The management of sweetpotato whitefly in the southeastern US

by

Thiago Rutz da Silva

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Approved by

Andre Luiz Biscaia Ribeiro da Silva, Chair, Extension Specialist Professor, Department of Horticulture
Alana L. Jacobson, Associate Professor, Department of Entomology and Plant Pathology
Ayanava Majumdar, Assistant Director for Grant and Program Development and Extension Professor, Department of Entomology and Plant Pathology
Edward J. Sikora, Extension Specialist Professor, Department of Entomology and Plant Pathology
Timothy Coolong, Professor, Extension Specialist, and SARE State Coordinator, Department of Horticulture, University of Georgia

Abstract

Sweetpotato whitefly (*Bemisia tabaci*) is a significant pest affecting vegetable crops in the southeastern United States, causing direct feeding damage and transmitting viruses that can lead to substantial yield losses. This dissertation investigates sustainable management strategies for whitefly control in zucchini and tomato production through two main approaches: integrated pest management (IPM) practices using insect exclusion row covers and reflective silver plastic mulching and the exploration of wild tomato (*Solanum habrochaites*) accessions for genetic resistance to whiteflies.

Field experiments in zucchini production demonstrated that using reflective silver plastic mulching and insect exclusion row covers significantly reduced whitefly populations. Silver reflective mulching decreased whitefly densities by up to 87%, leading to a 17% increase in total yield compared to traditional white plastic mulching. Row covers reduced whitefly populations to near zero during the critical early growth stages, resulting in a 14% yield increase. These findings highlight the effectiveness of combining physical barriers and reflective mulches to manage whitefly infestations and increase crop productivity without heavy reliance on chemical insecticides.

Also, the physical and chemical characterization of wild tomato accessions for resistance to whiteflies identified specific wild tomato accessions with high densities of glandular trichomes and elevated terpene production, both of which were associated with reduced whitefly infestation. These findings suggest that wild tomato accessions, particularly *S. habrochaites*, offer promising genetic traits for breeding whitefly-resistant cultivars. The chemical analysis revealed that terpenes such as zingiberenoids are critical in deterring whiteflies, providing a biochemical basis for resistance.

This research offers a dual approach to whitefly management in the southeastern U.S. by integrating immediate, field-based IPM strategies with long-term genetic resistance through plant breeding. The findings contribute valuable insights into developing sustainable solutions to mitigate the economic impact of whiteflies on vegetable crops.

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Chapter 1 - Literature Review

Characterization of species *Bemisia tabaci* (Gennadius)

Origin, history, distribution, and systematic

Whiteflies have long been considered an important insect pest on a global scale, attacking various agricultural commodities. There are 1556 accepted species names in 161 genera among the existent whiteflies worldwide. However, two species, the sweetpotato whitefly, *Bemisia tabaci* (Gennadius), and the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), cause most of the damage (1).

The geographic origin of many whitefly species is primarily discussed. The evolutionary affiliations of the Bemisia taxa within the family Aleyrodidae suggest that *B. tabaci* may have originated in tropical Africa and was introduced into the Neotropics and southern North America. Some evidence also suggests that *B. tabaci* may be native to India or Pakistan, where the remarkable diversity of the species parasitoids have been found, criteria that have been considered a good indication of a genus epicenter (2–4). Further extension of its geographical range from subtropical and tropical agriculture systems has occurred to include temperate climate areas; as the species is now globally distributed and found on all continents (4). The inadvertent transport of the B-biotype on ornamental plants beginning in 1985-1986 established *B. tabaci* throughout Europe, the Mediterranean Basin, Africa, Asia, Central America, North America (Mexico and the US), South America (Argentina, Brazil, Colombia, and Venezuela), and the Caribbean Basin (2).

The first description of *B. tabaci* was in 1889 as a tobacco pest in Greece and named *Aleyrodes tabaci*, the tobacco whitefly, and the first *B. tabaci* in the New World was collected in 1897 in the United States on sweetpotato. It was initially given the common name of sweetpotato whitefly, described as *Aleyrodes inconspicua* Quaintance. This species was moved into a new genus, *Bemisia*, in 1914, giving rise to *B. inconspicua* Quaintance, the type species for the genus (5). It was found in Brazil on *Euphorbia hirtella* and described as *B. costalimai* Bondar in 1928, and in 1933, the species was collected in Taiwan and described as *B. hibisci* (4). Over the years, 19 other species of whiteflies (that later were synonymized with *B. tabaci*) were described from 14 other countries on various host plants. Significant in this list of species was the placement of tabaci in the genus Bemisia in 1936 by Takahashi, resulting in *B. tabaci* Gennadius, which remains today (5).

After several decades of research into the nomenclature of *B. tabaci,* it can now be considered a complex of morphologically indistinguishable species (1). The different responses of two separate population explosions in the southwestern desert of the US suggested that possibly two species were involved under the name *B. tabaci*. In 1991, significant differences between these two populations eventually became known as biotype A for those in the southwestern desert areas of the US before 1990, and the B biotype for those in Florida starting in 1985–1986 and Arizona in 1986–87, leading to the species description of biotype B as the silverleaf whitefly, *Bemisia argentifolii* (6).

Currently, *B. tabaci* is considered a cryptic species complex containing 11 higher genetic groups and at least 43 morphologically indistinguishable species. Those genetic groups are classified based on genetic differences identified through the use of allozyme electrophoresis, random amplification of polymorphic DNA (RAPD), polymerase chain reaction (PCR), mitochondrial cytochrome oxidase 1 (CO1), ribosomal internal transcribed spacer (ITS), phylogenetic analysis, and pairwise comparisons of genetic distance between genetic groups of *B. tabaci* worldwide. (7–9). The major *B. tabaci* species on tomatoes are the New World whitefly (New World), previously known as Biotype A, the Middle East Asia Minor 1 whitefly (MEAM1), previously known as Biotype B or *B. argentifolii* Bellows and

Perring, and the Mediterranean (MED) whitefly, previously known as Biotype Q and now known to be the original *B. tabaci* (1).

