Molecular Matchmakers: Phytoplasma Effector SAP54 Targets MADS-Box Factor SVP to Enhance Attraction of Fecund Female Vectors by Modulating Leaf Responses to Male Presence

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Abstract

Obligate parasites often trigger significant changes in their hosts to facilitate transmission to new hosts. The molecular mechanisms behind these extended phenotypes - where genetic information of one organism is manifested as traits in another - remain largely unclear. This study explores the role of the virulence protein SAP54, produced by parasitic phytoplasmas, in attracting leafhopper vectors. SAP54 is responsible for the induction of leaf-like flowers in phytoplasma-infected plants. However, we previously demonstrated that the insects were attracted to leaves and the leaf-like flowers were not required. Here we made the surprising discovery that leaf exposure to leafhopper males is required for the attraction phenotype, suggesting a leaf response that distinguishes leafhopper sex in the presence of SAP54. In contrast, this phytoplasma effector alongside leafhopper females discourages further female colonization. We demonstrate that SAP54 effectively suppresses biotic stress response pathways in leaves exposed to the males. Critically, the host plant MADS-box transcription factor SHORT VEGETATIVE PHASE (SVP) emerges as a key element in the female leafhopper preference for plants exposed to males, with SAP54 promoting the degradation of SVP. This preference extends to female colonization of male-exposed *svp null* mutant plants over those not exposed to males. Our research underscores the dual role of the phytoplasma effector SAP54 in host development alteration and vector attraction - integral to the phytoplasma life cycle. Importantly, we clarify how SAP54, by targeting SVP, heightens leaf vulnerability to leafhopper males, thus facilitating female attraction and subsequent plant colonization by the insects. SAP54 essentially acts as a molecular "matchmaker," helping male leafhoppers more easily locate mates by degrading SVP-containing complexes in leaves. This study not only provides insights into the long reach of single parasite genes in extended phenotypes, but also opens avenues for understanding how transcription factors that regulate plant developmental processes intersect with and influence plant-insect interactions.

Introduction

Parasites often excel in altering the development and behaviour of their hosts, a trait essential for their survival and propagation. The trait is particularly pronounced in obligate parasites, which have substantial control over their hosts, earning them the nickname 'puppet masters'. The phenomenon exemplifies the concept of extended phenotypes (originally coined by Dawkins, 1982), where the impact of an organism's genes extends beyond its own physical form and affects other organisms. This concept is especially evident in obligate parasites that depend on alternate hosts for transmission, as they often do not only alter the conditions of their immediate hosts but also have far-reaching effects on other organisms in the ecosystem.

For instance, the protozoan parasite *Taxoplasma gondii* changes the behaviour of their intermediate rodent hosts by reducing their innate fear for cats, which are the definite hosts where the parasite undergoes sexual reproduction to produce oocysts (Tong *et al.*, 2021). Similarly, the rust fungus *Puccinia monoica* induces its host plant, *Boechera stricta*, to produce 'pseudoflowers'. These structures mimic real flowers and attract pollinators with their scent and sugary rewards, an essential strategy for the fungus to spread its spores between plants (Roy, 1993; Cano *et al.*, 2013). Although advancements have been made in identifying parasite virulence factors, our understanding of the specific host processes that are commandeered to produce these extended phenotypes is still developing.

Parallel to these discoveries are the studies on the extended phenotypes of phytoplasmas, which have revealed that their manipulative abilities stem from particular genes within these pathogens (Sugio *et al.*, 2011b; Tomkins *et al.*, 2018; Huang *et al.*, 2020; Wang *et al.*, 2024). Phytoplasmas cause disease in crops, ornamentals and native plants worldwide (Kumari *et al.*, 2019) and are often dependent on sap-feeding insects, including leafhoppers, plant hoppers and psyllids, for transmission (Weintraub & Beanland, 2006). As obligate bacterial parasites, phytoplasmas frequently trigger the emergence of unusual plant structures such as leaf-like floral parts (phyllody) and the excessive growth and clustering of leaves and branches (witch’s broom) (Lee *et al.*, 2000; Al-Subhi *et al.*, 2018; Kumari *et al.*, 2019). These alterations not only compromise plant health but also promote attraction and colonization of insect vectors that are primarily responsible for

phytoplasma spread and transmission (Sugio *et al.*, 2011a; Frost *et al.*, 2013; MacLean *et al.*, 2014; Orlovskis & Hogenhout, 2016; Clements *et al.*, 2021; Al-Subhi *et al.*, 2021; Huang & Hogenhout, 2022). The responsible phytoplasma genes encode for effector molecules that, once inside the plant cell cytoplasm, target and typically disrupt or degrade essential plant transcription factors involved in growth, development, and defence (review by Wang *et al.*, 2024 and references therein as well as Liu *et al.*, 2023; Suzuki *et al.*, 2024; Correa Marrero *et al.*, 2024; Yan *et al.*, 2024; Zhang *et al.*, 2024). This molecular interference exemplifies the extended phenotype reach, affecting not just the host appearance but also its physiological and biological processes.

The interaction between the Aster Yellows strain Witches Broom (AY-WB) phytoplasma and its vector, the aster leafhopper *Macrosteles quadrilineatus*, offers insights into the complex interplay between parasites and hosts. The effector protein secreted AY-WB protein (SAP) 11 binds to and destabilizes class II TCP transcription factors - this action leads to changes in leaf shapes and stem proliferation, reminiscent of witches' brooms, and altered root architecture resembling the hairy roots found in infected plants (Bai *et al.*, 2009; Sugio *et al.*, 2011a, 2014; Lu *et al.*, 2014; Chang *et al.*, 2018; Pecher *et al.*, 2019). Additionally, this effector reduces plant jasmonic acid and salicylic acid-mediated defence responses, alters volatile organic compounds, and promotes the reproduction rates of the insect vectors (Sugio *et al.*, 2011a; Lu *et al.*, 2014; Tan *et al.*, 2016). Another effector, SAP05, recruits the 26S proteasome component RPN10, instigating the breakdown of SPL and GATA transcription factors (Huang *et al.*, 2021; Liu *et al.*, 2023; Yan *et al.*, 2024; Zhang *et al.*, 2024). This process is linked to the typical leaf and stem proliferations of witches' brooms in phytoplasma-infected plants. The SAP05-mediated degradation of SPLs, but not GATAs, increases the attractiveness of these plants to insect vectors (Huang *et al.*, 2021).

However, the relationship between phytoplasma SAP54/PHYL1 effectors inducing leaf-like flowers and attracting insects seems more intricate. These effectors target and disrupt MADS-box transcription factors, akin to animal HOX genes, leading to a transformation of flowers into leaf-like structures, alterations that mirror the phyllody and virescence symptoms of phytoplasma infection (MacLean et al., 2011; MacLean et al., 2014; Wang *et al.*, 2024; Suzuki et al., 2024; Correa

Marrero et al., 2024). SAP54 recruits plant 26S proteasome shuttle factors called RADIATION SENSITIVE 23 (RAD23) to break down these transcription factors (MacLean et al., 2014; Kitazawa et al., 2022; Suzuki et al., 2024). Leafhoppers prefer plants that stably produce SAP54 for reproduction and this preference phenotype requires the presence of RAD23, which is essential for both breaking down MADS-box transcription factors and inducing leaf-like flowers (MacLean et al., 2014). However, the increased reproduction of insect vectors does not depend on the presence of leaf-like flowers. Our past research showed that insects prefer SAP54 plants during the vegetative developmental phase, before flowers emerge, and even flowering SAP54 plants upon removal of the leaf-like flowers (Orlovskis & Hogenhout, 2016). Therefore, it remains unclear whether plant MADS-box transcription factors play a role in the leafhopper attraction phenotype.

MADS-box transcription factors have been predominantly studied in relation to regulation of floral transition and flower organ development. Nonetheless, factors like SUPPRESSOR OF OVEREXPRESSION OF CO 1 (SOC1) and short vegetative phase (SVP) are also expressed in leaves and other organs during the *A. thaliana* vegetative developmental phase, and regulate processes other than orchestrating the flowering process, such as plant defence responses, as has been shown for SOC1 (Li *et al.*, 2020). Based on these insights, we hypothesize that leafhopper attraction is influenced by MADS-box transcription factors involved in processes other than flowering.

Here, we analysed the factors influencing leafhopper colonization preference in SAP54 plants. Surprisingly, we found that colonization preference on SAP54 plants only occurs in the presence of males on leaves during choice tests. Both SAP54 and males are essential for female preference, as females did not exhibit a preference for male-exposed versus non-exposed control plants, nor for SAP54 plants versus control plants in the absence of males. In contrast, female-only presence on SAP54 plants deters colonization by other females. We noted a clear downregulation of biotic stress response pathways in male-exposed SAP54 leaves, contrasting with the upregulation observed in female-exposed SAP54 leaves and control plants exposed to both males and females. Furthermore, we found that the MADS-box transcription factor SVP is essential for female preference in male-exposed SAP54 plants. Females also prefer colonizing male-exposed *svp null*

mutant plants over non-exposed ones. Our research sheds light on how the conserved MADS-box transcription factor SVP influences leaf susceptibility to male herbivorous insects, promoting female attraction and colonization.

Results

Attraction of SAP54 plants to fecund female leafhoppers relies on the simultaneous presence of leafhopper males on these plants.

Given our previous finding that SAP54 promotes fecundity of the AY-WB phytoplasma leafhopper vector *M. quadrilineatus*, we wished to further investigate what aspect of the insect-plant interaction is affected by SAP54. Females feed and lay eggs, while males only feed, and it is known that plants can induce different plant defence responses to insect feeding and egg laying (Little *et al.*, 2007). Hence, the first experiment was to assess the SAP54 effect on males and females separately using choice tests. In these tests, the insects were given a choice to feed and lay eggs on 35S:SAP54 or 35S:GFP transgenic plants (henceforth referred to as SAP54 and GFP plants) for 5 days, then adult leafhoppers were removed. Immediately after, plants from each choice test were individually caged to avoid the hatching nymphs from moving between plants. The number of progeny (nymphs) were counted 14 days later. When these choice tests were done with both males and females, the leafhoppers produced more progeny on SAP54 than on GFP plants (**Figure 1A, B; treatment 1; Figure supplement 1**), confirming previous findings (MacLean *et al.*, 2014; Orlovskis & Hogenhout, 2016). However, to our surprise, choice tests with fecund females alone, without males, did not result in more leafhopper progeny on the SAP54 versus GFP plants (**Figure 1A, B; treatment 2; Figure supplement 1**). The females also produced more progeny on the SAP54 compared to GFP plants when the males were caged and were not in direct contact with females (**Figure 1A, B; treatment 4; Figure supplement 1**). In contrast, the females did not produce more progeny on the SAP54 plants when males were left on the plant for only 48 hours and removed before the choice test with females alone (**Figure 1A, B; treatment 5; Figure supplement 1**) or when the plants were exposed to the conspecific females in the clip cages during fecund female-only choice tests (**Figure**

1A, B; treatment 6; Figure supplement 1). In the latter choice test, there was a reproduction preference for the GFP plants instead. These results indicate that the presence of males on the leaves are required for the leafhopper preference to reproduce more on SAP54 versus GFP plants. Importantly, we found no obvious difference in progeny when fecund females were given a choice between (non-transgenic) wild type plants with and without males **(Figure supplement 2**), indicating that SAP54 is required for the modulation of plant processes that results in the male-dependent reproduction preference of females.

Quantification of honeydew excretions provide a measure of how much phloem fluids the insects acquire from their host plant and therefore is a proxy for measuring feeding preferences (Hong & Rumei, 1993; Ammar *et al.*, 2013; Cameron *et al.*, 2014). Honeydew secretion tests showed that fecund females secreted more honeydew on SAP54 plants in the presence of males in clip-cages (**Figure 1A,C; treatment 4; Figure supplement 1**), whereas no increase in honeydew secretions of these females was observed on the SAP54 plants in absence of males (**Figure 1A,C; treatment 2; Figure supplement 1**) or when males were removed from the clip-cages prior to the choice test with females alone (**Figure 1A,C; treatment 5; Figure supplement 1**), indicating that females ingest a greater volume of plant sap from SAP54 than from GFP plants when males are present. There were slight increases in honeydew secretions on SAP54 versus GFP plants when the choice tests were done with both sexes, but these differences were not significant (**Figure 1A,C; treatment 1; Figure supplement 1**), in agreement with behaviour studies showing that female feeding is interrupted when in direct contact with males possibly due to mating (Beanland *et al*., 1999). Females did not produce more honeydew on the SAP54 plants when other conspecific females were caged on the same plant (**Figure 1A,C; treatment 6; Figure supplement 1),** indicating their enhanced feeding activity is specifically dependent on the presence of males. Males alone did not secrete more honeydew on SAP54 versus GFP plants (**Figure 1A,C; treatment 3; Figure supplement 1**), indicating that males alone do not show any feeding preference for SAP54 plants and that the females are primarily responsible for the increased honeydew production on male colonized SAP54 plants.

Together, the fecundity and honeydew secretion data indicate that attraction of SAP54 plants to fecund female leafhoppers relies on the simultaneous presence

of leafhopper males on the plants. The females produce more progeny and feed more on these plants only when males are present, even when the males are physically separated from the females.

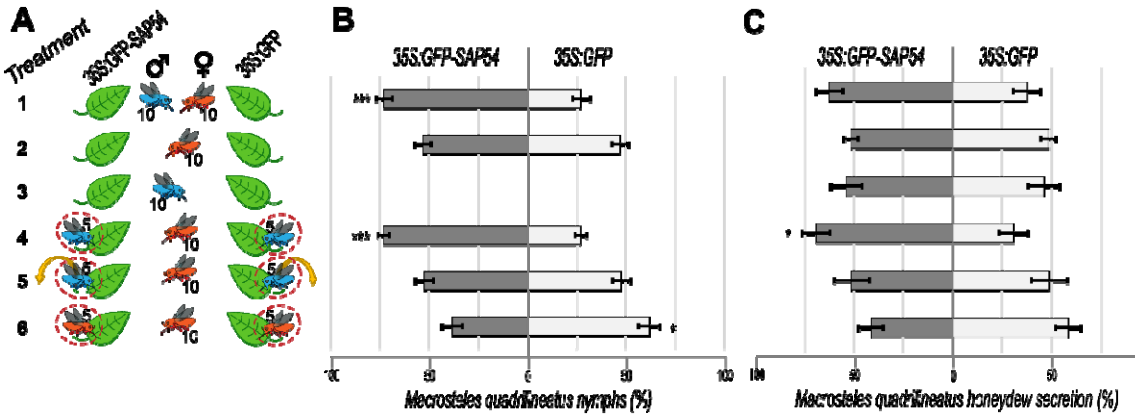

Figure 1. *M. quadrilineatus* leafhopper preference to reproduce and feed on SAP54 versus GFP plants is dependent on leaf exposure to leafhopper males. A. Experimental design of 6 choice tests (treatments) with 10 male and/or 10 female insects, as indicated, on 6 weeks old *A. thaliana* rosettes. Dashed circles indicate clip-cages and the arrow the removal of males before the start of the choice test. Each choice test (treatment) is placed in a separate cage. **B**. Percentages (%) of nymphs found on SAP54 versus GFP plants. **C**. Percentages (%) of leafhopper honeydew secretions in choice tests where insects are allowed to feed on either SAP54 or GFP plants. Horizontal bars in **B, C** indicate the mean ± 1 SEM. *p < 0.05, ***p<0,001. The entire series of choice tests 1 to 6 were performed in parallel and repeated independently 3 times for progeny count and 2 times for honeydew quantification - data presented in **B** and **C** include the pooled results of the independent choice test series.

The online version of this article includes the following source data and figure supplement(s) for figure 1:

Figure supplement 1: Data distributions of independent repeats that were used to generate graphs displayed in B and C.

Figure supplement 2: *Macrosteles quadrilineatus* female leafhoppers show no preference for *A. thaliana* Col-0 wild-type plants exposed to conspecific male leafhoppers.

Figure supplement 3: Female *M. quadrilineatus* preference for male-exposed SAP54 plants is unlikely to involve long-distance cues.

Female leafhopper preference for male-exposed SAP54 plants unlikely involves long-distance cues.

To investigate if the fecund females are attracted to the SAP54 plants via volatile cues released by male-exposed SAP54 plants or mating calls from males that may be perceived by females from a distance, we established a sticky-trap assay that capture females before they access the plants themselves. Interestingly, female leafhoppers were equally likely captured on GFP and SAP54 plants when both of these plants were placed in an odour and sound permeable black container, equipped with either transparent or green sticky traps (**Figure supplement 3A, choice tests 1 and 2**), suggesting that volatile or acoustic cues emitted from male-exposed GFP or SAP54 plants do not bias female landing choice at a distance when identical visual cues are present. To test whether the assay works, we compared different colour sticky traps as a positive control for insect visual choice. By doing so, female leafhoppers were more likely to be captured on green versus transparent sticky traps placed over the black containers, regardless of whether the green sticky traps were placed over male exposed GFP or SAP54 plants (**Figure supplement 3A, choice tests 3 and 4**), indicating that visual cues from the green traps were the primary determinant for female landing choice. Female insects also did not show an obvious landing preference for wild-type plants colonized by males compared to insect-free plants (**Figure supplement 3B)**. These results indicate that female choice for SAP54 versus GFP plants does not appear to depend solely on plant volatiles that are released by the SAP54 plants in the absence or presence of males on the leaves and that the females do not appear to respond to potential mating calls of the males that may be perceived by the females from a distance. Therefore, these results indicate that female reproductive preference for the male-exposed SAP54 versus GFP plants is dependent on the direct females access to the leaves of SAP54 plants and presence of males on these leaves.

SAP54 plants display a dramatic altered transcriptional response specifically to male leafhoppers.

