L in controlled environment testing by the USDA-ARS Crop Protection and Pest Control Research Unit in West Lafayette, IN (Murphy and Navarro 2011). Biotype L is the predominant biotype observed in North Carolina, and field observations suggested that LA03136E71 exhibits field resistance. The objectives of this study were to: (i) identify if there are significant differences within the LA03136E71 x Shirley population for Hessian fly for field resistance, (ii) conduct a quantitative trait locus (QTL) analysis to identify which regions of the genome were related to those significant differences, and (iii) identify single nucleotide polymorphic (SNP) markers for use in marker-assisted selection.

Methods

Plant materials

A population of 200 random $F_{4:5}$ lines was developed via single seed descent from a cross between the field resistant "LA03136E71" and susceptible "Shirley" soft red winter wheat genotypes. LA03136E71 is an advanced breeding line with the pedigree: ARLA97-1047–4-2(P2684/ 3 / N7840 // PARULA / VEERY#6)/ LA95125BUB73-1. LA03136E71 does not possess any known major genes for resistance to Hessian fly, and the resistance exhibited in the field is not evident in greenhouse assays.

Shirley (Reg. No. CV-1039, PI 656,753) was developed by the small grains breeding program at Virginia Polytechnic Institute and State University and released as a cultivar for grower use by DynaGro® (Griffey et al. 2010). Shirley was developed from the three-way cross of VA94-52–25 / Coker 9835// VA96-54–234 and has been noted for its resistance to Hessian fly biotype C. Shirley, however, has demonstrated a susceptibility to biotypes B, D, and L, which warrants its use as the susceptible parent for this population.

Experimental design and phenotyping

In 2017, the USDA-ARS Crop Protection and Pest Control Research Unit conducted greenhouse screenings for resistance on the two parental genotypes. Infestations were conducted in greenhouses at two temperature regimes; 16 and 20 °C. Seeds of each parental genotype were planted in separate flats, and each flat was infested with 150 gravid female Hessian fly to achieve a egg density of 12 to 15 eggs per plant. After hatching of the eggs and established feeding of the first larval instar, a binomial response of susceptible (S) or resistant (R) was recorded based on observed Hessian fly infestation symptoms of surviving seedlings. These methods were similar to those found in Cambron et al (2010) and Hao et al (2013).

In the 2018–2019 field season, a single replication of the $F_{4:5}$ lines was planted in an augmented block design with LA03136E71 and Shirley as the repeated checks in Wilson, NC (WIL19). Planting occurred in early October in 2018 to induce an infestation of Hessian fly. Each

genotype was planted in a four row 1.2 by 1.2 m plot with 0.3 m spacing between rows. In mid-December to late January, after Hessian fly infestation was evident, a 0.48 m subsample was dug from each plot in the WIL19 location for further analysis. Within each subsample, the total number of tillers (TT), number of infested tillers (IT), and the number of larve/pupae (NOP) present were measured. Any stem producing three or more leaves was considered a tiller. The percent infested tillers (PIT) were estimated by dividing the number of IT by the TT of a subsample. The number of larve/pupae per tiller (NOPPT) was estimated by dividing the NOP by the TT. The number of larve/pupae per infested tiller (NOPPIT) was estimated by taking the NOP and dividing by the IT.

In the 2019–2020 field season, reminant $F_{4:5}$ seed was planted in a randomized complete block design (RCBD) consisting of two replications in the Cunningham Research Station in Kinston, NC (CUN20) and Caswell Research Station in Kinston, NC (CAS20). Plots were planted in late September to early October. Due to seed limitations, genotypes were planted in a three row 0.9 by 0.9 m plot with 0.3 m spacing between rows. In December to late January, 0.48 m subsamples were dug and analyzed in the same way as the previous field season. A separate location of increase was planted in Kinston, NC, in late October, and $F_{4:6}$ seed was harvested in June of 2020.

In the 2020–2021 field season, $F_{4:6}$ seed of each line was planted in two replications, in a RCBD, at the Research Station in Hugo, NC (HUG21). A single replication of augmented block design was planted at Cunningham Research Station in Kinston, NC (CUN21). The $F_{4:6}$ lines were planted in 1.2 by 1.2 m plots with 0.3 m spacing between rows in late September to early October. All lines in the HUG21 and CUN21 locations were subsampled and measured as previously indicated for the 2018–2019 and 2019–2020 field season.

Genotyping

Five $F_{4:5}$ seeds were planted and tissue samples of each seedling from a single line were bulked. DNA was extracted using sbeadex plant maxi kits (LGC Genomics, Berlin, Germany), and tissue extraction was performed using the manufacturer's protocol. Genotyping-by-sequencing for all 200 $F_{4:5}$ and two parental checks was executed according to Poland et al. (2012) and sequenced on an Illumina HiSeq 2500 (San Diego, California, USA) using the manufacturer's protocol. Reads were aligned to the RefSeqv1.0 assembly via Tassel5GBSv2 pipeline version 5.2.35 using the Burrows-Wheeler aligner version 0.7.12 to call single nucleotide polymorphisms (SNPs) (Glaubitz et al. 2014).

Data analysis and statistical software

All analysis was performed in R statistical software version 4.0.5 (R Core Team 2013). Two tail student's t tests with unequal variances were conducted using all the observations across environments for Shirley and LA03136E71 to test significant differences in TT, IT and NOP between the two parents. Data collected for PIT, NOPPT, and NOPPIT exhibited non-normality due to their zero inflated count nature. To accommodate non-normality, a series of generalized mixed linear models with inverse Gaussian distributions were applied to analyze collected data within and across environments using the package "asreml" in R statistical software (Butler et al. 2009). To analyze data collected within an environment in a RCBD, the following model was used:

$$y = \mu + G_i + R_j + \varepsilon_{ij}$$

where y is the response, μ is the population mean, G is the fixed genotype effect, R is the random replication effect, and ε is the residual error $\varepsilon \sim N(0, \sigma_\varepsilon^2 E)$. For sites planted in augmented design, the following model was used:

$$y = \mu + G_i + B_j + \varepsilon_{ij}$$

where y is the response, μ is the population mean, G is the fixed genotype effect, B is the random column effect, and ε is the residual error $\varepsilon \sim N(0, \sigma_\varepsilon^2 E)$. Best linear unbiased estimations (BLUEs) were drawn using the above models for later use in QTL mapping.

