Supporting Information Appendices

Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and defense hormone biosynthesis

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Supplementary Materials and Methods

AY-WB Maintenance and Insect Rearing and Fecundity Experiments

All insect rearing and maintenance of AY-WB-infected plant materials occurred in 0.52 x 0.48 x 0.52 m^3 insect rearing cages in a growth room set at 16/8-hour light/dark period at 26/20ºC. Cages were started with ca. 50 female and male *M. quadrilineatus* adults and 2 oat (*Avena sativa* L.) plants. To obtain AY-WB-infected leafhoppers, ca 50 female and male *M. quadrilineatus* leafhoppers were transferred to AY-WB-infected China aster (*Callistephus chinensis* Nees) for two weeks to allow oviposition. Nymphs were kept on the infected plants until adulthood. The adults were used for experiments or were transferred to young China aster or Arabidopsis seedlings to create new batches of AY-WB-infected plants and leafhoppers.

Analysis of AY-WB symptoms in Arabidopsis

Arabidopsis Col-0 plants were grown in 10/14 hour light/dark at 22ºC. Trays of ten 4 week-old plants were exposed to twenty *M. quadrilineatus* leafhoppers (2 leafhoppers per plant) enclosed within perforated bags (300 x 400mm) for inoculation. A total of 20 plants were exposed to non-infected leafhoppers (referred to as healthy plants), and another 20 plants to AY-WB-infected leafhoppers (infected plants). Following addition of insects, all plants were transferred to a growth room in 16/8

hour light/dark at 23ºC/20ºC for the duration of the experiment. After one week, all insects were removed. All twenty plants exposed to AY-WB infected insects exhibited marked symptoms of infection, including stunted growth and yellowing of developing rosette leaves. Witches' broom symptoms were observed ca. 14 days after inoculation. Stems of healthy and infected plants were counted 3.5 weeks post-inoculation.

M. quadrilineatus survival, fecundity and oviposition experiments

Arabidopsis Col-0 plants were grown and the experiments were conducted in 10/14hour light/dark period at 22ºC. Three week old plants were exposed to three non-infected or AY-WB-infected adult male *M. quadrilineatus* (harvested from the rearing cages as described above) for one week. Plants started showing AY-WB symptoms ca. 14 days later at which stage they were used for the fecundity experiment. For other experiments (35S::*SAP11* or 35S::*LOX2* lines), the Arabidopsis plants were used for survival and fecundity assay without preceding exposure to *M. quadrilineatus.*

Fecundity was assayed for four days for a range of adult ages. To raise adults of similar age, adults were transferred to rearing cages with 6 Arabidopsis plants for a 5-day oviposition period and removed. For the fecundity assay of TCP silencing lines (**Fig. 7B**) only, *M. quadrilineatus* was reared on Oat (*Avena sativa*). The hatched nymphs were allowed to grow until two days after their final moult into adults. Plants were replaced with fresh plants every four days to prevent mixing of newly hatched nymphs with the existing leafhopper population. The fecundity experiments started with transferring a first set of two males and 8 females to each of three 7 week-old Arabidopsis plants for four days. All the *M. quadrilineatus* used for fecundity assay were anesthetized with CO_2 gas for sexing. The plants were placed in separate trays using a randomized design. Tray numbers were recorded to determine whether possible differences in conditions between trays account for variation in leafhopper survival and fecundity. After the four days, live adults on these plants were counted and removed, and nymphs were counted a further 15 days after removal of the adults. To avoid double counting a mouth aspirator was used to remove nymphs as they were counted. In order to assess the

successful hatch rate of eggs oviposited, eggs were counted on plants during two replicates of the fecundity trials. Eggs were carefully identified and counted the day after adults were removed and counted. Eggs were predominantly laid into stem tissue on the underside of the oldest Arabidopsis rosette leaves. Hatch rate was assessed by calculating the number of nymphs divided by the number of eggs found per plant. This was repeated for different ages of adults in which the leafhoppers were transferred to a fresh set of non-infected or AY-WB-infected Arabidopsis plants for four days.

To visualize *M. quadrilineatus* feeding/probing sites on Arabidopsis leaves, trypan blue, which stains dead cells, was used. Leaves were submerged in lactic acid–phenol–trypan blue solution (2.5 mg/ml trypan blue, 25% (vol/vol) lactic acid, 23% (vol/vol) water-saturated phenol, and 25% (vol/vol) glycerol) and boiled for 2 min. The leaves were destained with 70% (vol/vol) chloral hydrate in a shaker for 90 mins. The leaves were further destained with fresh chloral hydrate over night. The destained leaved were soaked in 80% (vol/vol) glycerol and mounted on microscope slides for photographs. The numbers of feeding/probing site per leaf were counted from the photographs.

Statistical analyses

All the statistical analysis was completed in Genstat v.13 (International Ltd, Hemel Hempstead, UK). For all data points of insect fecundity and oviposition assay, we recorded the biological replicate, adult age, and plant treatment or line as explanatory variables, and female survival as response variables. Analysis of Deviance was used to determine differences in female survival rates, egg hatch rate, fecundity, oviposition and number of feeding sites. All explanatory variables were described as factors and modelled to assess their contribution to the deviance in leafhopper survival, egg hatch rate, fecundity, oviposition rates and number of feeding sites. Female survival data and egg hatch data were analyzed using a binomial distribution within a generalized linear model (GLM). Fecundity, oviposition and feeding data were analyzed using a Poisson distribution within a GLM. When fecundity and oviposition data were analyzed, female survival in each experiment was added as a co-

variate. Means of nymph or egg numbers or female survival at each insect ages were compared using t-probabilities calculated within the GLM.

For all the other experiments, normal distributions of the data sets were examined by Genstat, and Analysis of Variance or Student's t-test (1 tail) was used.

Generation and analyses of 35S::*SAP11* Arabidopsis lines

All the intermediate DNA constructs were maintained in *Escherichia coli* DH5α cells. A codon-optimised version of the AY-WB SAP11 sequence (GenBank accession number gi: 85057280) without the sequence corresponding to the signal peptide and attB1 and attB2 adapters at the ends (***SI Appendix*, Fig. 2A**) was synthesized (GenScript, Piscataway, NJ, USA). This SAP11 fragment was amplified with attB1 and attB2 adapter primers (***SI Appendix*, Table 2**) and was cloned into pDONOR207 (Invitrogen) using Clonase LR (Invitrogen Ltd, Paisley, UK) following the manufacturer's instructions. Clones were sequenced to verify the origin and sequence of the insert. The SAP11 fragment in pDONOR207 was then Clonase LR cloned into the Gateway-adapted vector pB7WG2 that has a 35S promoter fragment cloned 5' end of the clonase compatible insertion site(1). The resulting plasmids were transformed into *Agrobacterium tumefaciens* strain GV3101. Arabidopsis Col-0 was transformed by floral dip as described (2). T1 seeds were germinated in soil and the transformants were BASTA selected. T2 seeds of BASTA-resistant lines were plated on MS media containing 20 μg/ml phosphinothricin. The lines showing a single insertion based on a 3:1 segregation ratio of live:dead seedlings were selected, and homozygous progeny of these plants were used for further analyses.

Detection of SAP11 in transgenic plants

Antibodies to SAP11 were raised against a partial peptide sequence of SAP11 (CEEGSSSKQPDDSKK) in rabbits (GenScript). Four seedlings of 10 days old Arabidopsis seedlings grown on Murashige and Skoog (MS) media were ground in 200µl of extraction buffer (50 mM sodium phosphate, pH 7.0, 10 mM Triton X-100, 10 mM N-lauroylsarcosine, 1 mM 2-mercaptoethanol). Twelve µl of the extracts were mixed with 4x NuPAGE LDS sample buffer (Invitrogen) and separated on 15% (w/v) sodium dodecyl sulfate (SDS) polyacrylamide gels (PAGE) and transferred to 0.45 µm Protran BA85 nitrocellulose membranes (Whatman®, Dassel, Germany) using the BioRad (Life Science, Hemel Hempstead, UK) minigel and blotting systems following standard procedures (3). The western blots were incubated with αSAP11 and peroxidase-conjugated anti-rabbit IgG (Sigma-Aldrich, Dorset, UK) and detection of bound antibodies was conducted with Immobilon Western Chemiluminescent HRP Substrate (Millipore, Watford, UK).

