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RESEARCH PAPER

A salivary ferritin in the whitefly suppresses plant defenses and facilitates host exploitation

Qi Su[1,*], Zhengke Peng[2,3,*], Hong Tong[1], Wen Xie[2], Shaoli Wang[2], Qingjun Wu[2], Jianmin Zhang[1], Chuanren Li[1] and Youjun Zhang[2,†]

[1] Institute of Insect Sciences, College of Agriculture, Yangtze University, Jingzhou, Hubei 434025, China
[2] Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[3] Department of Entomology, China Agricultural University, Beijing 100083, China

*These authors contributed equally to this work.
† Correspondence: zhangyoujun@caas.cn

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Abstract

The whitefly *Bemisia tabaci* is an important pest of worldwide agriculture. Previous work has shown that *B. tabaci* actively suppresses host plant defenses, but our knowledge of the specific mechanisms involved remains limited. Here we describe a *B. tabaci* salivary protein, the ferritin BtFer1, and its role in facilitating exploitation of host plants. We show that BtFer1 exhibits Fe^{2+} binding ability and ferroxidase activity, and that secretion of BtFer1 during *B. tabaci* feeding suppresses H_2O_2-generated oxidative signals in tomato (*Solanum lycopersicum*). Silencing *BtFer1* enhanced the induction of the jasmonic acid (JA)-mediated defense signaling pathway in response to whitefly feeding, and led to increased callose deposition and the production of proteinase inhibitors that prevent whiteflies from continuously ingesting and digesting phloem sap. Consistent with these effects, silencing *BtFer1* reduced whitefly survival on tomato but not on artificial diet. Using a JA-deficient *spr2* mutant plant further showed that suppression of JA defenses by BtFer1 is sufficient to increase *B. tabaci* survival. Taken together, these results demonstrate that BtFer1 acts as an effector protein that mediates whitefly–tomato interactions. These findings represent an important step forward in understanding the molecular mechanisms by which whiteflies and other insect herbivores suppress host plant defenses.

Keywords: *Bemisia tabaci*, defense suppression, effector protein, feeding behavior, host adaptation, plant–insect interactions, *Solanum lycopersicum*.

Introduction

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a cosmopolitan, phloem-feeding insect pest that causes extensive damage to crops in temperate climates around the world (De Barro *et al.*, 2011). Unlike chewing insects, which cause extensive tissue damage, whiteflies use their highly modified stylets to navigate the plant cuticle, epidermis, and mesophyll, and establish feeding sites in phloem sieve elements without causing substantial host cell damage (Walling, 2008). In addition to this 'stealthy' feeding strategy, whiteflies have evolved several adaptations that allow them to evade or suppress plant defenses (Walling, 2008). For example, previous studies using Arabidopsis and tomato demonstrated that whiteflies can suppress jasmonic acid (JA)-mediated plant defenses by inducing the JA-antagonistic salicylic acid (SA)

signaling pathway (Zarate *et al.*, 2007; Zhang *et al.*, 2013; Su *et al.*, 2015*a*). Whitefly feeding can also inhibit the production of JA-regulated defensive compounds and the expression of related biosynthesis/catabolism genes (Kempema *et al.*, 2007; Zhang *et al.*, 2009; Su *et al.*, 2018*a*). Furthermore, Kempema *et al.* (2007) observed that whiteflies did not induce a strong oxidative burst in Arabidopsis despite the fact that they induced expression of genes implicated in scavenging of reactive oxygen species (ROS) and in redox homeostasis. This suggests that whiteflies may disrupt the effective oxidative signal response of Arabidopsis. To date, however, the underlying molecular mechanisms by which whiteflies counter the induced defenses of their host are not well understood.

While some plant defense traits are expressed constitutively, others are induced in response to herbivore feeding (Felton and Tumlinson, 2008; Erb *et al.*, 2012; Schuman and Baldwin, 2016). Such induction is mediated by the recognition of specific cues, including herbivore-associated molecular patterns (HAMPs) in herbivore oral secretions, followed by the elicitation of complex signaling networks, involving mitogen-activated protein kinase (MAPK) cascades as well as signaling via the JA, SA, and ethylene pathways (Felton and Tumlinson, 2008; Acevedo *et al.*, 2015; Schmelz, 2015). This signaling, in turn, leads to a reconfiguration of the transcriptome and proteome, as well as the biosynthesis of defensive chemicals (Wu and Baldwin, 2010; Hogenhout and Bos, 2011).

HAMP-mediated plant defenses may be suppressed by other molecules secreted by herbivores, which are known as effectors (Felton and Tumlinson, 2008; Hogenhout and Bos, 2011). While effector molecules that suppress plant defense responses are well documented in plant–pathogen systems (Göhre and Robatzek, 2008), much less is known about effectors employed by arthropod antagonists of plants. However, there are indications that herbivorous insects that suppress plant defenses secrete effectors into their hosts via saliva (Hogenhout and Bos, 2011; Elzinga and Jander, 2013). Previous reports have implicated a number of salivary proteins in overcoming plant defenses and enhancing herbivore performance, including glucose oxidase from tomato fruitworm (Musser *et al.*, 2002); C002, Mp10, Mp55, Me10, Me23, Me47, Armet, and migration inhibitory factor from aphids (Mutti *et al.*, 2008; Bos *et al.*, 2010; Atamian *et al.*, 2013; Elzinga *et al.*, 2014; Naessens *et al.*, 2015; Wang *et al.*, 2015; Kettles and Kaloshian, 2016); flagellin from the orally secreted bacteria of Colorado potato beetles (Chung *et al.*, 2013); effector 28 and effector 84 from spider mites (Villarroel *et al.*, 2016; Schimmel *et al.*, 2017); and endo-β-1,4-glucanase from the rice brown planthopper (Ji *et al.*, 2017). Such effectors may play a central role in helping herbivores to overcome plant defenses and to colonize host plants successfully.

As the ability of whiteflies to suppress plant defenses is now well established (Zarate *et al.*, 2007; Zhang *et al.*, 2013; Su *et al.*, 2015*a*, 2018*a*), it seems likely that they have also evolved salivary effector proteins that are secreted into plant tissue during feeding (Kaloshian and Walling, 2016) to modulate host defense responses. However, little is currently known about whitefly effector proteins or the mechanisms by which they act. The current study focuses on a candidate effector gene, *Bt11666*, which we have named *BtFer1* because it encodes a salivary ferritin secreted into plant tissues during *B. tabaci* feeding. We selected it from the salivary gland transcriptome of *B. tabaci* (Su *et al.*, 2012), using a bioinformatics effector-mining pipeline similar to that of Bos *et al.* (2010), because we hypothesized that ferritins may interfere with oxidative signaling responses. Ferritins are iron storage proteins that bind ferrous iron [Fe(II)] and facilitate migration to the ferroxidase catalytic site where Fe(II) is oxidized to the ferric state [Fe(III)] (Arosio *et al.*, 2009). Because oxygen and hydrogen peroxide (H_2O_2) are the major cellular oxidants consumed during this oxidation reaction, ferritins may also protect against oxidative signals through their potential ability to detoxify excess H_2O_2 (Arosio *et al.*, 2009).

Oxidative signaling induced by insect feeding is believed to play an important role in plant resistance to arthropod herbivores (Bi and Felton, 1995; Orozco-Cárdenas and Ryan, 1999; Liu *et al.*, 2010; Lei *et al.*, 2014). Therefore, we hypothesized that the suppression of oxidative signaling responses by *BtFer1* might contribute to the suppression of host plant defenses. To explore this possibility and other potential effects of *BtFer1* on whitefly–host interactions, we employed a combination of molecular biology, chemical analyses, and bioassays. Our findings provide evidence that BtFer1 functions as an effector protein that promotes *B. tabaci* performance on tomato plants while suppressing several aspects of the induced plant defense response, including oxidative signals, callose deposition, proteinase inhibitor (PI) activation, and the JA-mediated signaling pathway.

Materials and methods

Plant growth and insect rearing

The following tomato (*Solanum lycopersicum*) cultivars or lines were used: cv. L402, cv. Castlemart (CM), and *suppressor of prosystemin-mediated responses 2* (*spr2*). Plants were grown as described by Su *et al.* (2015*a*), and used for experiments when they were 4–5 weeks old and had four fully expanded true leaves. A colony of *B. tabaci* Mediterranean (MED; formerly the 'Q' biotype) was maintained on tomato (cv. L402) at 26±2 °C and 60±10% relative humidity under a 14/10 h light/dark photoperiod in a growth chamber.

Cloning and sequence analysis of BtFer1

The full-length coding sequences for *BtFer1* (Bt11666) was amplified by reverse transcription–PCR (RT–PCR) with gene-specific primers (Supplementary Table S2 at *JXB* online) from total RNA isolated from salivary glands of adult *B. tabaci* females. The PCR products were cloned into the pMD18-T vector (TaKaRa, Dalian, China) and sequenced. The ORF of *BtFer1* was found by the ORF Finder tool at the NCBI website (https://www.ncbi.nlm.nih.gov/orffinder/). The nucleotide sequence similarity analyses were performed with the BLAST tool at the NCBI website (http://blast.ncbi.nlm.nih.gov/). The deduced protein sequence was obtained with an ExPASy translate tool (http://web.expasy.org/translate/), and the molecular weight and isoelectric point (pI) of the predicted protein were determined using Compute pI/Mw software (http://web.expasy.org/compute_pi/). The N-terminal signal peptides and transmembrane helices were predicted by using SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP/) and TMHMM 2.0 (http://www.cbs.dtu.dk/services/TMHMM/), respectively. The presence of *N*- and *O*-glycosylation sites on the predicted protein sequence was tested using the NetNGlyc 1.0 (http://www.cbs.dtu.dk/services/NetNGlyc/) and NetOGlyc 4.0 server (http://www.cbs.dtu.dk/services/NetOGlyc/),

respectively. The secondary structure was predicted by using PSIPRED 3.3 (http://bioinf.cs.ucl.ac.uk/psipred/). Domains of BtFer1 were searched using PROSITE (http://prosite.expasy.org/). Amino acid sequences of insect ferritin downloaded from NCBI (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov) were aligned using ClustalW2 (www.ebi.ac.uk/Tools/msa/clustalw2).

