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TESI DI LAUREA

**Can genome comparison provide insights into
how different plant pests succeed with
divergent lifestyles?**

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RIASSUNTO IN ITALIANO:

Nel corso dell'evoluzione, le piante si sono adattate agli attacchi costanti di vari organismi, inclusi gli insetti erbivori, attraverso la produzione di sostanze fitochimiche, composti chimici che svolgono funzioni difensive. Questi composti possono essere prodotti in risposta al singolo attacco (fitoalessine) o venire costitutivamente espressi (fitoanticipine). Tuttavia, la produzione costante di tali composti richiede energia e può essere tossica per la pianta stessa. Le piante producono anche composti volatili (HIPV) che attraggono i nemici naturali degli insetti nocivi, rappresentando una difesa indiretta.

Gli insetti erbivori, come la mosca bianca (*Bemisia tabaci*), hanno sviluppato strategie di sopravvivenza per detossificare i composti difensivi delle piante, innescando una coevoluzione che ha portato alla diversificazione dei metaboliti secondari vegetali, come ai meccanismi di attacco degli insetti. Lo studio dei genomi dei parassiti delle piante può fornire informazioni preziose sulle loro strategie di attacco e adattamento, utili per sviluppare meccanismi di gestione e migliorare la sostenibilità agricola.

Con la crescente domanda alimentare globale, è cruciale gestire le colture per minimizzare le perdite causate da parassiti come *B. tabaci*, uno degli insetti più distruttivi. Esistono diverse specie di *B. tabaci*, identificate tramite l'analisi del gene mitocondriale citocromo ossidasi 1 (mtCO1), che mostrano una significativa polifagia, alimentandosi su diverse piante. Le specie invasive MEAM1, MED e la mosca bianca delle serre (TV) sono tra le più distruttive, con differenze genetiche che influenzano vari aspetti biologici.

Le piante utilizzano metaboliti secondari come terpenoidi, glucosinolati e composti fenolici per difendersi dagli insetti erbivori. Ad esempio, i terpenoidi possono attaccare il sistema nervoso degli insetti o fungere da deterrenti. Alcuni glucosinolati alifatici possono ridurre significativamente la sopravvivenza della mosca bianca. Tuttavia, con l'evoluzione si sono sviluppati determinati geni nella mosca bianca, come BtPMT1, che permettono di detossificare i glucosidi fenolici delle piante, riducendo l'efficacia difensiva di queste ultime.

Oltre alle difese chimiche, le piante possono produrre proteine di difesa come CYS6, un inibitore della proteasi presente nel tabacco, che interferisce con la digestione degli insetti. Nonostante ciò, la mosca bianca ha evoluto proteine salivari che possono neutralizzare tali inibitori. Le piante possono anche emettere composti volatili in risposta all'attacco di *B. tabaci*, attirando predatori naturali come i parassitoidi.

Il pathway di segnalazione del jasmonato (JA) è fondamentale per la difesa delle piante contro gli insetti erbivori. La manipolazione di questo pathway ha dimostrato

che la sua attivazione può ridurre la popolazione della mosca bianca, regolando la sintesi di terpenoidi, glucosinolati e fenolici essenziali per la difesa delle piante.

La complessità genetica di *Bemisia tabaci* rappresenta una sfida nella gestione dei parassiti. L'uso estensivo di insetticidi ha portato allo sviluppo di resistenze. Sebbene le colture geneticamente modificate siano efficaci contro alcuni parassiti, sono necessarie strategie innovative ed ecocompatibili per controllare i fitofagi succhiatori di linfa come le mosche bianche. L'RNA interference (RNAi) rappresenta una promettente metodologia di controllo, sebbene la sua efficacia debba essere valutata per evitare effetti collaterali sugli insetti non target. L'uso di geni trasferiti orizzontalmente (HTGs) potrebbe limitare questi effetti, offrendo un vantaggio selettivo nel controllo dei parassiti.

In sintesi, comprendere i meccanismi genetici e biochimici della mosca bianca è cruciale per sviluppare strategie di controllo efficaci e sostenibili, migliorando la resistenza delle colture e mitigando le perdite agricole causate da questo parassita.

ABSTRACT:

Genome comparison can reveal critical insights into the divergent success strategies of plant pests. This dissertation focuses on whiteflies (*Bemisia tabaci*), a significant pest complex causing substantial crop losses. By comparative genomic and proteomic analyses, it is possible to identify genetic traits and mechanisms that enable whitefly's survival and adaptability to various phytochemicals produced by host plants. Understanding plant-pest interactions can inform pest management strategies and support agricultural sustainability. This dissertation aims to enhance crop resilience through targeted approaches, including RNA interference and transgenic methods, to mitigate the impact of whiteflies and other herbivorous insects.