Yeast Two-Hybrid Analysis

Yeast two-hybrid screening was performed by Hybrigenics Services SAS, Paris, France. The codon-optimised sequence of AY-WB SAP11 without signal peptide (aa 1-90, ***SI Appendix,* Fig. 2A**) was PCR-amplified and cloned into pB27 as a C-terminal fusion to LexA (N-LexA-SAP11-C) and into pB66 as a C-terminal fusion to Gal4 DNA-binding domain (N-Gal4-SAP11-C). The constructs were checked by sequencing the entire insert and used as a bait to screen a random-primed *Arabidopsis thaliana* seedlings cDNA library constructed into pP6. pB27, pB66 and pP6 derive from the original pBTM116 (4), pAS2ΔΔ (5) and pGADGH (6) plasmids, respectively. For the LexA bait construct, 130 million clones (13-fold the complexity of the library) were screened using a mating approach with Y187 (mat alpha) and L40DGal4 (mat a) yeast strains as previously described (5). For the Gal4 construct, 74 million clones (7-fold the complexity of the library) were screened using the same mating approach with Y187 and CG1945 (mat a) yeast strains. A total of 25 His+ colonies were selected on a medium lacking tryptophan, leucine and histidine. The prey fragments of the positive clones were amplified by PCR and sequenced at their 5' and 3' junctions. The resulting sequences

were used to identify the corresponding interacting proteins in the GenBank database (NCBI) using a fully automated procedure. A confidence score (PBS, for Predicted Biological Score) was attributed to each interaction as previously described (7).

35S::*miR319a*, 35S::*miR3TCP*, BLS::*rTCP4GFP*, 35S::*LOX2* and 35S::*LOX2* antisense lines

Seed for the 35S::*miR319a* (Col-0), 35S::*miR3TCP* (Col-0), 35S::*miR319a x* 35S::*miR3TCP* (Col-0) and pBLS::*rTCP4GFP* (Ler) lines were kindly provided by Dr. Idan Efroni (Department of Plant Sciences, Weizmann Institute of Science, Rehovot 76100, Israel) and the phenotypes of the transgenic lines were described previously (8). The BLS promoter was selected for expression of *rTCP4GFP*, because 35S::*rTCP4* plants show severe developmental phenotype. Activity of the BLS promoter is initiated and maintained solely in young lamina (P3 to P7 stages) (9). Transgenic pBLS:miR319a plants show crinkled leaves similarly those of 35S::*miR319a* plants (8).

The 35S::*LOX2* (NASC ID: N3748) and corresponding control line 35S::*LOX2* antisense (NASC ID: N3749) were requested from the European Arabidopsis Stock Centre and were described previously (10).

Generation of crosses

Flowers of transgenic lines derived from Col-0 were emasculated and pollinated with the stamens from the transgenic lines derived from Ler. The presence of the 35S::*SAP11* and pBLS::*rTCP4GFP* in the crosses and parent lines was verified with specific primers (SAP11_f and SAP11_r, pBLS_f and TCP4_r) using genomic DNA as template. PCR with actin primers (ActinFor and ActinRev) was used as positive control. Primer sequences are provided in ***SI Appendix,* Table 2**.

Co-expression and pull-down assays

For cloning the full-length Arabidopsis cDNAs corresponding to TCP2, 4, 7 13, 3, 5, 10, 7 and 24 specific primers sets were designed to add partial sequences of the attB1 or attB2 Gateway recombination sites (***SI Appendix,* Table 2**), and used in PCRs with a cDNA library generated from Col-0 *Arabidopsis* leaves as template. The fragments were further amplified using attB1 and attB2 adapter primers (***SI Appendix,* Table 2**) and were then Clonase LR cloned into pDONOR207 (Invitrogen). The inserts were then Clonase LR cloned into pGWB18 that has a 35S promoter and a 3xMyc fragment cloned at the 5' end of the Clonase LR compatible insertion site (11). The resulting 35S::*3xmyc-TCP* recombinant plasmids were electro-transformed into *A. tumefaciens* strain GV3101.

For generating the pTRBO:FLAG-RFP and pTRBO:FLAG-SAP11 constructs, the RFP fragment was synthesized along with the sequence corresponding to a N-terminal FLAG tag and *Pac*1 and *Not*1 restriction sites (GenScript) and cloned into the *Pac*1 and *Not*1 sites of pJL48 (also known as pTBRO) (12). The SAP11 fragment was amplified with primers PacI-FLAG-SAP11Wt-For and AvrII-SAP11Wt-Rev (***SI Appendix,* Table 2**) using the codon-optimized version of SAP11 (***SI Appendix,* Fig. 2A**) as template, and cloned into the *Pac*I and *Avr*II sites of pTBRO (12). The resulting pTRBO recombinant plasmids were electro-transformed into *A. tumefaciens* strain GV3101.

To generate the 35S::*GFP-SAP11* construct, the SAP11 fragment in pDONOR207 (described above) was Clonase LR cloned into the Gateway-adapted vector pB7WGF2 that has 35S promoter and GFP fragments cloned 5' end of the clonase compatible insertion site(1). To create 35S::*GFP*, eGFP fragment was amplified from pB7WGF2 by attB1foreGFP and attB2reveGFP primers (***SI Appendix,* Table 2**) and cloned into pDONOR207 and then into pB7WG2. The plasmids were transformed into *A. tumefaciens* strain GV3101.

The co-expression and pull-down assays were conducted in *N. benthamiana* leaves. Plants were grown in a controlled growth room in 16/8-hour light/dark at 22ºC with 55% humidity for both the co-expression and pull down experiments. The *A. tumefaciens* clones carrying the appropriate constructs were inoculated into 10 ml LB (Luria- Bertani) medium for overnight growth at 28ºC. The cultures were spun down and pellets were resuspended into 10 mM $MgCl_2$ to a final OD_{600}=0.1 for cultures

containing *pTRBO:FLAG-RFP* and *pTRBO:FLAG-SAP11*, and OD_{600}=0.5 for cultures containing all the other constructs. A 100 mM acetosyringone solution was added to the cultures to a 150 µM final concentration. Cultures were incubated for one hour at room temperature and then infiltrated into individual *N. benthamiana* leaves using a needle-less syringe. After three days, two 11-mm diameter leaf disks (one leaf disc per leaf) immediately adjacent to the mid vein were harvested and snap-frozen in liquid nitrogen along with the remaining leaf samples.

The two leaf disks were ground in liquid nitrogen in the presence of 50 µl 1x NuPage Sample Buffer (Invitrogen), and boiled for 5 minutes. Proteins were separated on 12.5% (w/v) SDS-PAGE gels. Western blot analyses and detection of proteins with antibodies were conducted as described above. In average three µl were loaded for detection of the FLAG-SAP11 and FLAG-RFP fusions with anti-FLAG (Sigma-Aldrich) and 15 µl for detection of 3xMyc-TCP proteins with anti-Myc (Santa Cruz Biotechnology, Inc., Heidelberg, Germany).

The remaining leaf sample was used in pull-down assays. The leaf was cut into one cm^2 pieces and crosslinked with 30 ml of 10mM tris(hydroxymethyl)aminomethane (Tris)-HCl (pH 8.0), 400mM sucrose, 3% w/v formaldehyde, 0.1 mM phenylmethanesulfonylfluoride (PMSF), 5 mM β-mercaptoethanol using vacuum infiltration for 10 min. The reaction was then quenched with 2.5ml 2 M glycine and vacuum infiltrated for a further 5 minutes. The entire extraction was conducted in a cold room at 4 °C. Samples were ground into a fine powder using a pestle and mortar in the presence of two ml GTEN buffer [10% (vol/vol) glycerol, 25mM Tris-HCl (pH 7.5), 1 mM EDTA, 150 mM NaCl], and centrifuged at 3000x g for 10 minutes. The supernatant was transferred to a fresh two ml Eppendorf tube and centrifuged at 14000x g for 10 minutes. The FLAG-tagged proteins were extracted by combining 250 µl of supernatant with 1.7 ml of GTEN buffer + 0.1% (vol/vol) Tween20 (IP buffer) and 20 µl anti-FLAG resin (Sigma-Aldrich), which was prewashed with IP buffer. The extraction was repeated four times for each sample. The four extractions were incubated end-over-end for one hour at 4 °C, pooled and then washed five times with IP buffer. The bound proteins were eluted in 40 µl of IP buffer containing 150 ng μl^{-1} 3X FLAG peptide (Sigma-Aldrich) for 30 minutes.