Protein expression, purification, and antibody preparation

The nucleotide sequence encoding BtFer1 lacking the N-terminal secretion signal was amplified by PCR using the primers listed in Supplementary Table S2, and then constructed into expression vector pET-28a. The recombinant plasmids and empty vector pET-28a (used as a control) were transformed into the *Escherichia coli* BL21 (DE3) strain for expression after sequence verification. Induced expression was conducted after adding 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 37 °C for 6 h. The products from the recombinant and empty vector were purified by using Ni-NTA columns (Qiagen, Valencia, CA, USA) and concentrated with a YM-10 Microcon centrifugal filter device (Millipore, Billerica, MA, USA) to remove imidazole. Based on the Optimum Antigen design tool, a polypeptide (CKRGGKMDFGFRKED) of Bt11666 was selected as the antigen to produce the rabbit polyclonal antibodies, and the polyclonal antibodies were made and purified by GenScript™ (GenScript, Nanjing, China).

Western blot

To test whether BtFer1 is secreted via saliva or honeydew, proteins extracted from *B. tabaci* heads (containing salivary glands), *B. tabaci* honeydew, and *B. tabaci*-infested and uninfested tomato leaves were used for western blot analysis. The heads of 200 newly emerged adult females were collected and homogenized in 1 ml of phosphate-buffered saline (PBS). The extract was centrifuged at 12 000 *g* for 10 min at 4 °C, and the supernatant was collected. Tomato plants were individually confined in a ventilated cage in which 5000 newly emerged *B. tabaci* adults were released, and Petri dishes were placed under tomato leaves. After 24 h, the whiteflies and their eggs were removed. Plants without whiteflies were used as controls. Honeydew was subsequently collected with a pipette by adding 1 ml of PBS to the Petri dishes, homogenized, and then the extract was filter-sterilized using 0.2 μm filters (Millipore). The top three leaves of each plant were harvested, and the collected leaves from three tomato plants (~1.0 g per plant) were combined and homogenized in 4 ml of PBS in liquid nitrogen. The extract was centrifuged at 12 000 *g* for 10 min at 4 °C, and the supernatant was collected as samples. After adding SDS loading buffer, the samples were then subjected to SDS–PAGE on a 12% gradient gel and transferred onto a polyvinylidene fluoride (PVDF) membrane. The blot was probed with the purified rabbit polyclonal anti-BtFer1 antibody (1:2000 dilution), visualized with a goat anti-rabbit IgG-conjugated horseradish peroxidase antibody (CWBIO, Beijing, China) at a 1:5000 dilution, and detected by ttttthe ECL Plus Detection System (Bio-Rad, Hercules, CA, USA).

Immunohistochemistry

Leaf samples infested or not infested by *B. tabaci*, treated as in the western blotting experiments, were fixed in 4% (w/v) paraformaldehyde in MSB buffer (50 mM PIPES, 5 mM EGTA, 5 mM $MgSO_4$, pH 6.9) at room temperature for 2 h. Fixed samples were rinsed several times with MSB buffer to remove any residual fixative and dehydrated through a graded ethanol series (supplemented with 10 mM DTT) from 10% to 100% (v/v). Dehydrated material was embedded in methacrylate mix (Sigma-Aldrich, St. Louis, MO, USA) and then cut into 3 μm sections on a Leica RM2165 microtome, mounted on pre-warmed silane-coated slides (Sigma-Aldrich), and used for immunofluorescent labeling. Slides were then incubated with the anti-BtFer1 antibody diluted 1:400 in Tris-buffered saline (TBS) for 1 h. After washing three times with TBST [TBS containing 0.05% (v/v) Tween-20], slides were incubated with secondary goat anti-rabbit antibody conjugated to Alexa Fluor 488 (Invitrogen) diluted 1:1000 in 2% (w/v) BSA in TBS for 1 h. After washing three times with TBST and distilled water, slides were observed using a Zeiss LSM 510 confocal microscope (Zeiss, Jena, Germany). Control experiments were conducted by replacing primary antibody with pre-immune rabbit serum.

Fe^{2+} binding assay

The Fe^{2+} binding ability of BtFer1 was confirmed by mixing purified BtFer1 (1.0 μg per 10 μl) with equal volumes of the following solutions: 0.5 mM (final concentration) EDTA or 0.05, 0.1, 0.2, or 0.5 mM $FeSO_4$. Each mixture was incubated for 30 min at 24 °C, added to 10 μl of Laemmli sample buffer, and then subjected to SDS–PAGE under reducing conditions.

Ferroxidase activity assay

The ferroxidase activity of BtFer1 was determined by the absorbance of Fe^{3+} (Ferric Gain Assay) as described previously (Minotti and Ikeda-Saito, 1992) with minor modifications. Briefly, reactions (200 μl) contained the purified recombinant protein BtFer1 (250 nM), and ferrous ammonium sulfate (125 μM) was added to HBS (50 mM HEPES, 150 mM NaCl, pH 7.2). Absorbance readings at 310 nm were kinetically monitored using a microplate spectrophotometer (SpectraMax M2e, Molecular Devices, San Jose, CA, USA) over 20 min at 30 s intervals with continual agitation and kept at 24 °C. Reactions were blanked against HBS only, and human ceruloplasmin (Sigma-Aldrich) was used as positive control. This experiment was repeated three times.

RNAi experiment

A 395 bp fragment of *BtFer1* and a 288 bp fragment of the control gene *EGFP* (enhanced green fluorescent protein) were amplified by RT–PCR with primers including a T7 promoter sequence (see Supplementary Table S2). The PCR products were used to synthesize dsRNA *in vitro* with the T7 RiboMax Express RNAi System (Promega, Madison, WI, USA). Newly emerged adult *B. tabaci* females were fed an artificial diet [5% yeast extract and 30% sucrose (w/v)] that was not supplemented with dsRNA or that was supplemented with either ~0.5 μg μl^{-1} dsRNA of *BtFer1* (*dsBtFer1*) or with ~0.5 μg μl^{-1} dsRNA of *EGFP* (*dsEGFP*). The diet was placed between two layers of Parafilm M stretched over the two open ends of glass cylinders (20 mm diameter×50 mm long), and the females were allowed to feed for 2 d as described by Su *et al.* (2018*b*). To assess the efficiency and duration of gene silencing after dsRNA feeding, we determined the levels of *BtFer1* transcripts in the salivary glands of *B. tabaci* females that had been subjected to these feeding treatments for 2 d and in the whole bodies of *B. tabaci* females after these treatments for 1, 3, 5, and 7 d.

Whitefly bioassays

To measure the effect of the knockdown of *BtFer1* on *B. tabaci* survival, newly emerged adult females previously fed a diet without dsRNA or with either *dsBtFer1* or *dsEGFP* (as described above) were allowed to feed on tomato plants or artificial diet. The treated adults were first allowed to recover on tomato plants at 27±1 °C with 70±10% relative humidity and a 14:10 h (light/dark) photoperiod for 1 d; only healthy adults were used for the following bioassay. Groups of 20 adult females were restricted to the first leaf (counted from the bottom) of individual plants within a clip cage (3 cm diameter, one plant per cage) as described previously (Su *et al.*, 2015*b*). In the artificial diet experiment, 20 adult females were introduced into individual feeding chambers (20 mm diameter×50 mm long) containing 5% yeast extract and 30% sucrose (w/v) as described in the RNAi experiment. The number of surviving adults in each clip cage or feeding chamber was recorded daily. The effect of *BtFer1* knockdown on whitefly fecundity was also investigated. Groups of 20 newly emerged adult females previously fed a diet without dsRNA or with either *dsBtFer1* or *dsEGFP* (as described earlier) were placed in clip cages on the first leaf of tomato plants. Whiteflies were removed after 7 d, and the number of the eggs laid by females in the clip cages for each

treatment was counted with a stereomicroscope. These experiments were repeated 15 times; in each repetition, the total numbers of eggs laid by females were determined for each treatment and used to calculate average individual fecundity.

Analysis of whitefly feeding behavior

Electrical penetration graphing (EPG) was used to analyze the feeding behavior of individual *B. tabaci* on tomato plants as previously described (Liu *et al.*, 2013). Individual newly emerged adult females that were previously fed a diet without dsRNA or with either *dsBtFer1* or *dsEGFP* (as described earlier) were moved to tomato plants, and their feeding was monitored continuously for 8 h on the third day. EPG waveforms were recorded using a Giga-8 direct-current EPG (DC-EPG) system (Wageningen University, The Netherlands). PROBE V. 3.4 software (Wageningen University) was used to analyze the recorded signals. Relationships between EPG waveforms and feeding behavior were described by Liu *et al.* (2013). Fresh plants and insects were used for each replicate, with 15–20 replicate females per treatment.

Measurement of H_2O_2

Fifty newly emerged adult female *B. tabaci* that had previously been fed a diet without dsRNA or with either *dsBtFer1* or *dsEGFP* (as described earlier) were placed into a clip cage on the first leaf of individual tomato plants. Leaves from a subset of plants from each treatment were harvested at 4, 8, and 24 h after infestation. Leaf samples were homogenized in liquid nitrogen and were individually completely mixed with 1 ml of deionized water, and the supernatants were collected following microcentrifugation of the extract. The H_2O_2 concentration was determined using an Amplex Red Hydrogen Peroxide/Peroxidase Assay kit (Invitrogen). Each treatment and time point was replicated five times.