Morphology, biology, and life cycle

B. tabaci is classified in the Order Homoptera, Family Aleyrodidae, and Subfamily Aleyrodinae (2). Five subfamilies within the extant Aleyrodidae have been established at various times, of which the Aleyrodinae are the most widespread. The Aleyrodinae consists of more than 140 described genera, but although 13 tribes have been proposed, at least half of the genera have not been allocated to tribes, and there is little consensus about the composition of those tribes (10).

Whiteflies have six life stages: the egg, four nymphal instars, and the adult stage. The egg stage of all whiteflies has a stalk at its larger end called the pedicel. During this time, the stalk is filled with protoplasm that dries up after fertilization and becomes a hollow tube. Most whiteflies insert the pedicel into leaf stomata, but *B. tabaci* pedicles are inserted directly into epidermal tissue. The apex of the pedicel has a porous fibrous structure that absorbs water and possibly nutrients from the leaf. The eggs of *B. tabaci* are typically scattered about the leaf surface, although sometimes they are laid in partial egg circles with a fecundity of well over 500 progeny per female has been demonstrated (1,11).

The nymphal stages of whiteflies exhibit an oval-shaped morphology with dorsoventral flattening. The initial nymphal instar, the "crawler" stage, displays exceptional mobility and actively explores the leaf surface to locate an appropriate location for inserting its stylets into the phloem sieve elements. Unlike the later instars, the lateral borders of crawlers possess a multitude of setae, which are believed to have mechanosensory functions, aiding in the detection of leaf hairs. Subsequently, upon identifying a suitable feeding site, the crawler undergoes a transition and becomes sessile, remaining stationary at the initially chosen location (1).

The second, third, and fourth nymphal instars constitute the final three stages experienced by the whitefly, characterized by their immobility and fixed position on the host plant. The fourth instar stage is occasionally called the "pupal" stage (1). However, during the early fourth instar, the insect continues to feed, indicating that it does not conform to the typical definition of a pupa in holometabolous insects. As the instar progresses, the insect undergoes a transitional substage characterized by apolysis, during which the adult cuticle is formed. During this stage, the insect possesses red eyes and a yellow body pigment, which recommends that if the term "pupa" is used, it should be reserved for the final nymphal stadium observed after apolysis (12).

Following the emergence of the adult whitefly from its last nymphal instar, the wings of *B. tabaci* become coated with a powdery white wax substance. This waxy covering is secreted shortly after the adult emerges. The wings of *B. tabaci* are positioned in a "roof-like" manner, extending over the abdomen, resulting in a long and narrow appearance when observed on leaves. Adult whiteflies of this species are commonly found on the undersides of tomato leaves. Sexual dimorphism is evident in *B. tabaci*, with females being more prominent in size compared to males and exhibiting a rounded abdomen, while males possess a more pointed abdomen (1). There are four distinct phases in the complex courtship behavior of MEAM1 whiteflies, ultimately leading to copulation. Additionally, MEAM1 males have been observed to interrupt courtship and influence the mating dynamics of other sibling species of *B. tabaci*. Whiteflies employ an arrhenotokous reproductive system, where unfertilized eggs give rise to haploid males, while fertilized eggs result in diploid females (13).

The development, survival, and reproductive success of *B. tabaci* are strongly influenced by temperature, as is characteristic of poikilothermic insects. Various studies have demonstrated that the time required to transition from egg to adult ranges from 105 days at 15°C to 14 days at 30°C. Fecundity, measured in terms of the number of eggs produced per female, also exhibited considerable variation, with an average of 324 eggs per female at 20°C and 22 eggs per female at 30°C. Alongside temperature, the host plant plays a significant role in the biology of *B. tabaci*. It was found that the influence of the host plant on egg development surpassed the effects of temperature, humidity, and photoperiod (1,11).

There was a notable disparity in the total duration of the pre-adult stage between New World whiteflies (22.3 days) and MEAM1 whiteflies (18.1 days), with a difference of 4 days. Similarly, the oviposition period also exhibits contrasting durations, with New World whiteflies having a period of 16.7 days compared to MEAM1 whiteflies with 2.25 days. Consequently, the average number of eggs laid per female differed significantly between the two variants, with New World whiteflies averaging 194.9 eggs per female and MEAM1 whiteflies averaging 94.5 eggs per female (14,15). Such substantial variations highlight the challenges of interpreting biological data concerning *B. tabaci*. It is crucial to consider the specific whitefly variant and the host plant used in the studies (1).

Significant damages caused by whitefly

Whiteflies have been recorded from more than 600 plant species, and their polyphagous nature has been documented worldwide (4). This insect species has risen in notoriety in past decades as a plant pest species, particularly in horticultural crops. *B. tabaci* has a broad host range which includes crop plants such as cassava, tomato, eggplant, cinnamon, cucurbits, muskmelon, okra, cucumber, black pepper, sunflower, pulses, tobacco, groundnut, cabbage, soybeans, potatoes, cauliflowers, cotton, lettuce, and numerous other crops of economic importance (16). *B. tabaci* can cause significant economic losses to crops by causing damage to the host plants during feeding through the secretion of honeydew and transmission of plant viruses (9).

Direct damage

Both nymphs and adults of *B. tabaci* inflict harm by inserting their mouthparts into plants while feeding, leading to the plant damage. This results in the removal of essential nutrients and consequent reduced photosynthetic activities in foliage. Consequently, leaves turn yellow, prematurely fall off, and overall plant vitality weakens. These symptoms are particularly prevalent during heavy infestations. In severe cases, die-back of branches can occur, and smaller plants may even experience death, although such cases are rare. It should be noted that dying plants are often weakened due to other factors, diminishing their value and fruit quality grade (17).

B. tabaci nymphs can introduce enzymes that induce alterations in plant physiology, consequently causing irregular fruit ripening and inhibiting internal coloration. The substantial quantities of honeydew secreted by *B. tabaci* negatively impact the quality of vegetables. The honeydew attracts ants, which disrupts the activities of natural enemies that could potentially control whiteflies and other pests (17). Furthermore, the honeydew secreted by *B. tabaci* serves as a growth medium for sooty mold on leaves and fruits, thereby diminishing photosynthetic activities and potentially compromising the quality of agricultural produce (9). This black, powdery fungus blocks sunlight from reaching the leaf surface, significantly reducing photosynthetic efficiency. As a result, plants exhibit diminished growth rates and reduced productivity. Moreover, the presence of sooty mold on fruits can compromise their market quality, making them less desirable to consumers. These combined effects can lead to substantial economic losses in agriculture (18).