In average 15 µl of the eluates were mixed with NuPAGE LDS sample buffer (Invitrogen), boiled for at least 30 minutes, and then separated on 12.5% (w/v) SDS-PAGE gels and analyzed on western blots as described above.

Quantitative RT-PCR experiments

For wounding experiments, mature leaves of 5 week old Arabidopsis were pinched twice by a pair of forceps. Samples were harvested 60 min after wounding. For infestation assay, a mature leaf of 5 week old Arabidopsis Col-0 wild type was caged (cylinder shape, 1cm radius, 1.5 cm deep) with 10 adult *M. quadrilineatus* anesthetized by CO_2 gas. As non-treated controls, Arabidopsis leaves were caged without *M.quadrilineatus.*

Arabidopsis leaves were snap frozen and used for RNA extraction with TRI® reagent (Sigma-Aldrich) and purified using Qiagen RNeasy columns (Qiagen Ltd., Crawley, West Sussex, UK). cDNA was synthesized from 0.5 microgram of total RNA using M-MLV reverse transcriptase (Invitrogen), and subjected to real time PCR SYBR® Green JumpStart™ Taq ReadyMix™ (Sigma-Aldrich) in a DNA Engine Opticon 2 (BioRad, Life Sciences) using gene specific primers (***SI Appendix,* Table 2**). Each reaction was triplicated, and average threshold cycle (Ct) was used to determine the fold change of gene expression. For the quantification of *LOX2* in 35S::*SAP11* transgenic lines (**Fig. 6A**) and 35S::*LOX2* line, fold differences of *LOX2* compared to that of the Col-0 non-transgenic line or control line which overexpresses antisense strand of LOX2 were calculated by using U-box housekeeping gene AT5G15400 transcript as a reference gene (13). Pfaffl method was used for this(14). Student's t-test was used to see whether the values are different from 0 (0 indicates no difference in *LOX2* expression compared to Col0 or the control line). In ***SI Appendix,* Figure 7**, relative expression level of *LOX2* compared to AT5G15400 transcript was determined by the comparative Ct method. Data were averaged from three independent biological replicates.

Extraction and quantification of jasmonic acid

Arabidopsis leaves were harvested carefully or wounded twice by forceps, and two to three leaves per treatment were collected at indicated time points and snap-frozen in liquid nitrogen. Four week old plants were used for the experiment shown in **Fig. 6B,** and 6 week old plants were used for the experiment shown in **Fig. 6C** and ***SI Appendix*, Fig. 6F**. To generate AY-WB infected plants, 6 plants (four-week old) were placed in a perforated bag with 20 AY-WB-carrier or non-carrier *M. quadrilineatus* males. The insects were removed 1 week later and the plants were wounded at 1, 2 and 3 weeks after insect removal. The samples were lyophilized and ground to powder with liquid nitrogen, and extracted at 4°C overnight with 5 mL of methanol containing 100 ng deuterated JA (d_5-JA, C/D/N isotopes) as an internal standard. The total extracts were centrifuged in a swing- bucket rotor (eppendorf 5810R, 4000 rpm, rotor radius 173 mm, 5 min), and the supernatants were dried under N_2 gas. The pellets were dissolved in diethyl ether:methanol (60:9) and loaded onto a Strata NH2 solid phase extraction column (Phenomenex) preequilibrated with 2mL diethyl ether. After two washes with 0.8mL chloroform:isopropanol (2:1), JA was eluted twice by 1 mL diethyl ether containing 4% (vol/vol) acetic acid. The combined eluates were dried with N_2 gas, and the pellets dissolved in methanol:water (1:4). Samples (10µL) were analysed using an Agilent 1100 single quadrupole LC-MS system. Samples were separated on a 50×2mm 3µ Luna C18(2) column (Phenomenex) using the following gradient of 0.1% (vol/vol) formic acid in water (Solvent A) versus methanol (Solvent B), run at 400 $\mu L.min^{-1}$ and 23°C: 0 min, 20% B; 6 min, 80% B; 7 min, 80% B; 7.2 min, 20% B; 9.5 min, 20% B. JA and d_5-JA were detected by negative electrospray mass spectrometry, using single ion monitoring of masses 209.1 and 214.1 with a fragmentor voltage of 70V and spray chamber conditions of 11 $L.min^{-1}$ drying gas at 350°C, 30 psi nebuliser pressure, and 3500V capillary voltage.

Supplemental Figures

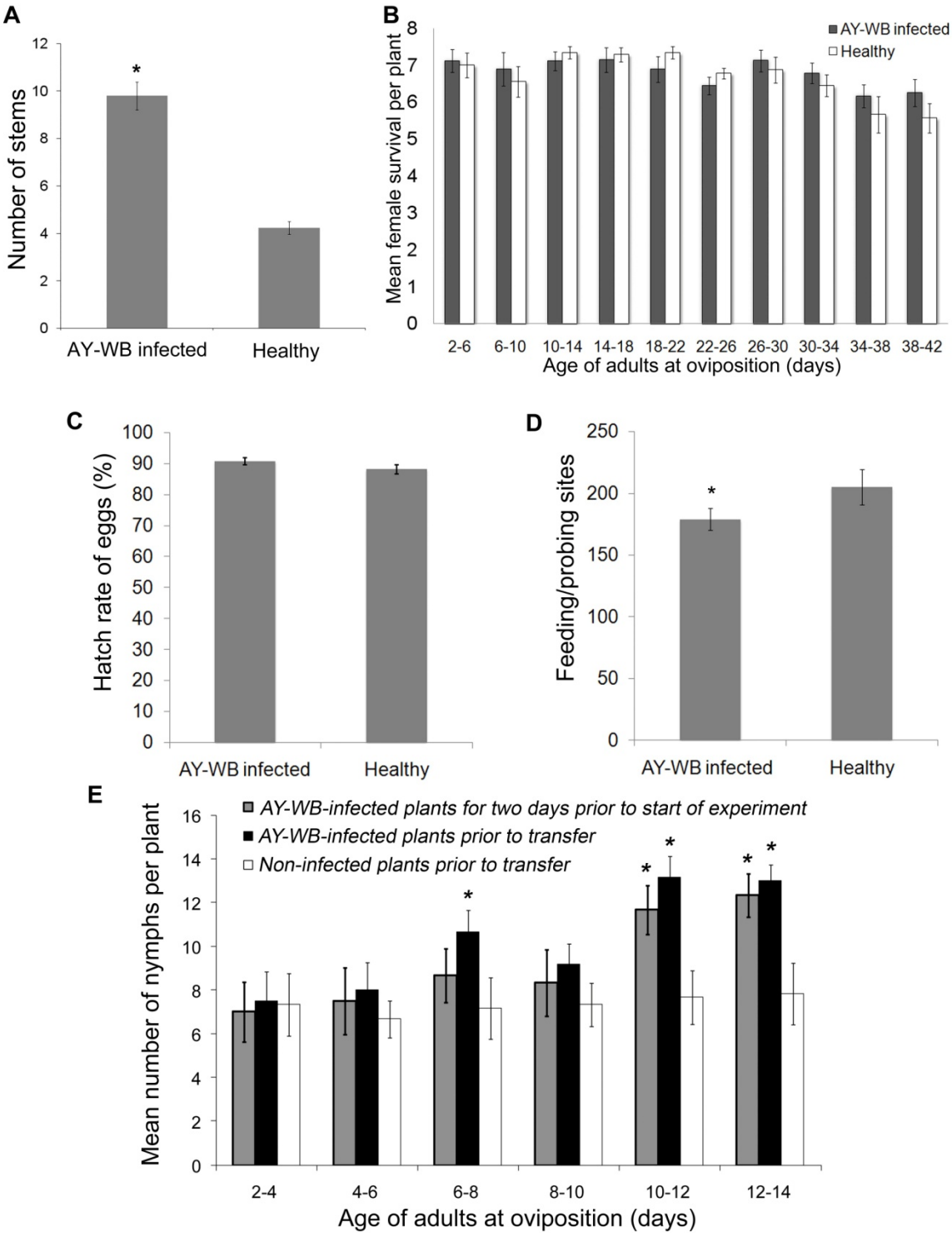

Fig. 1. **Effect of AY-WB infection on Arabidopsis and the insect vector.** (**A)** Infected plants produced more stems. Plants were grown and treated as described in **Fig. 1** legend. The data were collected at 3.5 weeks post infection. Bars present standard error from the mean. *P < 0.05 (Student's