The following multi-environmental model was used to calculate BLUEs and estimate variance parameters:

$$y = \mu + G_i + E_j + Y_k + R_l + GE_{ij} + GY_{ik} + EY_{jk} + GEY_{ijk} + \varepsilon_{ijkl}$$

where y is the response, μ is the population mean, G is the genotype effect and is treated as fixed for calculating BLUEs and random for estimating variance, E is the random environmental effect, Y is the random year effect, R is the random block effect, GE is the random genotype by environment interaction, GY is the random genotype by year interaction, EY is the random environment by year interaction, GEY is the random genotype by environment by year three-way interaction, and ε is the residual error where $\varepsilon \sim N\left(0, \sigma_\varepsilon^2 E\right)$. Per plot-sense heritability was calculated using the following formula:

$$H_{plot} = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GE}^2 + \sigma_{GY}^2 + \sigma_{GEY}^2 + \sigma_\varepsilon^2}$$

where σ_G^2 is the genotypic variance, σ_{GE}^2 is the genotype by environment variance, σ_{GY}^2 is the genotype by year interaction variance, σ_{GEY}^2 is the genotype by environment by year variance, and σ_ε^2 is the error variance. Entry-mean heritability was calculated using the following formula:

$$H_{entry-mean} = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{GE}^2}{e} + \frac{\sigma_{GY}^2}{y} + \frac{\sigma_{GEY}^2}{e*y} + \frac{\sigma_\varepsilon^2}{e*y*r}}$$

where σ_G^2 is the genotypic variance, σ_{GE}^2 is the genotype by environment variance, σ_{GY}^2 is the genotype by year interaction variance, σ_{GEY}^2 is the genotype by environment by year variance, σ_ε^2 is the error variance, e is the number of environments for which the response was recorded, y is the number of years for which the response was recorded, and r is the number of replications for which the response was recorded.

Molecular markers were arranged into linkage groups using the algorithm proposed by Wu et al (2008) in the package "ASMap" in R statistical software (Taylor and Butler 2017). The following parameters were used in the "mstmap()" function: significance threshold of $p = 0.0001$, missing marker data threshold of ten percent, and markers were mapped by chromosome. A second, higher coverage linkage map of chromosome 7D was made for further investigation. The following parameters were used in "mstmap()": significance threshold of $p = 0.0001$ and missing marker data threshold of ten percent. To increase coverage, a total 182 SNP markers on chromosome 7D were considered for inclusion in the map. Candidate markers on 7D were removed if they were significantly ($p \leq 0.000001$) distorted or if they were missing ten percent or more of their data. An algorithm to detect erroneous marker calls and false recombination events was written to curtail map expansion.

Interval mapping was conducted using the package "qtl" in R statistical software (Broman et al. 2003; Jansen 1993). Genotype probabilities were calculated with an error probability of $p = 0.0001$ using a step of two cM, a fixed step width, and the Kosambi function. The following options were used for the function "scanone()" in the "qtl" package: the model was set to normal, Haley-Knott regression was the method of LOD calculation, and LOD thresholds were set using 1000 permutation. The QTL regions were drawn using a Bayesian credible support interval via the function "bayesint()"; all support intervals were drawn using a p value of $p = 0.001$. Marker significance and percent variation accounted for were calculated via the "fitqtl()" function. Genotypes were simulated with 32 draws and an error probability of $p = 0.0001$. All graphics were drawn using the packages "ggplot2" and "psych" in R statistical software (Wickham et al. 2016; Revelle and Revelle 2015).

Results

Phenotypic evaluation

Greenhouse screening of the two parental genotypes against Hessian flies of known biotype revealed total susceptibility to Biotypes O, L, and vH13 for both parents at both temperature regimes (Table 1). For collected field data, in all environments for all traits, the genotype effect was significant ($p < 0.05$). The BLUEs of PIT, NOPPT, and NOPPIT for each environment were best approximated by a Gaussian distribution while still showing some characteristics of a bimodal distribution. The genotype effect was significant ($p < 0.05$) for all traits measured in the multi-environmental analyses. A pairwise Pearson's correlation was performed on the BLUEs derived from the multi-environmental models (Fig. 1). All traits were significantly correlated ($p < 0.05$). Significant differences in the TT, IT, and NOP measured were observed between the parental lines (Table 2).

Per plot-sense and entry-mean heritabilities as well as their standard errors were calculated for all traits in multi-environmental models (Table 3). Per plot-sense heritability ranged from 0.14 for NOPPIT to 0.49 for PIT, indicating that measurements for PIT are more replicable for a single observation of a genotype than NOPPT or NOPPIT in this population. Entry-mean heritability ranged from 0.87 in NOPPT to 0.98 for PIT, indicating that replicability of measurements across genotypes and environments is exceptionally high in this population. Standard errors for all heritability calculations ranged from 0.007 for entry-mean heritability of PIT to 0.034 for per plot-sense heritability of NOPPT.

Parental averages across environments revealed relatively consistent infestation pressures across locations and

Table 1 Table of observed resistance (R) or susceptible (S) responses to greenhouse application of Hessian flies with known biotype at a specific temperature regime

Biotype	Temperature (°C)	Response	LA03136E71	Shirley
Bio O	16	R	0	0
		S	33	34
	20	R	0	0
		S	33	34
Bio L	16	R	0	0
		S	32	30
	20	R	0	0
		S	33	39
vH13	16	R	0	0
		S	28	32
	20	R	0	1
		S	31	25

