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Identifying novel sources of resistance to wheat stem sawfly in five wild wheat species

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Abstract

BACKGROUND: The wheat stem sawfly (WSS, *Cephus cinctus*) is a major pest of wheat (*Triticum aestivum*) and can cause significant yield losses. WSS damage results from stem boring and/or cutting, leading to the lodging of wheat plants. Although solid-stem wheat genotypes can effectively reduce larval survival, they may have lower yields than hollow-stem genotypes and show inconsistent solidness expression. Because of limited resistance sources to WSS, evaluating diverse wheat germplasm for novel resistance genes is crucial. We evaluated 91 accessions across five wild wheat species (*Triticum monococcum*, *T. urartu*, *T. turgidum*, *T. timopheevii*, and *Aegilops tauschii*) and common wheat cultivars (*T. aestivum*) for antixenosis (host selection) and antibiosis (host suitability) to WSS. Host selection was measured as the number of eggs after adult oviposition, and host suitability was determined by examining the presence or absence of larval infestation within the stem. The plants were grown in the greenhouse and brought to the field for WSS infestation. In addition, a phylogenetic analysis was performed to determine the relationship between the WSS traits and phylogenetic clustering.

RESULTS: Overall, *Ae. tauschii*, *T. turgidum* and *T. urartu* had lower egg counts and larval infestation than *T. monococcum*, and *T. timopheevii*. *T. monococcum*, *T. timopheevii*, *T. turgidum*, and *T. urartu* had lower larval weights compared with *T. aestivum*.

CONCLUSION: This study shows that wild relatives of wheat could be a valuable source of alleles for enhancing resistance to WSS and identifies specific germplasm resources that may be useful for breeding.

Supporting information may be found in the online version of this article.

Keywords: host plant resistance; *Cephus cinctus*; genetic diversity; phylogenetic analysis; hollow stem

1 INTRODUCTION

Wheat stem sawfly (WSS), *Cephus cinctus* (Hymenoptera: Cephidae) is a native, grass-feeding insect and one of the most important insect pests of wheat in North America.[1–3] WSS has a univoltine life cycle, with adults that emerge in spring and lay eggs within host stems.[4] The entire larval development occurs within the stem as the larvae consume parenchyma tissue during the growing season, which can lead to yield reductions.[5] To complete development and survive the winter, WSS larvae create an overwintering chamber by cutting the stem near the root crown of the wheat and, in the process, cause wheat heads to lodge and fall to the ground.[4] The majority of the yield loss is attributed to stem cutting because lodged wheat is difficult to harvest.[6] Historically, areas of high WSS damage included Canada and the Northern Great Plains of the United States. However, since the late 2000s, WSS infestation has expanded south into Wyoming, Nebraska, and Colorado. Interestingly, WSS was first observed on native grass in Colorado in 1872, yet damage caused by the pest was not reported in cultivated wheat until 2010,[2] suggesting that southern outbreaks are likely caused by local host range shifts rather than movement from previously infested areas.

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Damage caused by WSS can be financially devastating to producers in affected areas. Widespread infestation (levels >80%) across the six most severely affected counties of Colorado involves roughly 303,500 ha of winter wheat.[7] In these fields, yield losses range from 20% to 50%, resulting in annual economic losses estimated at 40 million USD in 2022.[8] Sawfly incidence and infestation severity are predicted to rise in areas where it is not yet a severe problem and to move further eastward in Kansas. Producers face several challenges caused by WSS such as crop residue losses, soil organic matter depletion, and yield losses. These issues are particularly severe in crops following winter wheat in diversified crop rotations, and can have equally damaging effects.[1] To address these challenges several strategies have been proposed to manage WSS.

Among the adopted integrated pest management strategies for WSS, genetic resistance is regarded as one of the most effective management methods.[1] The most widely deployed form of genetic resistance to WSS is in the form of 'stem solidness'. The primary source of stem solidness in common wheat is under the genetic control of a quantitative trait locus (QTL) on chromosome 3B (*Qss.msub-3BL*),[9] originally derived from the Portuguese wheat landrace 'S-615'.[10] However, solid-stem expression is highly variable in common wheat and does not provide consistent control.[11]

Although new sources of resistance have been identified on chromosomes 2A, 3A and 5B (larval mortality),[12] 2D (decreased oviposition), and 4A (host plant attractiveness to females),[13] their effectiveness and durability have yet to be demonstrated in large populations. Given the limited sources of genetic resistance to WSS in common wheat germplasm, there is a need to evaluate more diverse wheat genetic resources, including close and distant wild relatives of wheat, for additional alleles that can enhance the development of WSS-resistant genotypes.

Hexaploid bread wheat (AABBDD; $2n = 6x = 42$) is an organism that contains three sub-genomes. Modern-day *T. aestivum* is the result of a hybridization event between *T. turgidum* ssp. *dicoccum* (emmer wheat; AABB; $2n = 4x = 28$) and *Aegilops tauschii* (DD; $2n = 2x = 14$).[14] The genetic bottleneck events caused by the polyploidization and domestication of hexaploid wheat have led to a significant loss of diversity in the hexaploid wheat genome. However, diverse germplasm of progenitor species is available for testing, which may provide a novel source of variation for genetic pest resistance that can be utilized through wide hybridization.

Although domesticated emmer wheat and *Ae. tauschii* are direct progenitors of the current hexaploid wheat genome, there are other *Triticum* species that share a similar genomic structure. Some other potential sources of genetic variation for the A subgenome can be found in *T. monococcum* (einkorn wheat; AA; $2n = 2x = 14$), *T. urartu* (AA; $2n = 2x = 14$), *T. turgidum* subsp. *dicoccoides* (wild emmer wheat; AABB; $2n = 4x = 28$), *T. urartu* (A_uA_u; $2n = 2x = 14$) and *T. timopheevii* (A_uA_uGG; $2n = 4x = 28$).[15]

Resistance to insect pest species has been identified in several wild wheat species. For instance, several studies demonstrated that *T. monococcum*,[16–19] *Triticum urartu*[17] and *T. turgidum*[20] accessions also showed resistance to bird cherry oat aphid (*Rhopalosiphum padi*). *Triticum timopheevii* accessions have exhibited resistance to both Russian wheat aphid (*Diuraphis noxia*)[21] and Hessian fly (*Mayetiola destructor*).[22] *Aegilops tauschii* accessions have provided resistance to Hessian fly[23,24] and greenbug (*Schizaphis graminum*).[25,26] Taken together, these studies suggest that common wheat progenitors and close relatives could serve as a potential source of novel genetic resistance to insect pests.

In this study, we examined host selection (antixenosis) and host suitability (antibiosis) of a collection of accessions of five wild relatives of wheat, *T. monococcum*, *T. urartu*, *T. turgidum* subsp. *dicoccoides*, *T. timopheevii* subsp. *armeniacum*, and *Ae. tauschii* held in the Wheat Genetic Resource Center (WGRC) at Kansas State University. Our goal was to evaluate host selection by quantifying the number of eggs deposited in stems following adult WSS oviposition in the field. We also assessed host survival by determining larval infestation (presence or absence) within the stems and measuring larval weight.

2 MATERIALS AND METHODS

2.1 Plant source and maintenance

In 2020 and 2021, host selection and suitability tests were performed on wild wheat species and cultivated wheat by growing plants in the greenhouse and exposing them to natural WSS infestation in the field. Because of limited seed availability and low germination rates, the plants were grown in cone-tainers in the greenhouse and then transported to the field for WSS natural infestation. Many of these species are not well-adapted to the climate conditions in Colorado. Cone-tainers (Stuewe & Sons, Tangent, OR, USA, SC10U UV-stabilized cones: 3.8 cm diameter × 20.9 cm depth, 164 mL volume) are ideal for increasing the number of replicates in limited space of the greenhouse. They have excellent drainage and are easy to bottom water, which helps minimize pathogens on the leaf surface. In addition, they are taller than other pots, providing ample room for the deeper root systems of some wild wheat species such as *Ae. tauschii*.[27]

In 2020, we compared 60 wild wheat accessions, and in 2021, we screened 49 accessions provided by the WGRC at Kansas State University (Supporting Information, Table S1). In both years, we also planted two winter wheat cultivars, 'Fortify SF' (semi-solid, resistant; PI 695152) and 'Avery' (hollow, susceptible; PI 676977).[28] In 2021 we also planted KS Ahearn (PI 701142) and KS Hamilton (PI 699003).[29] The WGRC expressed interest in using two Kansas cultivars to create synthetic hexaploids in the future. As a result, we included two cultivars from Kansas in our study. Only 18 accessions were common in both years.