t-test). (**B**, **C**) *M. quadrilineatus* female survival (**B**) and egg hatch rates (**C**) are similar on AY-WB infected and healthy plants. Data in **B** and **C** were obtained from experiments shown in **Fig. 2**. In **B**, upon the 4-day exposure of plants to various age ranges (x-axis) of 8 adult females and 2 adult males, numbers of surviving females were counted (y-axis). Each experiment included 3 plants per age range per treatment and the experiments were repeated three times. Columns show the mean numbers of surviving females per plant of all biological replicates for each leafhopper age range. Bars=s.e. In **C**, columns show the mean percentages of the hatch rates of eggs calculated from the number of nymphs (**Fig. 2A**) divided by the number of eggs laid (**Fig. 2B**) on each experimental plant for two biological replicates. Bars = s.e. (**D**) Leafhopper feed/probe less on AY-WB-infected plants. AY-WB-infected and healthy plants were obtained as described in the legend of **Fig. 2**. Twelve mature leaves were caged with 5 adult *M. quadrilineatus* for 5 days and then subjected to trypan blue staining to visualize the feeding/probing sites. Columns show the mean feeding/probing sites per leaf. *$P < 0.001$ by ANODE. Bars = s.e. (**E**) AY-WB phytoplasma does not affect leafhopper fecundity up to 6 days after acquisition. Eight females and two males of various age ranges (x-axis) were transferred to healthy experimental plants for two days and nymphs were counted 15 days later (y-axis). Each experiment included 3 plants per age range per treatment and the experiments were repeated two times. Columns show the mean numbers of nymphs per plant of two biological replicates for each leafhopper age range and treatment. Bars=s.e. Black and white columns represent nymph counts of adult leafhoppers that were kept on AY-WB-infected plants and non-infected plants, respectively, prior to transfer to experimental plants for 2 days, while the grey bars represent nymph counts from adult leafhoppers that were allowed access to AY-WB infected plants for maximally two days at the beginning of the experiment. These leafhoppers were quarantined on healthy plants before transfer to the experimental plants for 2 days. * $P < 0.05$ when the treatments were compared to the treatment that used the insects kept on non-infected plants (white bars) at each adult age (t probabilities calculated within GLM).

A

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Optimized     1 AAAAAGCAGGCTCCACCATGTCTCCAAAGAAGGAATCTTCAGATAAAAAGAGAGATATTC
Original      1                    ATGTCACCTAAAAAAGAATCTAGTGATAAAAAAAGAGATATTC

Optimized    61 CAAAAATTAACAAATCTGAAGAGAAGAACAAGAAGCAAAAAGAGGATATTAAGAGATTTT
Original     44 CGAAAATTAATAAATCAGAAGAAAAAAACAAAAAACAAAAAGAAGATATAAAAAGATTTT

Optimized   121 ATACTATTCATAAGGAGTTTAAGGAATATTCTATTGAAAAGAACAATGAGATCATTAAGA
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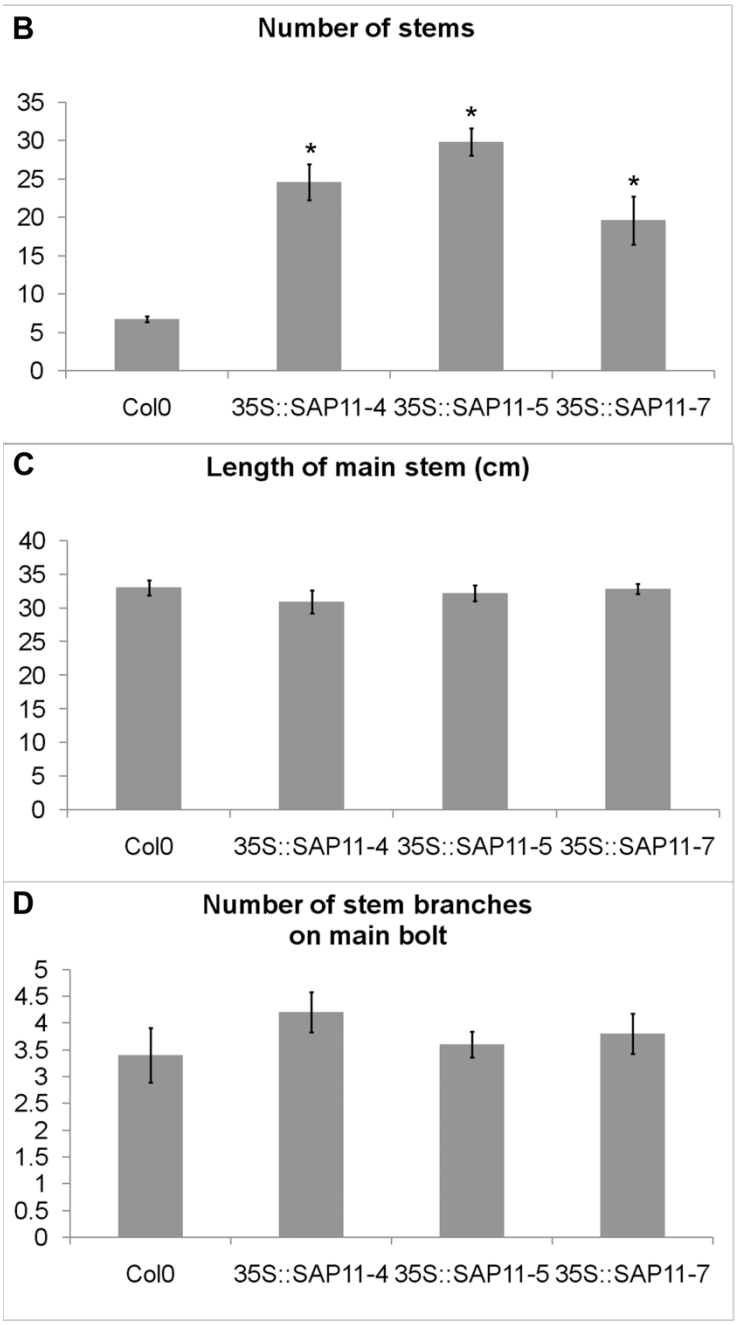

Fig. 2. **The 35S::*SAP11* lines produce more stems.** (**A**) The complete synthesized codon-optimized sequence of SAP11 with the additional attB1 and attB2 sites (underlined) for generating a fragment

compatible with the Gateway® BP Clonase™ II system (Invitrogen). Optimised codons are shown in red. (**B**) Three 35S::*SAP11* lines generate significantly more stems compared to Col-0. *P<0.05 compared to Col-0 (ANOVA and the Tukey's multiple comparison test). (**C** and **D**) The SAP11 lines did not generate taller stems (**C**) or more branches on the main bolt (**D**) at the time when most of the siliques had matured (9 weeks after seed sowing for Col-0 and 12 weeks for 35S::*SAP11*). Columns show the mean and standard errors (bars) calculated from 5 plants per line. Plants were grown at 16/8 hour light/dark period at 22ºC.