To further investigate how the combination of SAP54 and male-exposure may affect the preference of fecund females to reproduce on SAP54 versus GFP plants, we conducted RNA-seq experiments and compared the transcriptional responses of leaves of SAP54 and GFP plants that were exposed to five individual *M. quadrilineatus* females or males in clip cages or to clip cages without insects

(controls). Each treatment consisted of 4 biological replicates (**Figure supplement 4A**). After normalization, transcripts with coverage of <1 FPKM in all replicates in at least one of the treatments were removed, leaving 16'307 genes (**Figure supplement 4B**). In a separate analysis, the data were analysed for differentially expressed genes (DEGs) that display significant changes in transcription levels between any two treatments, amounting to 6'957 DEGs (**Figure supplement 4B**). Considering both categories, a total of 17'153 genes were identified (**Supplementary file 1**) of which 6'111 genes were shared between the categories (**Figure supplement 4B**). Based on these 17'153 genes, we performed multiple discriminant analysis (MDA) to visualize the separation between the treatment groups. While most treatments largely clustered together, SAP54_MALE_2 and GFP_MALE_2 appear to be switched based on the groupings of the other 3 replicates in the SAP54_male and GFP_male treatments (**Figure supplement 4C**). Analyses of the normalized reads that map to GFP versus GFP-SAP54 ruled out that the plant genotypes were accidentally swapped between the SAP54_MALE_2 and GFP_MALE_2 treatments or that the GFP or GFP-SAP54 were not expressed in the leaves (**Figure supplement 4D**). Moreover, plots of the median FRKM of all transcripts versus the GFP or GFP-SAP54 read counts did not flag the SAP54_MALE_2 and GFP_MALE_2 treatments as different from the others (**Figure supplement 4E**). Therefore, the apparent SAP54_MALE_2 and GFP_MALE_2 outliers may represent inherent variation of the biological system. Nonetheless, the MDA plot shows that in at least three out of four replicates per treatment, the leaf transcriptional responses are clearly distinct in male-treated SAP54 plants versus GFP plants, and more so than for the non-exposed and female-exposed SAP54 plants versus GFP plants (**Figure supplement 4C**).

When all samples were considered, a total of 136 DEGs were identified (**Figure supplement 5A**). Of these, 79 DEGs were derived from male-exposed leaves of SAP54 plants versus those of GFP plants and 69 DEGs from the non-exposed leaves of SAP54 versus those of GFP plants, with 12 DEGs overlapping between these two categories, whereas no DEGs were identified from female-exposed leaves of SAP54 versus those of GFP plants (**Figure supplement 5A**). This suggests that SAP54 has a bigger impact on leaf responses to males than to females. This was corroborated with the PCA analysis showing that insect exposure,

male or female, of leaves of either SAP54 or GFP plants explained 54% of the variance (PC1), whereas the male-exposed SAP54 leaves are predominantly responsible for the 25% variance of PC2 among the insect-exposed treatments (**Figure supplement 5B**). The impact of SAP54 on male-exposed leaves was more obvious when three outliers were removed (**Figure supplement 5C,D)**. Because the goal of this experiment was to assess how leaf responses of male-exposed plants differ from other treatments, we excluded these outliers, as including these obscures the transcriptional signal derived from the other three samples.

To further assess how SAP54 modulates leaf responses specifically to males, we compared DEGs between male or female exposed leaves of GFP versus those of SAP54 plants (**Figure 2A**; **Supplementary file 2**). The leaf response of GFP plants to female exposure amounted to 2'375 DEGs as opposed to 909 male-responsive DEGs of which the vast majority (894 DEGs) overlap with those of males in the GFP plants (**Figure 2A**; **Supplementary file 2, table tabs A-B**). A higher transcriptional response to females may be expected, because whereas both females and males feed, females also lay eggs on leaves, and plants induce defence responses associated with egg laying of insects (Stahl *et al.,* 2020; Little *et al*., 2007). In contrast, leaves of SAP54 plants display dramatically different transcriptional response to the insects, and with 2'456 male-specific and only 571 female-specific DEGs, the leaves particularly exhibit an altered transcriptional response to leafhopper males (**Figure 2B; Figure supplement 6A; Supplementary file 2, table tabs C-D**). This is striking particularly given that only minimal transcriptional differences (266 DEGs) are observed between leaves of SAP54 and GFP plants that were not exposed to insects (**Figure supplement 6A; Supplementary file 2, table tab E**). These data suggest that SAP54 modulates leaf responses of plants that are challenged by leafhopper herbivory, particularly leafhopper males.

SAP54 plants exposed to males display significant downregulation of biotic stress responses.

To better understand how SAP54 may alter leaf responses to the insects, *A. thaliana* genes were binned into functional groups and these groups were analysed for enrichment of DEGs among treatments using the MapMan built-in Wilcoxon rank

sum test with Benjamini-Hochberg correction (Usadel *et al.*, 2005). Leaves of GFP plants exposed to females show enrichment for DEGs in 30 functional groups compared to those of non-exposed GFP plants, as opposed to only 2 groups in GFP plants exposed to males (**Supplementary file 3**). However, strikingly, leaves of the SAP54 plants exposed to males show enrichment of DEGs in 42 functional groups compared to those of non-exposed SAP54 plants, as opposed to only 11 groups in leaves of SAP54 plants exposed to females (**Supplementary file 3**). The majority of the functional groups, and particularly in leaves of the male-exposed SAP54 plants, included genes with known functions in biotic stress responses, hormone metabolism, signalling, secondary metabolism and other plant defence-related processes (**Supplementary file 3**). Moreover, bins enriched for genes in respiration (glycolysis) and cell wall modification functions were uniquely enriched in the leaves of male-exposed SAP54 plants (**Supplementary file 3**). In the no-insect/cage-only SAP54 versus GFP treatments, only 1 functional group (biotic stress responses involving plant defensins) was significantly enriched in the leaves (**Supplementary file 3**) and the majority of genes were upregulated in leaves of the SAP54 plants (**Figure supplement 6B**). Therefore, SAP54 alone has a relatively minor impact on leaf responses. Visualization of the fold change of these genes in MapMan graphs focused on genes with roles in plant responses to pathogen and pest attack (Usadel *et al.*, 2005) show upregulation of the majority of biotic stress response DEGs in leaves of female- and male-exposed GFP and those of female-exposed SAP54 plants, as opposed to a prominent downregulation of the majority of DEGs in leaves of male-exposed SAP54 plants (**Figure 2C and 2D; Supplementary file 4**). Specifically, multiple genes with roles in abiotic stress, cell wall modification, proteolysis, respiratory burst, secondary metabolism and defence signalling (MAPKs) were downregulated in the male-exposed SAP54 leaves. Together these results indicate that SAP54 dramatically alters leaf biotic stress response to leafhoppers, and downregulates the majority of this response in the presence of males.

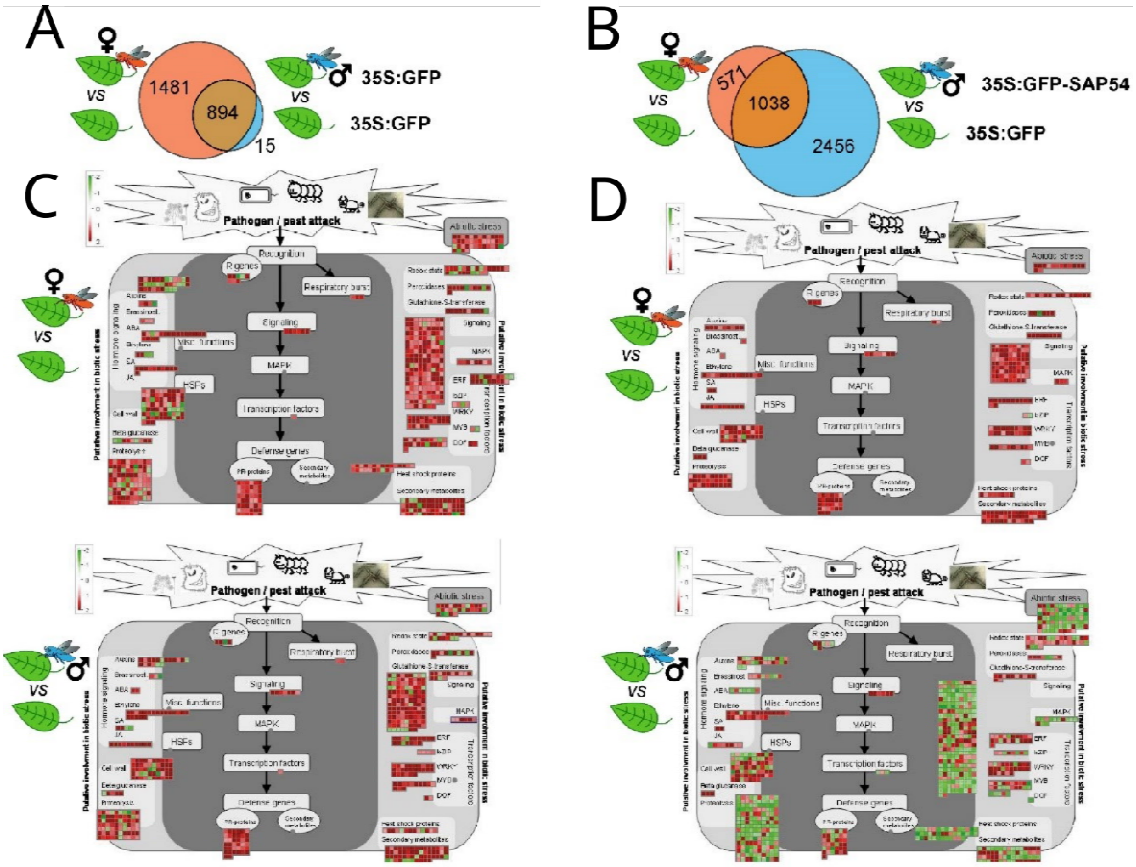

Figure 2. SAP54 plants display a dramatically altered leaf response to male leafhoppers by transcriptionally downregulating the majority of biotic stress and plant defence related processes. **A-B**. Euler-Venn diagrams illustrating DEGs in leaves of GFP plants (**A**) and SAP54 plants (**B**) exposed to female leafhoppers compared to male leafhoppers, versus leaves of plants in the control group (cage-only, non-exposed plants). DEG analysis was performed on 17'153 leaf-expressed genes available in **Supplementary file 1.** DEG IDs listed within each Venn diagram are provided in **Supplementary file 2. C-D.** MapMan diagrams of *A. thaliana* DEGs involved in biotic stress from female (red insect) or male (blue insect) exposed GFP (**C**) or SAP54 plants (**D**). Biotic stress related pathways were significantly enriched with DEGs from male exposed SAP54 plants compared to other functions listed in **Supplementary file 3.** Names of functional bins (e.g., respiratory burst or MAPK) are listed next to the corresponding colour boxes and fully listed in **Supplementary file 4** along with individual transcript names and their fold changes. Red colour boxes indicate upregulated, but green – downregulated DEGs based on $\log_2$(fold change).

The online version of this article includes the following source data and figure supplement(s) for **Figure 2**:

Supplementary file 1. FPKM and differential expression values of 17'153 genes included in the response analyses of plants to SAP54 vs GFP and male vs female leafhopper exposure.

Supplementary file 2. IDs and log2-fold changes of DEGs of male and female *M. quadrilineatus* leafhopper-exposed GFP and SAP54 plants compared to insect-free GFP plants.

Supplementary file 3. MapMan build-in functional bins enriched for DEGs in male and female *M. quadrilineatus* leafhopper-exposed GFP and SAP54 plants compared to insect free GFP plants.

Supplementary file 4. Source data for generating Fig. 2CD - Enrichment statistics of biotic stress bins and fold-change of DEGs in each bin.

We wished to further characterise which components of defence pathways were predominantly affected in male-exposed leaves of SAP54 plants. To achieve this, we performed a *de-novo* manual curation of *A. thaliana* defence signalling module, integrating published literature and public TAIR database annotations for known families of membrane receptors & receptor-like kinases (Shiu & Bleecker, 2001), cytoplasmic receptors such as NLR proteins (Hofberger *et al.*, 2014; Kroj *et al.*, 2016; Sarris *et al.*, 2016), CDPK-SnRK superfamily (Hrabak *et al.*, 2003), MAP kinases cascade (Asai *et al.*, 2002; Jonak, 2002) as well as known salicylic acid, jasmonic acid and ethylene biosynthesis and signalling genes (van Verk, 2010). These manually curated defence signalling modules are provided here as **Figure supplement 7** and **Supplementary file 5,** and were imported into the MapMan tool for visualisation and DEG analyses (**Figure 3**). Genes for LRR cell surface receptors, CC-NBS-LRRs, RLCKs, MAPKs, JA biosynthesis and ET biosynthesis and signalling were largely down regulated in leaves of the male-exposed SAP54 plants compared those of the other treatments (**Figure 3).** Among the DEG-enriched biotic stress or defence signalling pathway bins, the one for LRR receptors is most enriched and most of these transcripts were downregulated in male-exposed leaves of SAP54 plants compared to the other treatments (**Supplementary file 6)**.

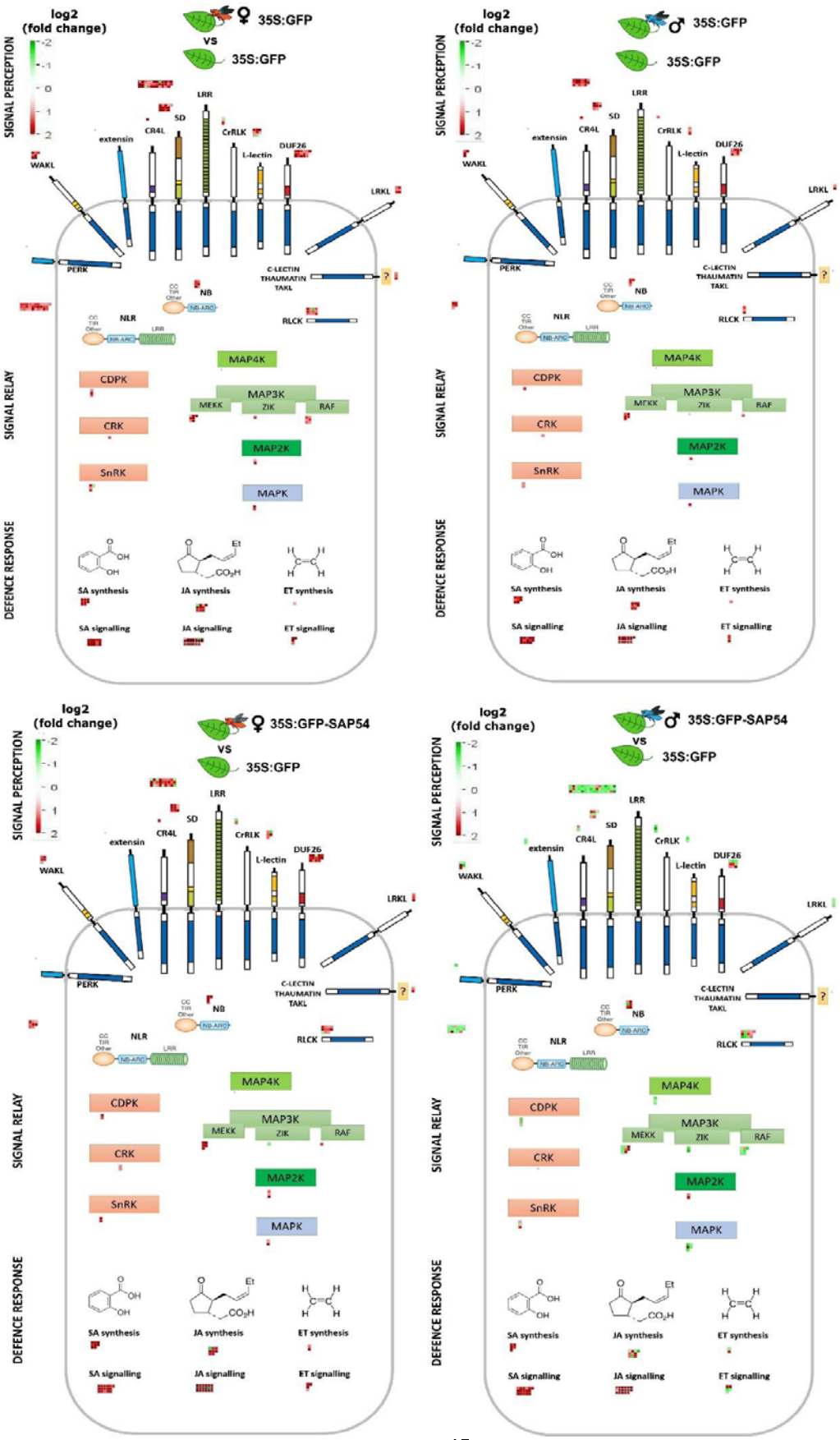
log2
(fold change)
♀ 35S:GFP
vs
35S:GFP
♂ 35S:GFP
35S:GFP
♀ 35S:GFP-SAP54
vs
35S:GFP
♂ 35S:GFP-SAP54
vs
35S:GFP
SIGNAL PERCEPTION
SIGNAL RELAY
DEFENCE RESPONSE
-2
-1
0
1
2
WAKL
extensin
CR4L
SD
LRR
CrRLK
L-lectin
DUF26
LRKL
PERK
C-LECTIN
THAUMATIN
TAKL
?
NB
NB-ARC
NLR
LRR
RLCK
MAP4K
CDPK
MAP3K
MEKK
ZIK
RAF
CRK
MAP2K
SnRK
MAPK
SA synthesis
JA synthesis
ET synthesis
SA signalling
JA signalling
ET signalling

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Figure 3. Genes involved in *A. thaliana* defence responses are predominantly down regulated in male-exposed leaves of SAP54 plants. MapMan diagrams with the manually curated bins for plant cell-surface receptors, NLRs, RLCKs, MAPKs and hormone biosynthesis and signalling proteins involved in plant responses to biotic stress. Full list of transcripts assigned to each bin can be retrieved from **Supplementary file 5.** DEGs are indicated as boxes above or adjacent to each protein category. Red colour boxes indicate upregulated, but green – downregulated DEGs based on $\log_2$(fold change). Female (red insect) or male (blue insect) exposed GFP or SAP54 plants are always compared to insect free plants for DEG analysis and indicated by the insect image above each panel. Individual transcript names and their fold changes within each panel are listed in **Supplementary file 6.** The manually drawn defence signalling background image can be downloaded as **Figure supplement 7.**

The online version of this article includes the following source data and figure supplements for **figure 3**:

Supplementary file 5: Manually curated and assigned defence signalling bins for MapMan import.

Supplementary file 6: Functional bins for manually annotated defence genes enriched for DEGs in male and female *M. quadrilineatus* leafhopper-exposed GFP and SAP54 plants compared to insect free GFP plants.

Figure supplement 7: Manually drawn MapMan image for defence signalling pathway visualisation.