Another problem seen in cucurbit plantations frequently encounters the influence of high whitefly populations and the consequential manifestation of squash silverleaf disorder due to their feeding activities. Squash silverleaf is a significant physiological disorder typified by the gradual silvering of the adaxial leaf surface. Leaves impacted by squash silverleaf exhibit diminished chlorophyll levels and augmented reflectance compared to unaffected foliage, potentially engendering diminished crop yields (19).

Whitefly as a virus vector

More than 200 plant viruses can be transmitted by *Bemisia* and *Trialeurodes* genera, with the majority of these viruses belonging to the genera Begomovirus (Geminiviridae), Ipomovirus (Potyviridae), Crinivirus (Closteroviridae), Carlavirus (Betafelxiviridae), and Torradovirus (Secoviridae) (9,20,21). *B. tabaci* has gained considerable significance as a pest and a vector of viral diseases in regions characterized by tropical, subtropical, arid, and Mediterranean climates. This insect is responsible for causing substantial crop losses in cultivating numerous food, fiber, and ornamental plants. Among the vulnerable crops are cassava, cotton, cowpea, cucurbits, crucifers, tobacco, tomato, potato, soybean, sweet potato, okra, lettuce, pea, bean, pepper, poinsettia, and chrysanthemum (20).

Whiteflies transmit viruses through two distinct modes: semipersistent and persistent transmission. Semipersistent transmission involves the acquisition of viruses within minutes to hours and a retention period in the foregut ranging from hours to days. On the other hand, persistent transmission involves a more extended acquisition period of hours, with the virus being retained in the hemolymph for days or throughout the insect's entire lifespan. In persistent transmission, the virus may either replicate within the insect (propagative transmission) or not replicate (circulative transmission). Among the virus diseases that have emerged in the past two decades, most are caused by viruses transmitted by whiteflies (21).

The genus Begomovirus (contraction of Bean golden mosaic virus), which includes about 200 accepted virus species, the most numerous of the *B. tabaci*-transmitted viruses and causes crop yield losses of between 20% and 100% (20,21). Members of this family have a circular single-stranded DNA (ssDNA) genome encapsidated in twinned quasi-icosahedral (geminate) virions. As with other members of the family Geminiviridae, most begomoviruses have a bipartite genome of circular, ssDNA, and both segments (referred to as DNA-A and DNA-B) are similar in size (2.5–2.7 kb); in contrast, monopartite begomoviruses contain only one genome component that is homologous to DNA-A Begomoviruses are transmitted in a persistent and circulative manner by *B. tabaci*, and most are restricted to the phloem of the infected plants (21).

In the southeastern United States, summer crops of significant importance include tomato (*Solanum lycopersicum*) and squash (*Cucurbita pepo* L.). Within this region, Tomato yellow leaf curl virus (TYLCV), which infects tomatoes, and Cucurbit leaf crumple virus (CuLCrV), which infects squash, are highly notable begomoviruses transmitted by *B. tabaci* MEAM1 (22). The Cucurbit leaf crumple virus (CuLCrV), an emergent and potentially economically significant bipartite begomovirus that primarily infects cucurbits such as cantaloupe, squash, and watermelon, was first identified in volunteer watermelon plants in the Imperial Valley of southern California in 1998 and are endemic to Americas (22,23).

CuLCrV is a full-length DNA-A and DNA-B phloem-limited virus and is not sap-transmissible. Symptoms of CuLCrV infection include stunted growth, leaf crumpling, curling, and chlorosis. Through host range studies, it was revealed that different cucurbit species exhibited varying levels of susceptibility. Squash, watermelon, cantaloupe, and honeydew melon were most to least susceptible, respectively. Genomic analysis of the CuLCrV showed a similar organization to other bipartite begomoviruses, and phylogenetic analysis placed CuLCrV in the Squash leaf curl virus (SLCV) cluster of New World bipartite begomoviruses (23).

Whitefly management

Integrated Pest Management (IPM) aims to reduce the harm caused by indiscriminate chemical pesticides. IPM approaches for whitefly management may involve different physical and mechanical methods, biological control, crop plant resistance, and selective chemical pesticides (9,16).

Chemical control

The management of whiteflies relies mainly on using insecticides that cannot eliminate the whitefly populations but significantly reduce them. The most common pesticides used to control whiteflies are pyriproxyfen, buprofezen (growth regulators), spiromesifen, spirotetramat (ketoenols), anthranilic diamides, cyantraniliprole, and chlorantraniliprole (diamides) (1,16).

However, for example, *B. tabaci*'s ability to develop resistance to pyriproxyfen and neonicotinoids seriously challenges farmers and pest control specialists. The rotation among different modes of action and nonchemical control methods are countermeasures in pest management programs to retain effective chemistries for as long as possible in the marketplace (24,25).

Cultural control

Floating row covers, a practice initially used to protect plants against frosts, has shown to be a potential strategy to avoid the whiteflies' direct and indirect damage to cucurbits. This technique consists of spun-bonded polyethylene row covers that protect plants from many foliar pests, such as whiteflies, aphids, and the pathogens they transmit, while also the horticultural yields can be increased significantly. Other benefits of using row covers include more favorable soil and air temperature regimes, improved water and fertilizer use efficiency, and higher and earlier crop production (19).

In crops where the row cover is hard to apply, an alternative practice to row covers is the use of ultraviolet-reflective mulches instead of the traditional white or black plastic mulch on the beds. UV-reflective mulches affect the whiteflies' behavior toward wavelengths, and with the positive phototactic responses to wavelengths other than visible light, the whitefly population is reduced (26,27). Silver plastic mulch can reduce the incidence of whitefly and virus symptoms in tomatoes, which leads to higher fruit yield and better quality fresh-market tomatoes (28).