INTRODUCTION:

Over the course of evolution, plants have had to adapt to constant attacks from a broad range of organisms, including microbes and animals, but the most harmful have always been (and still are) herbivorous insects.

The key feature of interactions between hostplants and insect-pests is the plants' adaptability to generate diverse chemical compounds and utilize them for their survival. The various chemicals synthesized by plants, called phytochemicals, have many functions, including defense against herbivorous insects.

Phytochemicals can be inducible or constitutively produced. When plants are attacked by pests, they must quickly react and generate defense chemicals where

the attack occurs. One way they achieve this is by detecting the attack and activating genes responsible for producing enzymes that create defense compounds, known as phytoalexins, that play important roles in deterring feeding by insects. They are defined “low molecular weight, antimicrobial compounds that are both synthesized by and accumulated in plants after exposure to microorganisms”. However, the drawback of inducible defense systems is the time it takes to produce new compounds, resulting in a delay in response. Another approach is to continually produce these compounds in the vulnerable tissues as a preemptive defense measure. Still, this strategy has disadvantages, such as the metabolic energy expended to produce the compounds even when there's no threat. Additionally, some compounds may be harmful not only to insects but also to the plant itself in their active form. To overcome this toxicity concern, a common alternative method is to store the compounds in inactive forms that can be readily activated upon insect attack. These compounds are known as phytoanticipins, “low molecular weight antimicrobial compounds present in plants before challenge by microorganisms or produced after infection solely from preexisting constituents” (VanEtten *et al.*, 1994).

Furthermore, plants produce an array of small-molecule volatile compounds, termed herbivore-induced plant volatiles (HIPVs), that includes terpenoids, volatile fatty and amino-acid derivatives, benzenoids and phenylpropanoids. Natural enemies of many plant pests can use HIPVs as cues to locate infested plants and, thus, their prey. HIPVs are, therefore, an indirect defense mechanism, as they attract predatory insects, which have a negative impact on the plant pest.

In response to these defensive strategies, insects have evolved functional survival tactics such as sequestration, detoxification, and repellence to counteract the harmful effects of plant secondary metabolites. The presence of toxic metabolites in plants created a selection pressure on insects, leading to the development of resistance to these phytochemicals. Consequently, this mutualistic coevolution transformed into a molecular arms race, driving the extensive diversification of plant secondary metabolites (Yactayo-Chang *et al.*, 2020).

Genome comparison between different pests can provide valuable insights into how they succeed with different attack strategies. By analysing the genomes of various plant pests, it is possible to identify specific genetic traits, gene clusters, and pathways that contribute to their ability to exploit different ecological niches and adapt to various plants' phytochemicals. By revealing the molecular basis of host interactions, metabolism and detoxification pathways, comparative genomics provides insights that can inform pest management strategies and contribute to agricultural sustainability.

In today's age of widespread consumerism, effectively managing extensive food crops is crucial to minimize wastage caused by crop diseases or damage from herbivorous insects. To prevent high levels of food loss (which could result in increased deforestation to establish new monoculture plantations), it's essential to

conduct genome-level studies on various pests to identify the diverse mechanisms they employ to harm plants. By comparing their genomes, we can gain insights into the varied defense reactions of plants against them. This information enables us to explore novel approaches for enhancing crop growth and development while mitigating damage caused by insect-pests. The dissertation will focus on a particular insect: the whitefly (*Bemisia tabaci*). This insect is recognized as one of the most destructive herbivorous pests, leading to substantial crop losses annually, up to 47% in nine East and Central African countries (Sani *et al.*, 2020). The discussion will delve into insights gained from genome and proteome comparisons with other arthropods, shedding light on the whitefly's mechanisms and the hostplants' mechanisms triggered in response. Additionally, various transgenic methods will be analysed to bolster plant resilience against this pest.

GENOMIC COMPARISON BETWEEN DIFFERENT GROUPS OF WHITEFLIES:

The whitefly *Aleyrodes tabaci*, first identified by Gennadius in 1889, has experienced numerous taxonomic revisions due to insufficiently distinct morphological features from closely related species. This led to the grouping of various biological species under the single name *Bemisia tabaci*. Recent studies have conclusively shown that *B. tabaci* actually encompasses over 40 distinct biological species. Consequently, this group is now collectively referred to as *B. tabaci sensu lato* to acknowledge its cryptic species nature.