Given our finding that females prefer to reproduce on male-exposed SAP54 leaves over those of male-exposed GFP plants in the choice experiments, and don't prefer the female-exposed leaves of SAP54 plants (**Figure 1**), we also compared leaf transcriptional profiles of the male-exposed and female-exposed SAP54 versus GFP treatments. Leaves of SAP54 plants respond more to males (2'857 male-specific DEGs) than to females (179 female-specific DEGs) compared to those of GFP plants with 957 DEGs shared between the two exposures (**Figure 4A**). Strikingly, none of the functional categories (bins) are enriched for DEGs in the female exposure treatment, as opposed to 51 categories in the male exposure treatment (**Supplementary file 7**). The 957 shared DEGs do not show enrichment for functional categories, though removing the shared DEGs from the male dataset by considering only the 2'857 male-specific DEGs reduced the enriched categories for the male treatment to 41. Among the latter DEG-enriched categories were protein synthesis, signalling involving receptor kinases, cell wall modification, hormone metabolism, including those of jasmonate and ethylene, and secondary metabolism of defence compounds. MapMan graphs of biotic stress responses show downregulation of all 957 shared DEGs and the majority of 2'857 male-specific DEGs, and only few downregulated genes in the 179 female-specific DEGs (**Figure**

4B; Supplementary file 8). Among the biotic stress response genes, those that are significantly enriched and display the greatest fold-change among male-specific DEGs in leaves of SAP54 plants encode cell surface receptors of LRR and WAKL family and MAP kinases, as well as those involved the ethylene synthesis and signalling, protein degradation and isoprenoid (relating to lignin, glucosinolate and flavonoid biosynthesis) pathways (**Figure 4B; Supplementary file 8**). In summary, protein translational processes and defence pathways predominantly downregulate in male-exposed leaves of SAP54 plants compared to those of GFP plants, and this response was not obvious in the female-exposed leaves. Therefore, leaf defence responses of SAP54 plants are downregulated specifically in response to male exposure and this could explain female preference for the male-exposed SAP54 plants.

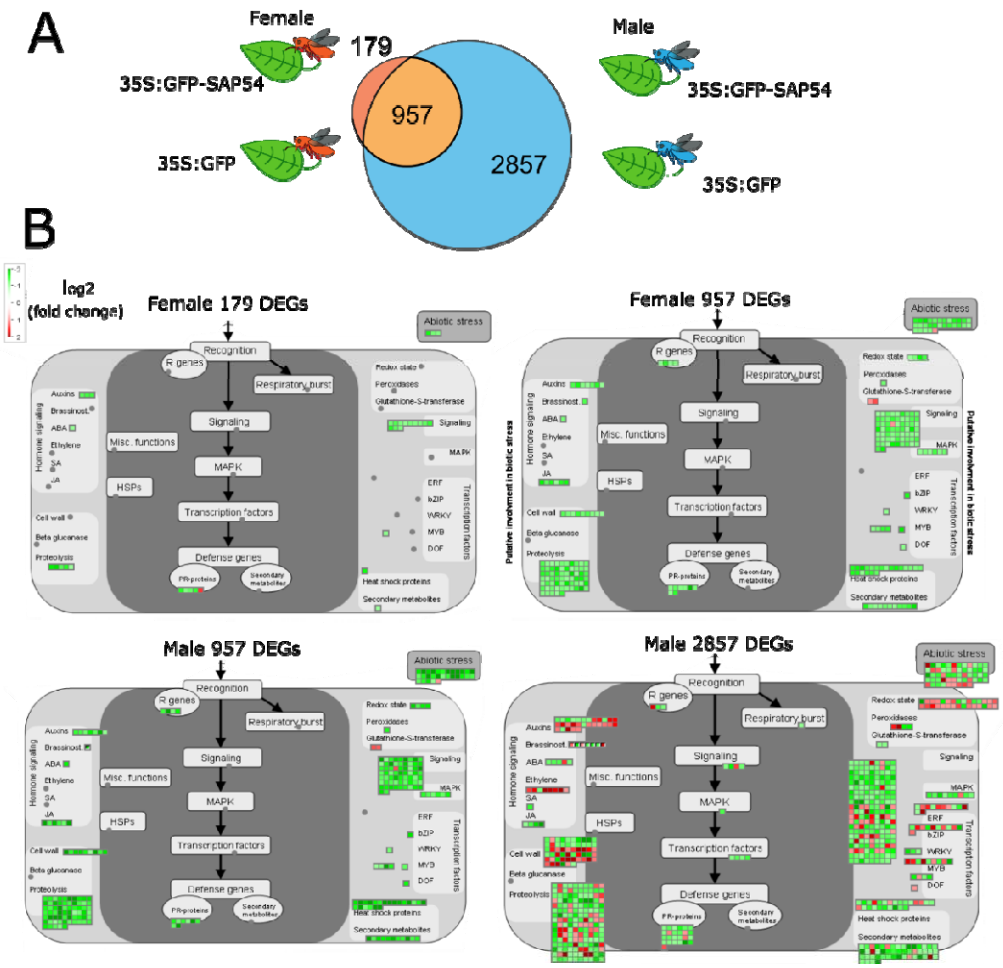

Figure 4. Biotic stress response genes are predominantly downregulated in male-exposed SAP54 versus GFP plants. Plant biotic stress is among the most enriched bins with male-specific responses in SAP54 leaves.

The online version of this article includes the following source data and figure supplement(s) for figure 4:

Supplementary file 7: MapMan build-in functional bins enriched for DEGs in SAP54 versus GFP plants with or without exposure to male and female *M. quadrilineatus* leafhoppers.

Supplementary file 8: Biotic stress bins from MapMan build-in and manually curated defence signalling pathway bins enriched for DEGs in GFP and SAP54 plants with or without exposure to male and female *M. quadrilineatus* leafhoppers.

The MADS-box transcription factor SVP is required for female preference of male-exposed SAP54 plants.

MADS-box transcription factors (MTFs) are predominantly investigated for their involvement in regulation of flowering. However, how MTFs may affect leaf responses to biotic stress, such as leafhopper colonization, is less well investigated. SAP54 mediates the degradation of many MTFs via the 26S proteasome by recruiting RAD23 proteins, which are 26S proteasome shuttle factors, and RAD23 proteins are required for both MTF degradation and leafhopper preference for SAP54 plants (MacLean *et al.*, 2014a). Therefore, we wished to investigate if the SAP54-mediated degradation of specific MTFs is required for the female preference for male-exposed SAP54 plants. The 17’153 leaf transcripts identified earlier (**Figure supplement 4B**) were filtered for the presence of 107 known MTFs (de Folter *et al.*, 2005) identifying 20 MTFs that are expressed in *A. thaliana* leaves independent from the presence of SAP54 (**Supplementary file 9**). The majority of the MTFs show differential expression among the male or female-exposed or cage-only leaves of SAP54 and GFP plants, including SVP, SOC1 and several of the MAFs (**Supplementary file 9**). The most striking included MAF5, which showed substantial downregulation in the male-exposed versus cage-only leaf responses of GFP plants and substantial upregulation in leaves that had combined exposure to SAP54 and leafhoppers (**Supplementary file 9**). Others, such SVP and AGL24, were mostly downregulated in the presence of insects or SAP54 and upregulated in the combined presence of insects and SAP54 (**Supplementary file 9**). The MADS-box transcription factor genes had similar expression levels between female-exposed and male-exposed leaves of SAP54 vs GFP plants, except MAF4 that was

substantially downregulated in leaves of male-exposed SAP54 vs GFP plants and substantially upregulated in female-exposed SAP54 vs GFP plants (**Supplementary file 9**). Therefore, MADS-box transcription factor genes are differentially expressed in leaves upon exposure to insects and/or presence of SAP54.

We and others previously found that SAP54 directly interacts with many MADS-box transcription factors and mediates their degradation via recruiting RAD23 (MacLean *et al.*, 2014b; Kitazawa *et al.*, 2022; Suzuki *et al.*, 2024). Here we found that SAP54 mediated the degradation of several MADS-box transcription factors for which we observed corresponding genes to be expressed in leaves (**Figure 5A**). SAP54 interacts directly with the majority of these (**Figure 5B**). However, it does not appear to interact directly with SVP (**Figure 5B**). Nonetheless, SVP interacts with other MTFs, including SOC1 and AGL6, which interact with SAP54 (**Figure 5B**) and SVP forms heteromeric complexes with SOC1, FUL, AGL6, AGL24 and several other MTFs (de Folter *et al.*, 2005; Gregis *et al.*, 2006, 2009; Balanzà *et al.*, 2014; Mateos *et al.*, 2015). Moreover, SAP54 mediates degradation of SOC1 and other MTFs via the 26S proteasome (MacLean *et al.*, 2014). Hence, SAP54-mediated destabilization of SVP could occur indirectly via SAP54 interactions with SOC1, AGL6 and other MADS-box transcription factors that form multimeric complexes with SVP.

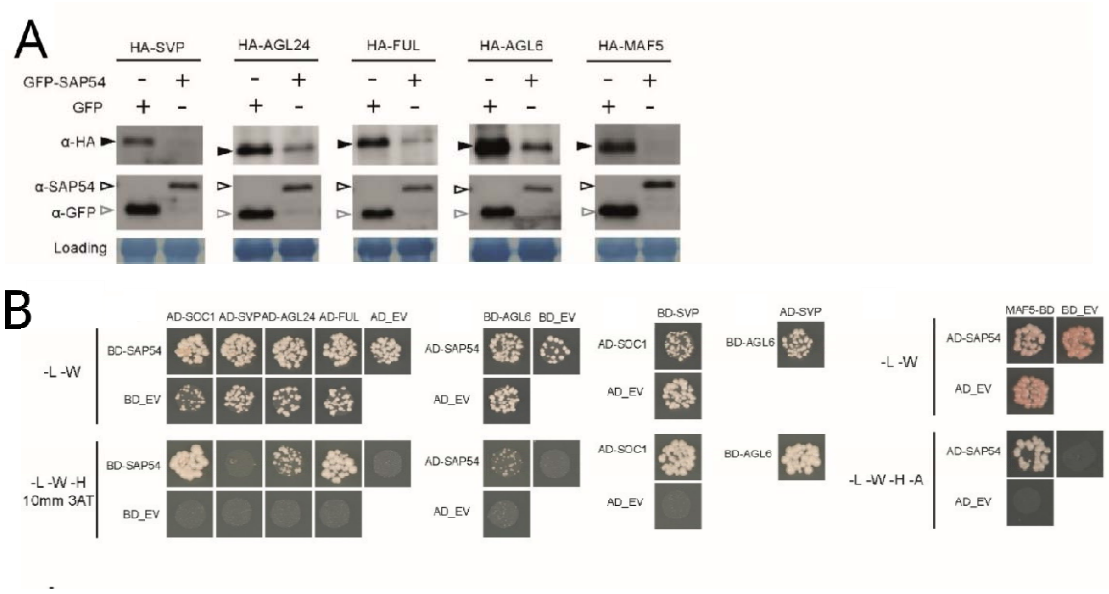

Figure 5. SAP54 interacts with multiple MADS-box transcription factors and mediates their degradation. A. Western blots showing degradation of MTFs in the presence of SAP54 in *A. thaliana* protoplasts. Assays were repeated twice with similar results and available in **Figure supplement 10**. **B**. Yeast two-hybrids assays with GAL4-activation (AD) and GAL4-DNA binding (BD) domains fused to the test proteins. -L-W-H-A denote auxotrophic SD media lacking leucine, tryptophan, histidine or adenine, conditionally supplemented with 3-Amino-1,2,4-triazole (3AT). EV, empty vector control.

The online version of this article includes the following figure supplement for figure 5:

Figure supplement 10: SAP54 mediates degradation of multiple MADS-box transcription factors.

Next, we conducted leafhopper choice tests with *null* mutants for several MADS-box transcription factor genes. These revealed that the leafhoppers preferred to reproduce on the *svp* and *maf5* mutants, whereas no clear preferences of the leafhoppers were observed for *A. thaliana agl24, sep4, maf1, maf4, ful* and *soc1* mutants compared to wild-type plants (**Figure 6A; Figure supplement 8**). Moreover, leafhopper egg-laying preference was abolished when SAP54 was introduced into the *svp* mutant background, but not when introduced into the *maf5* mutant background (**Figure 6B; Figure supplement 8**), suggesting that that plant SVP is the dominant MADS-box transcription factor required for the SAP54-dependent leafhopper preference. In parallel, choice tests were done with AY-WB-infected wild-type and mutant plants, and the leafhoppers did not display the colonization

preference for both *svp* and *maf5* mutants (**Figure 6C; Figure supplement 8**), indicating that AY-WB-infected wild type and mutant plants are equally attractive to the leafhoppers. While females showed a preference for male-exposed *svp* and *maf5* mutants versus male-exposed wild-type plants (**Figure 6A**) this preference for *svp* and *maf5* mutants was abolished in the absence of leafhopper males (**Figure 5D; Figure supplement 8**). Transcripts for *SVP* and *MAF5* were detected, and upregulated, in leaves in the combined presence of SAP54 and leafhoppers (**Figure 5E; Supplementary file 9**), consistent with previous findings of Yang et al. (2015) showing upregulation of *SVP* in *Catharanthus roseus* by peanut witches' broom phytoplasma effector PHYL1. Together, these data provide evidence that female preference for male-exposed SAP54 plants is dependent upon the presence of SVP, while MAF5 plays a role in female choice independently from SAP54.

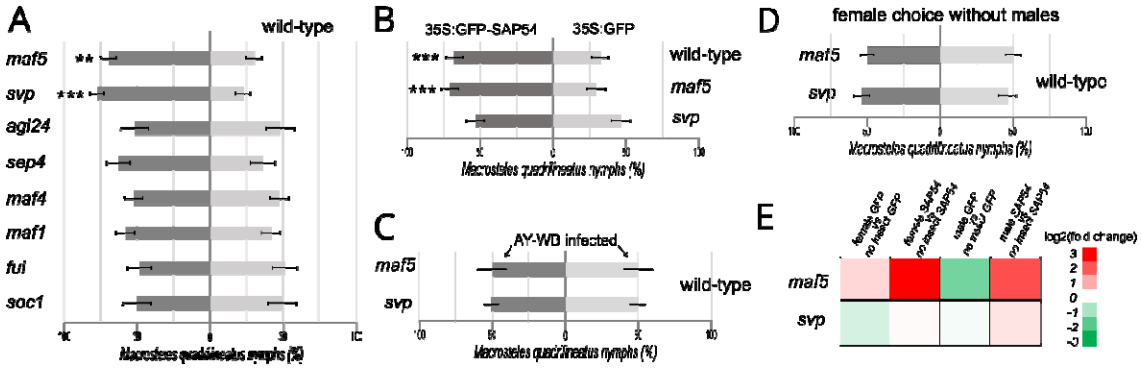

Figure 6. The MADS-box transcription factor SVP is required for female preference to reproduce on male-exposed SAP54 plants. A-C. Choice test with equal numbers of 10 males and 10 females on wild type and MTF *null* mutant *A. thaliana* rosettes (**panel A**), 35S:GFP-SAP54 *A. thaliana* rosettes (**panel B**) and AY-WB phytoplasma-infected plants (**panel C**). **D**. Choice tests with 10 females on *A. thaliana svp* and *maf5 null* mutants without males. The entire series of choice tests depicted in panels **A-D** were conducted in parallel and repeated independently 2 times. ** p<0.01, *** p<0.001. Bars are ±1 SEM. **E**. Expression levels of *SVP and MAF5* in leaves among treatments showing that SVP and MAF5 are upregulated in male-exposed SAP54 plants.

The online version of this article includes the following source data and figure supplement(s) for figure 6:

Figure supplement 8: Data distributions of independent repeats that were used to generate graphs displayed A-D.

Supplemental file 9. Fold-expression changes of MADS-box transcription factor genes insect-exposed SAP54 vs GFP leaves.

SVP differentially regulates plant responses to herbivores.

Considering the role of SVP in leafhopper reproduction preference, we conducted transcriptional analyses to assess how leaves of *svp* mutant plants respond to male and female leafhopper exposure and compared this to male or female exposed SAP54 plants (**Figure supplement 9A)**. A comparable number of DEGs was found in leaves exposed to both males and females, with 464 DEGs uniquely responding to females, 347 to males, and 460 to both sexes (**Figure supplement 9B**). Many of biotic stress related DEGs in male or female exposed *svp* mutant were upregulated (**Figure supplement 9C**). When searching for functions that were enriched with the 924 DEGs in the female exposed *svp* mutant versus female exposed wild-type plants, we found significant enrichment for abiotic stress and cell wall genes (**Supplementary file 10)** as well as DUF26 family cell surface receptors (**Supplementary file 11**). Furthermore, the 807 DEGs in *svp* leaves exposed to males compared to male colonized wild-type plants were predominantly involved in sulphur-containing glucosinolates and cell wall associated proteins (**Supplementary files 10 and 11**). Hence, the DEGs linked to cell wall structure and modulation were prominently enriched in leaves exposed to both male and female leafhoppers. There was no clear enrichment of functional categories among the 347 male-specific DEGs in *svp* mutant (**Supplementary files 10 and 11**). These findings suggest that SVP influences leaf responses to leaf-colonizing insects in a manner that is partially specific to the gender of the leafhoppers.

We further wished to explore the differentially expressed genes (DEGs) that are common between the male-exposed leaves of SAP54 and *svp* mutant plants. Firstly, we analysed the overlap between male-specific (not up- or down-regulated by female) SAP54 expressing plants and the *svp* mutant, where we found only 49 DEGs (**Figure supplement 9D**). Many of these genes display similar up- or down-regulation in SAP54 and *svp* plants, and some of the most downregulated DEGs include cytochrome P450s such as CYP81F, CYP78A as well as glutamate receptor GLR3 and calcium-binding transcription factor MYC5 (NIG1) (**Supplementary file 12, Table Tab A**).

However, due to the relatively small number (49) of shared DEGs for full pathway characterisation, we analysed functions encoded by all 155 shared DEGs in male exposed SAP54 and *svp* plants, including those that overlap with the female regulated DEGs in those plants (**Figure supplement 9E**). The 155 DEGs showed

significant GO term enrichment associated with various biological responses, including responses to stimuli, oxygen-containing compounds, lipids, hormones, and calcium signalling (**Supplementary file 12, Table Tab B**), suggesting common functions in biotic stress regulation between SAP54 and SVP in male exposed plants. Moreover, we also examined the full spectrum of MapMan biological functions enriched by DEGs in leaves of male-exposed SAP54 (3814 DEGs) and *svp* mutant (807 DEGs) plants separately before looking for the overlap in functions (rather than gene identities first). This comparison revealed that the similarities between the enriched functions in male exposed SAP54 and *svp* mutant plants predominantly pertain to cell wall modification and secondary (isoprenoid) metabolism (**Supplementary file 12, Table, Tab C**). Considering that female insects are more attracted to the male-exposed leaves of SAP54 and *svp* mutant plants for feeding and oviposition, alterations in cell wall structure and changes in secondary (isoprenoid) metabolism may potentially play significant roles in influencing female preferences.