The experimental protocol was similar to that used by Peirce *et al.*[30] Seeds were either hand-threshed or threshed using a custom single-head thresher and then germinated on 5 × 10 mm germination blotter paper (Anchor Paper Co., St. Paul, MN, USA) with 5 mL of Dividend XL RTA Seed Treatment (Syngenta, Greensboro, NC, USA) mixed in a 10 ppm aqueous solution to prevent fungal growth. Seeds were kept at an average temperature of 24 °C for 3 days. Germinated seeds were then vernalized at 4 °C for 7 weeks. After vernalization, a single seedling was planted in a cone-tainer with a soil mixture of seven parts soil (20B/30 V, Lambert, Québec, Canada), two parts perlite, and a cotton ball at the bottom to prevent soil loss.

Plants were grown in a greenhouse at Colorado State University with supplemental light [430 W high-pressure sodium fixtures (PL Light Systems, Beamsville, ON, Canada); bulbs, GE Lucalox lu400 series, 400 W]. The greenhouse had a 16:8 h light/dark photoperiod with supplemental light when necessary and a day/night temperature of 23:18 °C. Plastic trays were placed under racks of 42 cone-tainers, and all trays were bottom-watered as needed. Germination and planting dates were staggered over 3 weeks in both years to synchronize plant phenology/maturity and peak WSS adult flight. In each cone-tainer tray, all accessions and wheat genotypes were randomized, and trays were placed randomly in the greenhouse. Winter wheat checks were replicated in every rack and the wild wheat species accessions were replicated 5–10 times each week. The number of wild wheat replicates was dependent on germination rates. Plants were fertilized once a week, beginning 1 week after initial planting, with 300 mL of 15-16-17 Peters General Purpose Fertilizer (JR Peters, Allentown, PA, USA) at 296 ppm in an aqueous solution.

2.2 Wheat stem sawfly infestation and data collection

Once plants reached Zadok's growth stage 32–75,[31] they were transported to the field for WSS infestation. Typically, Zadok's 32 (when two to three nodes were visible) is considered the appropriate stage for WSS oviposition[32]; however, we also had plants at Zadok's 75 (ripening of kernels) stage because of differences in the maturity of all the species and accessions. We scouted for WSS weekly before bringing the plants to the field. Typically, peak WSS flight occurs around the end of May–early June in eastern Colorado.[2] Plants were brought out on calm sunny days, conditions favorable for adult sawfly activity. We performed 100 sweeps using 180° pendulum sweeps with a standard 38-cm diameter sweep net to confirm WSS adult population pressure in the field. The trays were placed in the midst of stubble from the previous WSS-infested crop and bordering growing wheat crop near Orchard, Colorado (104.0609900° W, 40.4659970° N). Trays were placed 10 m apart with 5 m within rows. The number of adult sawflies varied across infestation dates. In 2020, we collected 145 sawflies on 22 May, 54 sawflies on 29 May, and 4 sawflies on 5 June. In 2021, we collected 127 sawflies on 25 May, 506 on 1 June, and 318 on 2 June.

In 2020, cone-tainer trays from each single planting date were placed in the field for 2–4 h, whereas in 2021, the trays were placed in the field for 4 h. Exposure or infestation times were higher when WSS pressure was low and were included in the statistical analysis as a covariate or random effect, depending on the analysis used. Plants in each round of exposure were from the same planting date. All field exposures started at 9:00 am. Adult WSS were removed from the plants using a sweep net or aspirator before they were returned to the greenhouse. After removing females, the plants were left outside the greenhouse for another 2–3 h. We then checked to ensure that no females were brought inside.

Within 48 h of exposure, half of the plants were dissected to examine stems for the number of eggs oviposited; the other half remained in the greenhouse to allow larvae to mature. While examining stems for eggs, stem diameter was recorded using a digital caliper (Pittsburgh, Model 47257, Harbor Freight Tools, Camarillo, CA, USA) by measuring the first visible node's width, the stem's maturity on the Zadok's scale, and the number of eggs present. In 2021 we also examined stems for solidness. Evaluations were made by bisecting (lengthwise) each of the stems and scoring the internodes on a scale of 1–5 (1 = hollow, 5 = solid). Values for any internodes present up to five internodes from a stem were then added to give a total score for each stem (5 = hollow, 25 = solid). The score was standardized to a five-internode number if fewer than five internodes were present. Median Zadok's growth stages, stem diameters and solid-stem ratings are provided in Supporting Information, Tables S2 and S3 and Supporting Information, Figs S1, S2, and S3.

Thirty days after infestation, the remaining stems were dissected to assess WSS presence. We considered a stem as infested if there were frass and/or larvae present in the stem. If larvae were present, they were weighed using a (Sartorius, Goettingen, Germany) scale (model CPA2245). We recorded the Zadok's growth stage of the stem during dissections. In 2021, we measured the stem diameter of every dissected stem. However, stem diameter was not recorded for the 2020 infestation dissections. If multiple stems were in a cone-tainer, we dissected the oldest stem based on the Zadok's growth stage.

2.3 Statistical analysis

The data for host preference and suitability with Zadok's stage of <32 was removed before analysis. Data were analyzed using R (version 4.2.1., 2020; R Core Team, R Foundation for Statistical Computing, Vienna, Austria) and the R packages lme4,[33] ggplot2,[34] and emmeans.[35] Separate analyses were conducted for 2020 and 2021 because of variable infestation pressures. Model assumptions were checked using residual diagnostic plots. Leverage of values was plotted against the Pearson residuals to ensure that small sets of observations did not have an undue influence on the outcome of the models. All multiple comparisons were adjusted using the false discovery rate (FDR) with a significance level of 0.05.[36]

2.3.1 Host selection-number of eggs

We used a linear mixed model (LMM) with the WSS exposure date in the field as a random effect and Zadok's growth stage, accession, and stem diameter as fixed effects to analyze the number of eggs within a stem. For 2021 we also measured stem solidness and added this as a fixed effect to the model. We included year, Zadok's growth stage, stem solidness, and stem diameter in the model because all are known confounding variables for oviposition rates. The Kenward–Roger test was used to calculate *F* values.

To compare among the different wheat species, an LMM was used with WSS exposure date as a random effect and Zadok's growth stage, species, and stem diameter as fixed effects to analyze the number of eggs within a stem. Accession was also included as a random effect because the accessions tested in this study are a sample of the population of all possible accessions of the species.

2.3.2 Host suitability—larval infestation

Larval infestation was classified as a binomial variable, where the response of infested or not infested was based on the presence or absence of frass. This variable is based on the life history of the WSS, where often only one larva per stem can survive to maturity,[4] and was the basis for the collected data. The proportion of infested stems was calculated as the number divided by the total number of stems sampled per accession. These data were analyzed using a generalized linear model (GLM) with a binomial error distribution and a logit link function. In the GLM model, WSS exposure date, Zadok's growth stage, and accession were covariates. In 2021, stem diameter was also a covariate. We did not use a generalized mixed model (GLMM) to analyze larval infestation, because the models failed to converge when treating the exposure date as random.

To compare among the different wheat species, a GLM was used with WSS exposure date as a random effect and Zadok's growth stage, species, and stem diameter as fixed effects to analyze the larval infestation in the stem. Because the model failed to converge, we did not add accession as a covariate.

2.3.3 Host suitability—larval weights

We used a linear model to compare larval weights among wheat species. There was not enough replication to conduct this analysis for accession because larvae could not be weighed if they were desiccated or injured when dissecting the stems. We used species, WSS exposure date and Zadok's maturity as covariates.

2.4 Tissue collection and DNA extraction

Five seedlings of each accession were grown in a germination box until the first true leaf was fully emerged, approximately 2–3 weeks. A composite tissue sample containing tissue from all germinated seedlings was collected and placed in a 96-well tissue collection box. The tissue was then freeze-dried and stored at −80 °C until it was used for DNA extraction. DNA extraction was performed using a BioSprint 96 DNA Plant Kit (Qiagen, Hilden, Germany). Extracted DNA was then quantified in plates using Quant-iT Pico-Green dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). As a quality control measure, one random well per plate was left blank to ensure the plate had not been switched or mislabeled. The quantified DNA samples were genotyped using genotyping-by-sequencing (GBS)[37] using 73-plexing with two technical reps. The prepared GBS libraries were sequenced on a single lane using an Illumina HiSeq 2000 at the McGill University Genome Québec Innovation Centre.