The group of > 40 cryptic whitefly species is amongst the world's worst agricultural pests and plant-virus vectors, particularly in sub-Saharan Africa and Asia. The different species can be recognised based on their geographical separation and differences in their partial mitochondrial cytochrome oxidase 1 (mtCO1) sequences and are named using the mtCO1-marker naming framework. These include Sub-Saharan Africa1 (SSA1 with five sub-groups: SSA1-SG1, SG2, SG3, SG4, SG5), SSA2 and SSA3. In a study of the genetic diversity of whitefly on crop and uncultivated plants in Uganda, the most prevalent whiteflies were *B. tabaci* MED-ASL (30.5% of samples), *B. tabaci* SSA1 (22.7%) and *B. tabaci* Uganda-1 (12.1%). These species were also the most polyphagous, occurring on 33, 40 and 25 different plant species, respectively (Mugerwa *et al.*, 2018).

Invasive MEAM1 (Middle-East-Asia Minor1, or *B. argentifolii*), MED (the Mediterranean, or *B. tabaci s.s.*) and the greenhouse whitefly (*Trialeurodes vaporariorum*, TV) are the 3 most destructive cryptic species of the *B. tabaci* complex.

All these species vary in several aspects, including genetic traits and genome composition. As a result, they exhibit differences in TE-coverage, proteomics, hostplant range and metabolic processes.

To control and prevent whitefly pests, several whitefly genomic studies have been performed:

1. Genome composition:

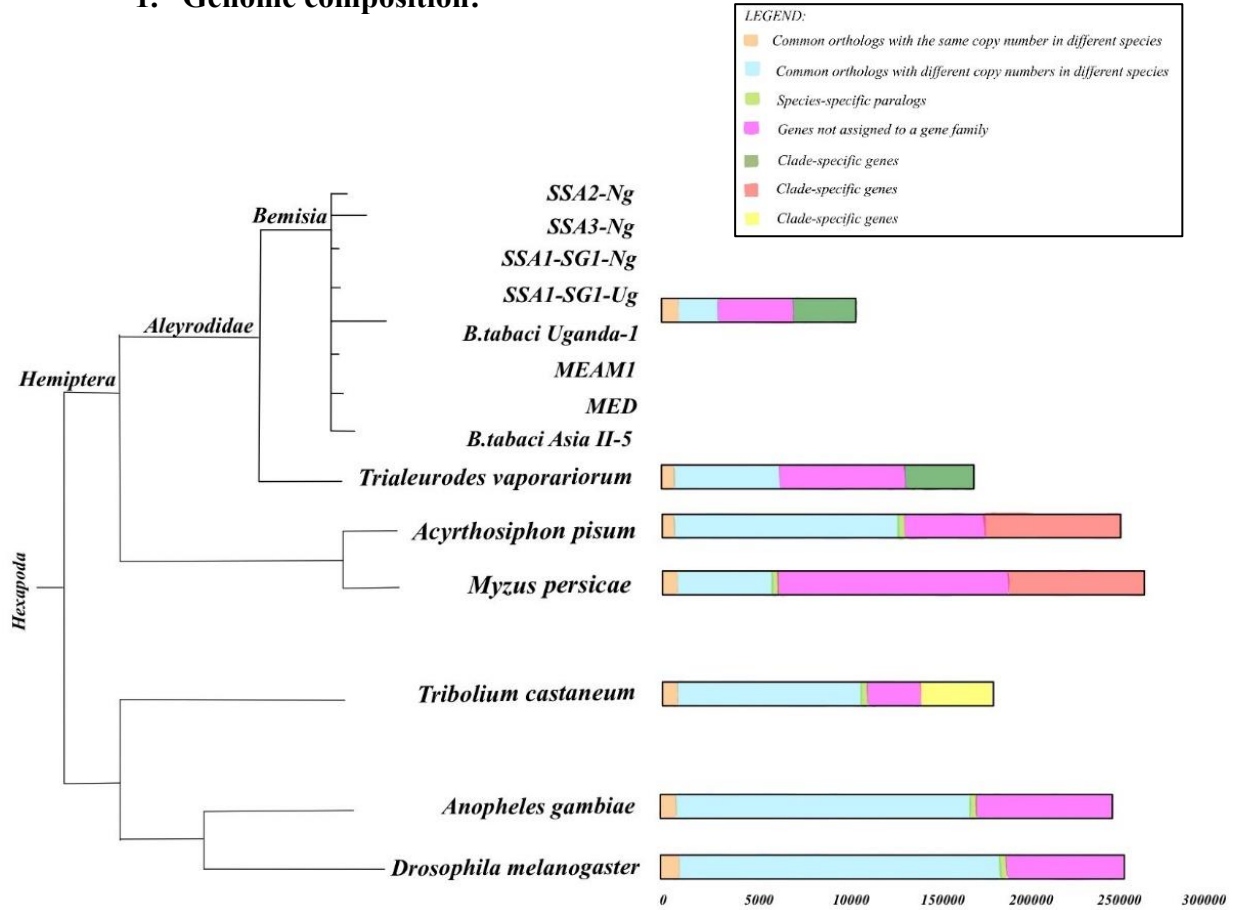


Fig.2: Whole genome comparative analysis. Species dated phylogenetic tree and gene family evolution and phylogenetic relationships of 14 species covering 655 OGCs (131,953 amino acids). Ortholog set delineation shown in relation to the main Arthropoda clades (Hexapoda, Hemiptera, and Aleyrodidae). Original diagram.