To visualize H_2O_2 accumulation, 3,3'-diaminobenzidine (DAB) staining was performed. Leaves from each treatment were incubated in 1 mg ml^{-1} DAB-HCl, pH 3.8 (Sigma-Aldrich) in the dark for 8 h, and then cleared by boiling in 95% ethanol for 15 min. Leaves were examined under bright-field microscopy, and images were then captured with a digital camera.

Proteinase inhibitor assays

Tomato leaves were collected 48 h after infestation with 50 newly emerged adult female *B. tabaci* that had previously been fed a diet without dsRNA or with either *dsBtFer1* or *dsEGFP* (as described earlier). PI activity was measured using the method as described by Sarmento *et al.* (2011). Trypsin activity was detected at 410 nm with a spectrophotometer. The difference between absorbance at 150 s and 60 s was used to determine trypsin activity. Measurements were performed in triplicate for each sample and were converted to milligrams of trypsin inhibited per gram of protein. Each treatment was represented by six replicate samples of tomato leaves.

Callose staining

Aniline blue staining was performed to detect callose deposition in tomato leaves treated as in the PI experiments following the method as described in Su *et al.* (2015*b*). Callose deposits were counted using QUANTITY ONE software (Bio-Rad). Counts from five adjacent fields of view along the length of the leaf (excluding the mid-vein or leaf edge) were averaged to generate a mean leaf value. Mean values from five leaves were averaged to generate a mean treatment value.

Phytohormone and gene expression experiments

Plants were subjected to the following treatments: (i) whitefly treatment. The first leaf of individual plants was infested with 50 newly emerged adult female *B. tabaci* that had previously been fed a diet without dsRNA or with either *dsBtFer1* or *dsEGFP* (as described earlier). (ii) BtFer1 treatment. The first leaf of individual plants was damaged by scratching a 9 mm^2 area of waxy cuticle with a razor blade. After the cuticle was scratched, 20 µl of either the purified recombinant protein BtFer1 (50 ng $µl^{-1}$) or the purified recombinant GFP (50 ng $µl^{-1}$) was infiltrated into the damaged sites using a syringe without a needle as described previously (Su *et al.*, 2015*a*). Wounding control plants received 20 µl of buffer only to the wounds. Leaf samples from a subset of plants were harvested at 48 h after the start of each treatment, and were stored at –80 °C until total RNA or phytohormones were extracted.

JA, JA-Ile, and SA analysis

Leaf samples were ground in liquid nitrogen, and the phytohormones were extracted with ethyl acetate spiked with labeled internal standards containing D_4-SA, D_6-JA, and D_6-JA-Ile (CDN Isotopes, Pointe-Claire, Canada), and analyzed with a triple–quadrupole LC-MS/MS system (Thermo Scientific, Waltham, MA, USA) following the method as described in Wu *et al.* (2007). Each treatment was replicated five times.

Reverse transcription real-time quantitative PCR (RT–qPCR)

Total RNA from whitefly tissues (primary salivary gland, midgut, cuticle, ovary, and fat body) and developmental stage samples (eggs, nymphs, psuedopupa, and newly emerged male and female adults) were collected and extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA from tomato leaves was extracted using an RNeasy plus Mini kit (Qiagen, Hilden, Germany). First-strand cDNA synthesis and RT–qPCR were performed using the SYBR Green PCR Master Mix (TaKaRa) on the 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) according to previously established methods (Su *et al.*, 2016, 2018*b*). Primers used for all tested genes are given in Supplementary Table S2. Expression levels of target genes were normalized with internal reference genes encoding *RPL29* and *EF-1α* from *B. tabaci*, and *Ubiquitin* and *Actin* from *S. lycopersicum*. Each gene was analyzed in triplicate for each of four biologically independent treatments.

Statistical analyses

Data were checked for normality and homogeneity of variance before analysis. ANOVAs were used to assess differences between treatments in *B. tabaci* performance, gene expression, H_2O_2 levels, callose deposition, and PI activity. If an ANOVA was significant, means were compared with a Tukey's honestly significant difference (HSD) test. The SPSS software package (ver.17, SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results

Isolation and characterization of BtFer1

The ORF of *BtFer1* (675 bp) was determined based on our previous *B. tabaci* genome data (Xie *et al.*, 2017) and by using RT–PCR (Supplementary Fig. S1, GenBank accession no. MF774484). Sequence analysis indicated that *BtFer1* encodes a 224 amino acid protein that has an extracellular signal peptide of 19 amino acids at its N-terminus and lacks transmembrane domains, suggesting that BtFer1 might be a secreted protein. The mass of the predicted mature protein is 23.45 kDa and the protein has a pI of 6.27. The protein has one possible *O*-glycosylation site (Supplementary Fig. S1). The predicted secondary structure of BtFer1 comprises 12 coil regions, 11 α-helixes, and two β-strands (Supplementary Fig. S2). According to a PROSITE scan, the protein sequence of BtFer1 includes one ferritin-like domain (^{44}Y–^{202}M, PS50905) (Supplementary Fig. S1). Based on protein alignment followed

by phylogenetic tree analysis, BtFer1 is homologous to insect ferritin and belongs to the ferritin-like superfamily. This superfamily is characterized by iron binding, storage, and transport region signatures, including: ferroxidase di-iron center sites (^{61}H, ^{68}Y, ^{95}T, ^{96}A, ^{99}D, ^{146}Q, and ^{184}H), ferrihydrite nucleation center sites (^{91}K, ^{94}D, ^{95}T, and ^{98}E), and iron ion channel sites (^{154}H, ^{174}S, and ^{177}E) (Arosio *et al.*, 2009) (Supplementary Figs S1, S3). BtFer1 shares the highest similarity (58%) with the ferritin from *Myzus persicae* (XP_022164719.1), followed by those from *Acyrthosiphon pisum* (XP_016664291.1) (57%), *Diuraphis noxia* (XP_015379107.1) (57%), and *Coptotermes formosanus* (AGM32344.1) (56%). RT–qPCR analysis revealed that BtFer1 was highly expressed in *B. tabaci* primary salivary glands and midguts (Fig. 1A) and that BtFer1 expression differed among *B. tabaci* developmental stages, with low levels in eggs and psuedopupae, and high levels in nymphs and adults (Fig. 1B).

To confirm that BtFer1 has Fe^{2+} binding ability and ferroxidase activity, the recombinant protein BtFer1 was successfully expressed in transformed *E. coli* BL21 (DE3) using the pET-28a vector (Supplementary Fig. S4). The molecular mass of purified BtFer1 was estimated to be ~27 kDa by SDS–PAGE (Supplementary Fig. S4). The Fe^{2+} binding ability of BtFer1 was verified by a gel mobility shift assay. Purified BtFer1 mixed with different concentrations of $FeSO_4$ was subjected to SDS–PAGE. Compared with the mobility of BtFer1 in the presence of 0.5 mM EDTA, the mobility of BtFer1 was decreased by the addition of $FeSO_4$ (Fig. 1C), indicating that BtFer1 has Fe^{2+} binding ability. We further monitored Fe^{2+} oxidation by measuring the increase in the absorbance at 310 nm resulting from the formation of Fe^{3+} in a reaction with a 1:500 protein:iron ratio. Enzyme activity assays demonstrated that BtFer1 was able to increase Fe^{3+} levels compared with blank control (HBS alone), although Fe^{2+} oxidation proceeded slower than that of ceruloplasmin, which was used as a positive control (Fig. 1D), indicating that BtFer1 has ferroxidase activity.