2.5 Single nucleotide polymorphism genotyping

Single nucleotide polymorphism (SNP) calling and genotyping were performed using the TASSEL *5 GBS V2* pipeline[38] after aligning sequence reads to the reference Chinese Spring Wheat Assembly v1.0.[39] Variant Call Format files were filtered to only contain SNPs that aligned to the A genome (1A, 2A, … 7A) and species that align to the A genome (*T. monococcum*, *T. turgidum*, *T. timopheevii*, *T. urartu*). SNPs were further filtered to remove sites with a minor allele frequency of less than 0.05. Similarly, the data were filtered to exclude SNPs that had more than 50% missing data. Lastly, because wheat is a self-pollinated plant and these lines are highly inbred, the heterozygosity of SNPs is assumed to be low. In addition, accessions that were genotyped were from individual plants, which further decreased the heterogeneity that is often part of wild collections. Therefore, SNPs with greater than 10% heterozygosity were discarded. Finally, missing SNP data were imputed using the Beagle algorithm v5.4 using the default parameters and a centimorgan distance of 1 Mbp to 1 cM.[40] After filtration, 15 975 A-genome-wide SNPs remained with 1916 on chromosome 1A; 2535 on 2A; 2566 on 3A; 1580 on 4A; 2728 on 5A; 1780 on 6A; and 2870 on 7A. *Ae. tauschii* accessions were not included in this analysis because they only have the D genome, and all other accessions share the A genome.

2.6 Hierarchical clustering of A genome relatives

Genotypic data were processed in the R statistical programming language (2023; R Core Team). Hierarchical clustering was performed on the imputed genotypic matrix using the R statistical software package ‘stats’. First, a Euclidean distance matrix was calculated using the ‘dist’ function with option of ‘method’ set to ‘Euclidean’. The resulting Euclidean distance matrix was processed using the hierarchical clustering method proposed by Murtagh and Legendre.[41]

We utilized a LMM to compare differences in eggs within species clades, as explained in the earlier statistical analysis section. In addition, we used a GLM to investigate infestation differences. Pairwise comparisons were conducted to identify any significant findings. Packages ‘ggtree’[42] and ‘ggplot2’[34] were used to visualize the output.

3 RESULTS

3.1 Host selection—variation in the number of eggs

Variation in number of eggs per stem was observed among wild wheat accessions and wheat genotypes in Zadok's maturity and stem diameter in both 2020 [Zadok's: $F(2, 577) = 2.18$, $P = 0.034$; stem diameter: $F(1, 577) = 5.2$, $P = 0.022$] and 2021 [Zadok's: $F(6, 601) = 4.41$, $P = 0.026$; stem diameter: $F(1, 601) = 20.82$, df = 1, $P < 0.001$; stem solidness: $F(1, 601) = 7.5$, $P = 0.006$].

Differences among accessions were found in the total number of eggs per stem in 2020 [$F(56, 577) = 1.74$, $P < 0.001$] and 2021 [$F(50, 601) = 5.33$, $P < 0.001$]. Across all accessions, we found an average of 1.39 ± 0.08 (mean ± SE) eggs per stem in 2020 (Fig. 1) and 6.51 ± 0.57 in 2021 (Fig. 2). Accessions were compared using estimated marginal means (emmeans) when the covariates Zadok's maturity and stem diameter were included in a LMM. The lowest emmeans for eggs per stem in 2020 included: *T. monococcum* (TA177, TA721); *T. turgidum* (TA1390); and *Ae. tauschii* (TA1707, TA2374, TA2413) (Supporting Information, Table S4). In 2021, the lowest emmeans for eggs per stem included: *T. turgidum* (TA1171) and *Ae. tauschii* (TA1707, TA2482, TA10113) (Supporting Information, Table S5).

Differences were found in the total number of eggs per stem among wheat species in 2020 [$F(5, 30) = 2.5$, df = 5, $P = 0.049$] and 2021 [$F(5, 28) = 19.03$, $P < 0.001$]. Pairwise comparisons using FDR adjustment found significant differences in eggs within stems among wheat species. In 2020, *Ae. tauschii* had fewer eggs per stem than *T. timopheevii*, but no other pairwise comparisons were significant. In 2021, *Ae. tauschii*, *T. urartu*, *T. turgidum*, and *T. monococcum* had fewer eggs per stem than *T. timopheevii* and *T. aestivum* (Fig. 3). Although there were differences in infestation pressure in 2020 and 2021, *Ae. tauschii* had the lowest number of eggs per stem, whereas *T. aestivum* and *T. timopheevii* had the highest. In both 2020 and 2021, *Ae. tauschii* accessions had a low number of eggs laid within stems. Pairwise comparisons and estimated marginal means are provided in Supporting Information, Fig. S4.

3.2 Host suitability—variation in larval infestation

Variation in larval infestation was observed among accessions in Zadok's maturity in 2020 ($X^2 = 38.72$, df = 4, $P < 0.001$) and for Zadok's maturity and stem diameter in 2021 (Zadok's: $X^2 = 2.70$, df = 4, $P < 0.001$; stem diameter: $X^2 = 44.94$, df = 1, $P \leq 0.001$).

The proportion of infested stems differed among accessions in both years (2020: $X^2 = 170.08$, df = 60, $P < 0.001$; 2021: $X^2 = 191.55$, df = 52, $P < 0.001$). However, no significant pairwise comparisons were observed among accessions in either year. Accessions were compared using proportional emmeans when covariates, Zadok's maturity, and stem diameter were included in a GLM with a logistic regression. Accessions with the lowest probability for infestation ($P < 0.01$) in 2020 included: *T. timopheevii* (TA2892), *T. turgidum* (TA1195), *T. urartu* (TA709), and *Ae. tauschii* (TA1596, TA1665, TA1667, TA1707, and TA2413) (Fig. 4 and Supporting Information, S6). In 2021, the accessions with the lowest probability for infestation ($P < 0.01$) were *Ae. tauschii* accessions TA1596, TA1634, TA1680, TA1697, TA1707, TA2374, TA2401, TA2458, TA2508, TA2512, TA10113, and TA10197 (Fig. 5 and Supporting Information, Table S7).

Wheat species differed in the probability of a stem being infested in 2020 ($X^2 = 71.74$, df = 5, $P < 0.001$) and 2021 ($X^2 = 242.91$, df = 5, $P < 0.001$). In 2020, *Ae. tauschii* and *T. urartu* had lower probabilities of being infested when compared