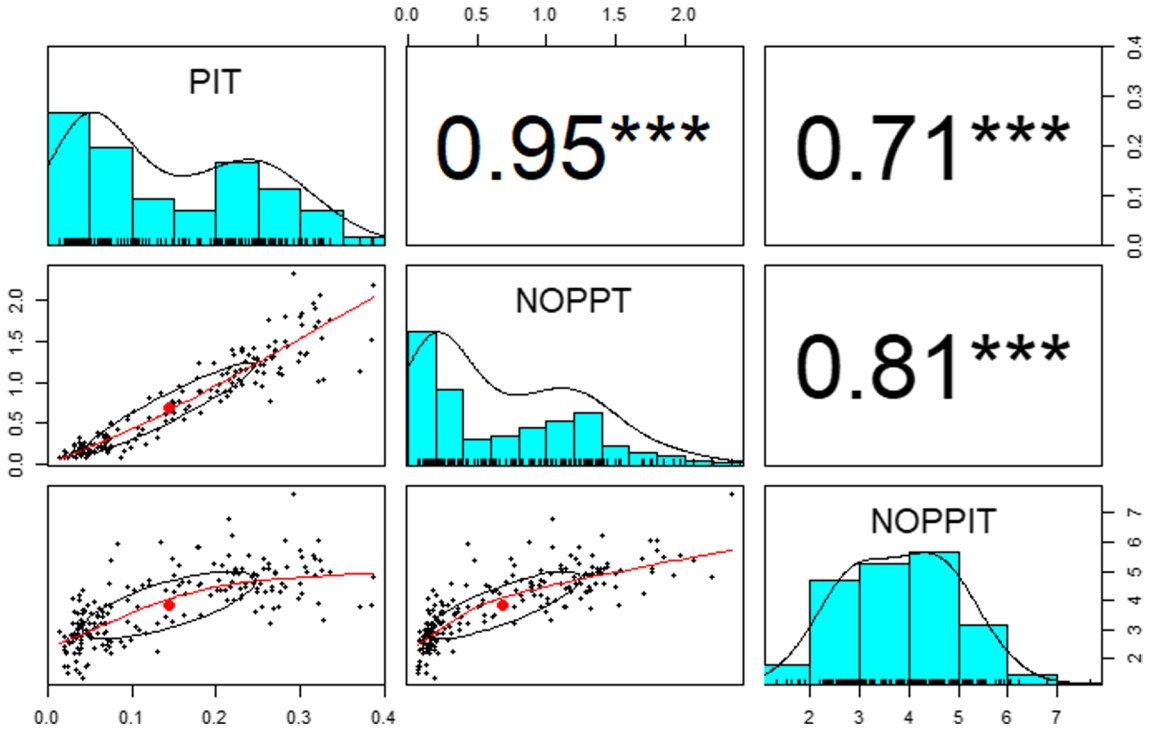


Fig. 1 Correlation study of multi-environment BLUEs. The diagonal of the matrix shows a histogram and density plot of the trait which is labeled within the square. The lower triangle shows a scatterplot of the two traits. The upper triangle of the matrix shows the Pearson's correlation coefficient with p value denoted by the number of asterisks: ∗= .05, ∗∗= 0.01, ∗∗∗= 0.001

Table 2 Table of student's t test statistics for parental values for total tillers, infested tillers, and number of larvae/pupae per tiller

Trait	T value	df	*P* value	$\overline{X}_{Shirley}$	$\overline{X}_{LA03136E71}$	95% Confidence interval
Total tillers	3.2208	136	0.0016	70.76	90.32	7.6–31.6
Infested tillers	− 9.0675	99	< 0.0001	17.9	4.7	(− 16.1) – (− 10.3)
Number of larvae/pupae	− 8.48	102	< 0.0001	68.2	14.1	(− 66.8) – (− 41.5)

Table 3 Table of estimated per plot-sense and entry-mean heritabilities with corresponding standard errors

Trait	$H_{plot\text{-}sense}$		$H_{entry\text{-}mean}$	
	Estimate	SE	Estimate	SE
PIT	0.489	0.03110	0.975	0.00767
NOPPT	0.344	0.03369	0.869	0.02111
NOPPIT	0.140	0.03424	0.912	0.03194

years (Table 4). LA03136E71 averages ranged from 5 to 7% across and within environments for PIT, while Shirley averages ranged from 23 to 30%. The same trend is observed for NOPPT and NOPPIT where LA03136E71 has a narrower distribution in comparison to Shirley. The environments CUN20, CUN21 and HUG21 all appeared to have a lower infestation pressure than the other environments, which is indicated by the generally lower values in NOPPT and NOPPIT for both lines in those respective sites.

Linkage mapping

Prior to filtering, 19,156 genome wide SNPs were aligned to the reference genome. Markers that had a minor allele frequency of 0.1 or less, had a heterozygosity frequency of 0.1 or higher, were significantly distorted ($p \leq 0.0001$), or were missing more than five percent of the marker data were removed. In post-filtering, there were a total of 1,167 high-quality genome wide SNPs remaining. A total of 21 linkage groups were assembled using the "mstmap()" function.

Twenty chromosomes were represented in the preliminary linkage map, measuring a total of 4,790 cM. Chromosome 4A was bifurcated into a short arm group and a long arm group due to a lack of linking markers spanning the centromere. Chromosome 4D did not form a linkage group due to poor coverage and a lack of polymorphism between the parents. Large regions of tightly linked markers were identified on chromosomes 1B, 2B, 3B, and 5B. Removal of distorted markers resolved the large tightly linked region on

Table 4 Table of parental means and corresponding standard errors

Trait	Environment	LA03136E71		Shirley	
		Value	SE	Value	SE
Percent Infested Tiller	Caswell: 2019–2020	7%	0.00031	29%	0.0026
	Cunningham: 2019–2020	5%	0.00053	31%	0.0059
	Cunningham: 2020–2021	4%	0.00022	24%	0.0042
	Hugo: 2020–2021	5%	0.00045	23%	0.0016
	Wilson: 2018–2019	6%	0.00089	30%	0.0028
	Multi-Environment	5%	0.00021	27%	0.0016
Number of Larvae/Pupae per Tiller	Caswell: 2019–2020	0.30	0.00962	2.32	0.2984
	Cunningham: 2019–2020	0.10	0.00218	0.76	0.0247
	Cunningham: 2020–2021	0.05	0.00053	0.47	0.0285
	Hugo: 2020–2021	0.14	0.00346	0.78	0.0199
	Wilson: 2018–2019	0.10	0.00206	1.82	0.2666
	Multi-Environment	0.15	0.00276	1.20	0.1020
Number of Larvae/Pupae per Infested Tiller	Caswell: 2019–2020	4.52	1.64358	8.21	3.2216
	Cunningham: 2019–2020	1.93	0.50940	2.65	0.1424
	Cunningham: 2020–2021	1.25	0.29484	2.10	0.2052
	Hugo: 2020–2021	2.41	0.35979	3.55	0.2554
	Wilson: 2018–2019	1.78	0.31294	6.04	1.3593
	Multi-Environment	2.59	0.47196	4.49	1.0221