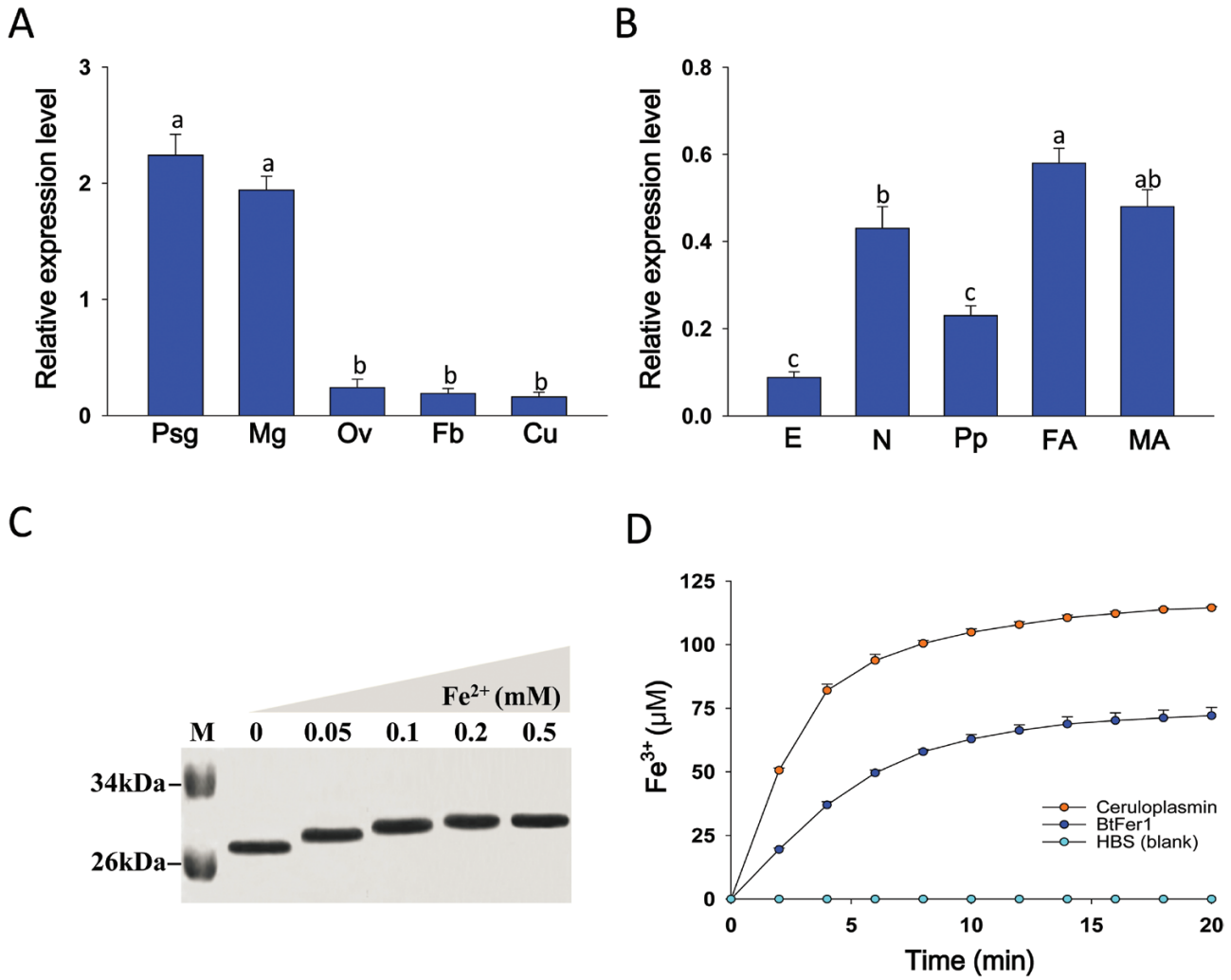

Fig. 1. Molecular characterization of *BtFer1*. Transcript levels of *BtFer1* in *B. tabaci* tissues (A) and developmental stages (B). Psg, primary salivary gland; Mg, midgut; Ov, ovary; Fb, fat body; Cu, cuticle; E, eggs; N, nymph; Pp, psuedopupa; FA, female adults; MA, male adults. Values are means ±SE (n=4). Means in (A) or (B) with different letters are significantly different (one-way ANOVA followed by HSD test, P<0.05). (C) Fe^{2+}-dependent mobility of purified BtFer1 under SDS–PAGE. The BtFer1 protein was incubated for 30 min at 24 °C with each of the following solutions: 0.5 mM EDTA (lane 0, used as a negative control) or 0.05, 0.1, 0.2, or 0.5 mM $FeSO_4$. (D) Kinetic measurements of purified BtFer1 ferroxidase activity were calculated by quantifying the amount of Fe^{3+} produced over 20 min. Individual data points shown are means ±SE (n=3). (This figure is available in color at *JXB* online.)

BtFer1 is secreted into tomato plants during B. tabaci *feeding*

Although we hypothesized that BtFer1 is secreted via saliva, it might also be present in honeydew. To explore this possibility, individual tomato plants were infested with 5000 newly emerged adult *B. tabaci* for 24 h, after which the honeydew excreted by *B. tabaci* was harvested, along with the top three heavily infested leaves, and proteins were extracted. Western blot analysis was performed using polyclonal anti-BtFer1 rabbit antibody. A band of ~23 kDa was detected in extracts from *B. tabaci* heads (containing the primary salivary glands) (Fig. 2A; lane 1). The same band was also detected in leaves infested by *B. tabaci* (Fig. 2A; lane 4). In contrast, the band of BtFer1 was not detected in *B. tabaci* honeydew or in leaves that had not been fed upon by *B. tabaci* (Fig. 2A; lanes 2 and 3). To confirm the results from our western blot analysis, we performed immunolocalization using anti-BtFer1 polyclonal antibody labeled with secondary antibody conjugated to green fluorescent dye. In *B. tabaci*-infested leaves, BtFer1 was detected in the phloem, a localization pattern reflecting the phloem-limited feeding of *B. tabaci* (Fig. 2B–E). In contrast, no signal was observed in *B. tabaci*-free leaves (Fig. 2F). Furthermore, control sections of *B. tabaci*-infested leaves incubated with pre-immune serum were also devoid of labeling (Fig. 2G). These results indicate that BtFer1 is a salivary component transferred from whitefly to plants during feeding.

BtFer1 decreases H_2O_2 levels in tomato

Ferritin plays a key role in the control of H_2O_2-generated oxidative signals (Arosio *et al.*, 2009). To investigate whether BtFer1 influences H_2O_2 levels in tomato plants induced by *B. tabaci* feeding, we used RNAi, as described by Su *et al.* (2018*b*), to obtain a *B. tabaci* population in which *BtFer1* had been silenced, then analyzed H_2O_2 levels in the leaves of plants infested by *B. tabaci* that had previously been fed a diet without dsRNA (used as feeding control) or with either dsRNA of EGFP (*dsEGFP*, used as an RNAi control) or dsRNA of *BtFer1* (*dsBtFer1*). Feeding with *dsBtFer1* decreased transcript levels of *BtFer1* in whole bodies and primary salivary glands of *B. tabaci* by 55–73% over a period of 7 d (Fig. 3A, B). A decrease in BtFer1 protein abundance was also observed in whitefly heads (containing the primary salivary glands) of the knockdown insects (Fig. 3C; Supplementary Fig. S5).

To measure potential non-target impacts of the RNAi treatment, we searched for closely related ferritin gene sequences in our annotated *B. tabaci* genome (Xie *et al.*, 2017), using tBLASTn analysis with Hemiptera ferritin transcripts as query and checking for the presence of conserved ferritin-like

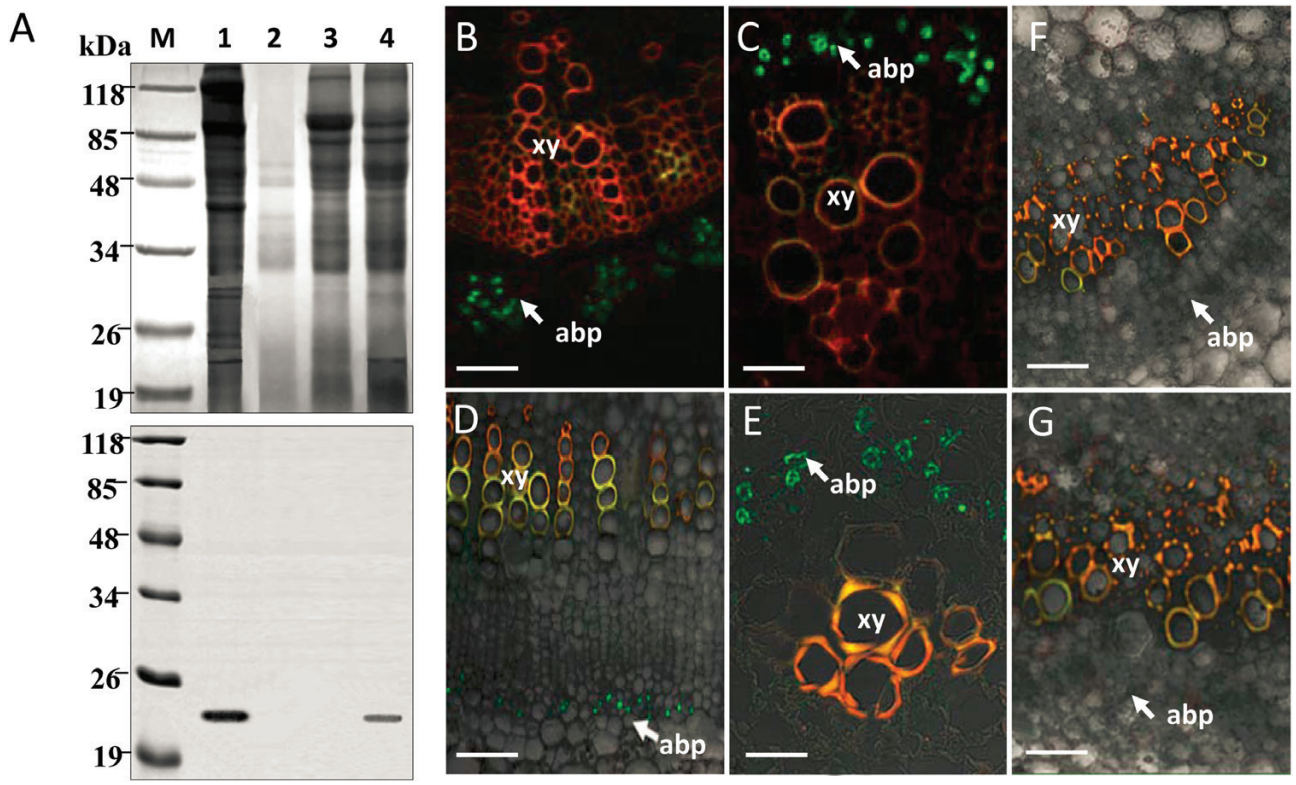

Fig. 2. Detection of BtFer1 in tomato. (A) SDS–PAGE (upper panel) and western blot (lower panel) analysis of extracts from *B. tabaci* heads (lane 1), *B. tabaci* honeydew (lane 2), uninfested tomato plants (lane 3), and plants infested by *B. tabaci* (lane 4). Rabbit anti-BtFer1 polyclonal antibody was used to develop the western blot. (B–G) Immunofluorescence localization of BtFer1 in tomato leaves. Sections of *B. tabaci*-infested (B–E) and non-infested (F) tomato leaves were incubated with anti-BtFer1 polyclonal antibodies and labeled with secondary antibody conjugated to green fluorescent Alexa Fluor 488 dye. (G) Sections of *B. tabaci*-infested tomato leaves were treated with pre-immune serum. Yellow and orange staining shows autofluorescence of phenolics in lignified cells; green staining shows BtFer1 localization. White light images and confocal images were superimposed in (D)–(G). Abbreviations: xy, xylem; abp, abaxial phloem. Scale bars=25 μm.