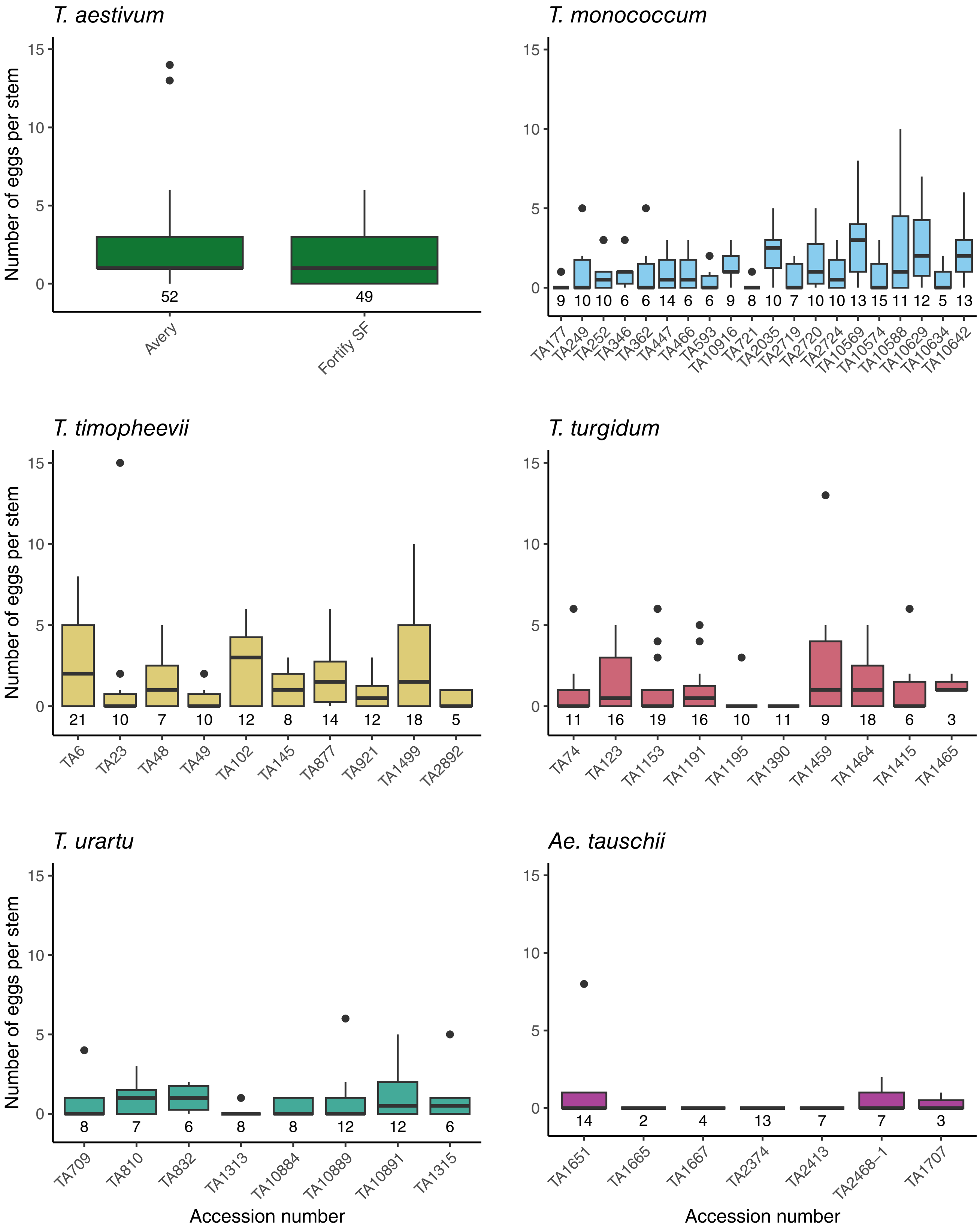

Figure 1. Number of wheat stem sawfly eggs found in each accession screened in 2020. Sample size is represented by the numbers beneath each boxplot (total stems cut). The boxes represent the 25th and 75th percentiles; the horizontal line in each box indicates the median. The whiskers signify maximum and minimum values, and circles indicate outliers. The *y*-axis is different for 2020 and 2021.

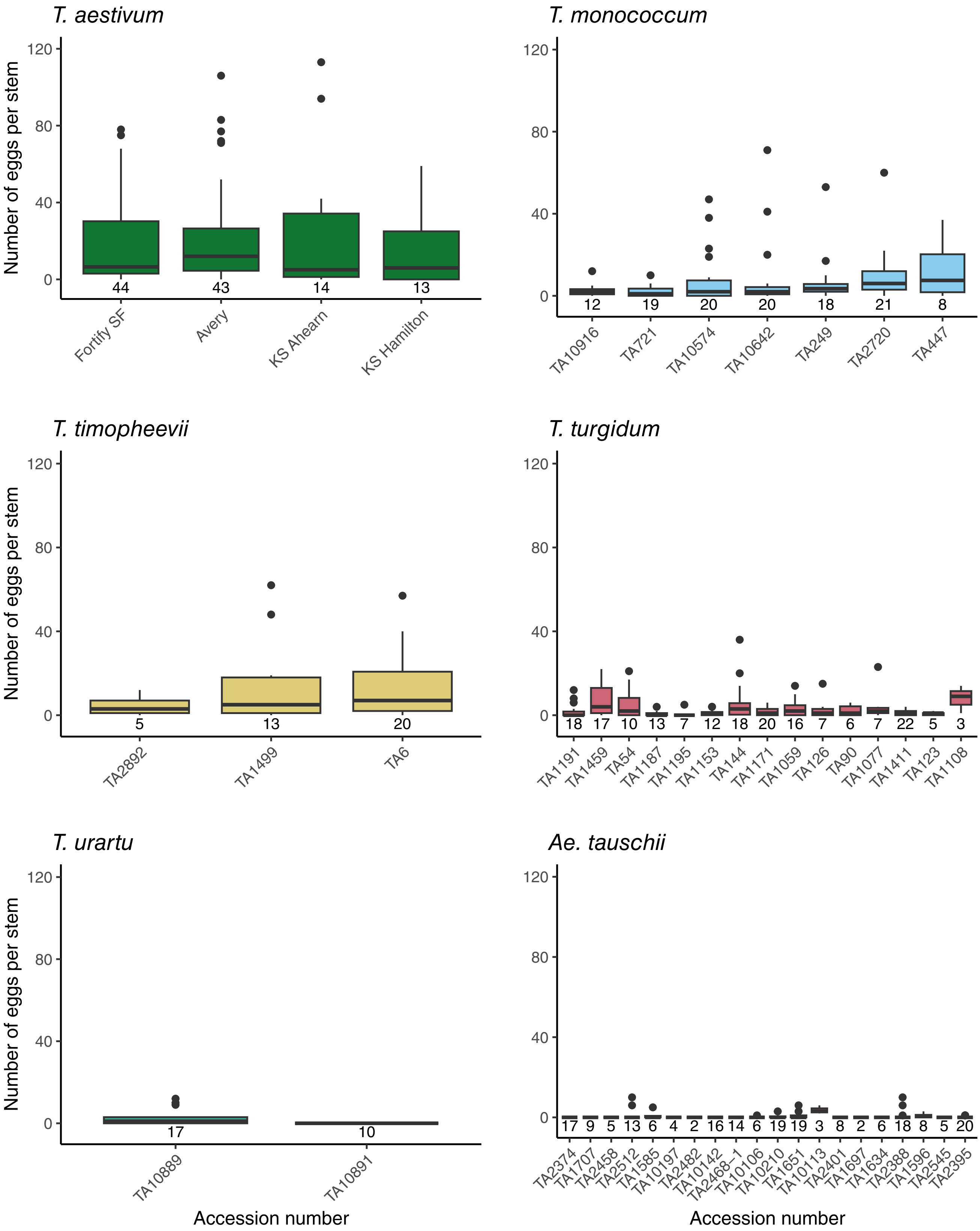

Figure 2. Number of wheat stem sawfly eggs found in each accession screened in 2021. Sample size is represented by the numbers beneath each boxplot (total stems cut). The boxes represent the 25th and 75th percentiles; the horizontal line in each box indicates the median. The whiskers signify maximum and minimum values, and circles indicate outliers. The *y*-axis is different for 2020 and 2021.