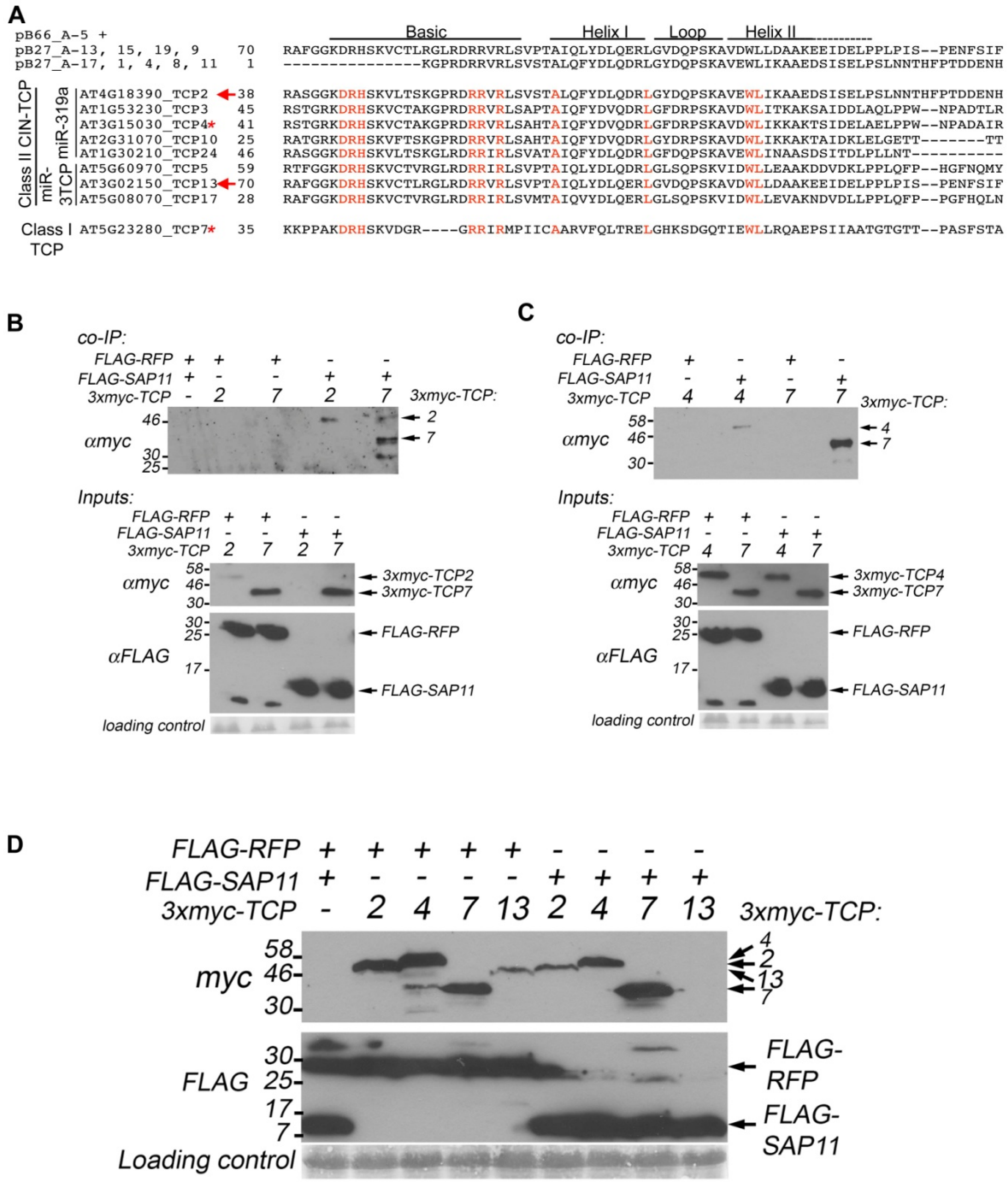

Fig. 3 SAP11 interacts with CIN-TCP transcription factors and destabilizes these transcription factors. (**A**) The DNA binding basic-Helix-Loop-Helix (bHLH) structure that is conserved among Class I and II TCP transcription factors is present in prey fragments of clones pB27 and pB66 that interact with N-LexA-SAP11-C and N-Gal4DBD-SAP11-C fusions, respectively, in the Hybrigenics services SAS yeast two-hybrid screens of Arabidopsis seedlings RP2 libraries. Closest matches of the

positive prey clones are TCP2 (clones pB27 A17, A1, A4, A8 and A11) and TCP13 (clones pB66 A-5 and pB27 A-13, A15, A19 and A9) and are indicated with red arrows. TCP4 and TCP7 were included in pull-down expression and are indicated with an asterisk. Conserved residues are highlighted in red font. (**B** and **C**) FLAG-SAP11 co-immunoprecipitates with myc-tagged TCP2, TCP4 and TCP7. *N. benthamiana* leaves were coinfiltrated with either *pTRBO::FLAG-SAP11* or *pTRBO::FLAG-RFP* (control) together with one of the 35S::*3xmyc-TCP2*, *13*, *4* or *7* constructs. Leaves were crosslinked with formaldehyde and protein extracts were run through an anti-FLAG resin to capture FLAG-SAP11 or FLAG-RFP and interacting proteins. The eluates were boiled for 30 minutes and then analyzed on western blots using antibodies to myc. Molecular weight markers are indicated in kDa at left of the blots. (**D**) SAP11 destabilizes CIN-TCP transcription factors. The abundance of TCP2, 4 and 13 are reduced in the presence of SAP11 compared to RFP. *N. benthamiana* leaves were coinfiltrated with either *pTRBO:FLAG-SAP11* or *pTRBO:FLAG-RFP* (control) together with one of the 35S::*3xmyc-TCP2*, *13*, *4* or *7* constructs. Protein extracts of leaf disks taken from the infiltrated leaves were transferred to western blots and probed with antibodies to myc and FLAG tags. Loading control stained by Ponceau Red shows equal loading. Molecular weight markers are indicated in kDa at left of the blots.