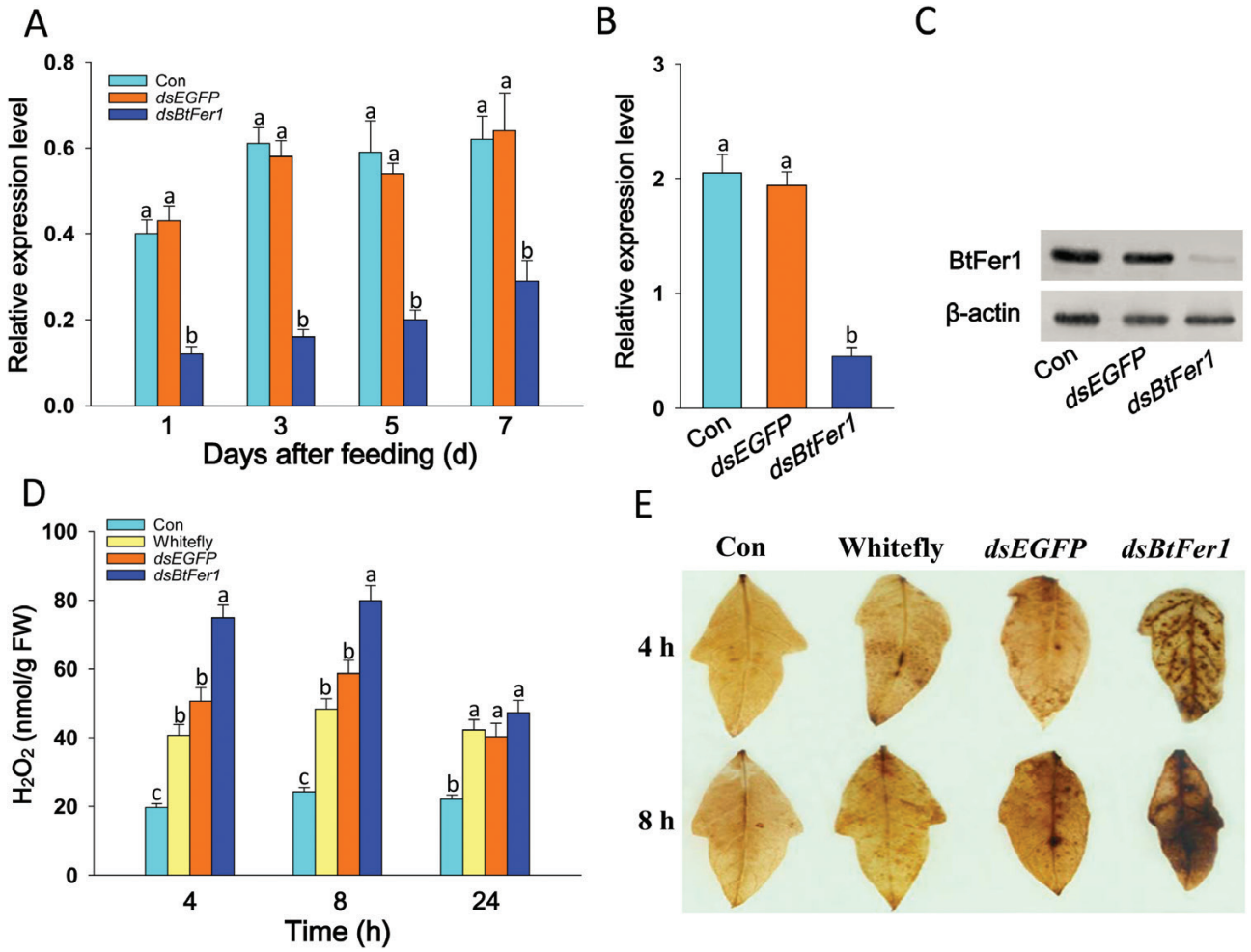

Fig. 3. BtFer1 decreases H_2O_2 levels in tomato. Transcript levels of *BtFer1* in (A) bodies of *B. tabaci* females after feeding on a diet with dsRNA of *BtFer1* (*dsBtFer1*), with dsRNA of *EGFP* (*dsEGFP*), or without dsRNA (Con) for 1, 3, 5, or 7 d, and in (B) primary salivary glands of *B. tabaci* females after feeding on the same three diets for 2 d. (C) Western blot analysis of BtFer1 in *B. tabaci* female heads that contain primary salivary glands after feeding with or without dsRNA as above for 2 d using anti-BtFer1 polyclonal antibody. β-Actin levels served as the loading control, and were detected using a β-actin-specific antibody. (D) Levels of H_2O_2 in tomato leaves at 4, 8, and 24 h, and (E) representative images of DAB staining (H_2O_2 indicator) of tomato leaves at 4 h and 8 h, after infestation with 50 newly emerged *B. tabaci* females previously fed (for 2 d) a diet with *dsBtFer1* (*dsBtFer1*), with *dsEGFP* (*dsEGFP*), or without dsRNA (Whitefly), as well as in uninfested control plants (Con). For (A), (B), and (D), values are means ±SE (n=4 for A and B and n=5 for D). In (B), means with different letters are significantly different; at each time interval in (A) and (D), means with different letters are significantly different (one-way ANOVA followed by HSD test, P<0.05).

domains. This approach revealed an additional four ferritin genes besides *BtFer1*. Full-length coding sequences of these four genes were amplified with gene-specific primers (Supplementary Table S2), analyzed by sequencing, and deposited as *BtFer2*, *BtFer3*, *BtFer4*, and *BtFer5* under the GenBank accession numbers MH324410, MH324411, MH324412, and MH324413, respectively. Analysis of the complete sequences of these genes revealed significant differences among genes of the ferritin family, and the highest similarity with *BtFer1* was only 19% (Supplementary Fig. S6). Interference efficiency and dynamics assays also showed that RNAi targeting of *BtFer1* did not statistically affect the transcript levels of the other four genes (Supplementary Fig. S7), suggesting that the effect of dsRNA on *BtFer1* is specific.

Down-regulation of *BtFer1* expression resulted in higher H_2O_2 levels in tomato plants at 4 h and 8 h following infestation by *B. tabaci* previously fed a diet with *dsBtFer1* (for 2 d) than in those infested by *B. tabaci* fed a diet with *dsEGFP* or without dsRNA (Fig. 3D). Using DAB staining, we also observed that leaves infested by *B. tabaci* previously fed a diet with *dsBtFer1* had much higher H_2O_2 accumulation at 4 h and 8 h than those infested by *B. tabaci* previously fed a diet with *dsEGFP* or without dsRNA (Fig. 3E). These results suggest that BtFer1 secreted during whitefly feeding decreases H_2O_2 levels in tomato.

Silencing BtFer1 *impairs the ability of* B. tabaci *to exploit host plants*

Silencing *BtFer1* did not affect the 7 d fecundity of adult *B. tabaci* females on tomato plants (Supplementary Fig. S8), but did result in a significant decrease in female survival (Fig. 4A). In contrast, no difference in survival was observed between *BtFer1*-silenced and control *B. tabaci* females reared on artificial diet (Fig. 4B), suggesting that the reduced survival of *B. tabaci* with silenced *BtFer1* was mediated by interaction with