Discussion

Here, we investigated colonization preferences of the leafhopper vector *M. quadrilineatus*, the predominant vector of AY-WB phytoplasma, for plants that produce the AY-WB effector SAP54. We made the surprising discovery that female preference for SAP54 plants only occurs in the presence of males. Both SAP54 and males are necessary for this preference, as females did not show a preference in their absence. As well, SAP54 plants exposed to males display significant downregulation of biotic stress responses. We identified the MADS-box transcription factor SVP to be essential for this female preference to male-exposed SAP54 plants. Leafhopper females exhibited a preference for reproducing on *svp null* mutant plants, a behaviour that is also contingent upon the presence of leafhopper males, and aligning with our observation that SAP54 mediates the degradation of SVP in leaves. Therefore, by characterizing a parasite effector, we revealed the crucial role of SVP in leaf susceptibility to male herbivorous insects, enhancing female attraction and colonization.

This work built on our previous findings that the leaf-like flowers are not required for the insect colonization preference and the insect vectors are predominantly attracted to the leaves and not flowers of plants (Orlovskis & Hogenhout, 2016). Here we independently confirm these data via experiments that show a preference of the insects for *A. thaliana* rosettes before the onset of flowering. Therefore, a morphological change in the host may not always be responsble for the extended phenotype effect on the alternative host, which is in this case the insect vector. It is possible that the attraction of insect vectors to leaves is the primary function of SAP54, and the induction of the leaf-like flowers an evolutionary side-effect of SAP54 adaptation to target MTFs that leads to degradation of SVP. However, the prevention of seed production through the formation of leaf-like flowers likely is likely to present an advantage to phytoplasma. AY-WB phytoplasma commonly infects annual plants, where leaves naturally senesce and die off after flowering and seed production. It is probable that prolonging the longevity of their plant hosts is advantageous for phytoplasmas, particularly considering that they are not known to be seed-transmitted. Instead, phytoplasmas rely on leafhoppers, which feed on vegetative tissues, for transmission.

We found that SAP54 mediates the destabilization of SVP. However, we did not find evidence of a direct interaction between SAP54 and SVP in our Y2H analyses. An explanation of this apparent discrepancy is that MTF proteins exhibit a propensity to form diverse complexes, including homo- and hetero-dimers, as well as higher order ternary and quaternary complexes (Davies *et al.*, 1996; Egea-Cortines, 1999; Hartmann *et al.*, 2000; Honma & Goto, 2001; Immink *et al.*, 2002; de Folter *et al.*, 2005). Notably, SVP is known to interact with other MTFs such as SOC1, FUL, and AGL24 (de Folter *et al.*, 2005; Gregis *et al.*, 2009; Balanzà *et al.*, 2014), and we previously established that SAP54 interacts with and facilitates the degradation of multiple MTFs, including SOC1, FUL, and AGL24 (MacLean *et al.*, 2014), as is also shown for FUL and AGL24 herein (**Figure 5**). Moreover, our research showed that these three MTFs are expressed in leaves, with SOC1 and AGL24 showing elevated expression in SAP54 plants exposed to males (**Supplementary file 9**). Hence, the destabilization of SVP by SAP54 may occur through its involvement with various SVP-containing protein complexes. This intricate interaction network also provides a

possible explanation for the absence of a leafhopper preference phenotype in *soc1*, *agl24*, and *ful* mutant plants. It suggests that the interaction of SAP54 with multiple SVP-containing ternary and quaternary MTFs, leading to their degradation, might be essential to trigger the observed phenotype.

SVP is recognized as a conserved regulator of flowering time across a variety of eudicot species, as evidenced by several studies (Sun *et al.*, 2016; Li *et al.*, 2016). It is expressed in the leaves during the vegetative growth stages of plants (Zheng *et al.*, 2009; Li *et al.*, 2016; Wu *et al.*, 2017), which correlates with the wide host range of AY-WB phytoplasmas. While the primary role of SVP and its homologs has been associated with flowering, their involvement in additional biological processes beyond this remains underexplored (Liu et al., 2018). However, emerging research has begun to shed light on the roles of SVP outside of flowering: for instance, it acts as a positive regulator of age-related resistance against bacterial pathogens in *Arabidopsis thaliana* (Wilson *et al.*, 2017) and serves as a negative regulator of various JASMONATE ZIM-domain (JAZ) genes during the vegetative growth phase (Gregis *et al.*, 2013). Our findings contribute to this expanding body of knowledge, suggesting that plant immunity may be intricately controlled by developmental regulators like SVP, which possesses multiple, diverse functions throughout plant development (Berry & Argueso, 2022, and references therein). The observation that female leafhoppers prefer to reproduce on both SAP54 and *svp* mutant plants exclusively in the presence of leafhopper males underscores a sophisticated regulatory effect of SVP and other MADS-box transcription factors targeted by SAP54 on plant responses to the different sexes of the herbivores. This intricate interaction is detailed in the mechanism proposed in **Figure 7**.

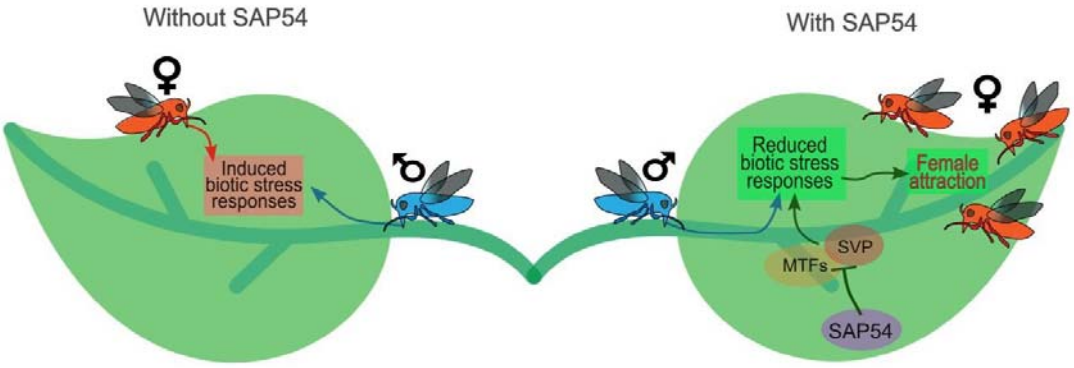

Figure 7. Model illustrating how the phytoplasma effector SAP54 facilitates leaf colonization by leafhopper vectors, which are essential for phytoplasma transmission and spread. During phytoplasma infection, the SAP54 effector is secreted and promotes the degradation of MADS-box transcription factors. This degradation of MADS-box transcription factor SHORT VEGETATIVE PHASE (SVP) specifically leads to the downregulation of biotic stress responses to male leafhoppers, thereby attracting female leafhoppers to the leaves. The females then lay eggs, and their offspring acquire phytoplasmas during feeding. Thus, SAP54 likely enhances phytoplasma spread by increasing female insect vector reproduction in a process that requires SVP and the presence of males. An additional implication of this model is that male leafhoppers might benefit from phytoplasma infection by attracting more mates. This work corroborates the previous findings that both the SAP54-mediated degradation of MADS-box transcription factors and the leafhopper attraction phenotype are dependent on the 26S proteasome shuttle factor RAD23 (MacLean et al., 2014) and that leafhoppers are specifically attracted to leaves and not the leaf-like flowers that are induced by SAP54 actions (Orlovskis and Hogenhout, 2016).

Considering that certain phytoplasmas can modify plant volatile production (Mayer *et al.*, 2008a,b; Rid *et al.*, 2016; Tan *et al.*, 2016), we examined whether male-induced volatile cues play a role in attracting females. Our results suggest that female leafhopper preference for laying eggs on male-exposed SAP54 plants is unlikely to depend solely on volatiles originating from the males or induced by them (**Figure supplement 3**). Thus, it is plausible that females are guided to male-occupied SAP54 and *svp* mutant plants through a different mechanism, possibly involving changes in leaf responses that are specifically induced to male insects. Male-exposed leaves of SAP54 and *svp* mutant plants shared transcriptional changes of genes involved cell wall structure processes and changes in secondary

(isoprenoid) metabolism. As such, these could influence female leafhoppers' host plant selection and their egg-laying preferences on plants exposed to males. However, given that the removal of males from SAP54 leaves prior to female choice does not enhance female colonization (comparison of Figure 1 treatment 4 with treatment 5), we cannot exclude that male produced volatiles or mating calls can enhance or supplement SAP54-dependent changes in biotic stress responses to males for enhanced female attraction.

Males and females of *M. quadrilineatus*, the primary vector of AY-WB phytoplasma, exhibit distinct movement and feeding behaviours, as documented by Beanland et al. (1999). Moreover, the leafhoppers, especially females, live longer on aster yellows infected plants compared to non-infected plants (Beanland *et al.*, 2000). Here we show that the combination of the aster yellows phytoplasma effector SAP54 and males attract females in a manner that depends on SVP. Moreover, plant biotic stress and defence responses are largely suppressed in male (compared to female) exposed SAP54 plants, and SVP also displays distinct plant transcriptional responses to male and female leafhoppers. Nevertheless, the mechanisms how male and female leafhoppers, in combination with SAP54, differentially affect plant defence responses will need further investigation. While the differences in male and female behaviour may play a role (Beanland *et al.*, 1999), there is also the possibility that effectors in the saliva of sap-feeding insects modulate plant processes, including defence (Snoeck *et al.*, 2022), and sex-specific differences exist in the proteome of sap-feeding insect saliva (Miao *et al.*, 2018), potentially influencing plant response. Moreover, plant immune responses initiated by the molecular patterns associated with insect eggs (EAMPs) that bear similarities to pattern-triggered immunity (PTI) activated by bacterial pathogens (Gouhier-Darimont *et al.*, 2013) may play a role in the observed gender-specific differences.

Pathogens uniquely exploit sexual dimorphism in their hosts to enhance their dispersal and survival in nature. For example, the bacterial parasite infecting the crustacean *Daphnia magna* selectively uses male hosts for horizontal transmission, while exploiting females for internal replication and spore production, thus facilitating widespread disease transmission (Nørgaard *et al.*, 2019). Toll signalling pathway contributes to the varied resistance observed between male and female *Drosophila* to both Gram-positive and Gram-negative bacteria (Duneau *et al.*, 2017). Similarly,

the endosymbiotic bacterium *Wolbachia* often exhibits distinct effects on the embryonic development of male and female gametes, showcasing another instance of pathogens leveraging host sexual differences (Serbus *et al.*, 2008). Recent discovery identified a novel *Wolbachia* factor Oscar which interacts with host protein Masculinizer to inhibit masculinization and lead to male-killing in two insect species (Katsuma *et al.*, 2022). Our study further expands the mechanistic understanding about insect gender specific factors targeted by parasites.

In our research, we have uncovered a mechanism whereby the phytoplasma effector SAP54 targets a conserved host regulator, SVP, to attract female leafhopper vectors to produce more progeny on plants exposed to leafhopper males. Given that phytoplasmas rely on leafhoppers for acquisition during feeding and subsequent transmission to other plants, SAP54 likely promotes the abundance of phytoplasma-carrying insect vectors and facilitates phytoplasma transmission. This illustrates how a bacterial gene extends its influence beyond its primary plant host to modulate the host choice and reproductive behaviour of the insect vector. Such findings underscore the complexity of tripartite plant-insect-microbe interactions, wherein parasitic genes exhibit what could be termed 'hyper-extended phenotypes,' manipulating the biology of one host to facilitate transmission through another host. This research advances our understanding of plant-microbe interactions and resonates with the concept of the Extended Evolutionary Synthesis (EES), as articulated by Hunter (2018). Within the EES framework, parasitic genes like SAP54 evolve adaptively through both natural selection and direct biochemical interactions with plant regulators, as well as through the extended functions these regulators serve in interactions with insect vectors. In this study, we found that the extended role includes enhancing the likelihood of male vectors to attract females for reproduction.

Materials and methods

Generation of plants for insect choice experiments

Generation of 35S:GFP-SAP54 and 35S:GFP transgenic *Arabidopsis* lines was done according to methods described in (MacLean *et al.*, 2011, 2014). *ful-1* line was provided by Lars Ostergaard Lab and described in (Gu *et al.*, 1998). *soc-1* and *sep4-*

1 by Richard Immink Lab and described in (Immink *et al.*, 2012). *maf4-2 and maf5-3* seeds were provided by Hao You Lab and described in (Shen *et al.*, 2014). *maf1* (also known as *flm-3)* was provided by Claus Schwechheimer Lab and described in (Lutz *et al.*, 2015). *svp1-41* was provided by Martin Karter Lab and described in (Hartmann *et al.*, 2000). *agl24* (N595007, SALK_095007) was obtained from The Nottingham Arabidopsis Stock Centre (NASC). Phytoplasma-infected plants were generated identical to methods described in (MacLean *et al.*, 2014). All plants were grown under short days (10h/14h light/dark) identical to methods described in (Orlovskis & Hogenhout, 2016)

Insect oviposition choice assays

All insect choice experiments were performed with a mixture of virgin and pre-mated females identical to methods described in (Orlovskis & Hogenhout, 2016) with the following modifications. Female-only oviposition choice experiments described in **Fig. 1** (*experiment 2*) were set up by releasing 10 *M. quadrilineatus* females, while the mixed-sex choice assay (experiment 1) was done with 10 male and 10 female insects*.* Five male leafhoppers were confined on test and another five on control plants using transparent clip-cage (2 cm diameter) before the release of 10 females in *experiments 3* and *4* displayed in **Fig. 1**. For the *experiment 5* in **Fig. 1**, five females were restricted on test and five on control plants prior the release of another 10 females in the choice cage. Notably, single-sex insects were selected from mixed-sex population of *M. quadrilineatus* stock. Adults were removed 5 days after release, nymphs counted 14 d after adult removal. Nymph counts analyzed using paired t-test.

Insect olfactory choice assay

Test and control plants were placed in a black (non-transparent) plastic container 12cm x 10cm x 10cm (H x W x D) with a perforated topside, permitting diffusion of plant odors but rendering plants invisible from the outside of the container. A colourless or a coloured sticky landing platform (OECO, Kimpton, UK) was fitted on the top of each container but not covering the perforations. 20 *M. quadrilineatus* females were released in the center of the arena (transparent polycarbonate 62cm x 30cm x 41cm (H x W x D)) equal distance from the test and the control containers. When coming in contact with the sticky landing platform, insects stuck permanently to its surface. Within the following 1h under the ambient

room light the majority of insects made their first landing choice, and were counted. Each experiment utilized clean choice arena, new containers and landing platforms to avoid any possible residual semiochemicals left by insects from the previous experiment which could bias the leafhopper choice in the next experiment. In selected experiments male leafhoppers were contained in clip-cages on the test plants placed inside the containers.

Western blotting

Proteins were separated on 12.5% (w/v) SDS-PAGE gels and transferred to 0.45 mm Protran BA85 nitrocellulose membranes (Whatman, UK) using the BioRad minigel and blotting systems following standard protocols. Blotted membranes were incubated in blocking buffer (5% w/v) milk powder in 1X phosphate buffered saline and 0.1% (v/v) Tween-20) with primary antibody at 4°C overnight. Peroxidase-conjugated anti-rabbit or anti-mouse secondary antibody (Sigma Aldrich) was added to washed blots and incubated at room temperature for 4h. Bound antibodies were detected using Immobilon Western Chemiluminescent HRP Substrate (Millipore, UK) when exposed to Super RX film (Fujifilm, Germany). Protein loading was visualized using Ponceau S solution (0.1% (w/v) in 5% acetic acid).

Yeast-two-hybrid assays

The Matchmaker Gold Yeast Two-Hybrid System (Clonetech) and the DUALhybrid system (Dualsystems Biotech) were used for detecting protein-protein interactions in yeasts. Yeast transformation were carried out following manufacturers' instructions. Successful transformants grew on medium lacking leucine and tryptophan (-L -W). Medium lacking leucine, tryptophan, histidine and Adenine (-L-W-H-A) or Medium lacking leucine, tryptophan, histidine and supplemented with 10 mM 3-AT (-L-W-H +10 mM 3-AT) were used to examine protein-protein interactions. The growth of yeast colonies was scored after 5 days of incubation at 28°C. Protoplast assays were carried out according to (Yoo *et al.*, 2007).

Protoplast assays

Transient expression protoplast assays were performed identical to methods in Huang *et al.,* 2021. *M*esophyll protoplasts isolated from 4 weeks-old *Arabidopsis*

leaves were resuspended at 4 × 105 mL−1 in MMG solution. 300 µL protoplast solution were then transfected with 24 µg plasmids (12 µg each for double transformation) using PEG–calcium-mediated transfection. pUGW15 plasmid was used for expressing HA-tagged genes, pB7WGF2 - for expressing GFP-tagged genes. pEarleyGate 202-GFP was used to express GFP. Protoplasts were kept at 22 °C for 16 hours in dark and then harvested for Western blot analysis.

Generation of plants for RNA-sequencing

The experiment used 8-weeks old *Arabidopsis thaliana* (Col-0) plants ectopically expressing Aster Yellows phytoplasma strain Withes' Broom effector SAP54 (35S:GFP-SAP54) or a control construct (35S:GFP). Plants were grown at short day photoperiod (10h/14h light/dark). Single fully expanded leaf of each plant was exposed to either 5 male or 5 female insects by placing them in a transparent 2cm diameter clip-cage which confines the leafhoppers to the leaf and prevents from escaping. An empty clip-cage was placed on a leaf for no-insect control. In order to prevent any insects from the room to land on the experimental plants and confound the results, we placed all plants with clip-caged female leafhoppers in a sealed larger cage separate from plants with clip-caged males or plants with empty clip-cages. Thus, all replicate plants with male, female or no-insect exposed leaves were maintained in similar micro-environment. The experiment with clip-caged males, females and empty clip-cages was done at the same time on the same shelf within the growth room to ensure similar conditions across all treatments. Plant tissue samples were collected 48h after exposure to insects and placed straight into liquid N_2. Insect number and exposure time of 48h was previously experimentally optimised by measuring number of feeding sites and eggs laid per clip-cage. The experiment with clip-caged male or female plants on *svp* mutant and *wild-type* Col-0 plants was performed at a different time than the experiment with 35S:GFP-SAP54 and 35S:GFP plants but with identical setup.