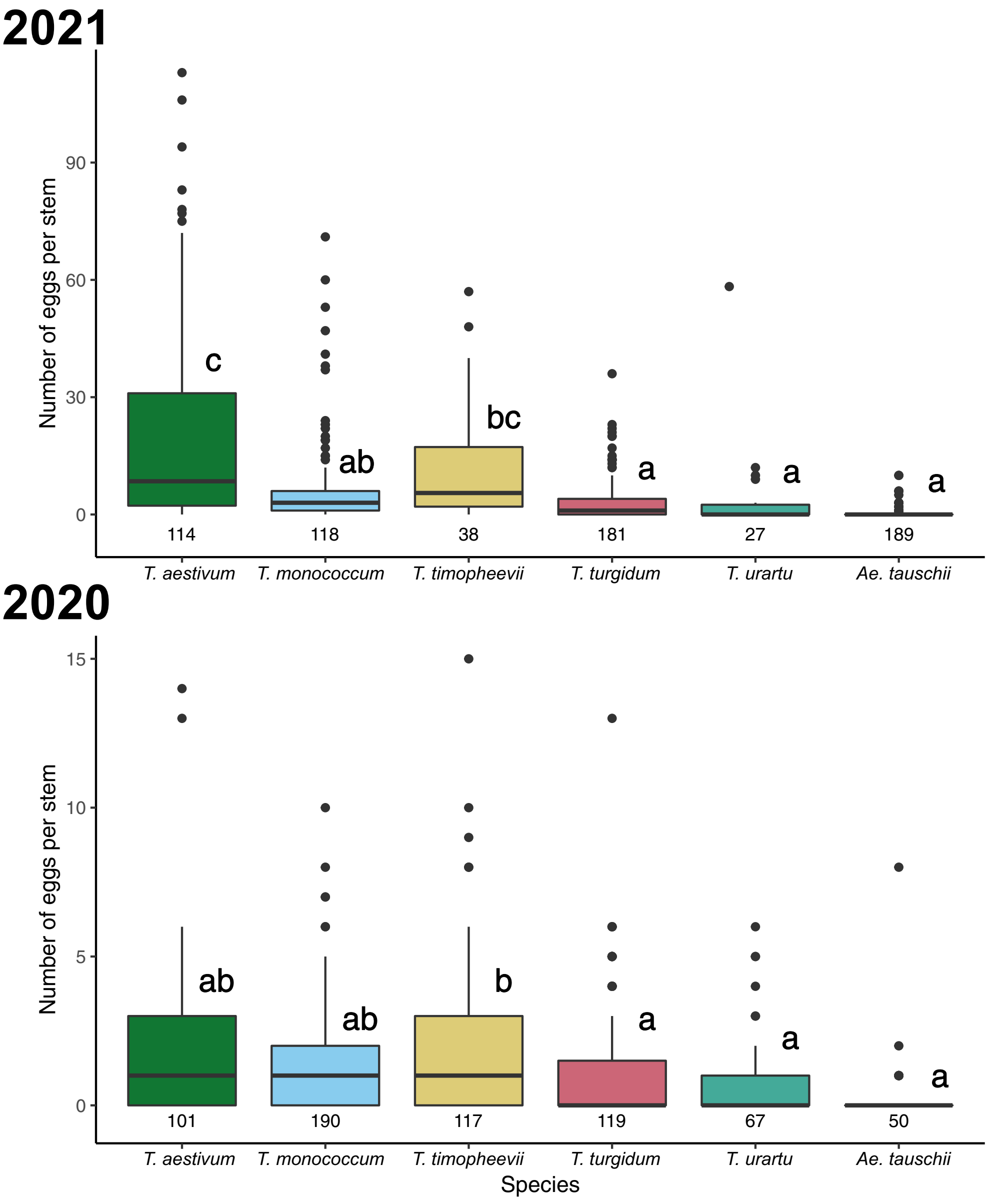

Figure 3. Number of wheat stem sawfly eggs found in each species screened in 2020 and 2021. Sample size is represented by the numbers beneath each boxplot (total stems cut). The boxes represent the 25th and 75th percentiles; the horizontal line in each box indicates the median. The whiskers signify maximum and minimum values, and circles indicate outliers. Letters indicate significant pairwise differences ($P < 0.05$, false discovery rate-corrected *post hoc* test). Pairwise comparisons and estimated marginal means, provided in Supporting Information, Fig. S4. The *y*-axis is different for 2020 and 2021.

with *T. monococcum* and *T. aestivum* (Fig. 6). In 2021, *Ae. tauschii* had a lower probability of being infested when compared with *T. aestivum*. *Triticum urartu*, *T. turgidum*, *T. timopheevii*, and *T. monococcum* had similar probabilities of being infested but less than that of *T. aestivum* (Fig. 6). In 2020 and 2021, *Ae. tauschii* exhibited the lowest probability of infestation, whereas *T. aestivum* and *T. monococcum* showed the highest probability of infestation. Pairwise comparisons and estimated marginal means are provided in Supporting Information, Fig. S5.

3.3 Host suitability—variation in larval weights

The wild wheat species varied in larval weights in 2020 ($F = 6.08$, df $= 4$, $P < 0.001$) and 2021 ($F = 7.44$, df $= 5$, $P = 0.003$). In 2020, *T. monococcum*, *T. timopheevii*, *T. turgidum*, and *T. urartu* had lower larval weights than *T. aestivum* (Fig. 7). In 2021, *T. aestivum*, *T. monococcum*, and *T. turgidum* had higher larval weights than *Ae. tauschii*. *T. timopheevii*, and *T. urartu*.

3.4 Hierarchical clustering of A genome relatives and associated WSS resistance

The relationship between the number of eggs and larval infestation rates and the accessions' phylogenetic clustering was compared (Fig. 8). The accessions belonging the wild tetraploid *T. turgidum* (AABB genome) clustered together (Fig. 8). These accessions had lower egg counts and larval infestation in both years. By contrast, *T. urartu* (AA) clustered together with *T. timopheevii* (AAGG genome). These accessions had higher egg counts and larval infestation (Fig. 8).

At the intraspecies level, *T. monococcum* could be roughly divided into two clades, A and B (Fig. 8). Clade B comprises

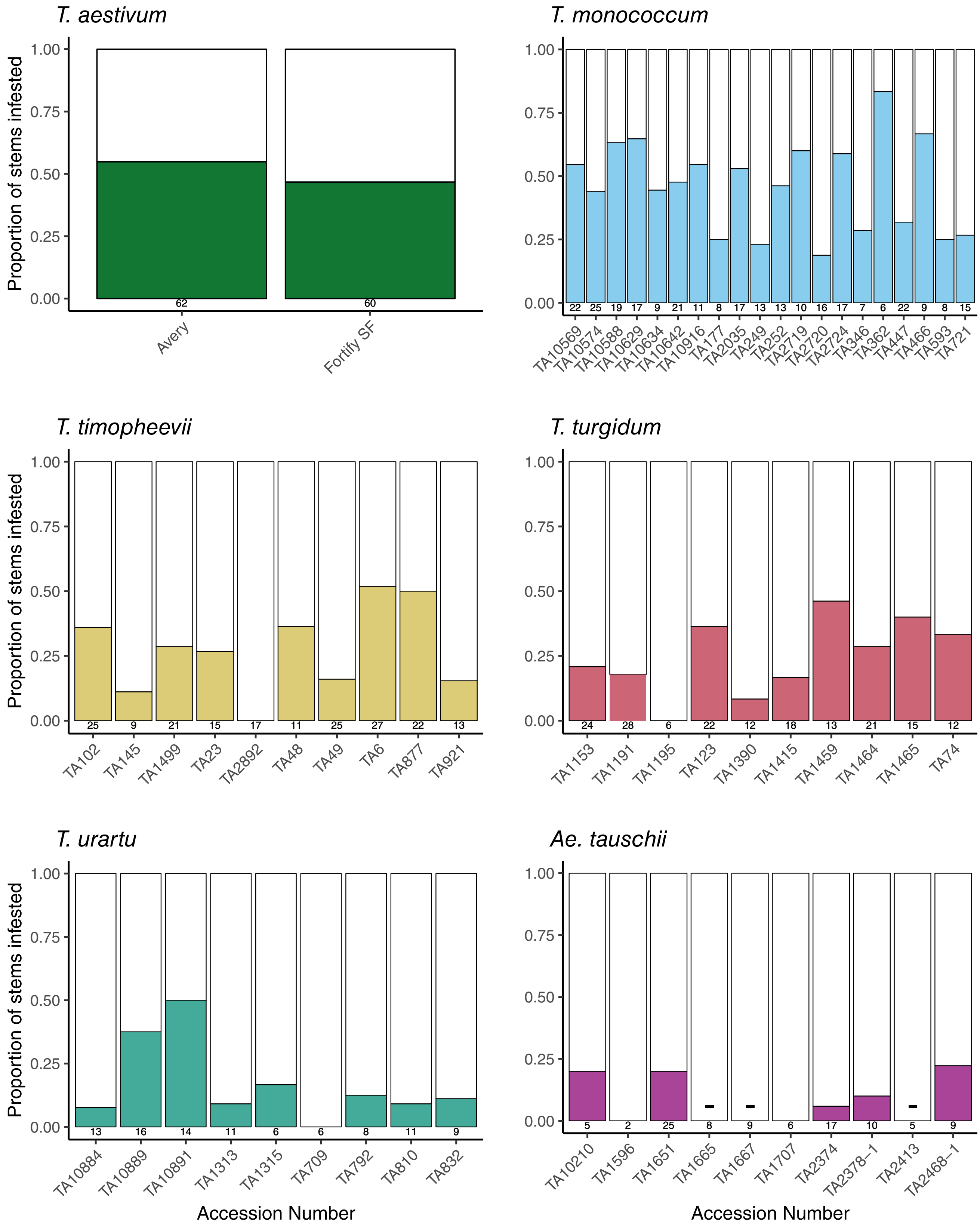

Figure 4. Proportion of wheat stem sawfly-infested stems in each accession screened in 2020. Filled-in bars represent the proportion of infested stems. *N* is represented by the numbers beneath each bar (total stems cut). Dashes indicate no eggs present when the accession was previously dissected.