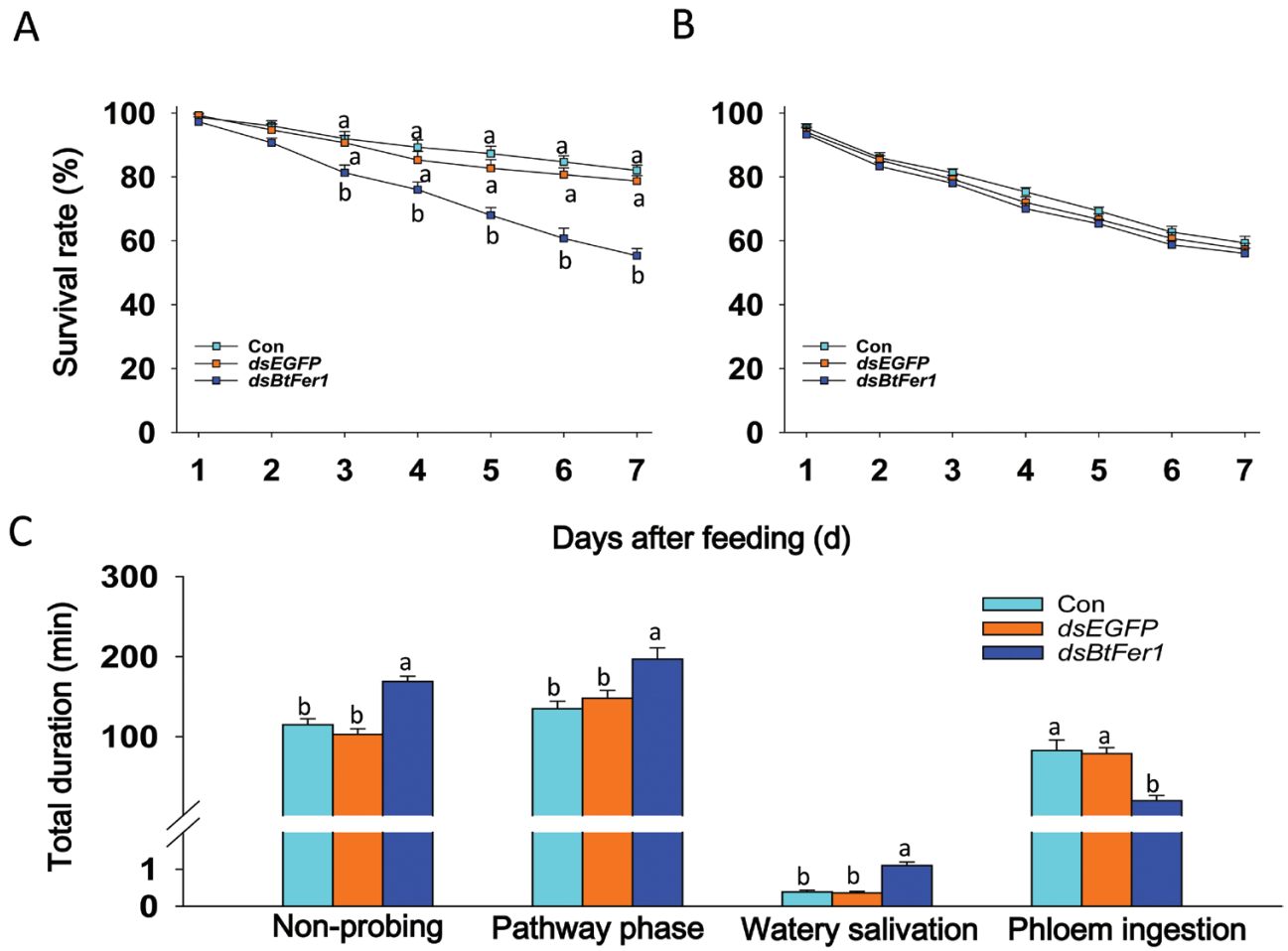

Fig. 4. BtFer1 is required for *B. tabaci* exploiting the host plants. (A and B) Survival rates of *B. tabaci* females previously fed (for 2 d) a diet with dsRNA of *BtFer1* (*dsBtFer1*), with dsRNA of *EGFP* (*dsEGFP*), or without dsRNA (Con), feeding on tomato plants (A) or artificial diet (B) for 7 d. (C) Total duration of EPG waveform types for *B. tabaci* females previously fed (for 2 d) a diet with dsRNA of *BtFer1* (*dsBtFer1*), with dsRNA of *EGFP* (*dsEGFP*), or without dsRNA (Con), over an 8 h recording period. For (A) and (B), values are means ±SE (n=15). For (C), values are means ±SE (n=15–20). In (A) and (B), means with different letters are significantly different, and the absence of letters indicates that differences were not significant; for each feeding phase in (C), means with different letters are significantly different (one-way ANOVA followed by HSD test, P<0.05). (This figure is available in color at *JXB* online.)

the host plant rather than direct effects of BtFer1 repression on the insect. We therefore explored the detailed effects of BtFer1 on the feeding behavior of *B. tabaci* in real time, using EPG techniques (Liu *et al.*, 2013). EPG recordings showed no variation between control and *dsBtFer1*-fed whiteflies from the initiation of probing to first phloem ingestion and the duration of probes. However, silencing of *BtFer1* significantly increased periods of non-probing, pathway phase, and watery salivation into phloem and significantly reduced the duration of phloem ingestion (Fig. 4C; Supplementary Table S1), thereby compromising the ability of *B. tabaci* to feed from phloem sap. Taken together, these results indicate that *B. tabaci* depends on BtFer1 to exploit host plants effectively, and further suggest that BtFer1 plays a role in repressing plant defense responses.

BtFer1 suppresses callose deposition and proteinase inhibitor production

To investigate how *BtFer1* affects host defenses, we quantified two markers of wound/JA-induced defenses, callose deposition and PI production, in tomato plants infested by *B. tabaci* that were previously fed a diet without dsRNA or with either *dsBtFer1* or *dsEGFP*. Both callose deposition and PI activity were significantly greater in plants infested by *B. tabaci* that had been fed a diet with *dsBtFer1* than in those infested by *B. tabaci* that had been fed a diet with *dsEGFP* or without dsRNA (Fig. 5A, B). We next assessed the expression patterns of callose synthase and hydrolyzing enzyme genes, along with corresponding PI genes, via real-time PCR. Two callose synthase genes, *CalS11-like* and *CalS12-like*, which encode proteins required for callose formation in tomato (Adkar-Purushothama *et al.*, 2015), were clearly up-regulated in plants after *B. tabaci* infestation. Silencing of *BtFer1* did not affect *B. tabaci*-induced expression of *CalS11-like* and *CalS12-like* genes in tomato (Fig. 5C). However, two callose-hydrolyzing enzyme genes, *Cel1* and *Cel2*, which are known to mediate the decomposition of tomato callose (Flors *et al.*, 2007) were not significantly induced in plants infested with *B. tabaci* that had been fed with *dsBtFer1* (Fig. 5C), which strongly suggests that BtFer1 prevents callose from decomposing. Furthermore, the expression of the PI genes *PI-Ic* and *PI-IIf* was stronger in plants infested by *B. tabaci* that had been fed a diet with *dsBtFer1* compared

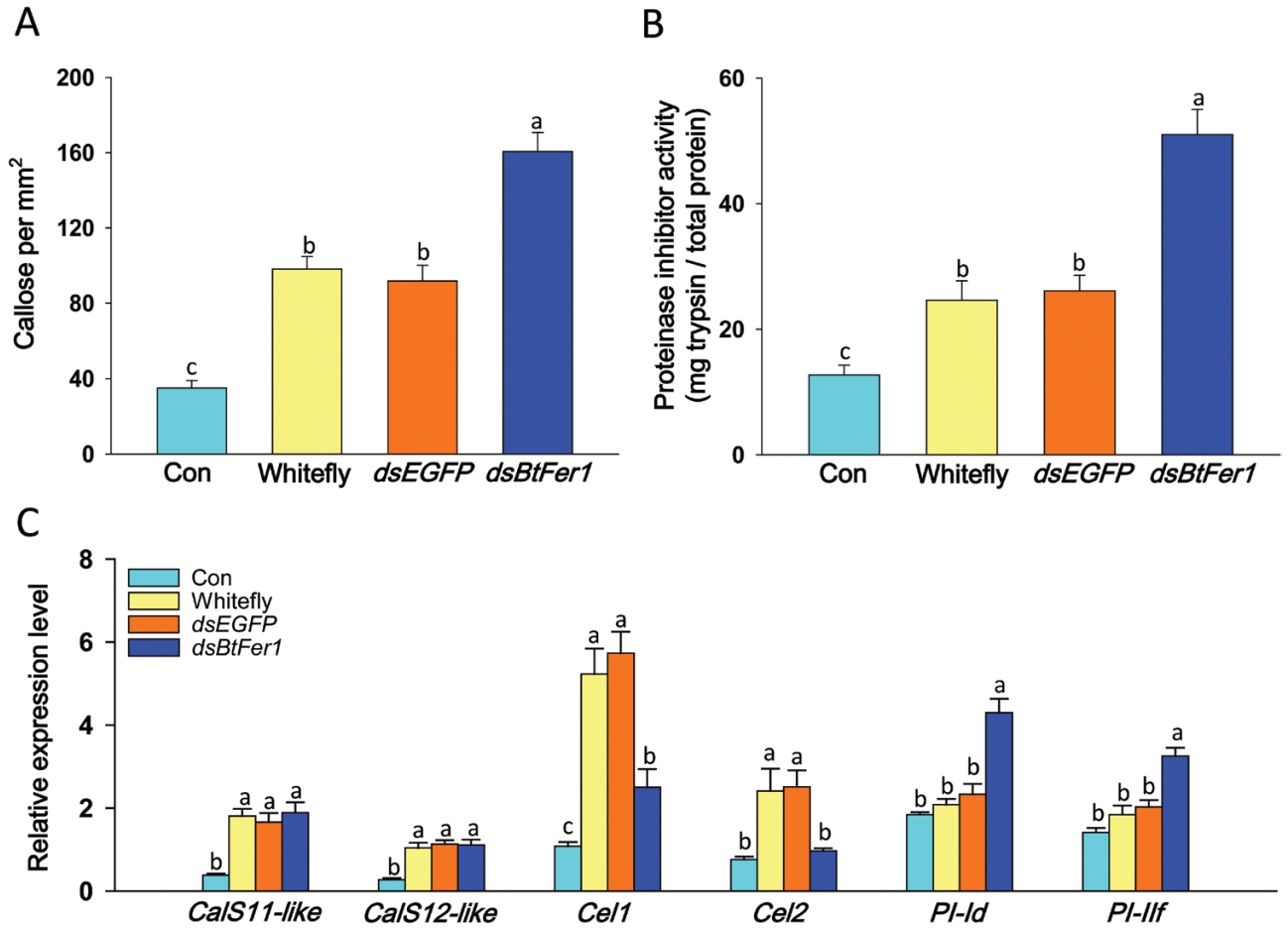

Fig. 5. Silencing of *BtFer1* increases callose deposition and proteinase inhibitor (PI) activity in tomato plants. (A) Callose accumulation, (B) PI activity, and (C) expression levels of callose-related genes and PI genes in tomato plants at 48 h after infestation with 50 newly emerged *B. tabaci* females previously fed (for 2 d) a diet with dsRNA of *BtFer1* (*dsBtFer1*), with dsRNA of *EGFP* (*dsEGFP*), or without dsRNA (Whitefly), as well as in uninfested control plants (Con). Values are means ±SE (n=5 in A, n=6 in B, and n=4 in C). In (A) and (B), means with different letters are significantly different; for each gene in (C), means with different letters are significantly different (one-way ANOVA followed by HSD test, P<0.05). (This figure is available in color at *JXB* online.)

with a diet with *dsEGFP* or without dsRNA (Fig. 5C). These results indicate that the BtFer1 secreted by *B. tabaci* suppresses callose deposition and PI production, benefitting whiteflies by allowing continuous ingestion and digestion of phloem sap.