Comparative genomics of *B. tabaci s.l.* was conducted by Campbell *et al.* (2023) using OrthoFinder (v 2.4.0) analysis, which included 14 *Hexapoda* genomes (**Fig.2**) with an emphasis on closely related *Hemiptera* taxa. From the 391,154 input genes assigned for orthology, 360,756 genes (92.2%) were clustered into 22,225 orthologous gene clusters (OGCs, groups of genes from different species that have evolved from a common ancestral gene and retain the same function). Of these, 2,297 OGCs (10.3%) were present in all 14 species, while 7,205 OGCs (32.4%) were species-specific, accounting for 9.1% of all genes analysed. Additionally, 2,404 OGCs (10.81%) were shared among all *B. tabaci* species, with an average of 7,620 OGCs containing one or more representative genes.

2. Comparative genomics:

		<i>Bemisia tabaci sensu lato</i>										<i>A. pisum</i>	<i>M. persicae</i>	<i>A. gambiae</i>	<i>D. melanogaster</i>	<i>T. castaneum</i>
		SSA1-SG1-Ug	SSA1-SG1-Ng	SSA2-Ng	SSA3-Ng	MED-ASL	Uganda-1	MEAM1	MED	TV						
Gene	Total genes	13,852	14,942	14,386	14,952	13,497	13,804	12,723	16,378	18,275	37,522	35,428	13,796	17,807	17,052	
	Protein coding	12,71	13,661	12,928	13,463	12,289	12,749	12,077	15,786	18,275	36,195	33,112	13,057	13,947	16,59	
Transcript	Total transcripts	29,757	29,919	29,035	30,073	28,862	25,101	26,475	30,266	18,275	37,522	35,006	15,718	34,92	18,996	
	Protein coding	28,022	27,923	26,825	27,844	26,928	23,614	25,522	29,609	18,275	36,195	33,112	14,979	30,588	18,534	
	% Prot. coding	94.17	93.33	92.39	92.59	93.30	94.08	96.40	97.83	100.00	96.46	95.34	95.30	87.59	97.57	
	Avg coding length	1,893	1,805	1,604	1,575	2,019	1,383	2,255	1,506	1,382	1,983	1,805	2,508	2,28	1,765	
Exon	Total coding exons	248,703	225,85	201,198	204,013	248,187	144,581	254,773	228,346	94,682	182,028	179,311	71,504	181,712	98,403	
	Avg translatable exon length	219.12	202.2	173.69	172.47	198.64	165.05	213.73	200.71	308	208.55	206	371.24	378.82	288.26	
	Avg exons per transcript	8.2	7.2	6.4	6.3	8.1	5.1	8.9	6.8	5.2	4.8	4.5	3.7	4.9	4.3	
Intron	Total introns	220,681	197,927	174,373	176,169	221,259	120,967	229,251	198,737	92,16	145,833	144,217	56,525	151,124	79,869	
	Avg length	3,661	3,051	3,325	3,148	3,641	2,113	3,409	2,143	1,846	1,658	1,715	1,862	1,606	1,424	
	Total length	807,954,736	603,944,323	579,736,570	554,533,025	805,703,415	255,575,888	781,519,935	425,841,115	170,187,655	241,821,138	223,943,227	105,259,352	242,737,713	113,707,069	

Table.1: Summary statistics for the *B. tabaci s.l.* genomes (Ng: Nigeria; Ug: Uganda) and five comparative insect genomes: *Acyrtosiphon pisum* (pea aphid), *Myzus persicae* (green peach aphid), *Anopheles gambiae* (African mosquito), *Drosophila melanogaster* (fruit fly), *Tribolium castaneum* (red flour beetle). Adapted from Campbell *et al.* (2023).

The **Table.1** presents genes number and constitution among various species of *B. tabaci*, plus five other arthropods. *B. tabaci* species show a range in total gene counts from 12,723 (MEAM1) to 18,275 (TV), and they have fewer total genes and transcripts compared to *A. pisum* and *M. persicae* but is comparable to or slightly less than *D. melanogaster* and *T. castaneum*. Furthermore, *B. tabaci* generally has shorter average coding lengths and exon lengths compared to *A. gambiae* and *D. melanogaster*, indicating potentially simpler protein structures, and it exhibits longer average intron lengths and total intron lengths compared to other species, suggesting greater regulatory complexity. This comparison highlights the genetic diversity and complexity of *B. tabaci* in relation to other significant insect species, offering insights into their evolutionary discrepancies.