Host plant resistance

Host plant resistance (HPR), in conjunction with natural enemies and cultural practices, constitutes a fundamental element within the framework of pest management strategies. Resistant varieties assume a paramount role as indispensable instruments, empowering agricultural systems to confront the global obstacles arising from population expansion, climate fluctuations, and ecological deterioration. Although many pests and diseases threaten most crops, resistance traits predominantly aim at particular or closely allied species (29,30).

HPR emerges through the manifestation of plant-specific traits associated with resistance, influencing various facets of the herbivore's interactions with the host plant and other organisms linked to the plant. The encompassing concept of plant resistance can be described as the cumulative expression of genetically inherited attributes that ultimately govern the extent of damage inflicted upon the plant by the herbivore, leading to yield loss (31).

The first step of the traditional approach to using HPR in IPM is screening or evaluating genotypes for resistance. Developing and standardizing resistance screening techniques is the key to an effective resistance-breeding program. This process involves the evaluation of crop germplasm to identify genotypes (lines, accessions, cultivars) that express resistance to the insect or express a phenotype putatively related to resistance (30,31).

Efforts to control the whitefly using insecticides have encountered significant challenges due to its predominant feeding and oviposition behavior on the abaxial leaf surfaces, coupled with its acquired resistance to most insecticide classes employed for its suppression. Given these limitations, HPR emerges as a promising alternative for effectively managing this pest (32).

Trichomes, particularly glandular trichomes, exert a significant impact on arthropod pests. Glandular trichomes can produce, store, or secrete many volatile and non-volatile specialized metabolites. The presence of glandular trichomes in wild tomatoes from the *Solanum* spp. Lycopersicon section plays a pivotal role in conferring resistance against arthropod pests, primarily attributed to the storage of secondary metabolites within their vesicles, which detrimentally affect the biology of pests. Notably, certain substances, such as acylsugars, including sugar esters, produced by glandular trichomes of types I and IV in *Solanum galapagense* and *Solanum pennelli* accessions and terpenoids, such as zingiberenoids, produced by type VI glandular trichomes in *Solanum habrochaites* accessions, represent noteworthy examples that contribute to pest resistance (33,34).

Rationale

Whitefly (*Bemisia tabaci*) is a polyphagous pest spread worldwide, causing crop yield losses by direct feeding damage or serving as a virus vector (21). An economic loss of more than 125 million US dollars in tomato production in Florida and a loss of up to 35% of the squash production in Georgia in 2016 are reports that demonstrate the impact of whitefly infestations may cause (16).

Among the more than 200 viruses that whitefly can transmit, the whitefly-transmitted geminiviruses (genus Begomovirus) is known to be a leading cause of yield losses, which can range from 20–100% in crops such as cotton, crucifers, eggplants, tobacco, tomato, potato, soybean, sweet potato, okra, zucchini, squash, cucumbers, and others (9,23). The bipartite begomovirus Cucurbit Leaf Crumple Virus (CuLCrV) is the most economically significant virus in the southeastern US (23,35). It was first reported in yellow squash and zucchini (*Cucurbita pepo*) fields in Florida in 2006 (36–38). Since then, it has caused progressive yield losses in zucchini production in the southeastern US (39).

Resistant cultivars to combat whiteflies should be the first step of adequately integrated pest management; however, no whitefly-resistant commercial yellow squash or zucchini cultivar are resistant to either whitefly or the virus. Furthermore, the extensive use of insecticides to prevent whiteflies in production fields allowed whiteflies to develop resistance to key chemical control agents such as organophosphates, carbamates, pyrethroids, neonicotinoids, diamides, and insect growth regulators (25,40). Therefore, alternative crop management practices have been studied to soften the impact of high whitefly populations. The use of row covers has shown the best control of whitefly and whitefly-transmitted viruses in crops such as snap bean (41), watermelon (42), tomato (28,43,44), and zucchini (45). The combination between reflective mulch and insecticides, such as imidacloprid, endosulfan, and insect growth regulator, has also decreased the whitefly population while reducing the number of spraying (19,45–48).

In the southeastern US, row covers have been used in large-scale production farms during the whitefly season. However, farmers have indicated a higher demand for irrigation water and nutrients during crop development due to the fast growth of plants into the row covers. Furthermore, the use of insect-resistant plants has been limited due to the time it takes to evaluate new materials for insertion into breeding programs for vegetable crops. The impact of different management practices on squash and tomato production in various regions or conditions to control whiteflies has not been reported yet.

Objectives

The general objective of this project is to evaluate sustainable alternatives for whitefly control in the southeastern US. Given that squash and tomato production are the most affected in this region, this study is divided into two sections. The first study is a short-term strategy for squash production because neither whitefly nor whitefly virus-resistant cultivars are available. Therefore, the specific objective for the first study is to assess the influence of the silver reflective plastic mulch and row cover on whiteflies on zucchini production during the whitefly season.

In the case of tomato production, as there are whitefly virus-resistant cultivars options, a long-term plan appropriate to obtain new potential accessions for the introduction of whitefly resistance into a breeding program for the crop. Thus, the specific objectives for the second and third studies are to characterize the repellence of wild accessions to whitefly, to categorize the types of trichomes present on the underside leaf surface, and to verify the presence of specific secondary metabolites in the leaves of wild tomatoes to identify the most promising accession.

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Chapter 2 - Use of insect exclusion row cover and reflective silver plastic mulching to manage whitefly in zucchini production

Introduction

Sweet potato whitefly (*Bemisia tabaci*) is one of the most significant challenges of vegetable production during the fall months in the southeastern US. The direct damage of the insect to vegetable crops and the whitefly's role as a vector of many plant viruses can lead to significant yield losses (1). The high insect pressure in vegetable crops is primarily due to the region's specialty and row crop production overlap. Crops such as yellow squash/zucchini (*Cucurbita pepo*), cucumber (*Cucumis sativus*), snap beans (*Phaseolus vulgaris*), tomato (*Solanum lycopersicum*), cotton (*Gossypium hirsutum*), and others are all grown in proximity throughout the southeastern US in the fall months. This may result in the widespread distribution of whiteflies among many regional crops (2).