BtFer1 represses JA-mediated defenses

Plant defense responses to whiteflies include the activation of pathways dependent on JA and SA signaling molecules (Kempema *et al.*, 2007; Zarate *et al.*, 2007). To identify the pathway involved in *BtFer1*-mediated defense suppression, we examined the effects of silencing *BtFer1* on levels of the phytohormones JA, JA-Ile, and SA, as well as transcript levels of defense-related genes in the JA- and SA-dependent pathways in tomato. In addition, we examined the effects of mechanical wounding followed by treatment with the recombinant protein BtFer1. *Bemisia tabaci* induced accumulation of SA, JA, and JA-Ile, and the expression of the SA marker genes *ICS* (isochorismate synthase), *PAL* (phenylalanine ammonia-lyase), and *PR-1a* (pathogenesis-related protein 1a), but not of the JA marker genes *AOS* (allene oxide synthase), *TD2* (threonine deaminase 2), and *LoxD* (lipoxygenase D) in tomato plants (Fig. 6A, B; Supplementary Fig. S9A). Silencing *BtFer1* did not alter hormone accumulation or the expression of SA marker genes, but now also led to the up-regulation of JA marker genes (Fig. 6A, B; Supplementary Fig. S9A). Transcript levels of *AOS* and *TD2* were significantly higher in plants after infestation by *B. tabaci* that had previously been fed a diet with *dsBtFer1* rather than a diet with *dsEGFP* or without dsRNA (Fig. 6B). Consistent with this observation, application of the recombinant BtFer1 protein had no influence on SA, JA, and JA-Ile biosynthesis or on SA-regulated *ICS*, *PAL*, and *PR-1a* transcripts, when compared with mechanical wounding or the application of the purified recombinant GFP protein, but suppressed the transcript levels of JA-responsive *LoxD*, *AOS*, and *TD2* (Fig. 6C, D; Supplementary Fig. S9B). These results show that BtFer1from *B. tabaci* represses the JA-mediated signaling pathway in tomato.

Suppression of JA defenses by BtFer1 enhances B. tabaci *survival*

The role of JA signaling in mediating defense responses that reduce *B. tabaci* performance is well established (Zarate *et al.*,

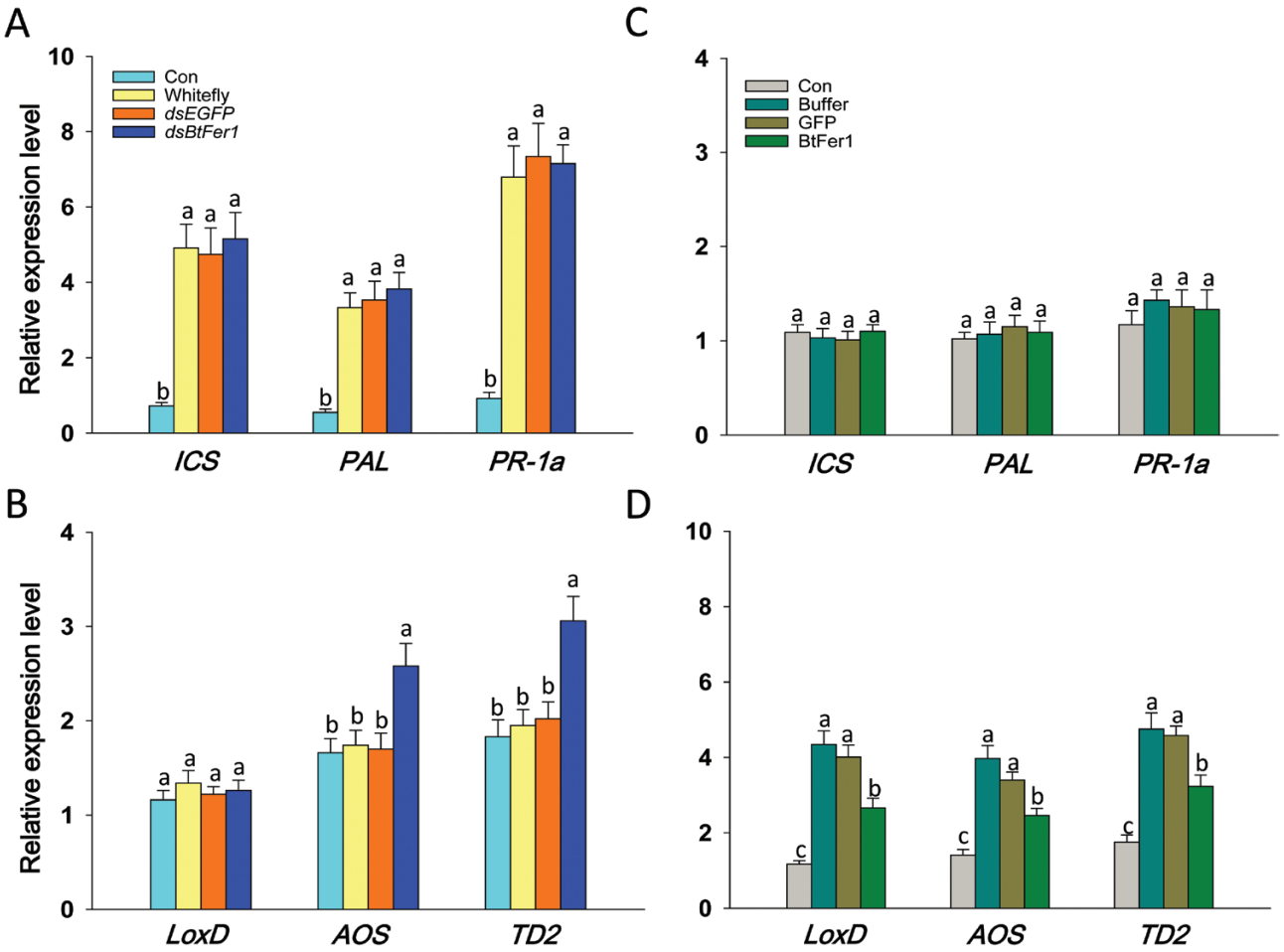

Fig. 6. BtFer1 suppresses the expression of JA-responsive genes in tomato plants. (A and B) Expression patterns of plant defense-response genes in tomato plants at 48 h after infestation with 50 newly emerged *B. tabaci* females previously fed (for 2 d) a diet with *dsBtFer1* (*dsBtFer1*), with *dsEGFP* (*dsEGFP*), or without dsRNA (Whitefly), as well as in uninfested control plants (Con). (C and D) Expression patterns of plant defense-response genes in tomato plants at 48 h after treatment with wounding plus 20 μl of the purified recombinant protein BtFer1 at 50 ng μl^{-1} (BtFer1), the purified recombinant GFP protein at 50 ng μl^{-1} (GFP), or buffer (Buffer), as well as kept non-manipulated (Con). Values are means ±SE (n=4). For each gene in each panel, means with different letters are significantly different (one-way ANOVA followed by HSD test, P<0.05). (This figure is available in color at *JXB* online.)

2007; Zhang *et al.*, 2013; Su *et al.*, 2015*a*). We therefore tested the extent to which the observed suppression of JA defenses by BtFer1 benefits *B. tabaci* by using a tomato *spr2* mutant line that is deficient in JA biosynthesis but otherwise exhibits normal growth, development, and reproduction (Li *et al.*, 2003). Whereas silencing *BtFer1* significantly decreased the survival of *B. tabaci* maintained on wild-type CM plants (Fig. 7A), survival was recovered for *BtFer1*-silenced *B. tabaci* fed on the *spr2* plants (Fig. 7B). These results demonstrated that suppression of JA defenses by BtFer1 is sufficient to increase *B. tabaci* survival.

Discussion

Our results demonstrate that *BtFer1* encodes a ferritin (Supplementary Fig. S1) with Fe^{2+} binding ability and ferroxidase activity (Fig. 1C, D) that is secreted into tomato during *B. tabaci* feeding (Fig. 2) and suppresses the induction of oxidative signals characterized by H_2O_2 production (Fig. 3D, E). These findings strongly suggest that BtFer1 is important in tomato–whitefly interactions. Consistent with this inference, the silencing of *BtFer1* resulted in the activation of the JA-mediated signaling pathway in tomato, leading to induced callose deposition and PI production (Figs 5, 6). These effects, in turn, prevented *B. tabaci* from continuously ingesting and digesting phloem sap, leading to reduced whitefly survival on tomato, but not on artificial diet (Fig. 4). Furthermore, using the tomato JA biosynthesis mutant *spr2* confirmed that suppression of JA defenses by BtFer1 is sufficient to increase *B. tabaci* survival (Fig. 7). Our findings thus demonstrate that BtFer1 functions as a herbivore-derived effector that suppresses host plant defense responses and thereby enhances *B. tabaci* performance.