3. Transposable elements:

Transposable elements (TE) are an important source of genomic variation and contributor not only to genome structural variation, but they can also influence changes to gene regulation. DNA TEs are part of class II type TEs, which mediate their movements via a DNA ‘cut and paste’ mechanism. Levels of TE-coverage in the genomes of MEAM1 (45%; 276.9 Mb) and MED (40.30%; 265.0 Mb) are intermediate. Apart from SSA1-SG1-Ug, all other *B. tabaci s.l.* genomes exhibit higher TE-coverage than the closely related *A. pisum* (38%), in agreement with recent reports. In contrast, TE-coverage is considerably lower in comparison to TV (56.60%) (Xie *et al.*, 2022). Differences in TE coverage reflects evolutionary divergence and genomic stability, influenced by environmental pressures. TE activity contributes to genome size and complexity, affecting species-specific traits. Future studies to understand TE coverage will aid conservation and biodiversity research by revealing genetic diversity and resilience of different insect species.

4. Proteomic of saliva:

Saliva is crucial in the interaction between herbivorous insects and hostplants. During feeding, insects inject salivary proteins into plant tissues, aiding in mouthpart lubrication, nutrient digestion, and plant defenses modification. However, plants can detect these insect feeding signals by recognizing specific salivary components and trigger their defense responses. Recent studies have shown that *B. tabaci*'s saliva plays a significant role in its feeding success and the extensive damage it causes. For instance, the salivary protein Bt56 enhances whitefly performance by modulating plant innate immunity, while BtFer1 improves feeding by reducing H₂O₂ levels, inhibiting callose deposition, and suppressing jasmonic acid(JA)-mediated defenses. Additionally, 698 unigenes enriched in *B. tabaci*'s salivary glands include 534 potential coding proteins, highlighting the complexity of its saliva's role in feeding. (Huang *et al.*, 2020).

	<i>Bemisia tabaci sensu lato</i>											<i>A. pisum</i>	<i>M. persicae</i>	<i>A. gambiae</i>	<i>D. melanogaster</i>	<i>T. castaneum</i>
	SSA1-SG1-Ug	SSA1-SG1-Ng	SSA2-Ng	SSA3-Ng	<i>B. tabaci</i> Asia II-5	<i>B. tabaci</i> Uganda-1	MEAMI	MED	TV							
14-3-3 protein epsilon																
actin																
aminopeptidase																
apolipoprotein																
arginine kinase																
Armet																
Bt56																
BtFer1																
C-1-tetrahydrofolate synthase																
calreticulin																
catalase																
csa-calmodulin																
elongation factor																
endoplasmic reticulum chaperone																
enolase																
glucose dehydrogenase																
glutathione S-transferase																
glycerol-3-phosphate dehydrogenase																
groEL																
heat shock protein 70																
histone catalase																
hydrolase																
lysosomal alpha-mannosidase																
maltase																
oxidoreductase																
peroxiredoxin																
puromycin-sensitive aminopeptidase																
putative Per a allergen																
ribosomal protein																
transitional endoplasmic reticulum ATPase																
tropomyosin																
tubulin																
ubiquitin domain containing protein																
vacuolar ATP synthase																
V-type proton ATPase																

Table.2: The main *B. tabaci* salivary proteins compared to those of five other arthropods, with *Bemisia* species-specific proteins in bold. Adapted from Huang *et al.*, 2020.

97 proteins in *B. tabaci* saliva have homologs in other herbivorous arthropods, indicating these proteins may have conserved functions in plant-feeding insects. However, 74 proteins are unique to *B. tabaci*, including 34 species-specific, with oxidoreductases and hydrolases being the most common (**Table.2**). Hydrolases are common salivary enzymes of many insect species, especially those with piercing–sucking mouthpart. They may aid in extra-oral, cell wall degradation and host tissue pH regulation. Catalase, a salivary effector, breaks down hydrogen peroxide,

mitigating plant defense responses. *B. tabaci* salivary oxidoreductases likely detoxify plant metabolites and manage reactive oxygen species, like other herbivorous insects (Huang *et al.*, 2020).