Total RNA extraction for RNA-seq and quality control

The collected leaf area enclosed by the clip-cage (~2 cm diam.) was stored at -80°C for subsequent RNA extraction using QIAGEN Plant RNeasy kit following the manufacturer's instructions. Initial RNA integrity was assessed by gel electrophoresis (1% Agarose) visualisation of ribosomal bands in the extracted dsRNA as well as ssRNA following 65°C denaturation and immediate transfer on ice to prevent hybridisation. Total RNA concentration and quality was assessed using Nanodrop (Thermofisher). Total ≥2µg of each RNA sample at ≥50ng/µL concentration, 260/280 ratio between 1.9 and 2.1, and 260/230 ratio between 1.5 and 2.0 was submitted for library preparation and RNA-sequencing.

RNA sequencing, read alignment and differential expression analysis

Experimental design consisted of 4 biological replicates for male- or female-exposed or insect-free transgenic 35S:GFP-SAP54 plants and transgenic 35S:GFP control plants as well as 3 biological replicates for *svp* null mutant and wild-type col-0 control plants. cDNA library construction for SAP54 and GFP was performed according to the IlluminaTruSeq protocol, followed by sequencing on Illumina HiSeq2000 platform and pooling 4 randomized libraries per lane, with 50bp single-end reads and 25M read coverage per sample, whereas *svp* null mutant and wild-type libraries were prepared with paired-end reads. Reads were trimmed for low quality and adapter contamination using Trim Galore! v0.4.0 with default settings (Krueger, 2012). Trimmed reads were aligned to *A. thaliana* reference genome (TAIR10) by HISat2 (Pertea *et al.*, 2016). We identified differentially expressed transcripts with DESeq2 package in R (version 1.2.10) (Love *et al.*, 2014) using expression count generated by Kallisto v0.42.3 (Bray *et al.*, 2016). Independent filtering was employed in in DESeq2 to remove very low expressing transcripts on the basis of normalized counts. Genes were considered differentially expressed if they had a p value less than 0.05 after accounting for a 5% FDR according to the Benjamini-Hochberg procedure and if a fold-change in expression of at least 2 was observed.

Functional analysis of transcriptome data

Enrichment of GO biological functions and GO molecular functions for DEGs were calculated using Fisher exact test (PANTHER) (http://geneontology.org/docs/go-enrichment-analysis). Graphical visualisation for functional catagorisation of differentially regulated transcripts was performed using MapMan 3.5.1R2 functional annotation tool (Thimm *et al.,* 2004). The tool, supporting resources and annotation database available from http://mapman.gabipd.org/web/guest/home. Statistical analysis on enrichment of differentially changed pathways was performed using Wilcoxon rank test and Benjamini-Hochberg (BH) p-value correction (Usadel, 2005). Methods for manual creation of new pathways and categorical filtering of expression data were adopted from (Usadel *et al*., 2009) and explained in sections below. The graphical overview of defence signalling cascades in **Figure 3** and *Figure supplement 7* were manually drawn and loaded as separate pathway image files into MapMan. The mapping file (**Supplementary file 5**) containing a list of gene identifiers and descriptions manually assigned to new functional bins previously absent from the MapMan annotation was generated from published literature and the public TAIR database (www.arabidopsis.org/browse/genefamily) for membrane-located receptor-like kinases and their classes (Shiu and Bleecker, 2001), cytoplasmic receptors such as NLR proteins (Hofberger et al., 2014; Kroj *et al.,* 2016; Sarris *et al*., 2016), CDPK-SnRK superfamily (Hrabak *et al.,* 2003), MAP kinases cascade (Asai *et al*., 2002; Jonak *et al.,* 2002) as well as SA, JA and ET biosynthesis and signalling genes (van Verk, 2010).

Data availability

RNA-seq raw data (fastq) are uploaded to NCBI under BioProject: PRJNA1090849.

Author contributions (The CRedit Roles and Number):

ZO: conceptualization (1), data curation (2) and formal analysis (3), investigation (4), methodology (5), data validation (11), statistical analysis and visualisation (12), writing, reviewing and editing the original manuscript (13,14)

WH: investigation (4), methodology (5), data visualisation (12), review of the original manuscript (14)

AK: data validation (11), review of the original manuscript (14)

AS: data curation (2), review of the original manuscript (14)

SH: conceptualization (1), data curation (2) and formal analyses (3), funding acquisition (4), supervision (10), data validation (11), writing, reviewing and editing the original manuscript (13,14).

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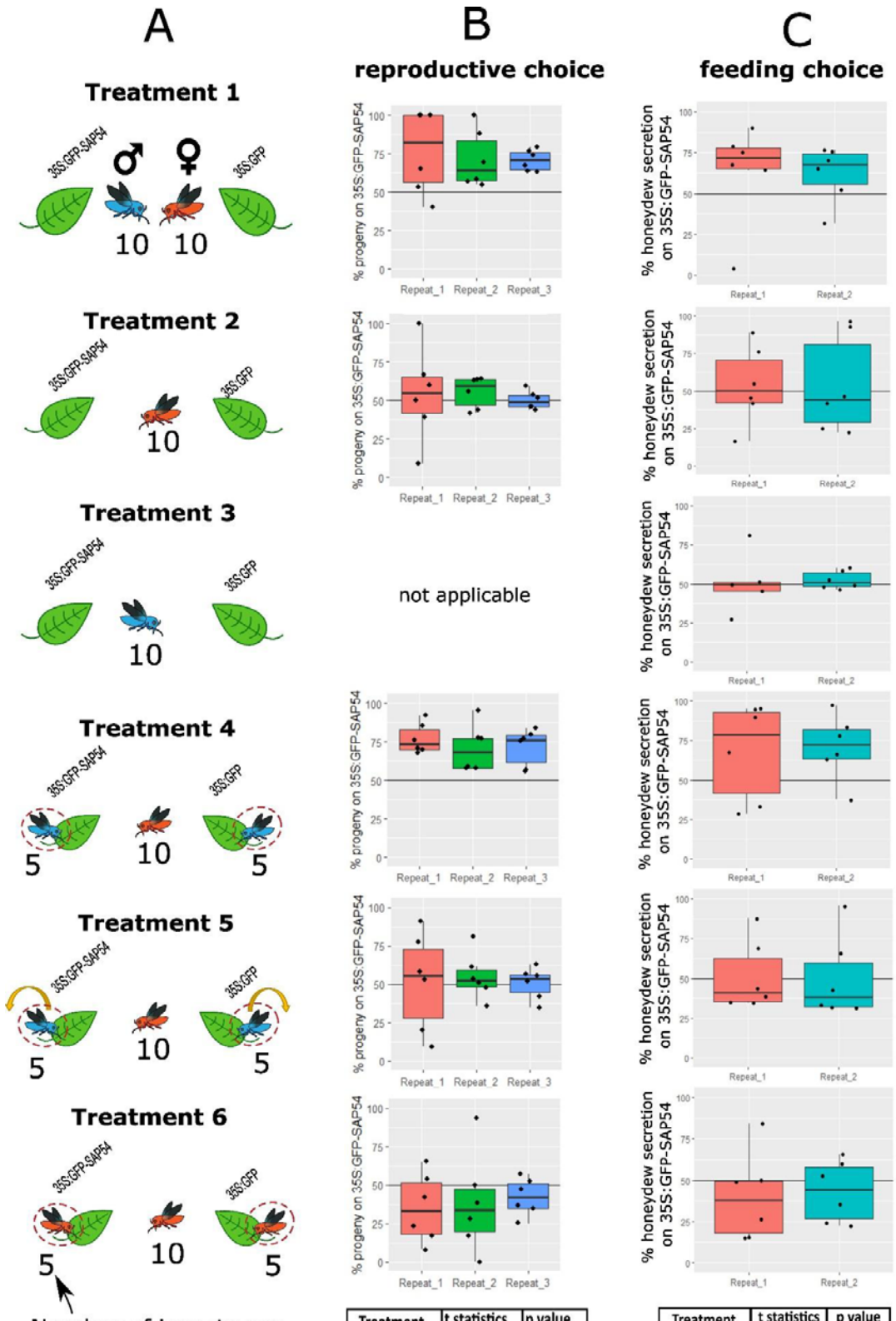
1281 **Figure supplements:**



Treatment	t statistics	p value
Experiment 1	5.2432	6.61E-05
Experiment 3	0.77136469	0.45108
Experiment 4	8.04400865	3.39E-07
Experiment 5	0.49580262	0.626382
Experiment 6	-2.19702566	0.042171

Treatment	t statistics	p value
Experiment 1	1.8504	0.091275
Experiment 2	-0.4779467	0.642045
Experiment 3	0.43710453	0.671324
Experiment 4	2.69865342	0.020707
Experiment 5	0.08967685	0.930156
Experiment 6	-0.8572801	0.400135

1282

Figure supplement 1. Data distributions of independent repeats that were used to generate graphs displayed in Fig. 1 B and C. Experimental design for choice tests (treatments) 1 to 6 represented in **Figure 1** of the main text is shown in **panel A**. Each choice test was performed in a separate choice cage (arena) but simultaneously with the other choice tests in the same room. Boxplots show variation among repeated experiments for progeny (**panel B)** and feeding (honeydew excretion) data (**panel C)**. Each data point in panel B represents oviposition choice of 10 female insects. Each data point in panel C corresponds to feeding choice of 20 adult insects within a single choice arena. No preference for either test or control plant in a choice cage is represented by the 50% reference line. Survived progeny or feeding preference for SAP54 plants is characterised by the skewed distribution above the 50% reference line. Each reproduction choice experiment consisted of 6 choice arenas and was performed independently 3 times (Repeat1, Repeat2, Repeat3 with corresponding bar colours), involving a total of 540 insects. Each feeding choice experiment consisted of 6 choice arenas and was performed independently 2 times (Repeat1 and Repeat2 with corresponding bar colours), involving a total of 480 insects). Paired t-test statistics for combined repeated experiments are summarised in the table below the boxplots.

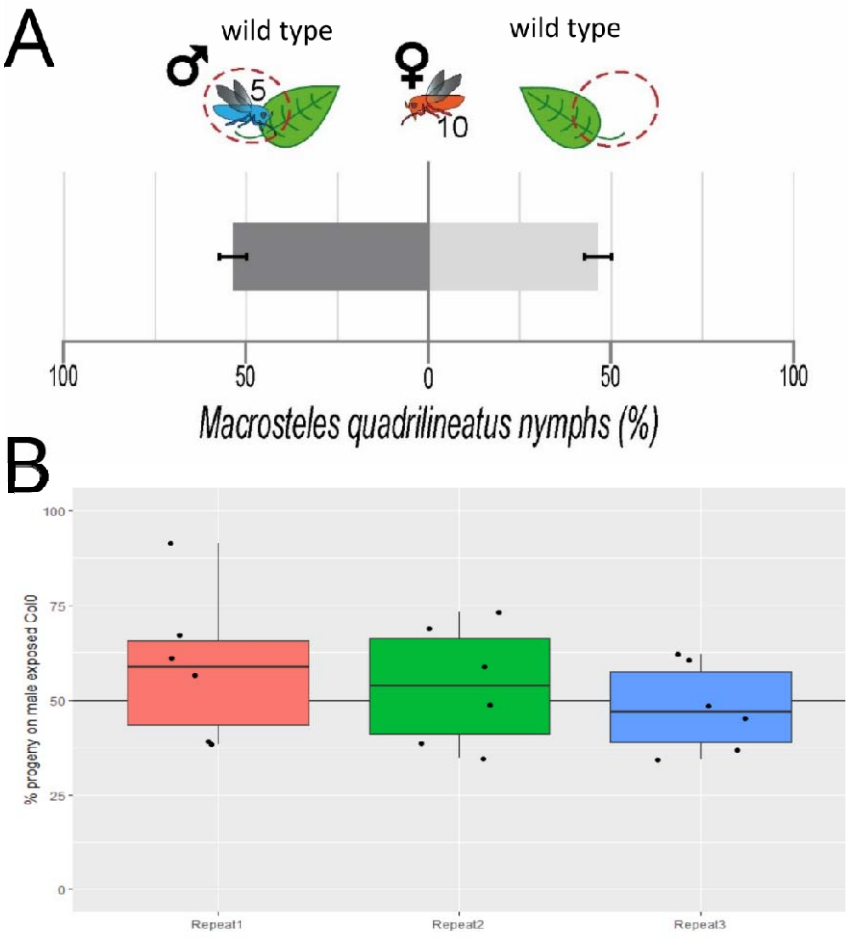


Figure supplement 2. *Macrosteles quadrilineatus* female leafhoppers show no preference for *A. thaliana* Col-0 wild-type plants exposed to conspecific male leafhoppers. **A**. In each choice arena 10 female leafhoppers were allowed to choose to lay eggs between insect-free *A. thaliana* Col-0 plants (with an empty clip-cage) and Col-0 plants with 10 male insects confined in clip-cages. Eggs laid by females were counted over the entire plant. Bars are 1 standard error of the mean**. B.** This experiment was independently repeated 3 times with combined total of 180 female insects. Paired t-test was performed on combined dataset considering all repeated experiments (t=1.09; p=0.325).

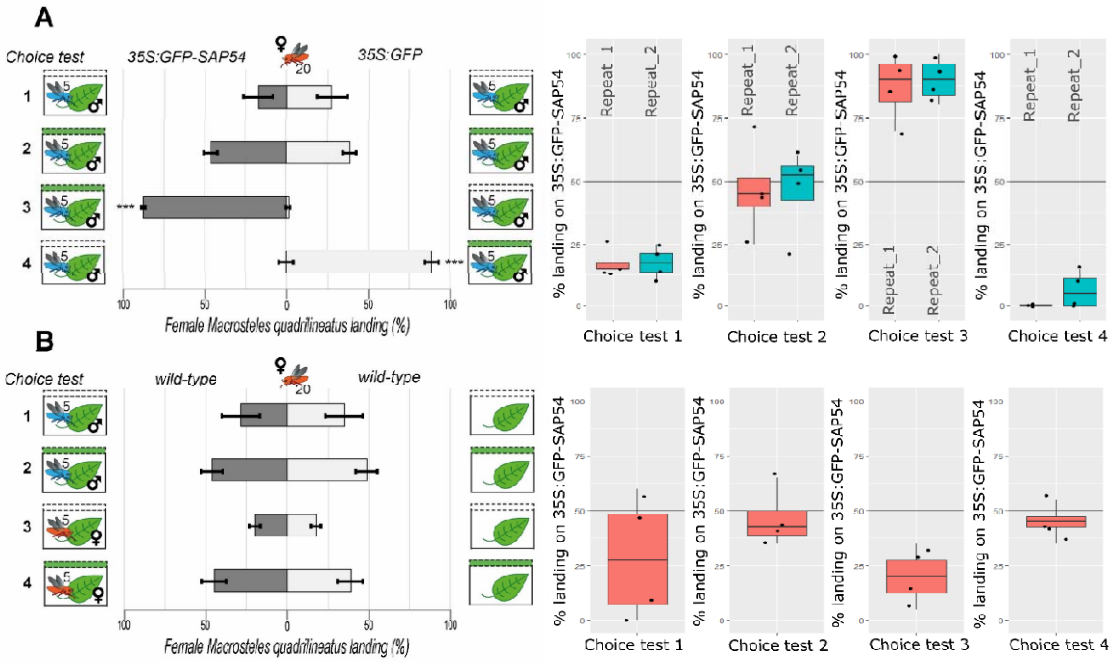


Figure supplement 3. Female *M. quadrilineatus* preference for male-exposed SAP54 plants are unlikely to involve long-distance cues. Choice tests were performed in separate cages, inside which two test plants were placed in non-transparent black plastic boxes that permit plant volatiles to escape but conceal the plants inside. Transparent or green sticky landing platforms were placed over each box (horizontal dashed bars). Twenty (20) female insects were released in a choice arena and females sticking to the transparent or sticky landing platforms were recorded after 1h. Bars indicate the percentage of recaptured females on each trap type. Boxplots show data distribution for recaptured females on SAP54 plants. **A**. Each choice test contained 4 choice arenas (cages) corresponding to a single datapoint in the boxplot. Each test was repeated independently 2 times. Females preferred the green over transparent platforms regardless of plant identity in within the trap. Figure shows the results of combined repeated choice tests. Choice test 1, t_7=1.521, p=0.172; Choice test 2, t_7=0.226, p=0.828; Choice test 3, t_7=21.826, p<0.001; Choice test 4, t_7=21.104, p<0.001. **B**. Females do not show preference for platforms of cages that contain male-exposed plants over insect free plants. Figure shows the results of single choice test with 4 choice arenas. Choice test 1, t_3=0.245, p=0.822; Choice test 2, t_3=0.322, p=0.769; Choice test 3, t_3=0.302, p=0.783; Choice test 4; t_3=0.555, p=0.617.