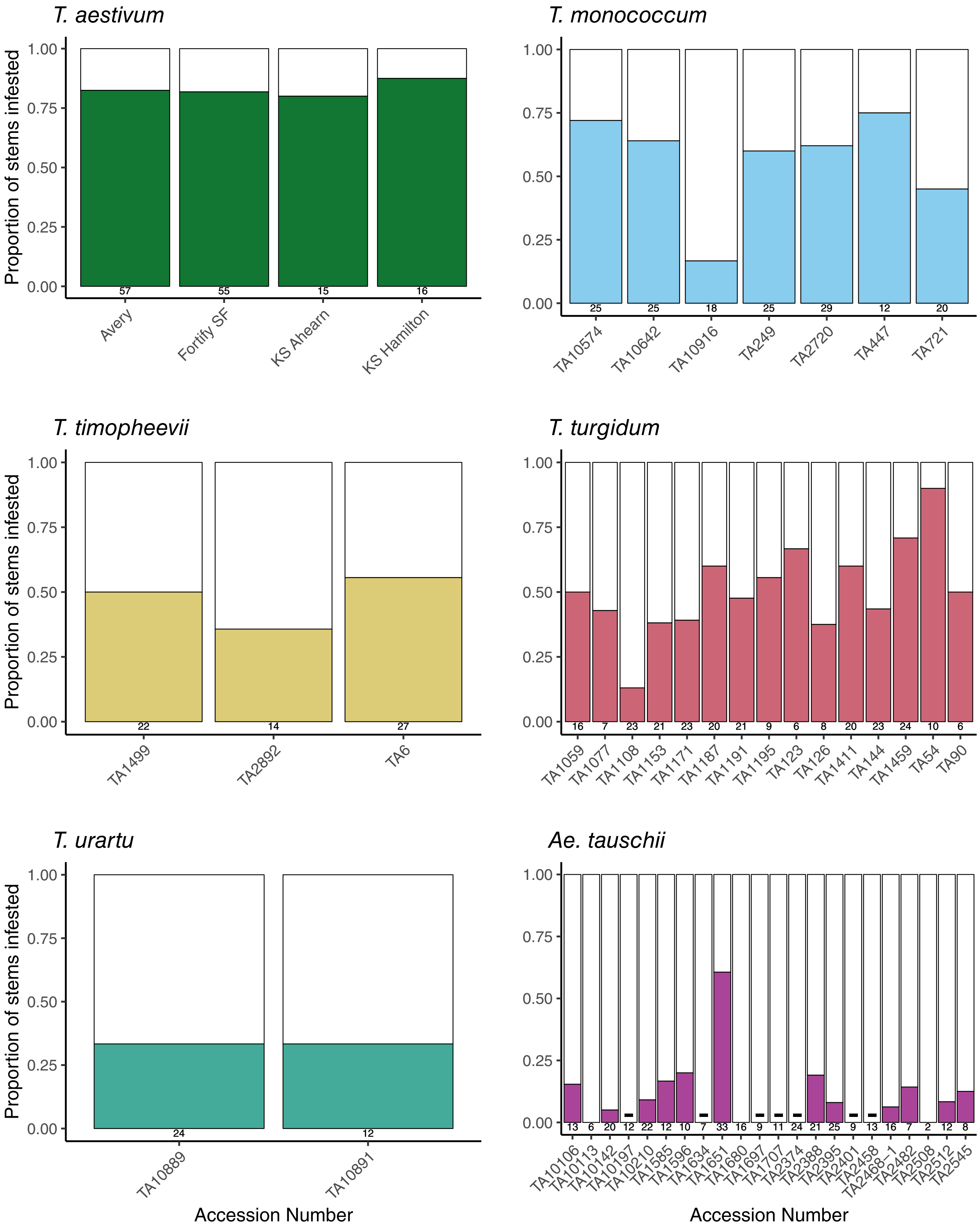

Figure 5. Proportion of wheat stem sawfly-infested stems in each accession screened in 2021. Filled-in bars represent the proportion of infested stems. *N* is represented by the numbers beneath each bar (total stems cut). Dashes indicate no eggs present when the accession was previously dissected.

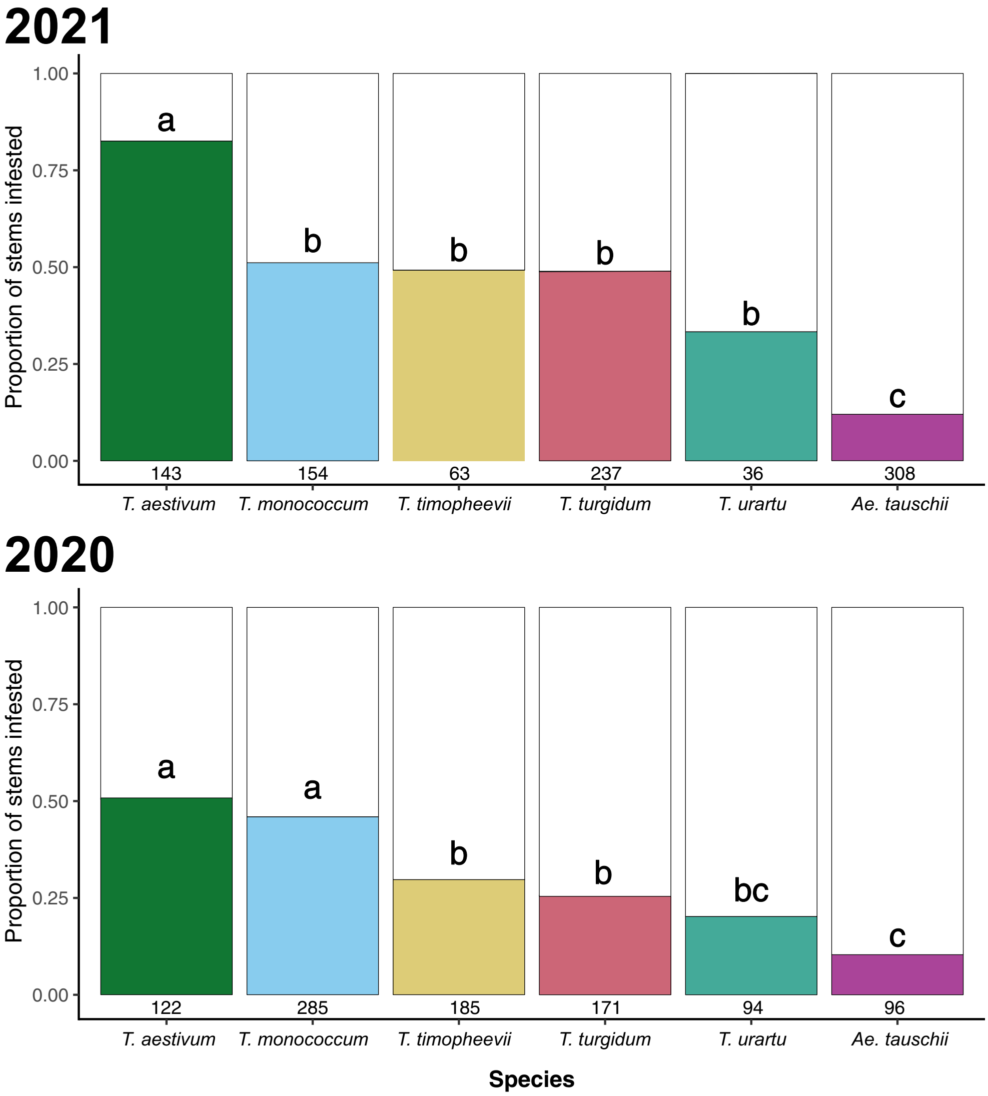

Figure 6. Proportion of wheat stem sawfly-infested stems in each species screened in 2020 and 2021. Filled-in bars represent the proportion of infested stems. *N* is represented by the numbers beneath each bar (total stems cut). Letters indicate significant pairwise differences ($P < 0.05$, false discovery rate-corrected *post hoc* test). Pairwise comparisons and estimated marginal means are provided in Supporting Information, Fig. S5.

accessions with lower egg counts and a lower larval infestation than clade A accession (in 2020: df = 180, $P = 0.008$; in 2021: df = 108, $P = 0.191$). However, fewer *T. monococcum* accessions were tested in 2021.