5. Types of hostplants:

Hierarchical clustering revealed clear host preferences of *B. tabaci* species for MED-ASL (pumpkin, *S. acuta* and sweet potato), MED (tobacco), SSA1 and SSA2 (cassava), SSA6 (African basil) and *B. tabaci* Uganda-1 (sweet potato) assisting in predicting probable identity of whiteflies on these plant species (Mugerwa *et al.*, 2018). In certain areas, MEAM1, MED, and TV whiteflies coexist and compete. Although all three species are capable of feeding on a variety of plants, MEAM1 and MED have a broader range of host plants compared to TV. In greenhouses where these three species are found together, they exhibit different patterns of distribution within the plants. (Arnó *et al.*, 2006).

6. Metabolism:

An analysis of sequence divergence in 2,944 orthologous genes indicates that they have been subject to strong purifying selection. Whiteflies which consume nutrient-poor phloem sap depend on endosymbionts for essential amino acids, vitamins, and cofactors. MED and TV whiteflies exhibit high sequence divergence in cofactor, vitamin, and amino acid metabolism. Genes with high divergence are notably over-represented in amino acid metabolism (26 out of 65) and vitamin metabolism (21 out of 37). At the expression level, several genes related to amino acids, vitamins, and cofactors show significant differential expression (Wang *et al.*, 2020).

7. Detoxification:

The most divergent category is xenobiotic metabolism, which might contribute to the differences in hostplant range and insecticide resistance between MED and TV. Several of the 2,944 orthologous genes related to xenobiotic metabolism exhibit high sequence divergence. For instance, the cytochrome P450 gene (CYP4C64) is linked to insecticide resistance in MED. Despite this high sequence divergence, the expression levels of detoxification-related genes are similar between MED and TV, indicating that these genes may influence hostplant range and insecticide resistance in a comparable way across both species (Wang *et al.*, 2020).

The comprehensive genomic and proteomic analysis of *B. tabaci s.l.* provides critical insights into the genetic and biochemical mechanisms driving its interactions with hostplants and its capacity as a pest and virus vector. The significant genetic diversity within the *B. tabaci* species has been highlighted, elucidating the roles of transposable elements, salivary proteins, and specific genes

related to metabolism and detoxification. Understanding these mechanisms is essential for learning about plant chemical defense mechanisms. Future genome studies will help to develop targeted and effective pest control strategies, that can aid in managing the substantial agricultural losses caused by this pervasive pest.

PLANT-WHITEFLY INTERACTION:

As a group of piercing-sucking insects, the feeding behaviour of whitefly differs significantly from that of insects with chewing mouthparts. Correspondingly, the responses of plants to whitefly infestation are markedly different from their responses to attacks by chewing insects. (Yactayo-Chang *et al.*, 2020).

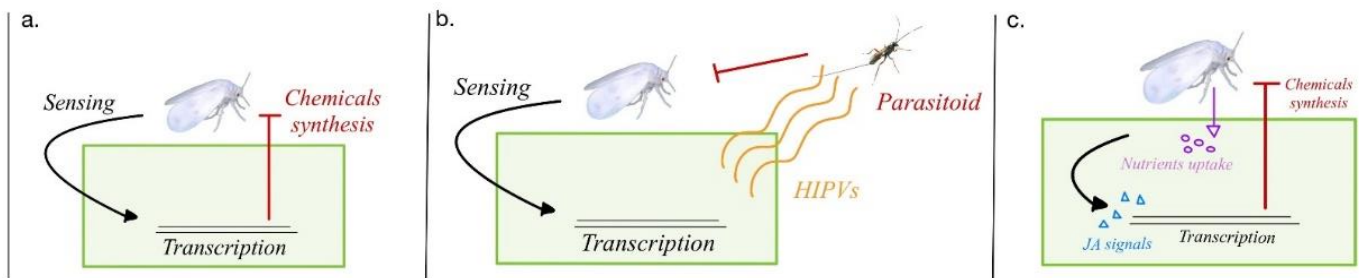


Fig.3: Plant resistance against whitefly. Schematic representation of plant resistance against whitefly at chemical **a.** direct and **b.** indirect levels, and **c.** signalling pathway of danger. Original diagram.

a. Plants may synthesise a repertoire of secondary metabolites such as terpenoids, glucosinolates, phenolic compounds and lignin, and defense proteins such as glucosidase, glucanase and chitinase to inhibit whitefly herbivory. **b.** Plants may also synthesise and release HIPVs such as ocimene, myrcene, methyl salicylate and tetradecane, to attract natural enemies of whitefly. **c.** Once plants sense danger, they activate jasmonate (JA) signaling pathway to regulate their own defense.

1. Direct chemical defense (Fig.3a):

Secondary metabolites play a major role in plant defense against insect herbivores. It was found that **terpenoids'** content increased with whitefly feeding. They can attack the nervous system and inhibit acetyl-choline esterase, or they can work as deterrents owing to physical barriers or act as a growth. The main defense mechanism is repression of the genes involved in biosynthetic pathways.