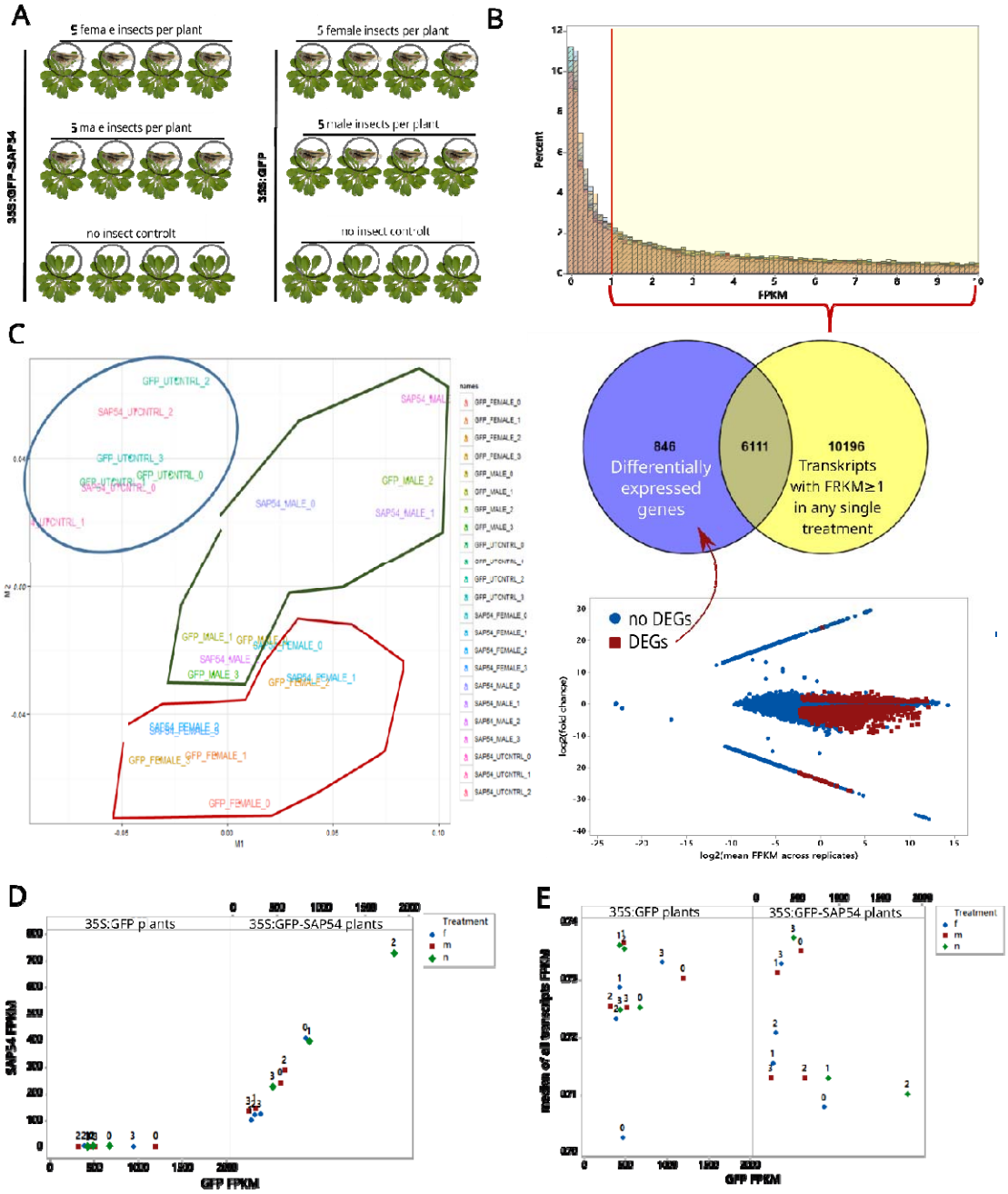


Figure supplement 4. Experimental design and selection of transcripts for downstream analysis. **A**. Five (5) male or 5 female *M. quadrilineatus* individuals were placed within a clip-cage onto a single rosette leaf of 35S:GFP-SAP54 or 35S:GFP plants. Empty clip-cages without insects served as controls. **B**. Mapped SAP54 reads plotted against GFP reads and colour coded for treatments (m=male; f=female; n=no insect) on GFP and SAP54 plants. **C**. Multi-dimensional analysis (MDA) plot demonstrates grouping of cDNA libraries according to treatment. **D.** Transcripts with normalized read count (FRKM) ≥1

in any of the sequenced libraries (10’196) and significantly differentially expressed transcripts (DEGs) from any of the treatment pairwise comparisons (6947) were considered for downstream analysis (total=17’153 transcripts). **E**. Median of all transcript FRKM plotted against GFP reads and colour coded for treatments (m=male; f=female; n=no insect) on GFP and SAP54 plants.

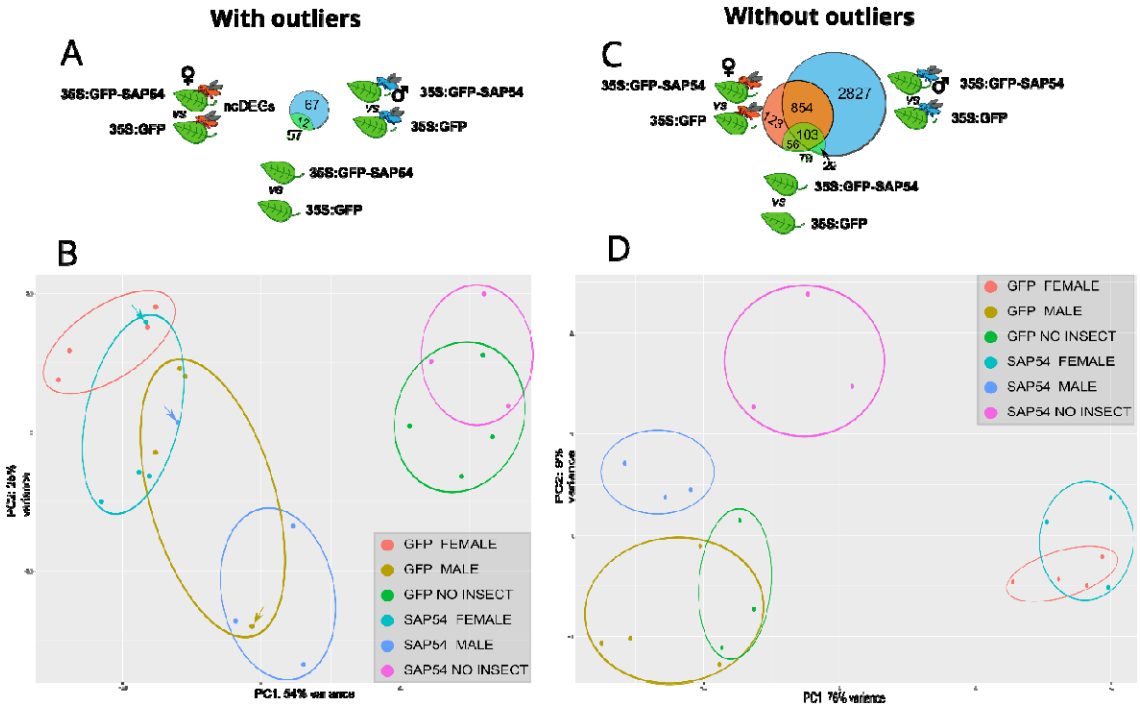


Figure supplement 5. Biological variation and role of outliers in separation of treatments and identification of differentially expressed genes. Euler-Venn diagrams illustrating DEGs that differentiate SAP54 and GFP plants exposed to female or male or no leafhoppers when outliers are retained (**A**) or removed (**B**). Principal component analysis (PCA) plots illustrate variation within and among treatments when outliers are retained (**C**) or removed (**D**). Arrows in panel **C** indicate the outliers (SAP54_male, GFP_male, SAP54_female) that were removed in panel **D**.

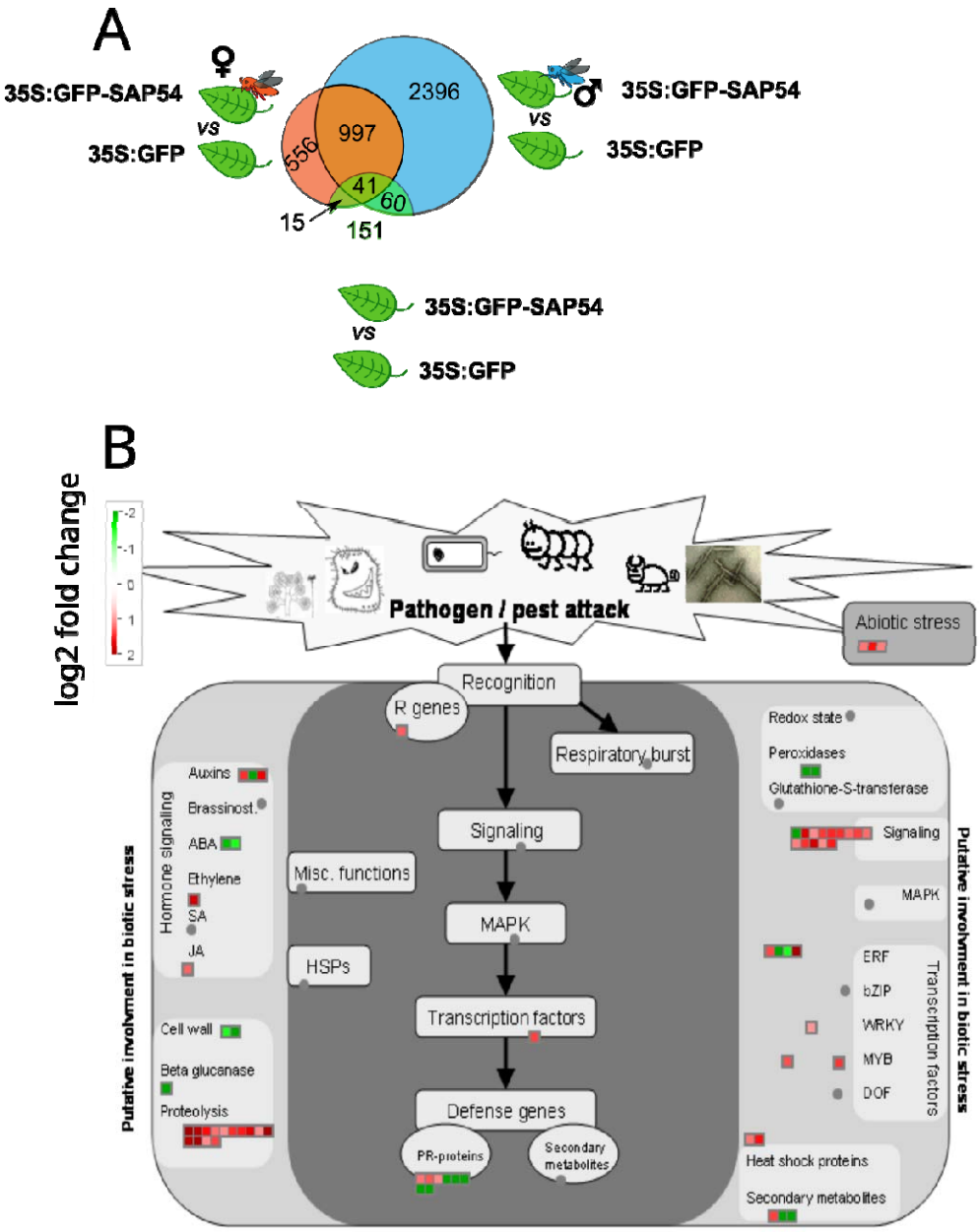


Figure supplement 6. The cage-only SAP54 vs cage-only GFP treatments show a limited number of biotic stress DEGs. A. Euler-Venn diagrams illustrating DEGs in SAP54 plants exposed to female or male or no leafhoppers compared to no insect exposed (empty cage-only) GFP plants. **B.** Mapman diagram of DEGs involved in biotic stress in the cage-only SAP54 vs cage-only GFP plants. Pathways are indicated and each square is a gene with red versus green shades illustrating the level of up- or downregulation.

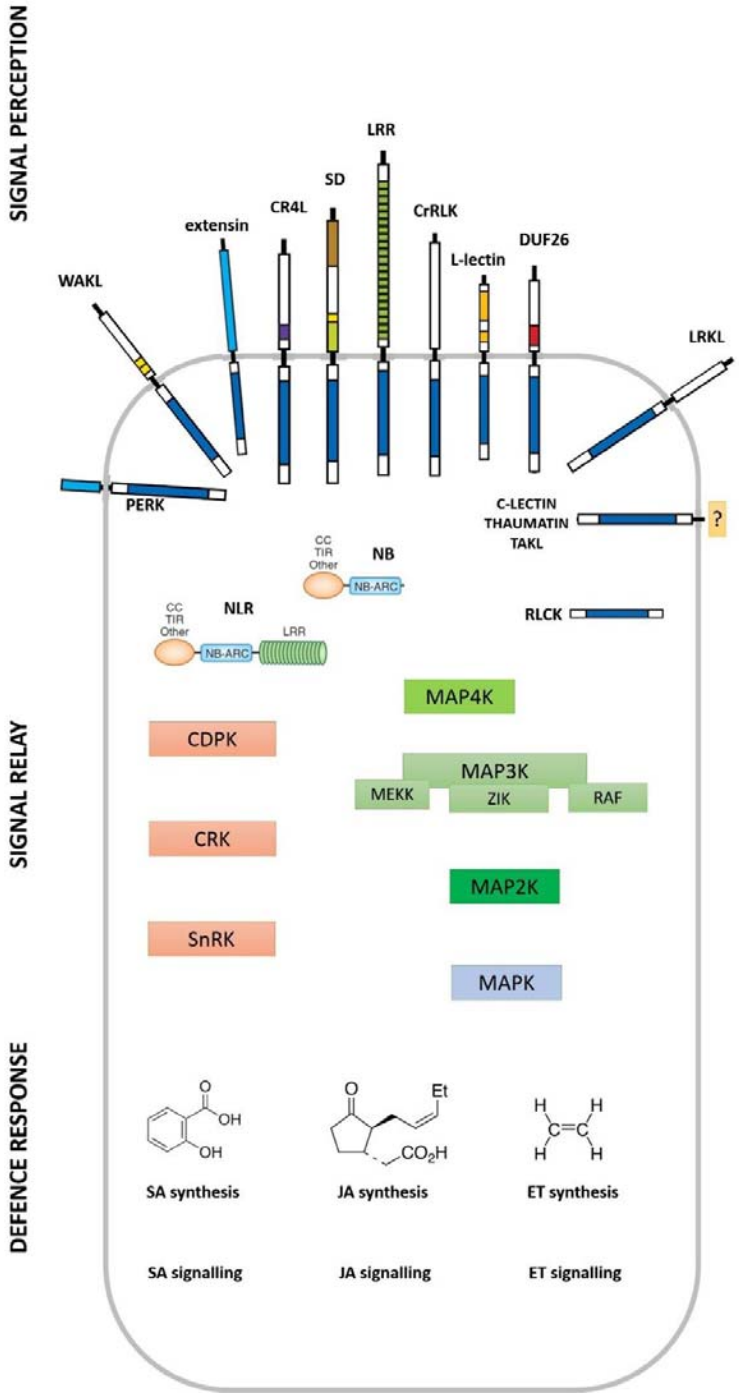
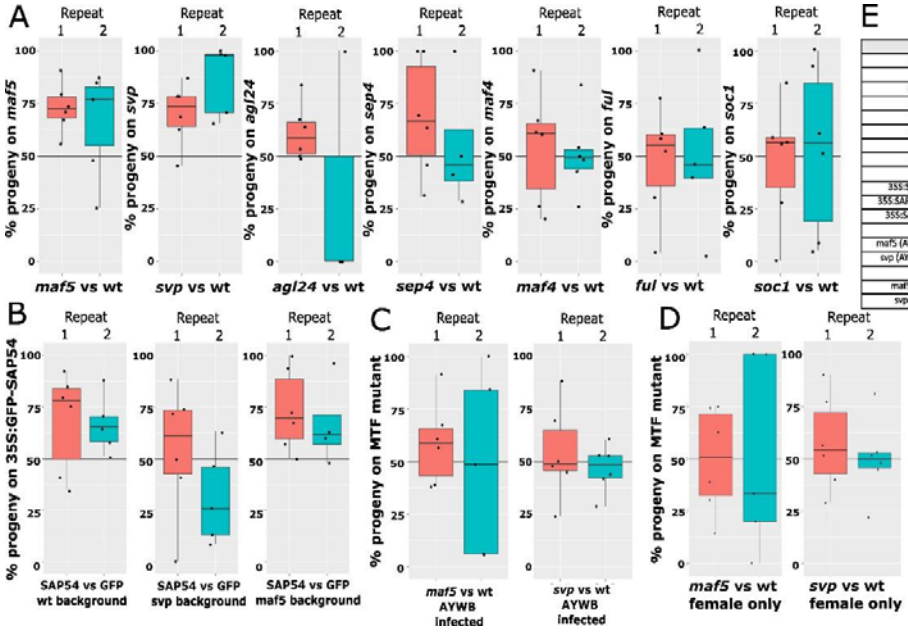


Figure supplement 7. Manually drawn MapMan image for defence signalling pathway visualisation.



E

Experiment	test statistics	p value
maf5 vs wild-type	3,6430	**0,0039**
svp vs wild-type	5,2720	**0,0004**
agl24 vs wild-type	0,1656	0,8726
sep4 vs wild-type	1,4857	0,1848
maf4 vs wild-type	0,3720	0,7168
maf1 vs wild-type	1,2659	0,2324
ful vs wild-type	0,1598	0,8686
soc1 vs wild-type	0,0182	0,9858
35S:SAP54 wt vs 35S:GFP wt	4,2445	**0,0004**
35S:SAP54 maf5 vs 35S:GFP maf5	4,7577	**0,0002**
35S:SAP54 svp vs 35S:GFP svp	1,0168	0,3214
maf5 (AYWB) vs wild-type (AYWB)	0,4514	0,6613
svp (AYWB) vs wild-type (AYWB)	0,0856	0,9333
maf5 vs Col0 (female only)	0,0178	0,9861
svp vs Col0 (female only)	0,6294	0,5419

Figure supplement 8. SVP enhances female egg-laying preference for male exposed plants in phytoplasma and SAP54-dependent manner. MTF mutants *maf5* and *svp* display preferential leafhopper reproduction over wild-type plants in mixed-sex choice tests **(A).** Preferential leafhopper reproduction on SAP54 plants is abolished in *svp* but not *maf5* mutant (**B**). Preferential leafhopper reproduction on *svp* and *maf5* mutants is abolished in AY-WB infected plants **(C)**. *svp* displays preferential leafhopper reproduction over wild-type plants in presence of males but not when males are removed (**D**). Each datapoint represents reproductive choice of 10 female and 10 male insects within single choice arena. Experiment consists of 6 choice arenas. Each experiment was repeated independently 2 times. No preference for either test or control plant in a choice cage is represented by the 50% reference line. Oviposition preference for test or control plant is characterised by significant deviation from the 50% reference line. All pairwise comparisons done with paired t-tests by combining all datapoints from the two repeated experiments for each choice test. Test statistics summarised in table (**E**).