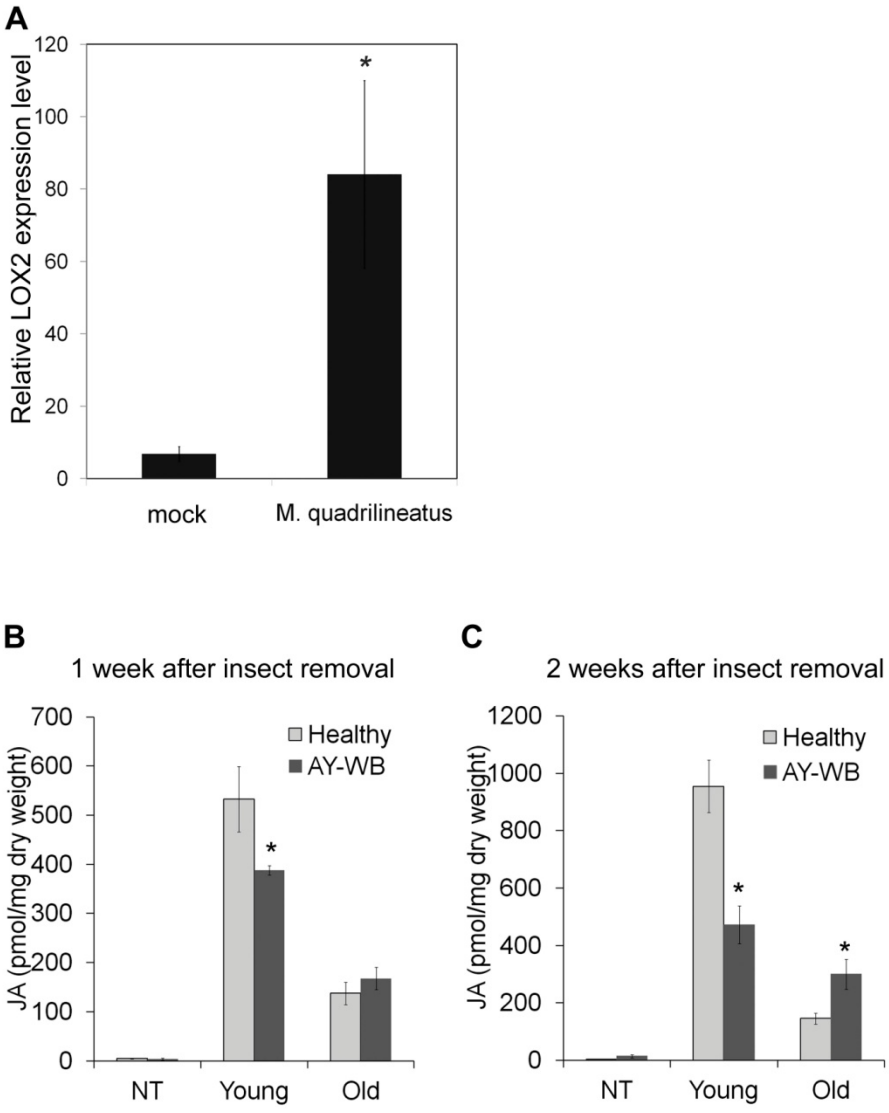

Fig. 4. JA production that is induces by *M. quadrilineatus* is reduced in AY-WB-infected plants. (**A**) Single five-week old mature Arabidopsis Col-0 leaves were clip-caged with 10 adult *M. quadrilineatus* or clip-caged without *M. quadrilineatus* (mock). In each experiment, three leaves per Arabidopsis line were individually analyzed and the experiment was repeated twice. The leaves were harvested 24 hours after *M. quadrilineatus* exposure and subjected to qRT-PCR to determine the expression level of *LOX2* compared to that of the U-box housekeeping gene AT5G15400 (13). The columns show the mean *LOX2* expression of 6 leaf samples relative to AT5G15400 (e.g. value 1 indicates expression levels of *LOX2* and AT5G15400 are equal). Bars = s.e. *P-value <0.05 (Student t-test). (**B** and **C**) The Arabidopsis were caged with AY-WB-carrier or non-carrier *M. quadrilineatus* males for 1 week. Leaves were wounded at 1 week (**B**) or 2 weeks (**C**) after insect removal. Young

symptomatic leaves of AY-WB infected Arabidopsis produce less JA 90 min after wounding compared to healthy control plants while old leaves showed no (**B**) or small differences (**C**). Columns show mean of 3 biological replicates. NT= non treated mature leaves. *P-value < 0.05 compared to corresponding healthy control (Student's t-test).

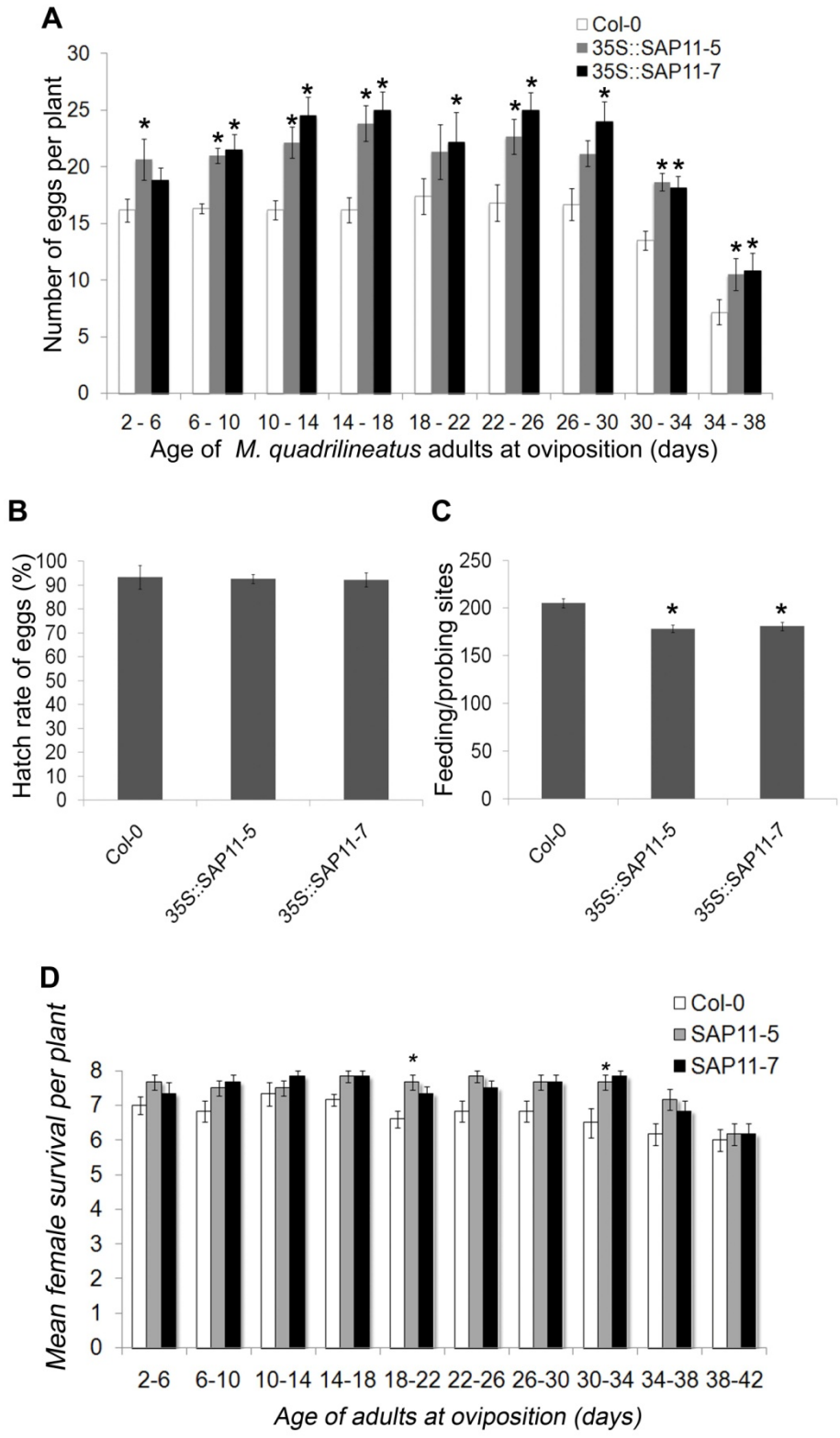

Fig. 5. *M. quadrilineatus* oviposition, egg hatch rate, feeding/probing and survival on *35S::SAP11* lines. (**A, B** and **C**) Data were obtained from experiment shown in **Fig. 7A**. Each experiment included 3 plants per age range per treatment and the whole experiments were repeated

two times. Each 7 week-old plant received 8 adult females and 2 adult males of different age ranges for 4 days (x-axes). (**A**) Oviposition rates. Numbers of eggs were counted one day (**A**) and nymphs 15 days (**Fig. 7A**) after removal of the adult insects. Columns show the means of egg number per plant calculated from two biological replicates for each leafhopper age range and line. *P < 0.05 when lines were compared to Col-0 at each adult age (t probabilities within GLM). (**B**) Egg hatch rates. Columns show the mean percentages of the hatch rates of eggs calculated from the number of nymphs (**Fig. 7A**) divided by the number of eggs laid (***SI Appendix*, Fig. 5A**) on each experimental plant for two biological replicates. (**C**) *M. quadrilineatus* leafhoppers feeding/probing. A mature leaf of 4-week old Arabidopsis lines was caged with 5 adult *M. quadrilineatus*. Twelve leaves for Col-0 and 8 leaves for two 35S::SAP11 lines were analyzed. The leaves were harvested 5 days upon *M. quadrilineatus* exposure and subjected to trypan blue staining to visualize the feeding/probing sites. Columns show the mean feeding/probing sites per leaf. Bars=s.e *P<0.05, (t probabilities calculated within GLM). (**D**) *M. quadrilineatus* female survival rates. Columns show the mean numbers of surviving females per plant of all biological replicates for each leafhopper age range. *P < 0.05 when lines were compared to Col-0 or the control line at each adult age (t probabilities calculated within GLM). Bars = s.e.