Furthermore, scientists found that the survival and developmental rate of whitefly significantly decreased with the accumulation of **aliphatic glucosinolate** through *AtMYB29* overexpression. Whitefly oviposition is significantly reduced when the contents of aliphatic glucosinolates are increased to unnaturally high levels. They work as respiration inhibitors, but pests evolved mechanisms of detoxification, especially the cytochrome P450 gene.

Studies have reported a positive correlation between the total content of **phenolic components** and whitefly resistance in eggplant and tomato. As revealed by metabolites profiling and feeding assays, many phenolic glycosides work as respiratory and growth inhibitors. However, through a horizontal gene transfer

event, the most important virus-vector species (MEAM1 and MED) have inexplicably acquired the plant-derived phenolic glycoside malonyl-transferase gene BtPMT1, that enables whiteflies to neutralize phenolic glucosides (Xia *et al.*, 2021). Numerous studies have emphasized the importance of plant phenolics in resistance metabolism. These compounds disrupt insect gut metabolism and prevent nutrient absorption by binding covalently to dietary proteins, lipids, and digestive enzymes. Additionally, plant phenolics inhibit insect growth by inducing oxidative stress through the production of oxygen and phenoxy radicals.

When infested by insect herbivores, plants can induce the production of defense proteins, known as phytoalexins, which can interfere with the insects' physiological processes, such as digestion and nutrient absorption. A particular example is CYS6, a protease inhibitor found in tobacco plants. Genetic modifications of CYS6 have shown its significant role in plant-defending against whitefly. Conversely, the Armet effector protein (**Table.2**), secreted by *B. tabaci*, is highly expressed in its primary salivary gland and introduces into tobacco plants during feeding. This protein interacts with CYS6 and mitigates the detrimental effects of CYS6 on the whitefly.

2. Indirect defense (Fig.3b):

Beyond direct defense mechanisms, whitefly feeding can trigger the release of HIPVs, which attract the natural enemies of whiteflies and aid in plant protection. For example, it was discovered that two predatory mites, *Typhlodromips swirskii* and *Euseius scutalis*, showed a marked preference for whitefly-infested cucumber plants over non-infested ones, with this preference being influenced by the volatiles emitted by the plants. Similarly, it was also reported that whitefly infestation in *Arabidopsis* plants resulted in the buildup of ocimene/myrcene, which effectively attracted the whitefly parasitoid *Encarsia formosa*. In response to whitefly herbivory, melon plants release methyl salicylate and tetradecane, facilitating the attraction of the whitefly parasitoid *E. desantisi*.

3. Plant signalling pathway against whitefly (Fig.3c):

The jasmonate (JA) signalling pathway is a fundamental mechanism regulating plant defenses against herbivorous insects, including whiteflies. This pathway is critical for mounting an effective defense response to whitefly infestations. Experiments have shown that disrupting JA signalling in tobacco plants, whether through virus-induced gene silencing or genetic mutations in the MYC2 gene, results in increased whitefly survival and reproduction rates. In tomato plants, similar effects were observed: whitefly survival and fecundity were higher on JA-deficient spr2 mutant plants and lower on JA-overexpressing 35S-prosystemin transgenic plants compared to wild-type controls. Moreover, exogenous application of JA to tomato plants significantly reduced whitefly survival and reproduction. Several defense genes, particularly those involved in terpenoid, glucosinolates and

phenolic components synthesis, are upregulated by the JA pathway and are essential for the plants' defense against whiteflies.

The specific interactions between whiteflies and their hostplants necessitate targeted approaches for enhancing resistance. Traditional breeding methods that confer resistance to other insect herbivores might not be effective against whiteflies. Therefore, innovative strategies tailored to boost plant resistance specifically against whiteflies are crucial for sustaining the productivity of susceptible crops.

INDUCED RESISTANCE THROUGH INSECTICIDES AND TRANSGENICS:

Members of the *B. tabaci* complex exhibit significant differences in several key aspects, including their range of hostplants, the induction of phytotoxic disorders, resistance to insecticides, invasiveness, and the specificity of viral transmission. These variations present significant challenges in identifying and effectively managing these pests. The annual economic losses caused by cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) in cassava crops are estimated at US\$1.9–2.7 billion for CMD and US\$0.1 billion for CBSD, and it is equally relevant the economical and physical damage caused to the plants by their herbivory diet. In Africa, small-scale farmers are increasingly resorting to pesticides to manage whitefly populations on high-value crops such as tomatoes and cabbage (Mugerwa *et al.*, 2018). Globally, the extensive use of insecticides, including neonicotinoids, has resulted in the development of resistance in *B. tabaci*. Over 60 active ingredients in insecticides are now less effective against whitefly, all available on Arthropod Pesticide Resistance Database (APRD), www.pesticideresistance.org. Some whitefly species, like MED-Q1 and TV, have shown a rapid development of resistance following insecticide use. It is essential to prevent the development of insecticide resistance in species as MED-ASL, given its widespread distribution and ability to feed on various plant species (Mugerwa *et al.*, 2018). Genetically engineered crops effectively control certain pests without long-term environmental impacts, but they have limited success against hemipterans. Therefore, innovative and eco-friendly transgenic strategies are needed to control phloem-feeding pests like aphids and whiteflies.