2B. The parental line Shirley is positive for the 1RS:1BL rye translocation, which caused large regions of linkage on1B (Brown-Guedira 2015). Shirley has also been shown to contain the stem rust (*Pucciniagraminis tritici*) resistance locus *Sr36* located on the 2G:2B translocation, which causes segregation distortion and preferential transmission of alleles (Brown-Guedira 2015).

Initial investigations with the genome wide preliminary linkage map were conducted to identify QTL contributing to PIT, NOPPT, and NOPPIT. All QTL scans implicated a single, highly significant peak on the distal portion of the short arm of chromosome 7D. Due to this, a second and final linkage map, specific to the 7D chromosome, was made for further investigation. This resulted in a single linkage group of 30 markers measuring approximately 300 cM long with consistent base pair alignment (Fig. 2).

Quantitative trait loci mapping

Interval mapping was conducted using the BLUEs of the within-environment models and the multi-environmental models. For all environments and trait combinations, a single high likelihood-of-odds (LOD) peak was seen only on chromosome 7D (Fig. 3A). Single marker regression analysis was performed using the function "fitqtl()," and flanking markers were identified using "bayesint()" function (Table 5). QTL identified for PIT had the highest LOD score and percent variation accounted for by the peak marker (LOD ≈ 76, %VAR ≈ 86%, multi-environmental model). The trait with the lowest LOD score and percent variation accounted for by the peak position was NOPPIT (LOD ≈ 8.5, %VAR ≈ 18%, CUN20).

The 7D QTL spanned on average 22.6 cM over a physical distance of 11.2 mega-base pairs. The left and right flanking markers identified for support intervals (Table 5) may be different in physical position (denoted by the number attached to the chromosome name) and may not be in order according to physical distance due to differences in recombination frequencies among markers (Fig. 3B). An interval comprised of the most distal marker and the most proximal marker drawn for PIT among and within environments was used to provide sequence information of pertinant SNPs which may be targeted for use as markers in marker-assisted selection (Table 6).

Discussion

The major R genes for Hessian fly act according to antibiosis, where an avirulent pest and a resistant host produce an incompatible reaction that results in the death of the pest (Hatchett and Gallun 1970). However, in the present study, we found live healthy pupae surviving on the resistant line LA03136E71 ($NOPPT : 0 - 0.81, \overline{X}_{NOPPT} \approx 0.15$), which may indicate that antibiosis is not at play in this case. This suggested that instead of resistance, we observed a partial resistance or tolerance like in the cultivar "Marquillo," which is defined by the ability of the plant to recuperate after a Hessian fly infestation (Painter et al. 1940). Live Hessian fly pupae have also been identified on plants exhibiting the

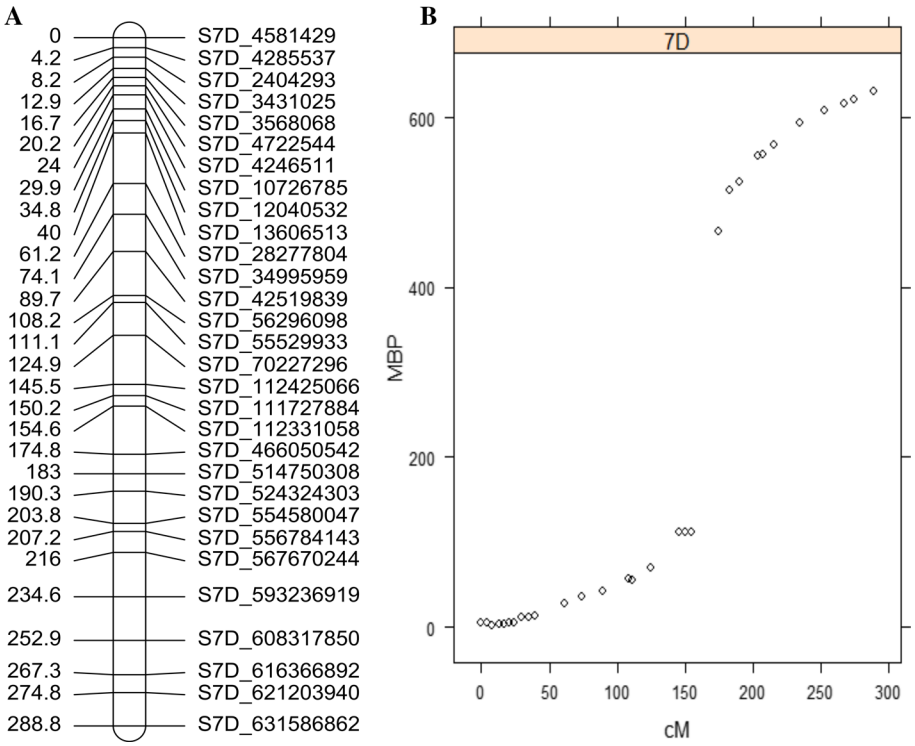


Fig. 2 Map visualizations. **A** a graph depicting the spacing and length of the 7D linkage group with marker names on the right and cM position on the left. **B** a graph showing the position of a marker in cM (X-axis) relative to the markers mega-base pair (BP) position (Y-axis) on the reference genome

Marquillo type tolerance, and the reason for Marquillo's tolerance has been cited as increased tillering post-infestation (Johnson et al. 1984). However, this hypothesis of increased tillers, post-infestation, has yet to be formally tested.