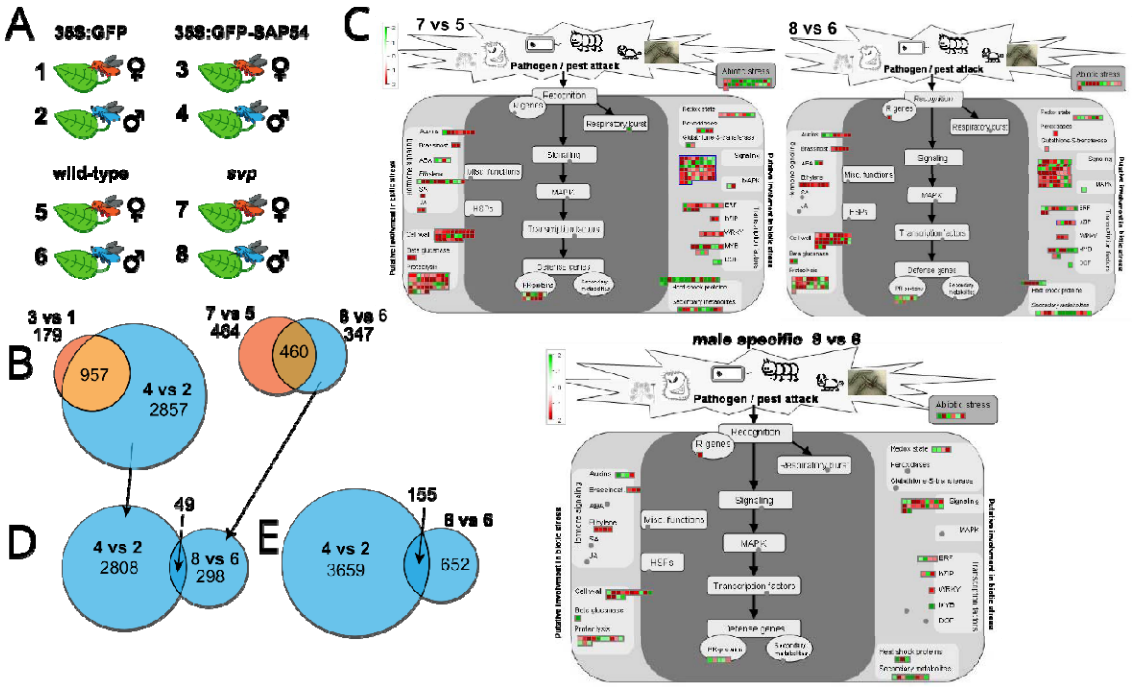


Figure supplement 9. Comparison between male and female colonized *svp* plants reveal SVP dependent insect regulated biotic stress genes. Pairwise comparisons are schematically depicted and abbreviated in **panel A** and correspond to the Venn diagrams and MapMan biotic stress graphs in **panels B-E**. The two Venn diagrams in **panel B** display the differences in the magnitude of response to male and female leafhopper exposure of SAP54 plants and *svp* mutants compared to insect-exposed GFP and wild-type controls, respectively. **Panel C** represents log2 fold change of biotic stress DEGs in male-and female-exposed *svp* mutants according to the MapMan annotation. 8 vs 6 includes male 347+460 DEGs, 7 vs 5 includes female 464+460 DEGs, and male specific 8 vs 6 includes male 347DEGs. **Panel D** displays the overlap between 49 male-specific (not regulated in female) DEGs of SAP54 plants and *svp* mutants while the **panel E** shows 155 DEGs that are shared by male-exposed SAP54 and male-exposed *svp* plants regardless of their regulation in female-exposed SAP54 or *svp* plants.

Includes the following source data and figure supplement(s) for Figure supplement 9:

Supplementary file 10: DEG encoded function enrichment for all MapMan built-in bins in female- or male-exposed *svp* plants vs female- or male-exposed wild type plants.

Supplementary file 11: DEG encoded function enrichment for MapMan built-in built-in biotic stress and manually designed defence signalling pathways in female- or male-exposed *svp* plants vs female- or male-exposed wild type plants.

Supplementary file 12: GO-term and MapMan functional enrichment of shared 155 DEGs between ''male-exposed SAP54 vs male-exposed GFP'' comparison and ''male-exposed *svp* vs male exposed wild type'' comparison.

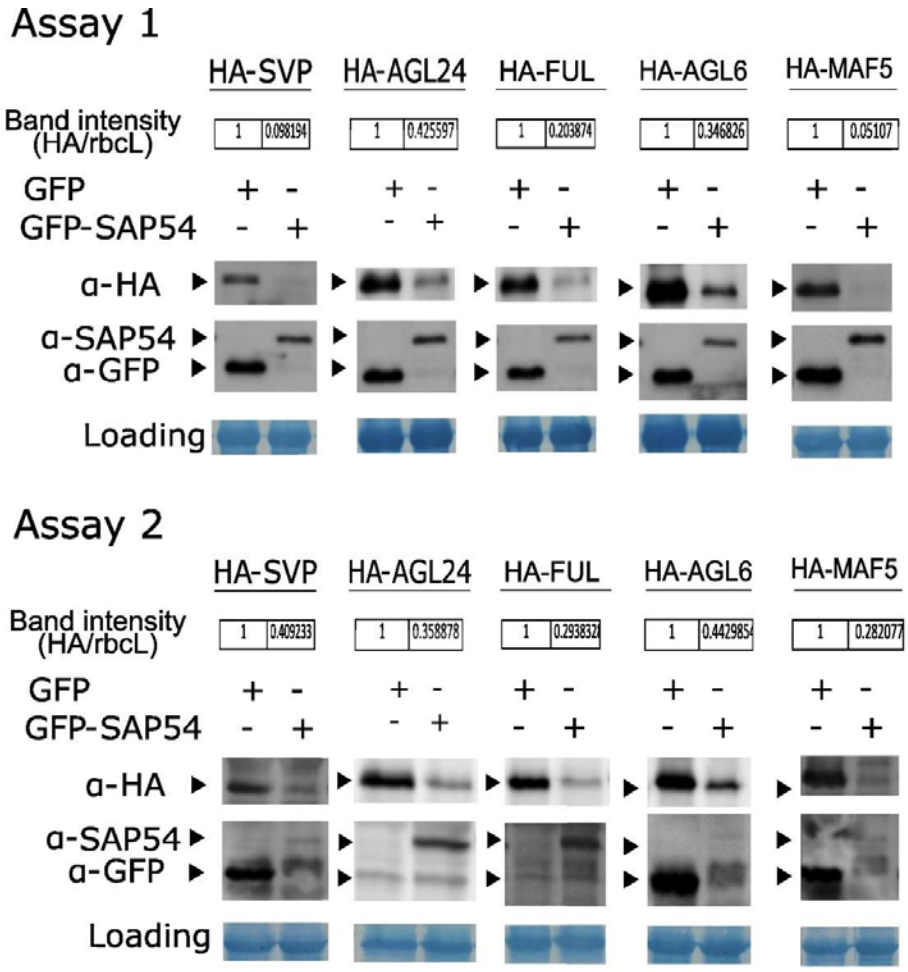


Figure supplement 10. SAP54 mediates degradation of multiple MADS-box transcription factors. Two independent transient expression experiments (assays) showing degradation of MTFs in the presence of SAP54 in *A. thaliana* protoplasts. The destabilization efficiency was calculated as the HA peak intensity divided by the RuBisCo large subunit (rbcL) peak intensity from the same sample using ImageJ. MTF levels in presence of SAP54 expressed as ratio relative to MTF intensity in GFP lanes which was normalized to 1.

Supplementary files:

Supplementary file 1. Supplementary Table 1. FPKM and differential expression values of 17'153 genes included in the response analyses of plants to SAP54 vs GFP and male vs female leafhopper exposure.

Supplementary file 2. Supplementary Table 2. IDs and log2-fold changes of differentially expressed genes (DEGs) of male and female *M. quadrilineatus* leafhopper-exposed GFP and SAP54 plants compared to insect free GFP plants.

Tab A: GFP plants exposed to caged females versus cages alone.

Tab B: GFP plants exposed to caged males versus cages alone.

Tab C: SAP54 plants exposed to caged females versus cages alone.

Tab D: SAP54 plants exposed to caged males versus cages alone.

Tab E: SAP54 versus GFP plants exposed to cages alone.

Supplementary file 3. Supplementary Table 3. MapMan build-in functional bins enriched for DEGs in male and female *M. quadrilineatus* leafhopper-exposed GFP and SAP54 plants compared to insect-free GFP plants.

Supplementary file 4. Supplementary Table 4. MapMan build-in biotic stress functional bins enriched for DEGs in male and female *M. quadrilineatus* leafhopper-exposed GFP and SAP54 plants compared to insect-free/cage-only GFP plants.

Tab A: MapMan build-in biotic stress functional bins enriched for DEGs. *p*-values based on Wilcoxon rank test with BH correction. Bins highlighted in bold are enriched for DEGs. Individual transcript identities within significantly enriched bins are provided in the tabs B, C, D, E.

Tab B: list of DEGs within significantly enriched biotic stress bins for GFP plants exposed to caged females versus cages alone.

Tab C: list of DEGs within significantly enriched biotic stress bins for GFP plants exposed to caged males versus cages alone.

Tab D: list of DEGs within significantly enriched biotic stress bins for SAP54 plants exposed to caged females versus cages alone.

Tab E: list of DEGs within significantly enriched biotic stress bins for SAP54 plants exposed to caged males versus cages alone.

Supplementary file 5. Supplementary Table 5. Manually curated and assigned defence signalling bins for MapMan import.

Tab A, Functional bin categories.

Tab B, IDs of genes assigned to each of the functional bins listed in Tab A. ''Type T'' means ''transcript'', nomenclature used according to in-built MapMan pathway files.

Supplementary file 6. Supplementary Table 6. Functional bins for manually annotated defence genes enriched for DEGs in male and female *M. quadrilineatus* leafhopper-exposed GFP and SAP54 plants compared to insect free GFP plants.

Tab A: Functional bins for manually annotated defense genes enriched for DEGs. P-values based on Wilcoxon rank test with BH correction. Bins highlighted in bold are enriched for DEGs. Individual transcript identities within significantly enriched bins are provided in the tabs B, C, D, E.

Tab B: list of DEGs within significantly enriched biotic stress bins for GFP plants exposed to caged females versus cages alone.

Tab C: list of DEGs within significantly enriched biotic stress bins for GFP plants exposed to caged males versus cages alone.

Tab D: list of DEGs within significantly enriched biotic stress bins for SAP54 plants exposed to caged females versus cages alone.

Tab E: list of DEGs within significantly enriched biotic stress bins for SAP54 plants exposed to caged males versus cages alone.

Supplementary file 7. Supplementary Table 7. MapMan build-in functional bins enriched for DEGs in SAP54 versus GFP plants with or without exposure to male and female *M. quadrilineatus* leafhoppers.

Supplementary file 8. Supplementary Table 8. Biotic stress bins from MapMan build-in and manually curated defence signalling pathway bins enriched for DEGs in GFP and SAP54 plants with or without exposure to male and female *M. quadrilineatus* leafhoppers.

Supplementary file 9. Supplementary Table 9. Fold-expression changes of MADS-box transcription factor genes insect-exposed SAP54 vs GFP leaves.

Tab A: List of 20 genes encoding MADS-box transcription factors that are expressed in leaves in the (insect-exposed) SAP54 and GFP plants.

Tab B: List of all 107 genes annotated as MADS-box transcription factors in *A. thaliana* (de Folter et al., 2005).

Supplementary file 10. Supplementary Table 10. DEG encoded function enrichment for all MapMan built-in bins in female- or male-exposed *svp* plants vs female- or male-exposed wild type plants.

p-values based on Wilcoxon rank test and have BH correction.

Supplementary file 11. Supplementary Table 11. DEG encoded function enrichment for MapMan built-in built-in biotic stress and manually designed defence signalling pathways in female- or male-exposed *svp* plants vs female- or male-exposed wild type plants.

Tab A: biotic stress and defence signalling pathway bins enriched with female- or male-specific DEGs. *p*-value based on Wilcoxon rank test and BH correction.

Tab B: list if DEGs within enriched bins for clip-caged females on *svp* plants vs clip-caged females on wild type plants.

Tab C: list if DEGs within enriched bins for clip-caged males on *svp* plants vs clip-caged males on wild type plants.

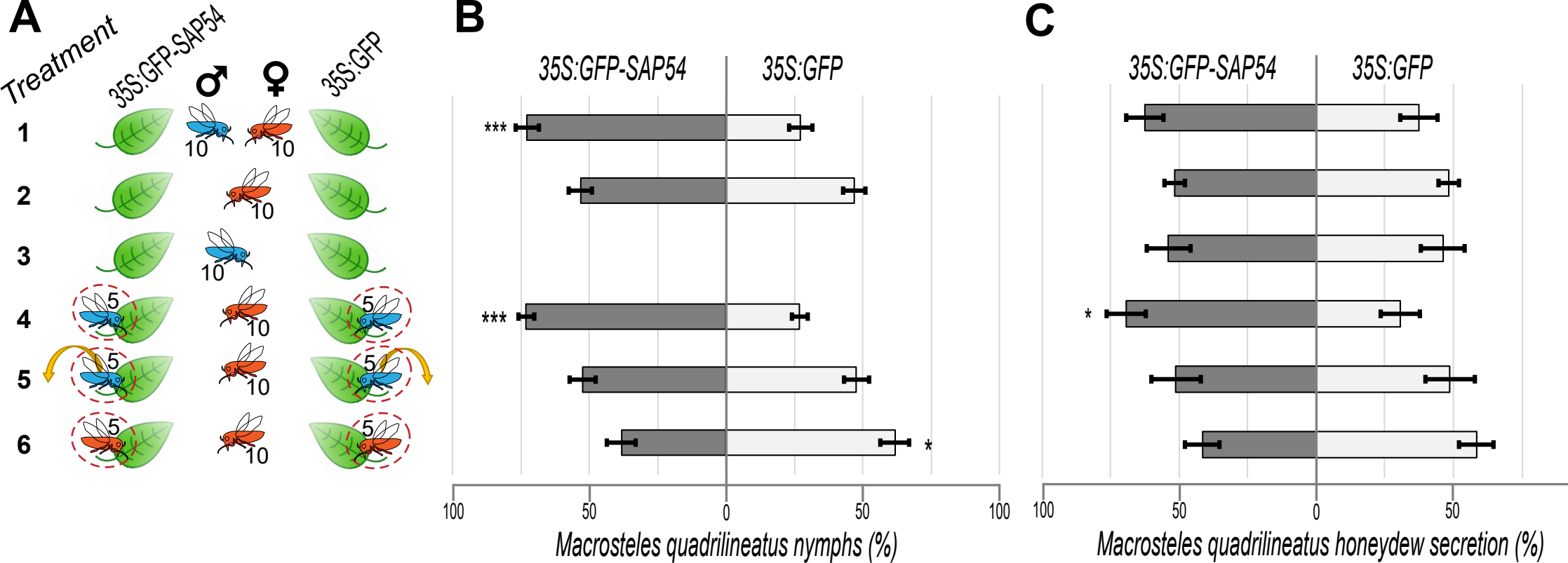
Tab D: since no enriched bins found for male-specific DEGs in tab A, no transcripts highlighted here.

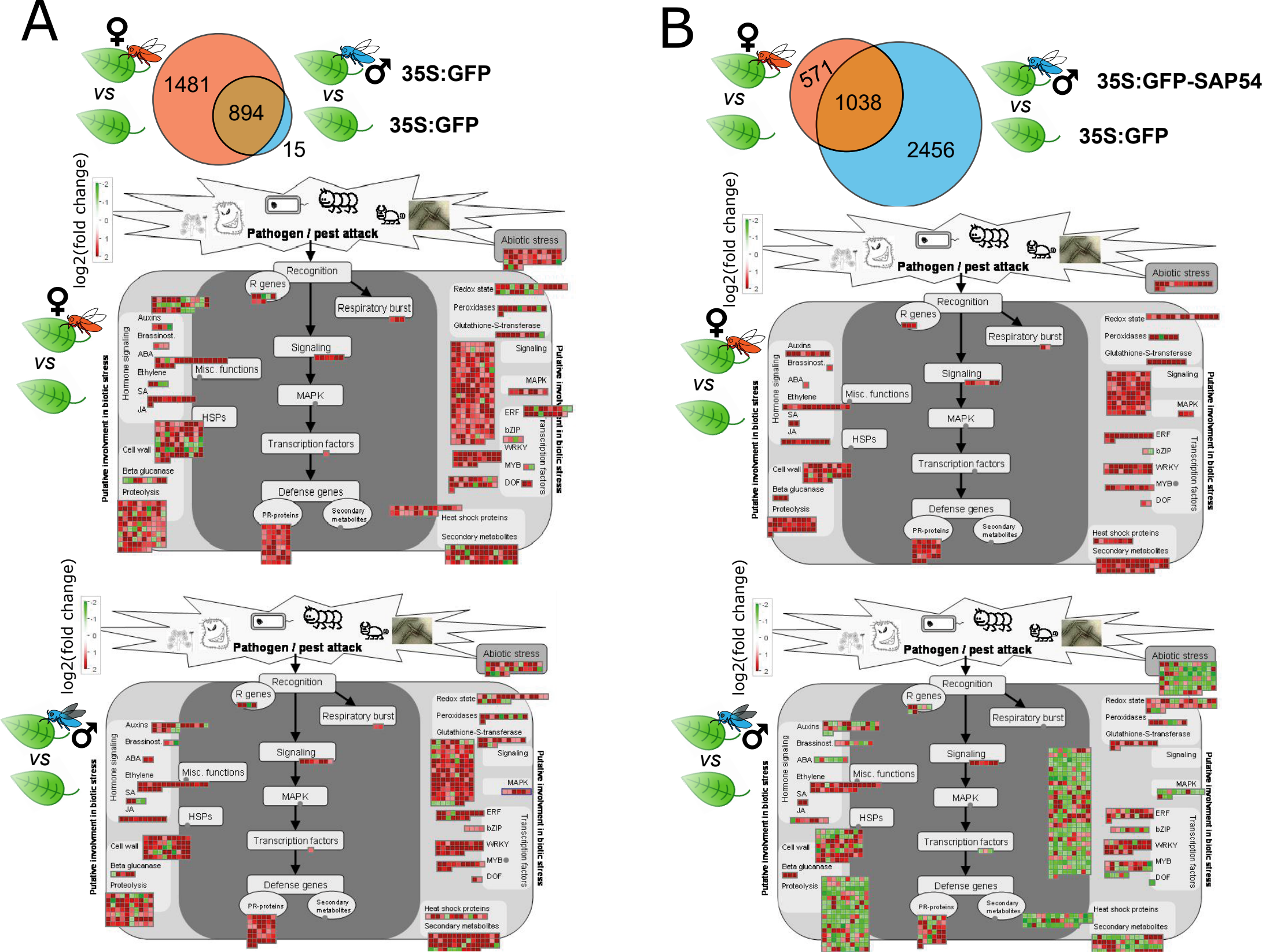
Supplementary file 12. Supplementary Table 12. GO-term and MapMan functional enrichment on shared 155 DEGs between ''male-exposed SAP54 vs male-exposed GFP'' comparison and ''male-exposed *svp* vs male-exposed wild type'' comparison.