1. RNA interference (RNAi):

RNA interference (RNAi) is a promising pest control method. While surface sprays of RNAi constructs are less effective for phloem feeders, plant-mediated RNAi has shown success in targeting genes in these pests. Several aphid genes have been inhibited using plant-mediated RNAi, such as C002, receptor of activated kinase C (Rack-1) and MpPInt01 (Feng *et al.*, 2022). Similarly, genes in *B. tabaci*, like cytochrome P450, heat shock protein 70 and phenolic glucoside

malonyltransferase (BtPMT1) (Xia *et al.*, 2021) have been targeted using plant-mediated RNAi. However, RNAi-based transgenic plants must be assessed for biological risks, including effects on non-target insects with similar gene sequences. Essential genes targeted by RNAi are often conserved across insect species, making it challenging to design RNAi constructs without off-target effects (Jain *et al.*, 2020), but genomic-comparison analyses as the one carried out in this essay could help address this problem.

2. Horizontally transferred genes (HTGs):

Horizontally transferred genes (HTGs) can limit potential off-target effects in RNAi-mediated pest control. The presence of HTGs in recipient genomes suggest these genes offer a selective advantage. Successful gene expression in recipient species can be influenced by factors like introns, GC content, codon preferences, and transcriptional promoter differences.

Aphids have acquired HTGs from fungi, bacteria, and viruses. For instance, an analysis of the *B. tabaci* MEAM1 genome revealed 142 HTGs (Chen *et al.*, 2016), some of which may influence the whitefly's broad host range and insecticide resistance. As explained hereabove, whiteflies possess a detoxifying gene, BtPMT1, likely from plants, which helps neutralize toxic phenolic glucosides produced by plants. Using plant-mediated RNAi to silence BtPMT1 in whiteflies disrupts their detoxification process, leading to increased mortality when they feed on transgenic tomato plants expressing dsBtPMT1. These genetically modified plants show strong resistance to whiteflies while not affecting non-target insects like peach-potato aphids, *Coccinella septempunctata* (seven-spotted ladybugs) and spider mites (Xia *et al.*, 2021). This illustrates that RNAi can effectively target whiteflies without harming other organisms, revealing an evolutionary mechanism where herbivores adopt hostplant genes to overcome plant defenses. These findings support the potential of HTGs as effective and safe targets for plant genetic engineering to control aphid populations (Chen *et al.*, 2016). Nonetheless, careful selection of controls for RNAi experiments is essential to avoid non-specific effects. Overall, the exploration of HTGs in agricultural pests provides valuable insights into their adaptation and potential for pest control strategies that minimize harm to beneficial organisms.

CONCLUSION:

To fully understand the interactions between plants and pests, numerous factors have been examined. The coevolution between the two has led to the diversification and gradual improvement of attack mechanisms for pests and defense mechanisms for plants, resulting in various lifestyles among pests (e.g., how they eat, such as chewing or piercing-and-sucking) and different defense chemicals among plants (phytoanticipins, phytoalexins, HIPVs). The underlying question and the main

theme of the essay was whether, through genomic comparison of pest insects, it would be possible to distinguish various interaction styles with plants, such as components of saliva or detoxification strategies.

Using these informations, it was possible to study in more detail the natural defense responses of plants when under attack. However, the issue of coevolution resurfaces like a snake biting its own tail: as plants evolve their defense systems, insects evolve and improve their attack and resistance strategies. A striking example of this is that some species of whitefly have developed genes resistant to many insecticide components in just a few decades. This is also due to the constitution of the whitefly genome itself, which is rich in transposable elements (TEs) and prone to quickly fixing mutations. One solution to this problem could be innovative and eco-friendly transgenic strategies, such as RNAi and HTGs techniques, which have shown promising results in trials.

In conclusion, it can be stated that genomic comparison between pests has led to greater insights into how these pests, despite having different strategies, often overcome plant defense systems. However, it is possible to expand studies precisely on these natural defense systems and, through transgenics, help nature take a step further against its (and our) enemies.

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