4 DISCUSSION

WSS continues to be a major threat to wheat production in North America. Over the past 70 years, solid-stem resistance has been the main management method for WSS. The expression of stem solidness in common wheat is often inconsistent.[43] By contrast, solid-stem expression in durum wheat exhibits greater genetic stability.[44] Another example of resistance was exhibited in a study by Varella *et al*.,[45] where using landraces from around the world they identified that 14% of the accessions screened showed some WSS resistance, and 7% of those had non-solid stems. Resistance genes have been found in wild wheat species to other pests, such as the Hessian fly (*Mayetiola destructor*),[24] greenbug (*Schizaphis graminum*)[25] and wheat curl mite (*Aceria tosichella*).[46] Hence, exploring the genetic diversity in wild wheat species could lead to the discovery of additional alleles that can enhance the development of WSS-resistant genotypes.

In this study, we measured WSS host preference defined by the number of eggs per stem in 91 accessions across five wild wheat species and four common wheat cultivars. Among the species tested, *Ae. tauschii*, *T. urartu* and *T. turgidum* had lower egg counts than those in other wild species and common wheat cultivars. Females often choose hosts that are taller, mature earlier, and have larger stem diameters,[47,48] but evidence suggests that WSS also chooses a host based on volatile organic compounds (VOCs) produced by the plants.[49–51] In addition to volatiles, wheat cultivars also produce variable epicuticular leaf wax, which may influence WSS behaviors such as ovipositor insertions and oviposition rates.[52] Using recombinant inbred lines of spring wheat lines 'Reeder' (PI 613586; attractive), 'Conan' (semi-solid stem, resistant), 'Scholar' (PI 607557; semi-solid stem), and 'Choteau' (PI 633974; solid stem, resistant), Varella and colleagues found several QTL associated with WSS behavior that were located on

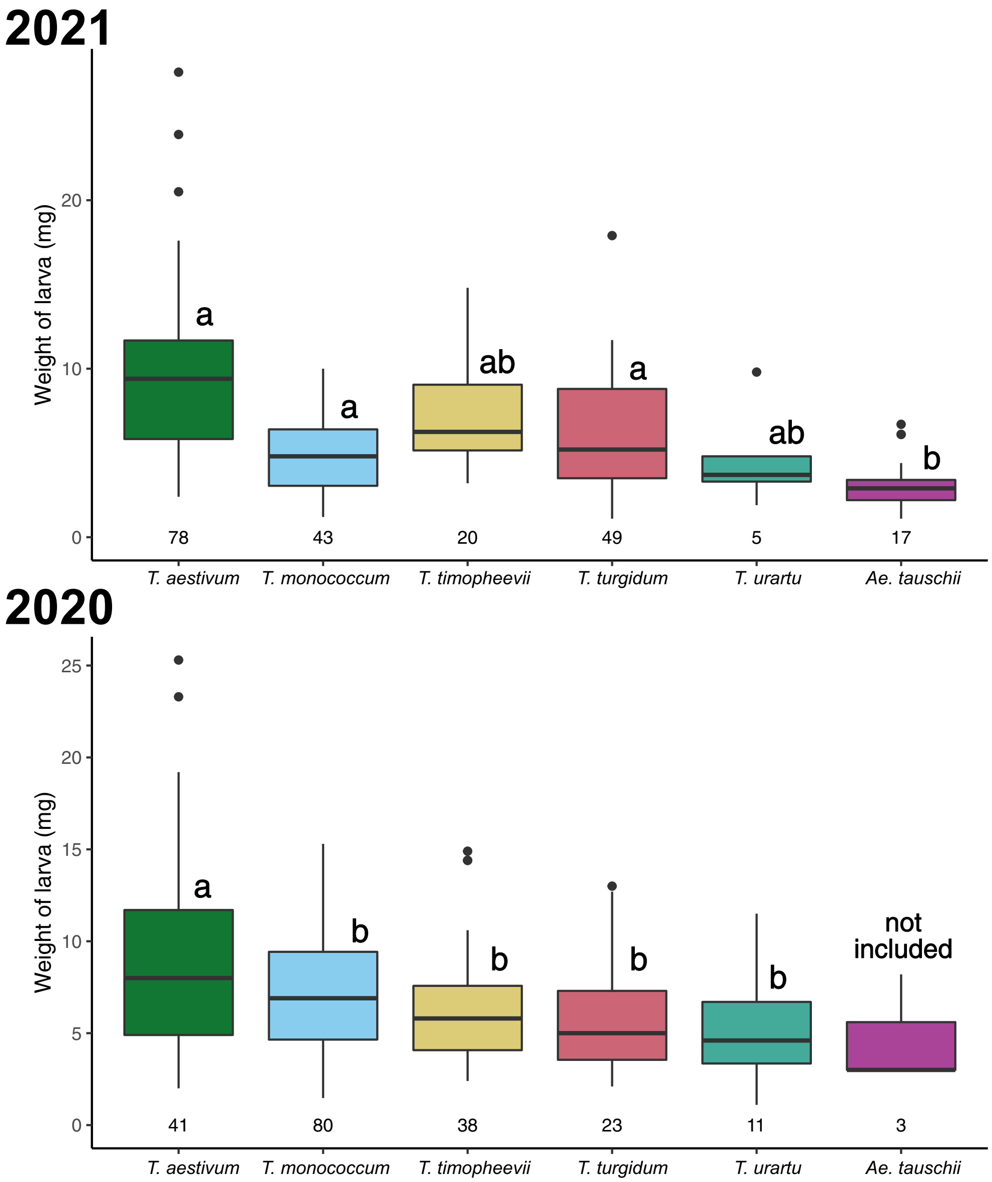

Figure 7. Wheat stem sawfly larval weights recorded in each wild wheat species screened in 2020 and 2021. Sample size is represented by the numbers beneath each boxplot (total stems cut). The boxes represent the 25th and 75th percentiles; the horizontal line in each box indicates the median. The whiskers signify maximum and minimum values, and circles indicate outliers. Letters indicate significant pairwise differences ($P < 0.05$, false discovery rate-corrected *post hoc* test).

3B, 2D and 4A.[13] WSS non-preference traits will likely have the most impact when fields are planted with combinations of preferred and non-preferred cultivars and when the WSS infestation is low to moderate.[53,54] Although VOCs or leaf structures of the wheat species were not examined in this study, they may play a part in WSS preferences. To ensure that potential VOCs released by different wheat species would not influence WSS preferences, we randomized the plants within trays. However, there is still potential that other leaf structures played a part in WSS preferences.

For WSS, host suitability refers to the ability of a host plant to support the growth and development of the insect. In our study, we found *Ae. tauschii*, *T. urartu*, and *T. turgidum* have the lowest infestation rates compared with those of other two wild wheat species and the four common wheat cultivars across 2020 and 2021. Previous studies have found smaller WSS adult sizes result in in lower fecundity for future populations because of reduced egg load.[55,56] It is difficult to rear WSS larvae after they have been removed from the stem, so we infer that smaller larvae result in smaller adults. Larvae found in common wheat were larger than those found in other species in 2020. Larger stem diameters of common wheat may have played a role in this. In 2021, larvae in *Ae. tauschii* were smaller than those in *T. aestivum*, *T. monococcum*, and *T. turgidum*. Smaller larvae and lower infestations suggest the *Ae. tauschii* accessions tested are not a suitable host for WSS. According to population models proposed by Rand *et al.* in 2020, resistant cultivars can reduce WSS performance for all life stages.[57] If we can create cultivars that produce adults that have low fecundity this can contribute to lowering the WSS population in future generations. In addition, if we can pair resistance with biological control by WSS