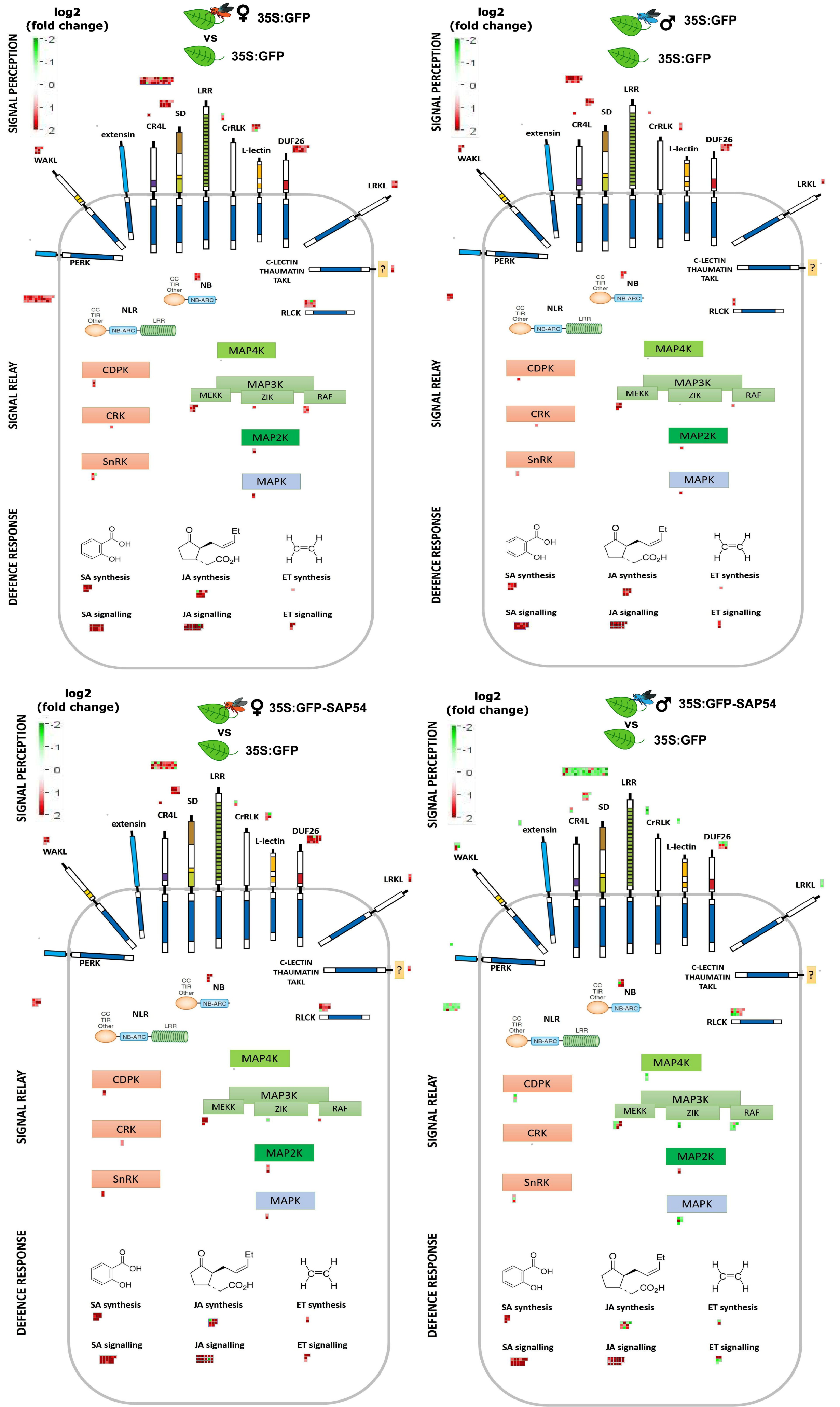
Tab A: GO-term enrichment with 155 DEGs shared by male-exposed SAP54 plants (versus male-exposed GFP plants) and male-exposed *svp* plants (vs male-exposed wild type plants) and depicted in Figure supplement 9D.

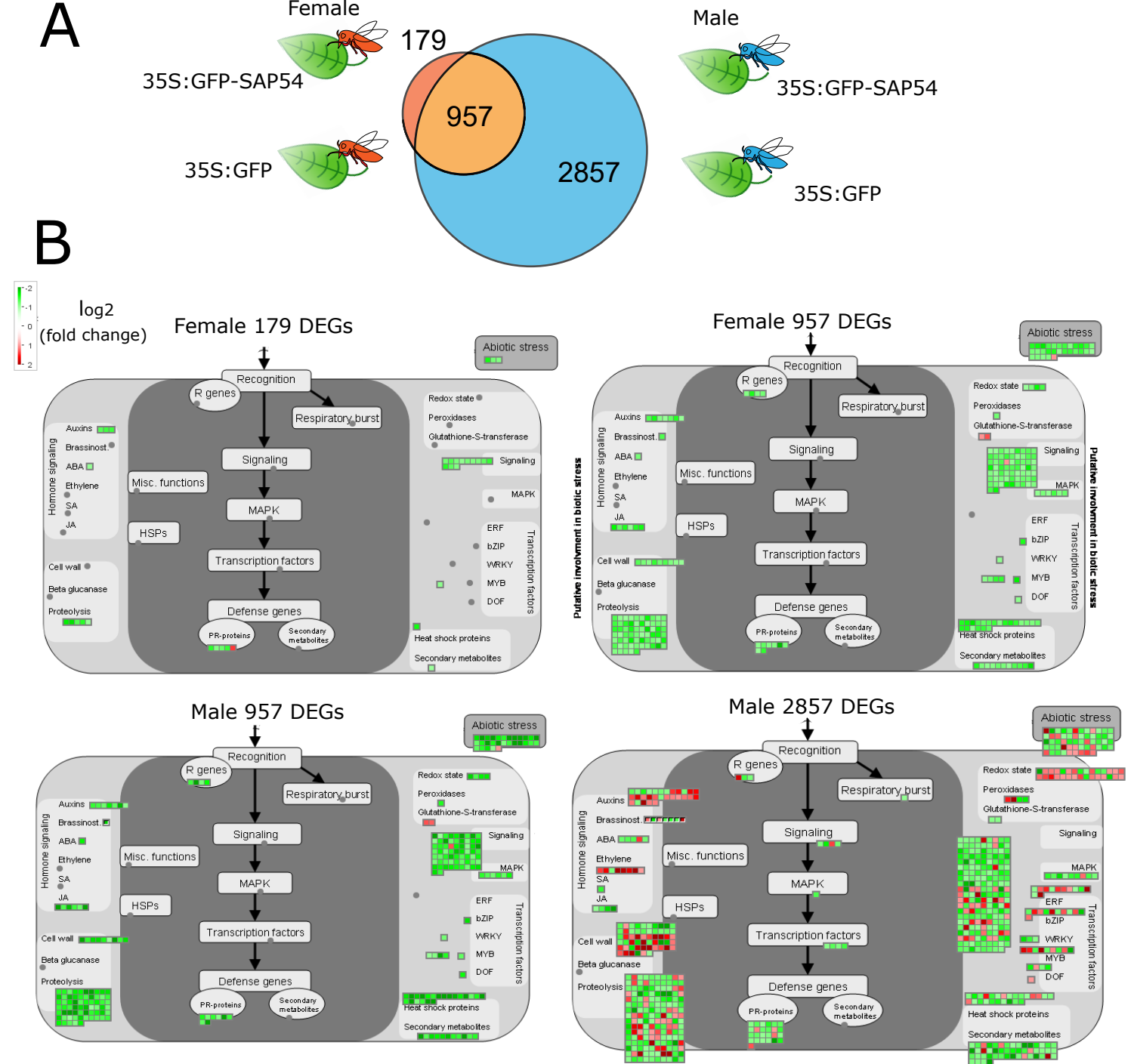
Tab B: gene names, encoded function description and log2(fold) change for the 155 DEGs analysed in tab A and depicted in Figure supplement 9D.

Tab C: Common functions identified between (1) MapMan function enrichment for 3816 DEGs on male-exposed SAP54 plants vs male-exposed GFP plants and (2) MapMan function enrichment for 807 DEGs on male-exposed *svp* plants vs male-exposed wild type plants; Venn diagram of the DEGs in Figure supplement 9D.


A
Treatment
35S:GFP-SAP54
♂
♀
35S:GFP
1
2
3
4
5
6
10
5
B
35S:GFP-SAP54
35S:GFP
***
***
*
100
50
0
50
100
Macrosteles quadrilineatus nymphs (%)
C
35S:GFP-SAP54
35S:GFP
*
100
50
0
50
Macrosteles quadrilineatus honeydew secretion (%)


A
1481
894
15
35S:GFP
35S:GFP
VS
VS
VS
VS
log2(fold change)
Pathogen / pest attack
Abiotic stress
Recognition
R genes
Respiratory burst
Signaling
MAPK
Transcription factors
Defense genes
PR-proteins
Secondary metabolites
Misc. functions
HSPs
Hormone signaling
Auxins
Brassinost.
ABA
Ethylene
SA
JA
Cell wall
Beta glucanase
Proteolysis
Redox state
Peroxidases
Glutathione-S-transferase
Signaling
MAPK
ERF
bZIP
WRKY
MYB
DOF
Transcription factors
Heat shock proteins
Secondary metabolites
Putative involvment in biotic stress
B
571
1038
2456
35S:GFP-SAP54
35S:GFP


log2
(fold change)
♀ 35S:GFP
vs
35S:GFP
♂ 35S:GFP
35S:GFP
♀ 35S:GFP-SAP54
vs
35S:GFP
♂ 35S:GFP-SAP54
vs
35S:GFP
SIGNAL PERCEPTION
SIGNAL RELAY
DEFENCE RESPONSE
-2
-1
0
1
2
WAKL
extensin
CR4L
SD
LRR
CrRLK
L-lectin
DUF26
LRKL
PERK
C-LECTIN
THAUMATIN
TAKL
?
CC
TIR
Other
NB
NB-ARC
NLR
LRR
RLCK
MAP4K
CDPK
MAP3K
MEKK
ZIK
RAF
CRK
MAP2K
SnRK
MAPK
SA synthesis
JA synthesis
ET synthesis
SA signalling
JA signalling
ET signalling


A
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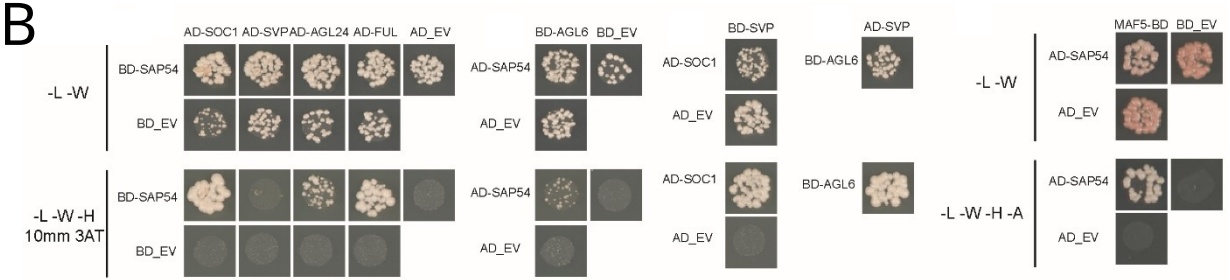
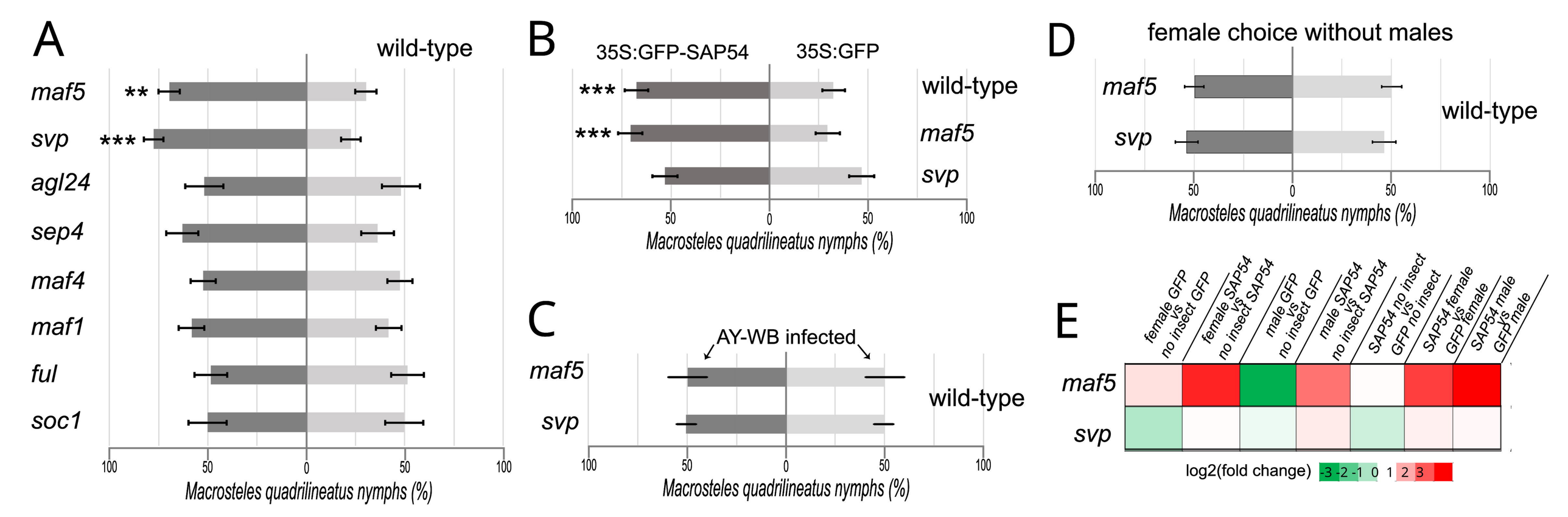
35S:GFP
179
957
2857
Male
35S:GFP-SAP54
35S:GFP
B
log2
(fold change)
Female 179 DEGs
Female 957 DEGs
Male 957 DEGs
Male 2857 DEGs
Abiotic stress
Recognition
R genes
Respiratory burst
Signaling
MAPK
Transcription factors
Defense genes
PR-proteins
Secondary metabolites
Misc. functions
HSPs
Hormone signaling
Auxins
Brassinost.
ABA
Ethylene
SA
JA
Cell wall
Beta glucanase
Proteolysis
Redox state
Peroxidases
Glutathione-S-transferase
ERF
bZIP
WRKY
MYB
DOF
Heat shock proteins
Putative involvment in biotic stress


A
HA-SVP
HA-AGL24
HA-FUL
HA-AGL6
HA-MAF5
GFP-SAP54
GFP
α-HA
α-SAP54
α-GFP
Loading
B
AD-SOC1
AD-SVP
AD-AGL24
AD-FUL
AD_EV
BD-AGL6
BD_EV
BD-SVP
AD-SVP
MAF5-BD
BD_EV
-L -W
BD-SAP54
BD_EV
AD-SAP54
AD_EV
AD-SOC1
AD_EV
BD-AGL6
-L -W
AD-SAP54
AD_EV
-L -W -H
10mm 3AT
BD-SAP54
BD_EV
AD-SAP54
AD_EV
AD-SOC1
AD_EV
BD-AGL6
-L -W -H -A
AD-SAP54
AD_EV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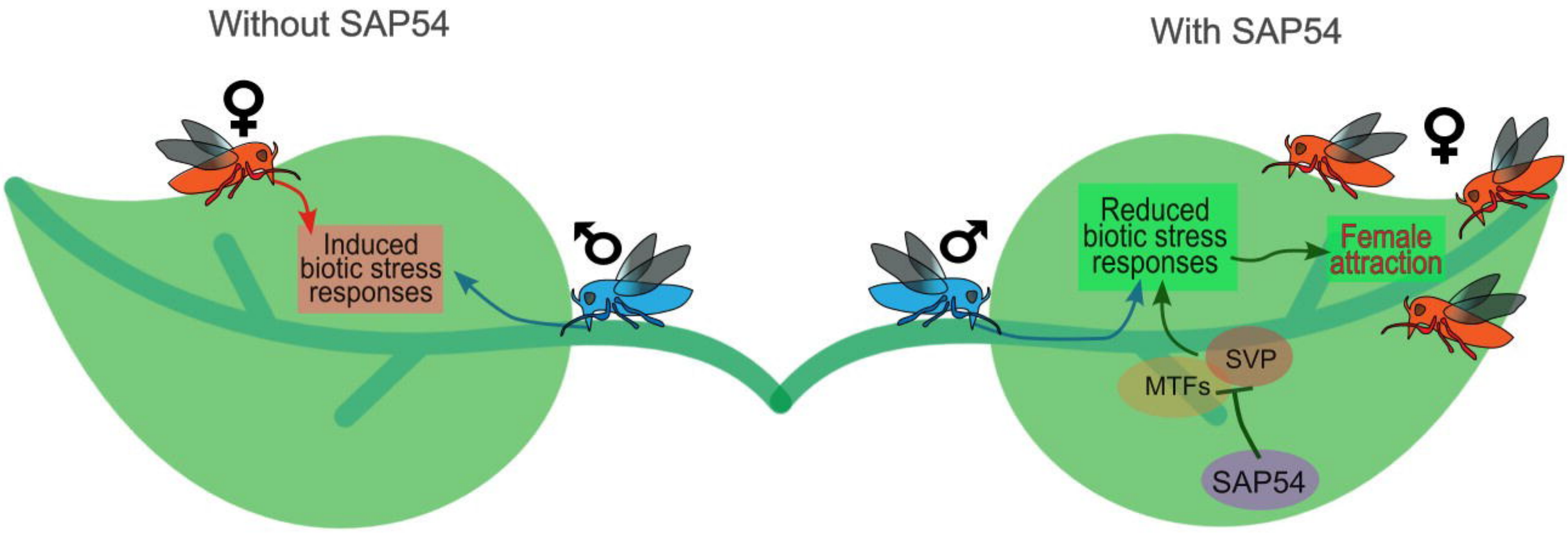
wild-type
maf5
svp
agl24
sep4
maf4
maf1
ful
soc1
**
***
100
50
0
50
100
Macrosteles quadrilineatus nymphs (%)
B
35S:GFP-SAP54
35S:GFP
wild-type
maf5
svp
***
***
100
50
0
50
100
Macrosteles quadrilineatus nymphs (%)
C
AY-WB infected
maf5
svp
wild-type
100
50
0
50
100
Macrosteles quadrilineatus nymphs (%)
D
female choice without males
maf5
svp
wild-type
100
50
0
50
100
Macrosteles quadrilineatus nymphs (%)
E
female GFP vs no insect GFP
female SAP54 vs no insect SAP54
male GFP vs no insect GFP
male SAP54 vs no insect SAP54
SAP54 no insect vs GFP no insect
SAP54 female vs GFP female
SAP54 male vs GFP male
maf5
svp
log2(fold change)
-3 -2 -1 0 1 2 3


Without SAP54
With SAP54
Induced biotic stress responses
Reduced biotic stress responses
Female attraction
SVP
MTFs
SAP54