Consequently, growers rely heavily on insecticide spray programs to control whitefly populations, which negatively impacts production costs and may increase environmental concerns. Furthermore, the elevated insecticide exposure has increased the resistance of whiteflies to key chemical control agents, such as organophosphates, carbamates, pyrethroids, neonicotinoids, diamides, and insect growth regulators (3,4). Sustainable crop management practices are required to help growers with effective integrated pest management (IPM).

Alternative crop management practices to control virus vectors, such as aphids (*Aphis sp.*), thrips, and even whiteflies, have been continuously evaluated on specialty crops (5–9). A few examples of IPM strategies against whiteflies include the use of silver reflective plastic mulch, which was previously reported to reduce the insect numbers on snap beans (10), watermelons (11), tomatoes (12–15), and zucchini. Combining a reflective plastic mulching plus imidacloprid resulted in a three-fold reduction in whitefly numbers on zucchini compared to a white plastic mulch control. In addition, it also reduced the incidence of cucurbit leaf crumple virus (CuLCrV) infected zucchini plants (5).

The use of insect row covers has also been successfully used to protect plants against whiteflies in cantaloupe (*Cucumis melon*) (16,17), tomato (18,19), and zucchini (9,20,21). Row covers combined with insect growth regulators reduced fruit damage and increased fruit size, weight, and quality in zucchini plants due to fewer adults, eggs, and pupae/nymphs per leaf compared with no cover treatments (9). The temporary pest exclusion systems that separate insects from host plants provide short-term solutions to insect damage and avoid an infestation in critical stages of crop development (22).

The effectiveness of silver reflective plastic mulching and row covers in managing insect pests for vegetable production has been previously reported; however, their impact on crop development (i.e., zucchini) during whitefly management in the southeastern US remains poorly studied. Thus, the objective of this study was to evaluate the use of reflective plastic mulching and insect row cover as an alternative method to the current grower practices against whiteflies for zucchini production during the fall season.

Materials and Methods

Experimental design and crop management

Field experiments were conducted on a commercial vegetable farm in Ty Ty, Georgia, US in 2020 (31°44’59” N, 83°59′04” W) and 2021 (31°41′59” N, 83°78’59” W) and at the Wiregrass Research and Extension Center from Auburn University, located in Headland, AL, US (31°21’11” N, 85°19’17” W) in 2021.

In each location, a two-level factorial experimental design with the type of plastic mulch and row cover treatments was arranged in a randomized complete block design (r = 4). Plastic mulching treatments consist of using a white plastic mulching (Vaporsafe RM, Raven Industries, Sioux Falls, SD, US), which is the standard practice for fall vegetable production in the region, or a silver reflective plastic mulching (metalized low-density polyethylene 0.2 mm, 2.0 OD, Intergro, Clearwater, FL, US). Cover treatments included an insect row cover treatment, installed at a low gothic tunnel shape (1.2 m tall, 1.8 m wide) with a white polypropylene fabric (Agribon AG-15, Berry Global**,** Evansville, IN, US) in each bed and a no-cover treatment.

Zucchini seeds, cultivar Paycheck (Syngenta US, Greensboro, NC, US), were planted into 200-cell trays filled with soilless media on 25 Aug. 2020 and 26 Aug. 2021 in Georgia and 02 Aug. 2021 in Alabama. Seedlings were greenhouse-grown until transplanting on 09 Sep. 2020 and 13 Sep. 2021 in Georgia, and 19 Aug. 2021 in Alabama. Plants were grown on 15-cm-tall, raised beds spaced at 1.8 m center-to-center, with an in-row plant spacing of 30 cm. Cover treatments were installed at transplanting and removed at the first sign of anthesis, which was 20 days after transplanting (DAT) in Georgia 2020, 21 DAT in Georgia 2021, and 18 DAT in Alabama 2021. During the entire growing season, zucchini plants receiving the no-cover treatment were sprayed weekly with 205 g/ha of flupyradifurone (Sivanto 200 SL; Bayer CropScience, Research Triangle Park, NC, USA), 30 g/ha of pyriproxyfen (Knack; Valent, Walnut Creek, CA, USA), or 150 g/ha cyantraniliprole (Exirel; DuPont, Wilmington, DE, USA) to control whiteflies. Plots with the insect row cover treatments were not sprayed during the cover period but received the same spraying program as the no-cover treatment after cover removal. Crop management practices of fertilizer application, irrigation events, and disease and weed control were similar in all treatments and followed the University of Georgia Extension Cooperative recommendations (23).

Weather parameters and data collection

Weather conditions of maximum, minimum, and average air temperature and rainfall events were recorded daily in all locations using the closest weather station from the Georgia Automated Weather Network (24) in Georgia and the Auburn University Mesonet in Alabama (25).

During the growing season, yellow sticky pest monitor cards (7.6 × 12.7 cm; BASF, Research Triangle Park, NC, USA) monitored the whitefly population weekly in each experimental unit at a density of 1,794 traps per hectare. Yellow pest monitor cards were installed 15 cm above the ground and vertically oriented. The numbers of whiteflies were counted in an area of 77.4 cm^2 in the center of each card. The number of whiteflies per trap was transformed to 1 m^2.

Above-ground plant tissue samples were collected at six points during crop development. Samples comprised two representative plants of each plot and oven-dried at 65.5°C until constant weight. Subsequently, a dry biomass accumulation logistic curve was fitted by adapting the following equation (26).

Equation 2. 1. Crop biomass accumulation equation. Logistic growth function used to model the accumulation of biomass in crops over time, where NM is the maximum crop dry biomass accumulation, k is the crop dry biomass accumulation rate constant, and l is the days to half biomass accumulation.

$$\text{Crop biomass accumulation} = \frac{\mathrm{NM}}{1 + \mathrm{e}^{-\mathrm{k}(\mathrm{x}-\mathrm{l})}}$$

Zucchini fruits were harvested when they met commercial standards for crop maturity and initiated at 27 DAT in all locations. Fruit was harvested thrice weekly for 4 weeks and 12 harvests. By the end of the last harvest, the total yield was estimated.