We detected significant difference in the total tillers subsampled between Shirley and LA03136E71. The confidence interval drawn for total tillers indicated that there were between 7.6 and 31.6 more tillers on LA03136E71 in comparison to Shirley. However, the total tillers sampled per plot represents a single observation pulled at a single timepoint post-infestation, and longitudinal samples at specific time intervals post-infestation would be most appropriate to test if this resistance is like Marquillo's tolerance. Furthermore, these samples were derived from Hessian fly damaged plots. Hessian fly are known to cause premature death of heavily infested tillers (Shukle et al. 2016), which may have affected the final count of tillers in susceptible plots.

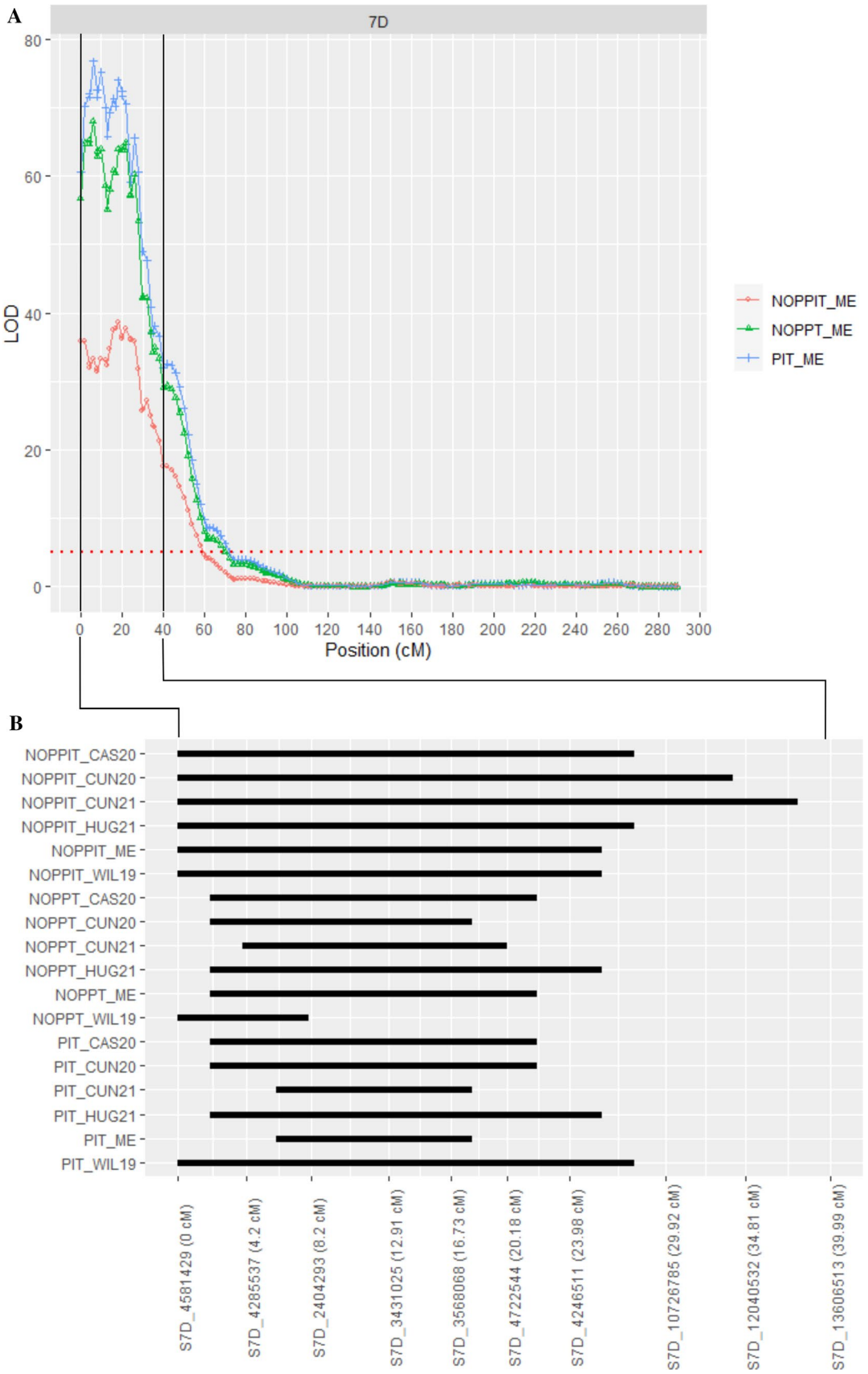
The Marquillo tolerance, *H18*, is thought to reside on any of the chromosomes in the A or B sub-genomes (Maas III et al. 1987; Painter et al. 1940). Because the locus we found resides on chromosome 7D, this new locus is most likely not the same as the one identified in Marquillo. Despite this, increased tillering post-infestation cannot be dismissed as a possible mechanism for this resistance, and further investigation is required.

There are many avenues of defenses, both indirect and direct, that plants utilize to deter unwanted insects (Belete 2018). The most frequently studied of these defense mechanisms for Hessian fly are plant defense proteins. However, because we did not observe dead first-instar larva on the resistant genotype, but rather healthy pupae on the resistant genotype, the resistance we observe may not be a plant defense protein. Considering that live pupae were observed on resistant phenotypes, this may suggest that there is a difference in preference of Hessian fly among the resistant and susceptible genotypes for oviposition, and that the deterrent phenotype is conferred by the LA03136E71 allele of the 7D locus.

The estimated average cM length for the 7D locus and its position on the distal short arm of 7D indicate high recombination frequencies in the region. Consequently, the region may be a candidate for further fine mapping and positional cloning of the causal polymorphism. There are several high confidence gene annotations in the region of the 7D locus; however, delimiting of the region is required before target putative genes may be identified for further analysis (Appels et al. 2018).

In a previous study conducted by Hoa et al. (2013), both H genes and tolerance loci were identified in a cross of the cultivars "AGS 2000" and "Pioneer 26R61." A multinomial response of resistant, susceptible, and tolerant was recorded for each line assayed, and three separate loci were identified for the tolerant category on 1AL, 1AS, and 3DL (Hao et al. 2013). However, beyond the previously identified study, the literature for tolerance to Hessian fly is sparse.