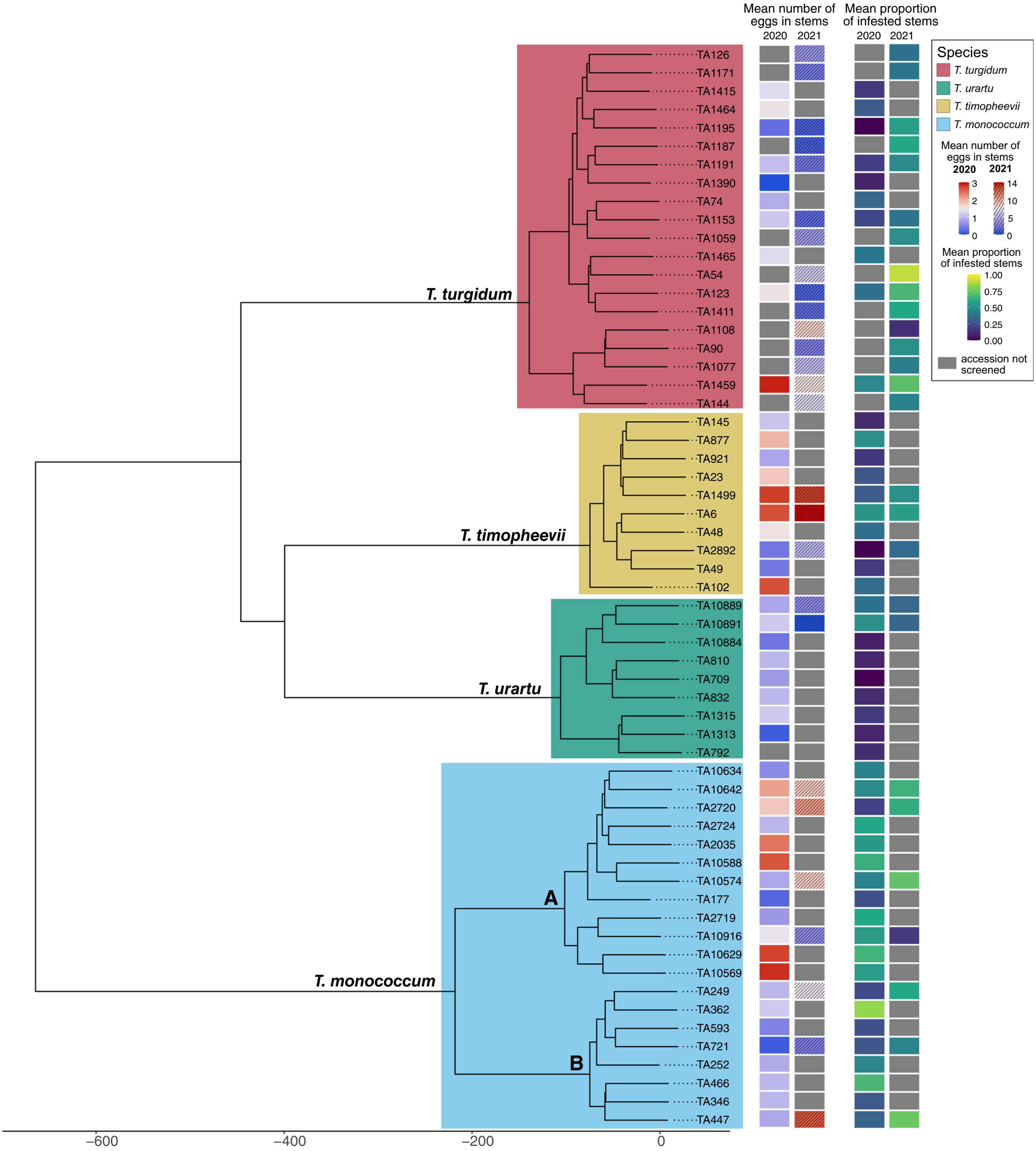

Figure 8. An unrooted phylogenetic tree of accessions of four wild wheat species. Only the A genome and accessions genotyped by the Wheat Genetic Resource Center are included. A and B denote significantly different clades of *Triticum monococcum*. The number of wheat stem sawfly eggs and larval infestation rates were evaluated in 2020 and 2021. number of eggs was much higher in 2021, so it has a separate scale.

parasitoids *Bracon cephi* and *B. lissogaster*, models suggest that 22% parasitism is needed to reduce WSS population growth rate.[57] Currently, stem solidity is thought to reduce parasitism.[58] Hence, exploring alternative resistance mechanisms can optimize the synergy with biological control.

Aegilops tauschii accessions have been identified with resistance genes for several insect pests, such as Hessian fly (*Mayetiola destructor*) and greenbug (*Schizaphis graminum*). Some of these genes have been successfully used in cultivated wheat. The Hessian fly is a pest of wheat that can lead to extensive crop damage. Six genes, namely *H13*, *H22*, *H23*, *H24*, *H26* and *H32*, were identified in *Ae. tauschii* and subsequently introduced into hexaploid wheat.[24] Evaluation of the donor *Ae. tauschii* accessions, where the resistance genes were identified, found that plants used an early defense strategy consisting of the production of anti-feedant proteins (lectins), secondary metabolites (such as phenylpropanoids), and reactive oxygen species (ROS).[23] The ROS radicals may counter any larval degradation of plant cells and prevent the larva from establishing feeding sites, whereas secondary metabolites affect larval performance. The greenbug is a phloem-feeding insect pest of wheat commonly controlled with host plant resistance. The gene *Gb3* from *Ae. tauschii* also provides resistance to greenbug in common wheat.[25] *Ae. tauschii*-derived lines showed a reduction of greenbug weight compared with susceptible controls, suggesting antibiosis is the main type of resistance in these lines.[26]

In our hierarchical clustering of ancestral A genome progenitors and wild relatives, our results indicate that the wild tetraploid *T. turgidum* (AABB genome) and the diploid A genome donor *T. urartu* grouped together with *T. timopheevii* (AAGG), whereas *T. monococcum* remained in its own clade. When comparing our clustering analysis with observed phenotypic data, we observed that *T. monococcum* was not significantly different from *T. timopheevii*, *T. turgidum*, or *T. urartu* in 2020 or 2021 for the number of eggs per stem. This may indicate that polymorphisms on the A genome may not be related to increased host selection.

By contrast, we did observe significant differences between *T. monococcum* and the other A genome relatives, where *T. monococcum* had a significantly higher portion of stems infested. In the context of our hierarchical clustering analysis, there is marked genetic distance between *T. monococcum* and the other A genome relatives; however, the source of the genetic resistance found in *T. aestivum* progenitors and relatives may not be purely defined by the polymorphisms on the A genome alone, as demonstrated by the superior performance of *Ae. tauschii*, which is the progenitor of the D genome in *T. aestivum*. Thus, novel sources of resistance may be found in either the A, B, or D genome progenitor and relatives. Therefore, further investigation into what regions of the genome contribute to host selection and suitability in these progenitors and relatives of *T. aestivum* is warranted.

Nevertheless, our study identifies specific wild wheat species and accessions that have the potential to influence WSS host selection and reduce WSS populations through host suitability. Future directions may include the development of synthetic hexaploids to incorporate pest resistance into common wheat from wild ancestors.[59] Research is underway to screen synthetic hexaploids that include several *Ae. tauschii* accessions (TA 2374 and TA 2468) and *T. turgidum* accessions (TA 1464 and TA 1195) that were screened in this study as part of their pedigree for WSS resistance. Only a handful of studies have investigated the molecular mechanisms underlying the wheat–WSS interaction.[60,61] Known mechanisms of resistance to stem-boring insects in wheat will inform hypothesis testing on conserved signaling pathways and secondary plant metabolites. Novel metabolic compositions could result from wild introgressions to wheat and can be evaluated for their potential for WSS resistance traits.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Novel sources of resistance to wheat stem sawfly at https://figshare.com/articles/dataset/_b_Novel_sources_of_resistance_to_wheat_stem_sawfly_in_five_wild_wheat_species_b_/24977937, reference number 10.6084/m9.figshare.24977937.

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AUTHOR CONTRIBUTIONS

ESP was responsible for study conceptualization, investigation, formal analysis, visualization, and writing the original draft. BE and ZJW conducted formal analysis and reviewed and edited the manuscript. WJR, MG, AFK, SH and EM were responsible for resources and reviewed and edited the manuscript. JP and EA were responsible for resources, funding acquisition and project administration, and reviewed and edited the manuscript. EM was responsible for resources, and reviewed and edited the manuscript. PN was responsible for study conceptualization, funding acquisition, project administration, reviewing and editing the manuscript, and supervision.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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