Statistical analyses

All data were analyzed using linear mixed techniques implemented in the SAS PROC GLIMMIX (SAS/STAT 9.4; SAS Institute Inc., Cary, NC). Whitefly counts and dry biomass accumulation over time were analyzed as repeated measures, accounting for the correlation structure induced by the repeated sampling. According to the smallest Akaike's information criterion, the variance-covariance matrix was adequately modeled as an ante-dependence structure of order 1. The total yield of zucchini was analyzed using treatments individually and their interactions as fixed effects. When the F value of the analysis of variance was significant, multiple mean comparisons were performed using Tukey's test with a p-value of 0.05.

Results

Weather conditions

Daily average air temperatures were similar across the three locations and decreased with plant development (Figure 1). Average air temperatures during the study period were 23.6°C in Georgia 2020, 22.9°C in Georgia 2021, and 27.5°C in Alabama 2021. Rainfall accumulation was similar among the three locations, totaling 183.3 mm in Georgia 2020, 191.4 mm in Georgia 2021, and 194.6 mm in Alabama 2021. Mainly, rainfall events were concentrated in the early season in Georgia 2020 and Georgia 2021 but more evenly distributed across the growing season in Alabama 2021.

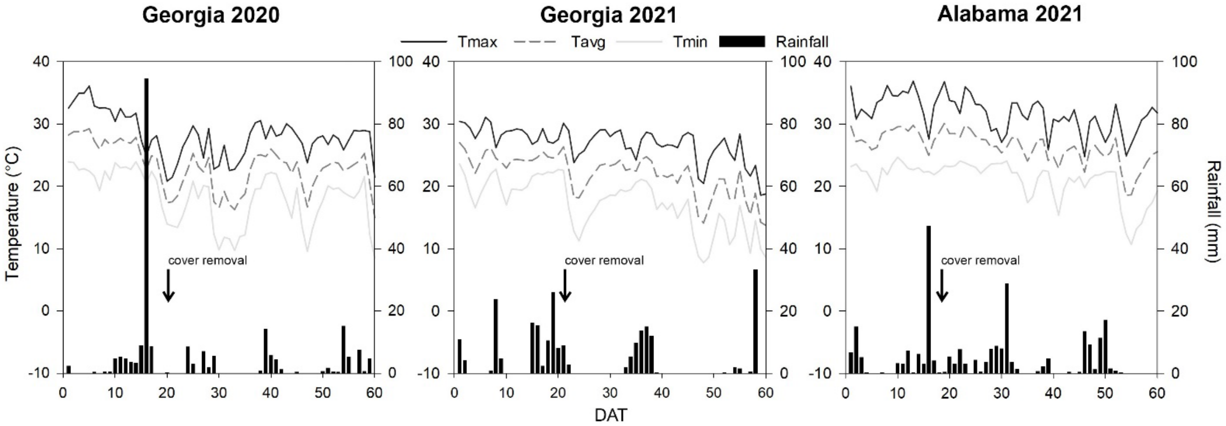

Figure 2. 1. Whether conditions of daily rainfall events and maximum (Tmax), average (Tavg), and minimum (Tmin) air temperature during the zucchini growing season in Georgia 2020, Georgia 2021, and Alabama 2021.

Whitefly population

For all growing seasons, whitefly populations were significantly impacted by the main effect of plastic mulching during crop development and the main effect of row cover during crop development (Figure 2). Whitefly populations were the highest during the early season in

Georgia 2020 and Georgia 2021 but the highest late season in Alabama 2021. Regardless of location, the silver plastic mulching reduced the number of whiteflies compared to the white plastic mulching in all locations throughout the growing season, while the row cover treatments decreased the number of whiteflies compared to the no-cover treatment early in the season. On average, the average whitefly population for the silver reflective plastic mulching was 3 whiteflies per trap in Georgia 2020 (Figure 2 A), 6 whiteflies per trap in Georgia 2021 (Figure 2 B), and 12 whiteflies per trap in Alabama 2021 (Figure 2 C). Whitefly population for the white plastic mulching averaged 24 whiteflies per trap in Georgia 2020, 9 whiteflies per trap in Georgia 2021, and 17 whiteflies per trap in Alabama 2020 (Figure 2 D, E, and F).

At a given sampling time, the number of whiteflies in the insect row cover treatment was significantly lower than in the no-cover treatments until cover removal. Overall, the whitefly population in the insect row cover treatment until cover removal averaged 2 whiteflies per trap in Georgia 2020 (Figure 2 D), 1 whitefly per trap in Georgia 2021 (Figure 2 E), and 12 whiteflies per trap in Alabama 2021 (Figure 2 F). Contrarily, the whitefly population in the no-cover treatment averaged 26 whiteflies per trap in Georgia 2020, 13 whiteflies per trap in Georgia 2021, and 17 whiteflies per trap in Alabama 2020 (Figure 2 D, E, F). After cover removal, the whitefly populations were not significantly different between cover treatments.