Oxidative signaling induced by insect feeding is an important component of plant resistance to invading insects. For example, detoxification of ROS following herbivory by the corn earworm, *Helicoverpa zea*, decreased antioxidant levels and increased H_2O_2 and other toxic oxidation products that directly damage the insect midgut and reduce insect growth (Bi and Felton, 1995). Similarly, consumption of artificial diets containing even relatively low concentrations of H_2O_2 caused

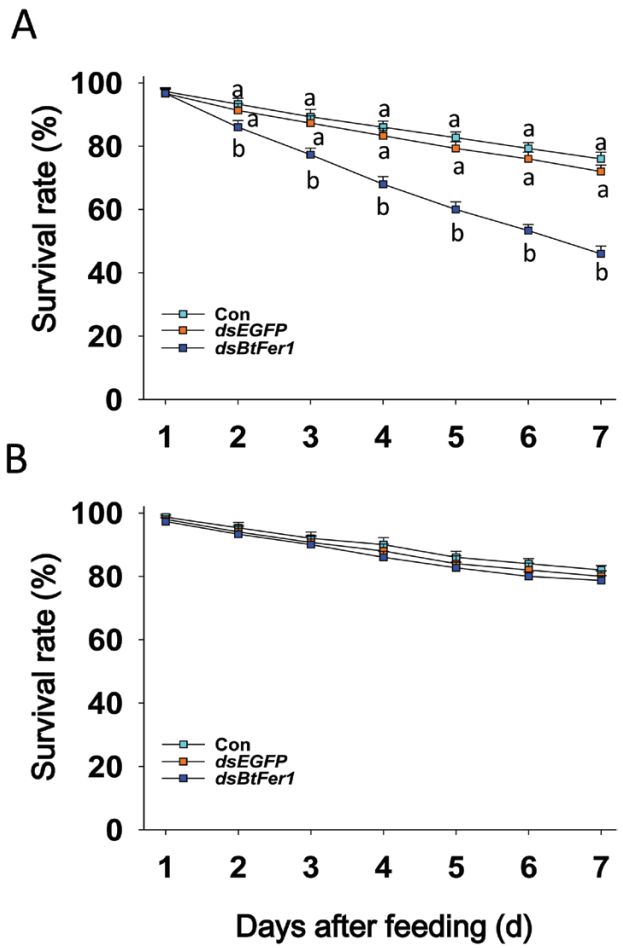

Fig. 7. *BtFer1*-silenced *B. tabaci* restores survival on JA-deficient *spr2* tomato plants. Survival rates of *B. tabaci* females previously fed (for 2 d) a diet with dsRNA of *BtFer1* (*dsBtFer1*), with dsRNA of *EGFP* (*dsEGFP*), or without dsRNA (Con), feeding on the wild-type CM (A) and JA-deficient *spr2* (B) tomato plants for 7 d. Values are means ±SE (n=15). In each panel, means with different letters are significantly different, and the absence of letters indicates that differences were not significant (one-way ANOVA followed by HSD test, P<0.05). (This figure is available in color at *JXB* online.)

high mortality of the Hessian fly *Mayetiola destructor* (Liu *et al.*, 2010), while enhanced resistance of Arabidopsis against the green peach aphid, *M. persicae*, is also accompanied by increased H_2O_2 levels that impede the function of BOTRYTIS-INDUCED KINASE1 (Lei *et al.*, 2014). Furthermore, genes whose transcription is regulated by JA have been shown to be modulated by H_2O_2 (Orozco-Cárdenas *et al.*, 2001), suggesting a potential link between the suppression of oxidative signaling responses by BtFer1 and the effects on defense genes regulated by the JA signaling pathway observed in the current study (Fig. 6).

Evidence that BtFer1 secreted via whitefly saliva affects H_2O_2-mediated signaling pathways in tomato is provided by the observation that plants exhibited higher H_2O_2 levels when infested by *B. tabaci* with silenced *BtFer1* compared with controls (Fig. 3D, E). The elevated H_2O_2 levels observed in tomato plants infested with *B. tabaci* with silenced *BtFer1* may, in turn, lead to decreased quantities and quality of nutrients and antioxidants, and damage to *B. tabaci* tissues. Our bioassays also revealed that knockdown of *BtFer1* significantly reduced *B. tabaci* survival on tomato but not on artificial diet (Fig. 4A, B), suggesting that the reduced survival of *B. tabaci* with silenced *BtFer1* is likely to be explained by higher levels of H_2O_2 and downstream effects on interactions with the host plants, rather than by general deleterious effects of *BtFer1* silencing. Interestingly, silencing *BtFer1* did not affect the egg production by the individual surviving whiteflies for 7 d, but had significant influence on egg production after 14 d (Supplementary Fig. S8). Thus, the effect of *BtFer1* silencing on whitefly fecundity would become apparent over longer time spans than the 7 d course of this experiment.

This interpretation is further supported by knockdown of the *BtFer1* transcript in *B. tabaci* via feeding with artificial diet supplemented with *BtFer1* dsRNA, using methods described by Su *et al.* (2018*b*). This method was effective in knocking down *BtFer1* transcript levels in the whole body and in the primary salivary glands (Fig. 3A, B) while having limited effects on four other ferritin genes (*BtFer2*–*BtFer5*), which also exhibit relatively high transcript levels but did not incur a statistically significant knockdown, presumably because of significant sequence differences from *BtFer1* (Supplementary Figs S6, S7). Thus, the observed phenotype in *BtFer1*-silenced *B. tabaci* (i.e. altered EPG patterns and reduced survival) is likely to be due to the knockdown of *BtFer1* itself.

Phloem plugging, including plugging via callose deposition, is thought to be important in interactions between plants and whiteflies (Kempema *et al.*, 2007; Walling, 2008). We found more callose deposits in tomato plants infested by *B. tabaci* with silenced *BtFer1* than in tomato plants infested by *B. tabaci* with non-silenced *BtFer1* (Fig. 5A). Moreover, silencing of *BtFer1* repressed the expression of *Cel1* and *Cel2*, genes encoding β-1,4-glucanase (Fig. 5C), which is important in the breakdown of callose in tomato infested by whiteflies (Flors *et al.*, 2007). Thus, the physical barriers imposed by callose may have contributed to the reduced performance of *B. tabaci* with silenced *BtFer1*. Because large amounts of callose on sieve plates can reduce phloem translocation, or even block it completely (Will and Bel, 2006), our results strongly suggest that silencing of *BtFer1* reduces the ability of *B. tabaci* to feed on phloem sap.

EPG recordings revealed that *B. tabaci* with silenced *BtFer1* exhibited significantly more time in non-probing and stylet pathway phases, as well as more time during salivation into the phloem, but less time feeding (Fig. 4C; Supplementary Table S1). Salivation always precedes phloem ingestion, and a longer duration of salivation is associated with difficulty in the initiation of phloem sap ingestion (Tjallingii, 2006). These results therefore suggest that secreted BtFer1 can prevent phloem plugging, which otherwise impedes continuous ingestion of phloem sap. As noted, somewhat similar effects have been documented for the aphid–broad bean system, in which *Megoura viciae* saliva can prevent sieve tube plugging by forisomes (Will *et al.*, 2007).

Increased PI activity also characterizes induced plant defense responses against chewing herbivores (Lawrence *et al.*, 2007; Chung *et al.*, 2013), and JA is known to mediate the induction of PIs (Turner *et al.*, 2002). For example, tomato *def-1* mutants, which are deficient in wound-induced JA accumulation

and the expression of downstream target genes, do not show increased PI activity upon herbivore attack (Li *et al.*, 2002). In the current study, PI activity and the expression of PI genes were higher in plants infested by *B. tabaci* with silenced *BtFer1* than in plants infested by *B. tabaci* with non-silenced *BtFer1* (Fig. 5B, C). This increase in PI activity coincided with the up-regulation of *AOS* and *TD2*, which function in two JA-dependent pathways (Fig. 6B). However, silencing *BtFer1* did not significantly alter SA, JA, and JA-Ile levels or transcript levels of *ICS*, *PAL*, and *PR-1a*, three marker genes of the SA signaling pathway (Fig. 6A; Supplementary Fig. S9A). Consistent with this, exogenous application of recombinant protein BtFer1 to mechanically wounded plants suppressed transcript levels of the JA-responsive *LoxD*, *AOS*, and *TD2* genes, but had no influence on phytohormone biosynthesis or on levels of SA-regulated *ICS*, *PAL*, and *PR-1a* transcripts (Fig. 6C, D; Supplementary Fig. S9B). Thus, our data indicate that BtFer1 secreted by *B. tabaci* suppresses the JA-mediated signaling pathway. These findings are consistent with previous reports indicating that whiteflies can suppress JA-mediated plant defenses in plants (Zarate *et al.*, 2007; Zhang *et al.*, 2013; Su *et al.*, 2015*a*). We also found that the performance of silenced *B. tabaci* was recovered on the JA-deficient *spr2* tomato plants compared with wild-type CM plants (Fig. 7), suggesting that JA-related processes related to the production of defenses such as PI may also impair whitefly performance.

Conclusions

In summary, our results show that BtFer1 is a salivary ferritin secreted into tomato plants during whitefly feeding that reduces H_2O_2 levels, callose deposition, and PI production, and represses JA-mediated defense responses, thereby enhancing whitefly performance. These findings further suggest that the secreted salivary protein BtFer1 functions as an effector, suppressing defense response in tomato plants and allowing *B. tabaci* to ingest phloem continuously. This study thus provides insight into the mechanism by which host plant manipulation by a herbivore involves the secretion of a salivary ferritin and broadens our understanding of the role of effector proteins in plant–herbivore interactions.

Supplementary data

Supplementary data are available at *JXB* online.

Fig. S1. Nucleotide sequence of *BtFer1* and its deduced amino acid sequence.

Fig. S2. Secondary structure analysis of BtFer1.

Fig. S3. Sequence alignment of homologous ferritins from insects.

Fig. S4. Expression and purification of BtFer1.

Fig. S5. The complete gel for detection of BtFer1 and β-actin in *B. tabaci* female heads.

Fig. S6. Alignment of BtFer proteins from *B. tabaci*.

Fig. S7. RNAi targeting of *BtFer1* did not statistically affect the expression of the other four ferritin members.

Fig. S8. Effect of silencing *BtFer1* on the fecundity of individual adult *B. tabaci* females.

Fig. S9. BtFer1 does not affect the levels of SA, JA, and JA-Ile in tomato plants.

Table S1. Feeding behavior parameters from whiteflies fed a diet with dsRNA of *BtFer1* (*dsBtFer1*) or *EGFP* (*dsEGFP*) or without dsRNA (Con).

Table S2. Primers used in this study.

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