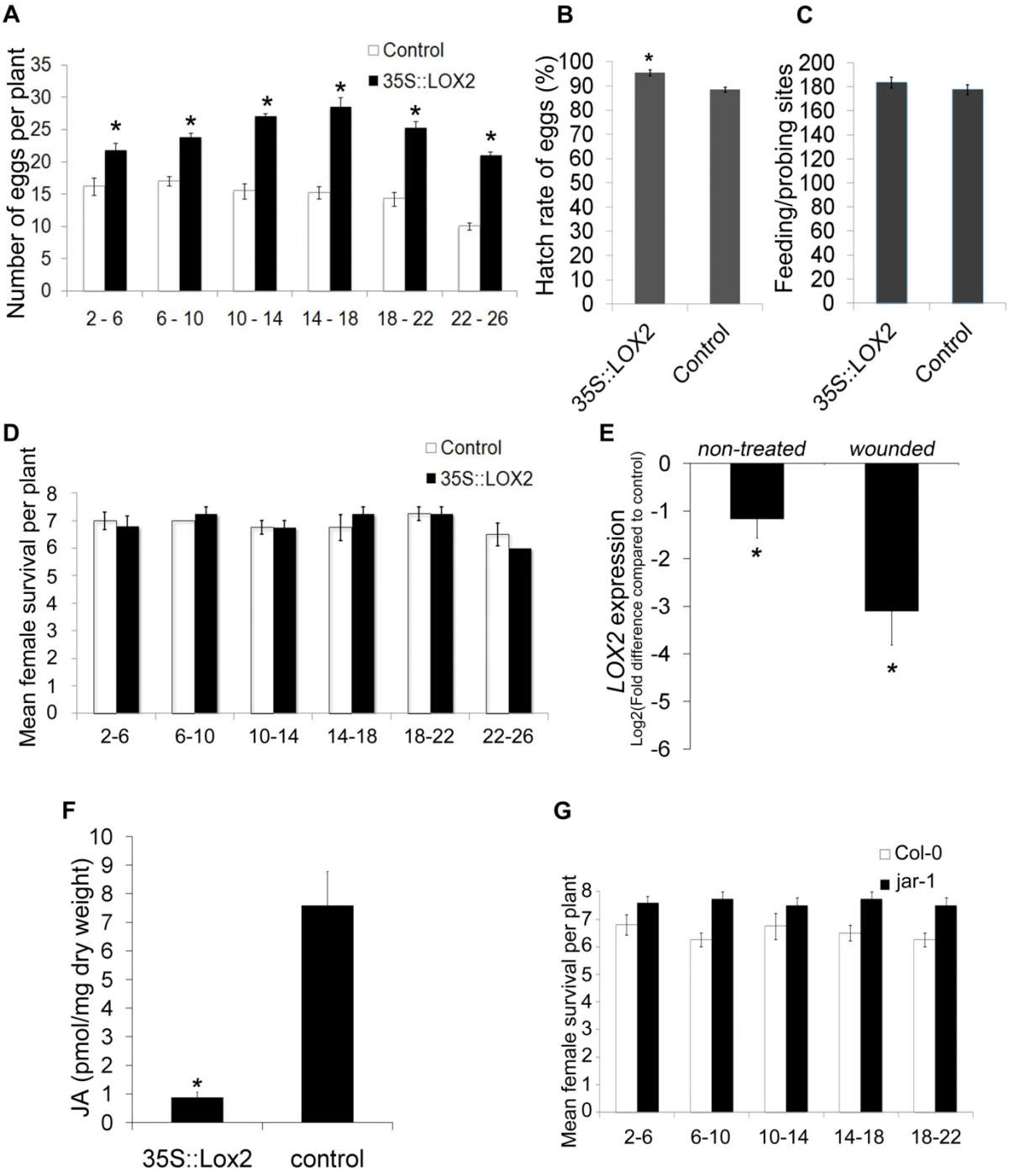

Fig. 6. *M. quadrilineatus* oviposition, hatch rates, feeding/probing and survival on Arabidopsis deficient in JA synthesis. (**A, B** and **C**) Data obtained from experiments shown in **Fig. 7C**. (**A**) *M. quadrilineatus* oviposition on *LOX2*-silenced line and the control line (10), which over-expresses antisense strand of *LOX2*. Columns show the mean numbers of eggs per plant calculated from all biological replicates for each leafhopper age range. *P < 0.05 compared to the control line at each adult age, t probabilities calculated within GLM. (**B**) Egg hatching rates on *LOX2*-silenced and

control lines. *P < 0.001 by ANODE compared to the control line. Columns show the mean percentages of the hatch rates of eggs calculated from the number of nymphs (**Fig. 7C**) divided by the number of eggs laid (***SI Appendix*, Fig. 6A**) on each experimental plant for two biological replicates. (**C**) *M. quadrilineatus* feeding/probing sites on *LOX2*-silenced and control lines. Mature leaves (8 per Arabidopsis line) of 4-week old Arabidopsis lines were caged with 5 adult *M. quadrilineatus*. The leaves were harvested 5 days after *M. quadrilineatus* were added and subjected to trypan blue staining to visualize the feeding/probing sites. Bars = s.e. (**D**) *M. quadrilineatus* female survival rates on *LOX2*-silenced and control lines. Columns show the mean numbers of surviving females per plant of all biological replicates for each leafhopper age range. Bars = s.e. (**E** and **F**) 35S::*LOX2* line accumulates less *LOX2* transcripts and JA. *LOX2* expression (**E**) and JA production (**F**) on 35S::*LOX2* lines compared to the control line. In **E**, log2-transformed fold differences of *LOX2* expression were compared before and 60 min after wounding. U-box housekeeping gene AT5G15400 transcript was used as a reference gene (13). Columns present data and standard errors (bars) from 3 biological replicates. *P < 0.05 by Student's t-test and shows significant difference from the control line. In **F**, JA was extracted from the leaves that are harvested 60 min after wounding and quantified by HPLC. Averages of four biological replicates are presented. JA was not detected from non-treated leaves. Columns present standard error of the mean. 35S::*LOX2* and its control lines are constructed in Columbia (gl1) background. *P < 0.05 compared to the control line (Student's t-test). (**G**) *M. quadrilineatus* female survival rates on Arabidopsis *jar1* mutant and Col-0. Columns show the mean numbers of surviving females per plant of all biological replicates for each leafhopper age range. Data were obtained from experiments shown in **Fig. 7D**. Bars = s.e.