In the current study, we reported one value to measure pest instance (percent infested tillers) and two values to measure pest severity (number of larvae/pupae per tiller


A
7D
LOD
Position (cM)
NOPPIT_ME
NOPPT_ME
PIT_ME
B
NOPPIT_CAS20
NOPPIT_CUN20
NOPPIT_CUN21
NOPPIT_HUG21
NOPPIT_ME
NOPPIT_WIL19
NOPPT_CAS20
NOPPT_CUN20
NOPPT_CUN21
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PIT_CUN20
PIT_CUN21
PIT_HUG21
PIT_ME
PIT_WIL19
S7D_4581429 (0 cM)
S7D_4285537 (4.2 cM)
S7D_2404293 (8.2 cM)
S7D_3431025 (12.91 cM)
S7D_3568068 (16.73 cM)
S7D_4722544 (20.18 cM)
S7D_4246511 (23.98 cM)
S7D_10726785 (29.92 cM)
S7D_12040532 (34.81 cM)
S7D_13606513 (39.99 cM)

◂**Fig. 3** Multiscan plot and Bayesian credible support interval visualization. **A** a multiscan plot of each multi-environmental interval mapping scan on chromosome 7D for PIT, NOPPT, NOPPIT. **B** a visualization of the confidence intervals drawn for the 7D QTL for each environment-trait combination. Marker names are placed on the x-axis in cM order. The Y-axis denotes what bar corresponds to what trait for which environment. Environment-trait combinations labeled with "ME" refer to the multi-environmental interval mapping scan

and number of larvae/pupae per infested tiller). The per plot-sense and entry-mean heritabilities observed indicated that our most consistent measurement of this resistance was percent infested tillers ($H_{\text{plot}} = 0.49$, $H_{\text{entry-mean}} = 0.98$), and therefore, we suggest this method of assessment of incidence for future field trials. The per plot-sense heritabilities observed for the indices of severity indicated that number of larvae/pupae per tiller is better replicated within environments ($H_{\text{plot}} = 0.34$, $H_{\text{entry-mean}} = 0.86$). Furthermore, LOD scores for number of pupae/larvae were higher than NOPPIT, where the lowest LOD score for NOPPT was 25.16 in HUG21 and 8.51 in CUN20 for NOPPIT. Therefore, we suggest NOPPT as the ideal measurement of pest severity.

Biotype L Hessian fly has been identified as the preeminent biotype affecting the southeastern USA, and the *H13* locus derived from *Aegilops tauschii* is effective against this Biotype for now (Ratcliffe et al. 2000). However, the *H13* virulent biotype *v*H13 has been observed in the southeast, indicating that the locus' efficacy is breaking over time due to selection pressure (Cambron et al. 2010). More recently a newly discovered allele of the resistance gene *H13* found in the line "NC09MDD14" shows efficacy in control of *v*H13 biotype (Miranda et al. 2010).

We were unable to report biotype of Hessian fly within our test-sites; however, the most recent biotyping assay performed by the USDA-ARS Crop Protection and Pest Control Research Unit on pupae collected from Wilson county in North Carolina in 2018 indicates that biotype L is the most prevalent (Cambron 2021). Hessian fly biotype specific H genes are known to lose efficacy at temperatures at or above 20 °C (Chen et al. 2014); however, considering that greenhouse screenings conducted at 16 and 20 °C indicated susceptibility to several biotypes, including biotype L, at both temperatures, we hypothesize that this major effect resistance locus is not a race-specific H gene confounded by heightened temperature.

Considering that biotype L is the most frequent biotype in the southeastern USA, and that live pupae were observed on the resistant genotype, we hypothesize that this resistance is not a biotype-specific resistance, but rather a broad partial resistance, resemblant of tolerance, that is non-biotype specific. The boom-and-bust cycle of major H genes is evident with the arise of new virulent biotypes and the defeat of H genes (Shukle et al. 2016). However, this new avenue of partial resistance may prove more durable like in other pest of wheat. We suggest future research on this new Hessian fly partial-resistance locus, which is termed *QHft.nc-7D*, be gaged toward its efficacy versus common insecticide seed treatments, its effect on yield, its biotype specificity, and its mode of action.

Availability of data

All data may be found at < https://github.com/zjwinn/Mapping-of-a-Novel-Major-Effect-Hessian-Fly-Partial-Resistance-Locus-in-Southern-SRWW-LA03136E71 > .

Table 5 Table of QTL Locations, LOD scores, percent variation, and estimated effects with resultant standard errors. Estimated effects are in reference to the allele carried by the resistant line LA03136E71

Trait	Environment	Distal flanking marker	Approximate peak marker	Proximal flanking marker	Length (cM)	Peak LOD	Percent variation	Estimated effect	SE
Percent Infested Tillers	Caswell: 2019–2020	S7D_4581429	S7D_4285537	S7D_4722544	20.18	40.2	60.9%	− 11.5%	0.00661
	Cunningham: 2019–2020	S7D_4581429	S7D_3568068	S7D_4722544	20.18	39.25	60.1%	− 11.8%	0.00693
	Cunningham: 2020–2021	S7D_4285537	S7D_2404293	S7D_3568068	12.53	50.74	71.0%	− 10.8%	0.00522
	Hugo: 2020–2021	S7D_4581429	S7D_2404293	S7D_4246511	23.98	30.59	51.1%	− 6.0%	0.00422
	Wilson: 2018–2019	S7D_4581429	S7D_4285537	S7D_10726785	29.92	36.17	60.8%	− 9.5%	0.00584
	Multi− Environment	S7D_4285537	S7D_4285537	S7D_3568068	12.53	76.41	83.2%	− 9.7%	0.00323
Number of Larvae/ Pupae Per Tiller	Caswell: 2019–2020	S7D_4581429	S7D_4722544	S7D_4722544	20.18	38.85	59.7%	− 0.99	0.05839
	Cunningham: 2019–2020	S7D_4581429	S7D_2404293	S7D_3568068	16.73	42.45	62.9%	− 0.34	0.01914
	Cunningham: 2020–2021	S7D_4285537	S7D_4285537	S7D_4722544	15.99	45.15	66.7%	− 0.26	0.01379
	Hugo: 2020–2021	S7D_4581429	S7D_2404293	S7D_4246511	23.98	25.16	44.5%	− 0.22	0.01780
	Wilson: 2018–2019	S7D_4581429	S7D_4581429	S7D_2404293	8.2	38.43	63.0%	− 0.65	0.03801
	Multi-Environment	S7D_4581429	S7D_4285537	S7D_4722544	20.18	66.59	78.9%	− 0.52	0.01995
Number of Larvae/ Pupae Per Infested Tiller	Caswell: 2019–2020	S7D_4581429	S7D_3568068	S7D_10726785	29.92	14.82	29.3%	− 1.40	0.16598
	Cunningham: 2019–2020	S7D_4581429	S7D_4246511	S7D_12040532	34.81	8.51	18.0%	− 0.44	0.06937
	Cunningham: 2020–2021	S7D_4581429	S7D_4246511	S7D_13606513	39.99	10.02	21.7%	− 0.53	0.07686
	Hugo: 2020–2021	S7D_4581429	S7D_3568068	S7D_10726785	29.92	11.42	23.4%	− 0.64	0.08447
	Wilson: 2018–2019	S7D_4581429	S7D_4581429	S7D_4246511	23.98	28.17	51.8%	− 1.89	0.13706
	Multi-Environment	S7D_4581429	S7D_3568068	S7D_4246511	23.98	38.28	59.1%	− 0.91	0.05636