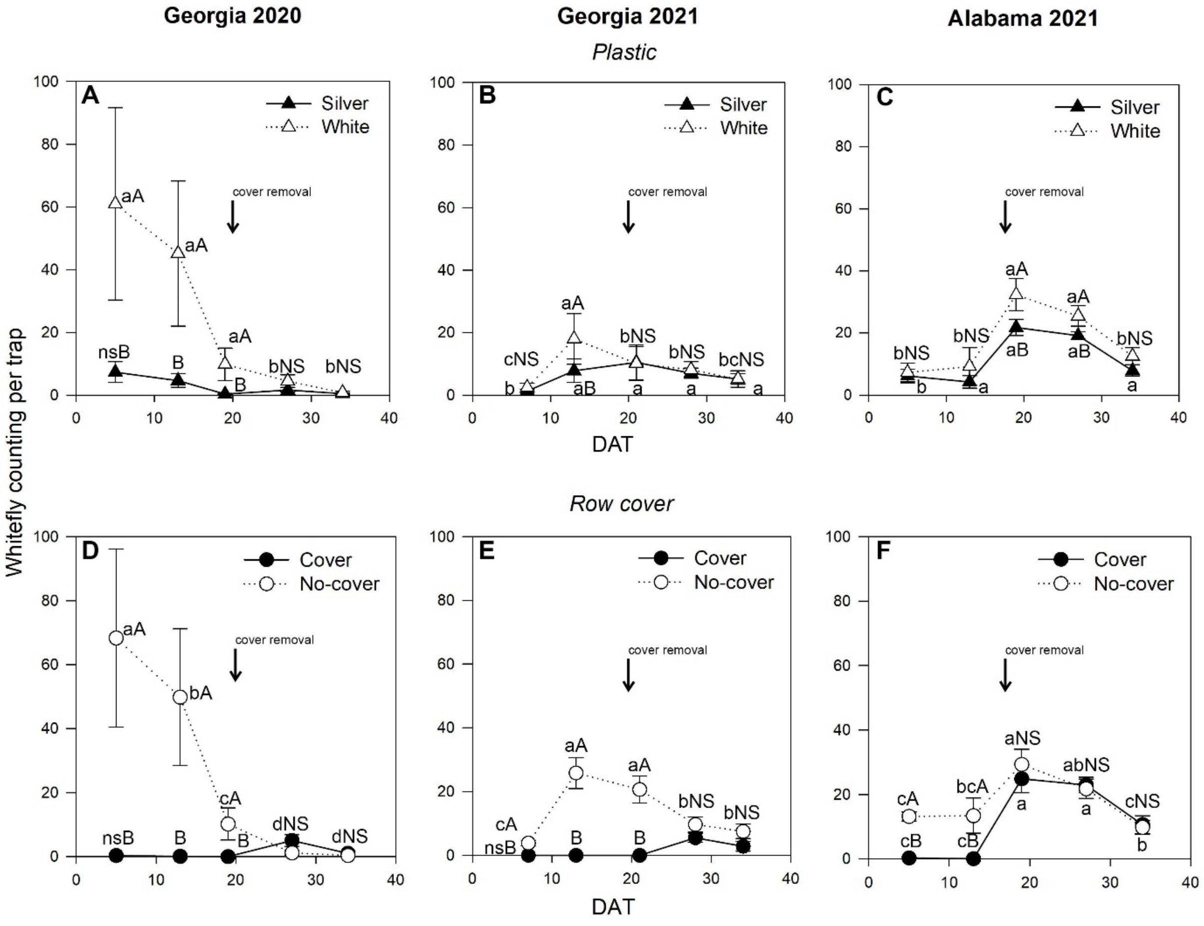

Figure 2. 2. Effect of plastic mulching treatment within sampling time on whitefly population during the zucchini's growing season for Georgia 2020 (A), Georgia 2021 (B), and Alabama 2021 (C), and row cover treatments within sampling time for Georgia 2020 (D), Georgia 2021 (E), and Alabama 2021 (F). Note: Different uppercase letters indicate significant differences ($p \leq 0.05$) among plastic mulching and row cover treatments within sampling time according to the Tukey mean test. According to the Tukey mean test, different lowercase letters indicate a significant difference ($p \leq 0.05$) among sampling time within plastic mulching or row cover treatments. ns and NS indicate no significant difference.

Zucchini plant development and total yield

Dry biomass accumulation data was used to determine maximum crop dry biomass accumulation, crop dry biomass accumulation rate, and days to half biomass accumulation for plastic mulching and row cover treatments within each location (Figure 3).

The estimated maximum crop dry biomass accumulation was higher for the reflective silver plastic mulching (NM = 3,318.8 kg ha^{-1}) than for the white plastic mulching (NM = 2,713.2 kg ha^{-1}) when zucchini was grown in Georgia in 2021 (Figure 3B). In contrast, white plastic mulch had a higher crop dry biomass accumulation when mulch treatments were compared in Georgia 2020 (NM = 2,042.6 kg ha^{-1} for the reflective silver plastic mulching and NM = 2288.9 kg ha^{-1} for the white plastic mulching) (Figure 3A) and Alabama 2021 (NM = 2,733.3 kg ha^{-1} for the reflective silver plastic mulching and 3075.8 kg ha^{-1} for white plastic mulching) (Figure 3C). Nevertheless, the reflective silver plastic mulching had a higher crop dry biomass accumulation rate and reduced the time zucchini plants took to reach half of the dry biomass in Georgia 2020 (k = 0.3028 and l = 27) and Georgia 2021 (k = 0.2495 and l = 32 days) compared to white plastic mulching in Georgia 2020 (k = 0.2044 and l = 29 days) and Georgia 2021 (k = 0.1891 and l = 31 days). In Alabama 2021, the crop dry biomass accumulation rate was lower for the reflective silver plastic mulch (k = 0.2659) compared to the white plastic mulch treatment (k = 0.3003), and the time for plants to reach half of the dry biomass accumulation was similar between plastic mulching treatments.

The response of zucchini plants grown under row cover treatments (Figures 3 D, E, and F) indicated that the maximum crop dry biomass accumulation was higher in the covered treatment in Georgia 2020 (NM = 2,343.6 kg ha^{-1}) and Georgia 2021 (NM = 3,120.3 kg ha^{-1}) compared to the no-cover treatment in Georgia 2020 (NM = 1,916.7 kg ha^{-1}) and Georgia 2021 (NM = 2,989.5 kg ha^{-1}). In contrast, the row cover treatment had a reduced estimated maximum crop dry biomass accumulation (NM = 2,708.2 kg ha^{-1}) than the no-cover treatment (NM = 3091.5 kg ha^{-1}) in Alabama 2021.