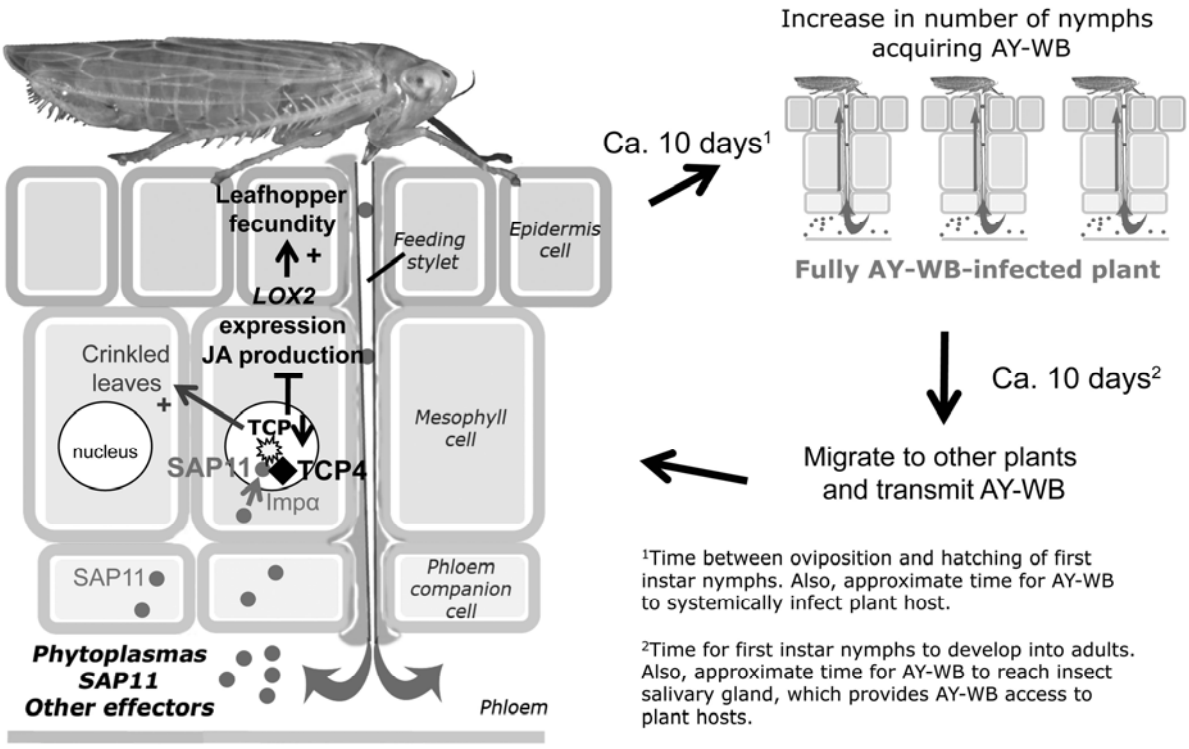

Fig. 7. **Model showing how SAP11 can positively affect AY-WB fitness.** SAP11 is secreted by AY-WB phytoplasma and migrates to plant cell nuclei in an importin α dependent manner (15). In this work we found that SAP11 binds TCPs (***SI Appendix*, Fig. 3**) and destabilizes Class II *CIN*-TCPs (**Fig. 4 and *SI Appendix*, Fig. 3**). TCP4 positively regulates the expression of *LOX2* (16). This is consistent with our finding that JA production is downregulated in 35S::*SAP11* (**Fig. 6, A, B and C**) and AY-WB infected plants (**Fig. 6D, *SI Appendix*, Fig. 4B and C**). *M. quadrilineatus* nymph production is higher on the Arabidopsis 35S::*SAP11*, TCP-silenced, *LOX2*-silenced lines and *jar1* mutant (**Fig. 7**). The newly born nymphs will acquire AY-WB phytoplasmas and become vectors as adults. Thus, an increase in the number of nymphs will boost AY-WB transmission rates.

Supplementary tables

Table 1. The list of clones identified in yeast hybrid screens

Clone Name	Genbank ID	Additional Gene Notes	Frame	Orientation
ATH_RP_hgx2189v1_pB27_A-12	30684297	Acyl-activating enzyme 7	IF	Sense
ATH_RP_hgx2189v1_pB27_A-7	30684297	Acyl-activating enzyme 7	IF	Sense
ATH_RP_hgx2189v1_pB27_A-16	145334952	Acyl-activating enzyme 7	OOF	Sense
ATH_RP_hgx2189v1_pB27_A-10	145361764	carbonate dehydratase	OOF	Sense
ATH_RP_hgx2189v1_pB27_A-6	145361729	late embryogenesis abundant family protein	IF	Sense
ATH_RP2_hgx2189v1_pB66_A-5	186509679	TCP13	IF	Sense
ATH_RP_hgx2189v1_pB27_A-13	186509679	TCP13	IF	Sense
ATH_RP_hgx2189v1_pB27_A-15	186509679	TCP13	IF	Sense
ATH_RP_hgx2189v1_pB27_A-19	186509679	TCP13	IF	Sense
ATH_RP_hgx2189v1_pB27_A-9	186509679	TCP13	IF	Sense
ATH_RP_hgx2189v1_pB27_A-17	42566927	TCP2	OOF	Sense
ATH_RP_hgx2189v1_pB27_A-1	42566927	TCP2	IF	Sense
ATH_RP_hgx2189v1_pB27_A-4	42566927	TCP2	IF	Sense
ATH_RP_hgx2189v1_pB27_A-8	42566927	TCP2	IF	Sense
ATH_RP_hgx2189v1_pB27_A-11	42566927	TCP2	IF	Sense
ATH_RP2_hgx2189v1_pB66_A-2	145361936	glutamate-tRNA ligase	IF	Sense
ATH_RP2_hgx2189v1_pB66_A-3	145361936	glutamate-tRNA ligase	IF	Sense
ATH_RP2_hgx2189v1_pB66_A-8	145361936	glutamate-tRNA ligase	IF	Sense
ATH_RP2_hgx2189v1_pB66_A-1	145361936	glutamate-tRNA ligase	IF	Sense
ATH_RP_hgx2189v1_pB27_A-2	6735358	Unknown	IF	Sense
ATH_RP_hgx2189v1_pB27_A-14	6735358	Unknown	IF	Sense
ATH_RP_hgx2189v1_pB27_A-20	6735358	Unknown	IF	Sense

IF: in frame, OOF: out of frame

Table 2. List of primer sequences.

For cloning	Sequence 5′ – 3′ *
attB1 adapter	GGGGACAAGTTTGTACAAAAAAGCAGGCT
attB2 adapter	GGGGACCACTTTGTACAAGAAAGCTGGGT
attB1foreGFP	AAAAAGCAGGCTccaccatgGTGAGCAAGGGCGAGGAGCTG
attB1reveGFP	AGAAAGCTGGGTgTTACTTGTACAGCTCGTCCATGCCGAG
attB1TCP13F	AAAAAGCAGGCTccaccatgAATATCGTCTCTTGGAAAG
attB2TCP13R	AGAAAGCTGGGTgTCACATATGGTGATCACTTCCTC
attB1TCP4F	AAAAAGCAGGCTccaccatgTCTGACGACCAATTCCATC
attB2TCP4R	AGAAAGCTGGGTgTCAATGGCGAGAAATAGAGGAAGC
attB1TCP2F	AAAAAGCAGGCTccaccatgATTGGAGATCTAATGAAG
attB1TCP2R	AGAAAGCTGGGTgTCAGTTCTTGCCTTTACCCTTATG
attB1TCP7F	AAAAAGCAGGCTccaccatgTCTATTAACAACAACAAC
attB1TCP7R	AGAAAGCTGGGTgTTAACGTGGATCTTCCTCTCTTCG
attB1TCP5F	AAAAAGCAGGCTccaccatgAGATCAGGAGAATGTGATGAAG
attB2TCP5R	AGAAAGCTGGGTgTCAAGAATCTGATTCATTATCGCTAC
attB1TCP17F	AAAAAGCAGGCTccaccatgGGAATAAAAAAAGAAGATCAG
attB2TCP17R	AGAAAGCTGGGTgCTACTCGATATGGTCTGGTTGTG
attB1TCP3F	AAAAAGCAGGCTccaccatgGCACCAGATAACGACCATTTC
attB2TCP3R	AGAAAGCTGGGTgTTAATGGCGAGAATCGGATGAAGC
attB1TCP10F	AAAAAGCAGGCTccaccatgGGACTTAAAGGATATAGCGTC
attB2TCP10R	AGAAAGCTGGGTgTTAGAGGTGTGAGTTTGGAG
attB1TCP24F	AAAAAGCAGGCTccaccatgGAGGTTGACGAAGACATTGAG
attB2TCP24R	AGAAAGCTGGGTgCTATCTCCTTTCCTTTGCCTTG
PacI-FLAG-SAP11Wt-For	gg**ttaattaa**c*ATGGACTACAAGGACGACGATG* ACAAATCTCCAAAGAAGGAATCTTC
AvrII-SAP11Wt-Rev	gg**cctagg**tcattacTTCTTAGAATCATCAGG

For qRTPCR		Sequence 5′ – 3′
	RTSAP11optF2	TTCTTGAAAATCCAGAGCTTATGGA
SAP11	RTSAP11optR2	CTTCTTAGAATCATCAGGTTGCTTTG
	qUBQF	TGCGCTGCCAGATAATACACTATT
AT5G15400	qUBQR	TGCTGCCCAACATCAGGTT
	AT3G45140-lox2-RTF	GCAAGCTCCAATATCTAGAAGGAGTG
AT3g45140 (Lox2)	AT3G45140-lox2-RTR	CGGTAACACCATGCTCAGAGGTAG

For verification of crosses		Sequence 5′ – 3′
rTCP4GFP	pBLS_f	CCTTTAAGCACCCCCTTCAC
construct	TCP_r	GTAAAACTGAATCGCCGTGTG
SAP11	SAP11_f	CTGAAGAGAAGAACAAGGAATATTCTATTGAAAAGAAC
	SAP11_r	AGAAAGCTGGGTCTTACTTCTTAGAATC
Actin	ActinFor	GCACCCTGTTCTTCTTACCG
	ActinRev	AACCCTCGTAGATTGGCACA

*Added sequences used for Gateway cloning are underlined, added sequences including those for adding start and stop codons and clone PCR fragments in frame are in lower case, restriction enzyme sites are in lower case and bolt font, and the fragment encoding the FLAG tag is in uppercase and italics font

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