Table 6 Sequences of SNP markers in and around the 7D QTL region with 200 base pair flanking sequences. The polymorphic site is flanked by brackets and the single base pair substitution is denoted by a backslash

Marker	Sequence
7D_443294	ACTTTAAAGGTGTAATCCCTAGAGTAGAGGCCCGCTGCACGGGCTCTATAACACTGGAAGTGGTCTTCGGATCCC CGGATAATTTCCGGAGCGAAGAGTTAATCTTCGATATCGTCCCTTTTTGTAGTGGTTATCACGCACTGCTCGGAC GAACCGCATTCGCTACATTGAATGCGGTACCACATTATGCATACCTCAAG[C/T]TCAAGATGCCAGGTCCGCGCG GGGTCATAACAGTCAACGGAAACATGGACCGCTCTCTCCGTACAGAAGAGCATACTGCAACCCTCTCCGCAGAAG TACAATGCGACCTCTTCCGCCAGGCCATCAATTCGGCGACGACAACCCCAAGCACCGTCAAGCGCGTCCGGGGCA CCCTGCGATAGGATCATCAGGAACGCCAA
7D_4285537	CACCACCCGCTTCATGACATCCTTCGGTTGAGAATACAACAACTCGTCATGGCAGAACTGGGTCCGGAGCGCCCA ATCGAGCACCTTGGCACGTAGAAGTACACCGTCAGTTGTTTGTTGCCACCGTTGGGGGCAGCGTGGACGAGGAGG CCAAAGGGGACAAGCGCACCATTGGCTGAGGCCCGTTGCTTACTGCAGTT[A/C]TAGTTGACGTCGGTCTCATTG ATGATGGCAATAGCCAATAGCGGAAGTGTCTACCGAAAGGAGGCCCCGATGTGGAACTGATTGGCTTGGCAGAGA CACAGAGATGCGATGGATCCATTGAAGTCGGTGTTATTGAAGCGGAGGGTCTCATTCTCCTCCACCGGCCATTGG AGGAGGTTCGTCTGGGTCTTCTCGTCCAA
7D_2404293	GACGAGCCATGCATTAATTTTCATGACCTCGAAATCTGGTTCTCCAACATGTACGTGTTCCTACGTATACAAACTGGG CTCCAGCAAGTGACTGCGGCAGTAACATAACATTTACTCTGGCCAGCCGATGCATAGACATGCAGTTGTCCGTGT GTGAACGATCGGGACGACGTGCATTCATATTCTCATCTGTCTGCAGC[G/A]GTGTTATAAGAGTAACTCCAATGG GGCGACCCATTTCGCCCGTTTGGGTTGGCACGGATTTAAAAGGTGCCCCAATACGTTGACCCAAATGGGCGCGTC CGCGGGCGACCCATTTTCGGCCTCATTTTTTAGCTGGATTCGCGTCGGCGCAGAAACAAGACGGACGCGCGCGCG CCTACTCCTCTCCCCGAGCCCGCCGG
7D_3431025	GCCCCTTTGGATCACCATCCCATCTAACCAGTTCAATCGACAGGATCTCCCCGGCTAACTCAGACACAAAATCACTAT TCTTCCAAGCTTTGCGGATGCACTCGGGCACTCCCTCCAATTCTGAAAGCTGGCCTGCCTCCCAGGGTAGTACAG CGATGGTCTCACTCAATTTCAAAGTCTTCAGCTTCTTCAGCCTCCCA[A/G]TTTCTCTTGGTAGCTTTACCACCCCCG TAGCACTCAAGAGCAGTTGTTCCAGATTCTGCAGATTCCCAATCTCACGAGGCAGTCCTGTGAGCCCGCGGCTGT AAATCAGGTCAAGAGTCTCCAAATTCTGGAGGTTCCCCATCTCACGAGGCAGCTCTGTGATCCATGTCCAACACG CCTTGAGTGTTTTCAAACGCTGC
7D_3433772	AATGCCTCACATAGGTTGACTATATGATGGTCAGTGAAGTCAATATGCGGACGAGAGAGGGAGGACTCTTGTCGG AGTACTCCTTGGCTTACCATTGCCACCGTGGCCAAGTGCGGCCACAACCGCACTACCACAGCAGGGCTGCTCGTG ATGCAGTACGGCTTCAGTAGTGCCACAAGGAGGCTGCAGCAGACCAGACG[C/T]TCCCAAGACATCTACAAGCAT CGGCTCCAGCGGCGGAGCTATGTACAACGTTGGGGCCATAGTGTGAAGAAAATTTGGAGGAGAGGGCTTAGATGA GATTTTTTTTGGCATTTTGCACCCCAAAACACTCATGTTTTAGTGTTGGCCCCCCAGCATTTTCTACCTAGCTCAACC GGTGATGTGCTCCAAAGTTCAAATAT
7D_3842577	GCAACGGCGCCCATTTCGTCCGGTGCCATGGACCCTGATTTAAGCGATGCCCCACGACATGTTCACCGGCCCGAG CCTCCCCTCTCTGCCCTCTCCTTCGCTACATCCCCCATTCCCCCTCAGTCCTTTCCTCTGCTATCTGCAGGCGTGTGC TGCTGCCTGCTGCTCCGCGTGCGTGCGGCGTGCTTAGATTAGTGGGC[G/A]TTGGCTGACGTACGAGCGCGACGG AGAGAGGGCCAGAGGAGAAGCGATTTCAGGCCACCGACCAAAGTACGTGCAGACGGGGAGAGGGCCGCCGTG CGGGACGAGGCAGCTTCGTTGTCGCTGGGATGCATGGGAGGAAGATCTTGGAGCAGCGACCGGAGGCCGCTCAAC GCAGAGTGGTCGGCCATGTGCGACTGAGG
7D_4722544	AGAACTAGCACCATTTCCAACTATCCAGGAGACAAGGGATCAATGTTTCTTATATTTAAGCGTTCGCGAGTGGGCCGG CCTGTTACTATAGGTGCTTCACGTCAGAGATCCTTCGGTCTTGCGTGAAGCGAGATATAACCTTCGCGGGCGACC TCGACACCAGCTTTTTCTCGTTTGCTAAAAAAATAAAACCAGCTTTC[A/C]CTCAAAGAAATGAAAGATATGATA ACCAGCTGAAAGTGGAGCTTGCATGTCGTCCACAGACATGCATACGCATGCTGCAGACACATGCCACGCTACAAT TAATCAAGTCGATCGATGGCACTAGGAAGGGAATCAAATTTCAGCTAAAAGTAGTGGAGGATGCACACATGCATG TGCCGGCGCGGCGGCGCCGCGAAAGT
7D_4246511	TGATGGGCCTTTGGTCCCGGTTCGTGTTAAACCGGTACTAAAGGAGGGGACTTTTAGTCCCCATTACAAACCGGTGAT AAAACTCTGTTTTCTACTAGTGGATGGATCGATGCAGGAGGGAGGATCCTGCAGCTGAACGCGTGGGTGATGGCC ACAGCAACGACGGAGGTTGATTTGTAGTACTCCTTGGCTGCGTGCCT[G/A]CCTACCTGTCCACGATCGAGGAGG TACGCCTTGCCAGGGAGTGTGGTGAAGCCGAGATTATTTGGAGGTTCGGTCGGCAAACTCCTCCGGCCACTCCAA CTCTCCGGCATACAATCGTGCTAATTCAAACAACATATTACAAGGGAAAACAAATGGAAGTGTTATGACTGTGCG CGGTGATTTACTATGTTCTGGCTTCG
7D_5673004	GAGTACTATTGCGCCGAGTTCGAGACGAGGTCTTCTACTGGAGGCGGTTTATCTACCACGTTCTCTGAGGCCGAC AAAAGTTCAGGTGATCCGTCCGACCGCAGGTTCCGCCGAGTTGTCGTAACCACGGCTCTTGGCCCGTTTTACCCG GTGGAAAATCCCTAAAGGCTGCAGCCGTCCTGGCTGTTCCGACGCGTTGC[A/T]ATTGCTGCTCCGTACGCAGCG CCCTGCCGCTCACAAGAGAGTTAAGTCCCGAAGCAAGCATTGATCTACCGCTGCTGCTTTATGTGTGAAGACCAA AGTCAAATCATCAGTACCTACTACGTACAAGGTATATCACCACCATCACCTTGAGCCGCCTCTCCTTGCTGTGTC GCAAAGGCATTTTCAGCTGTAGGCAAGCC

Table 6 (continued)

Marker	Sequence
7D_7559557	GCCGGGGCAATGGAGGGATCCGGTGCCAAACTACGAAAAAGTGAATTTGGCACCTCTTTTGGCCTACTAAGTGGA GGGCCTAAAGGACAGCTCGGTCAGGGAACGGATCTGACCTGATCGTGTCCATCCTTAATACTGACCCATAATGGT CCCGTCATCCTACTTTAACTACACAAAATGGAGTGGTTGATGAGAGTTAG[G/C]TCATCAATTAGGTTGACGAGC TGAAGATGGCAGGACTCGGTGCTAAGCAAGTATGTCTTCTTCCGTGCAGGCCGGCAGAGGGTGGTTGATGAGGGG AGCTTATCGATCAGTTAAATCTAATTGAAAAGTTGGCAGGATGTGTAGCTAAGAGACGAGGTAGCAGGTTGGAAG GGATTGGATTTAGAATATACATCGCCATC
7D_8071275	TCATACAATGTAGCTTGAGGCGAGTCGTCCATTTGTTTCTGCACATCCATGAGTTTCTGCTGCCCGAGTCGCACTATT TTTCGGGCCTTAGTGAAATACTCTGGGTACTCGGCTTTCGGTGCTATTCCTGTAGTGGATACACTACATGTAGTCTCT ACTCAACTCTCAAGGGGCATTTTCAATCCAGTAGTTCACTGCAG[C/G]TAGCTGTAGGGGTCTCAGGTTTGTAGT AAAGTGCATTTGCATCAATTAATTAGTTACTGCTATAGTGTAGTACCGTCCCAACAAATGGGGGTATTGGGTCGA TCCGACCATAACCACCATGAACATGAATACAGGAAATCCGGCAAGCAAATGGGGGAAAAGAAATGAAATGGCAAA CAGGTGCACATAAAAATCATAGA

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Code availability All code may be found at < https://github.com/zjwinn/Mapping-of-a-Novel-Major-Effect-Hessian-Fly-Partial-Resistance-Locus-in-Southern-SRWW-LA03136E71 > .

Declarations

Conflicts of interest The authors declare that they do not have any conflict of interest.

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