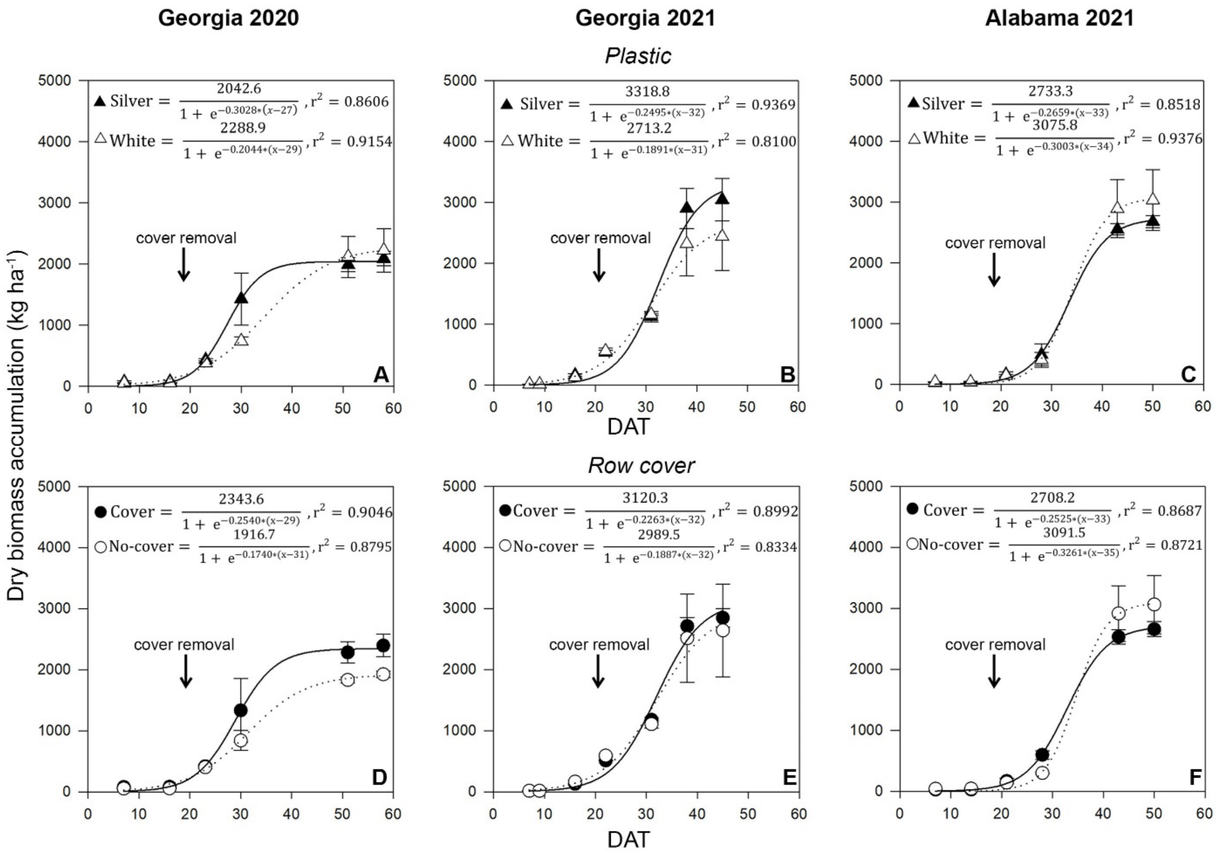

Figure 2. 3. Effect of plastic mulch treatments for Georgia 2020 (A), Georgia 2021 (B), and Alabama 2021 (C) and row cover treatments for Georgia 2020 (D), Georgia 2021 (E), and Alabama 2021 (F) on biomass accumulation during the zucchini's growing season.

In addition, the covered treatment had greater crop dry biomass accumulation rate estimates in Georgia 2020 (k = 0.2044) and Georgia 2021 (k = 0.2263) than the no-cover treatment in Georgia 2020 (k = 0.1740) and Georgia 2021 (k = 0.1887). In Alabama 2021, the crop dry biomass accumulation rate was lower for the covered treatment compared to the no-cover treatment (k = 0.2525 and k = 0.3261, respectively). The days to half of dry biomass accumulation estimation in Georgia 2020 (l = 29 days) and Alabama 2021 (l = 33 days) was shorter under the covered treatment compared to no-cover treatment (l = 31 days and l = 35 days, respectively). In Georgia in 2021, the days to half of dry biomass accumulation estimation was similar for both cover treatments (l = 32).

Fruit total yield was significantly impacted by location, plastic mulching, and cover treatments (Table 1). For the main effect of location, the total yield was higher in Alabama 2021 (15,248 kg ha^{-1}) and Georgia 2021 (15,177 kg ha^{-1}) compared to Georgia 2020 (11,451 kg ha^{-1}). The total yield for zucchini grown under the silver reflective plastic mulching (15,246 kg ha^{-1}) was higher than zucchini plants grown under white plastic mulching (12,672 kg ha^{-1}). Ultimately, the total yield was higher for plants grown under the row cover treatment (15,030 kg ha^{-1}) than those grown without row covers (12,887 kg ha^{-1}).

Table 2. 1. The main effect of location, plastic color mulch, and row cover treatments and their interaction on zucchini's total yield (kg ha^{-1}).

Effects	**Total yield (kg ha^{-1})**
Location	
Georgia 2020	11,451 b†
Georgia 2021	15,177 a
Alabama 2021	15,248 a
p-value	**
Plastic mulching treatment	
Silver	15,245 a
White	12,672 b
p-value	*
Cover treatment	
Row cover	15,030 a
No-cover	12,887 b
p-value	*
Location x Plastic mulching	
p-value	ns
Location x Cover	
p-value	ns
Plastic mulching x Cover	
p-value	ns
Location x Plastic mulching x Cover	
p-value	ns

ns, *, and ** Nonsignificant or significant at $p \leq 0.05$ or 0.01, respectively.

†According to the Tukey mean test, values followed by different letters indicate a significant difference ($p \leq 0.05$) among treatments.

Discussion

Daily air temperatures during the early part of the production season were within the optimum range of 20 to 30°C for whitefly development in the three locations studied, which may have resulted in ideal conditions for the growth and reproduction of whiteflies (27–30). Rainfall events likely harmed whitefly populations (30); the average whitefly population fell from 50 whiteflies per trap to 10 whiteflies per trap following a single rainfall event of 96 mm at 18 DAT in Georgia 2020. Similarly, rainfall events in Georgia 2021 (from 0 to 10 DAT) and in Alabama 2021 (from 0 to 16 DAT) totaled 45 mm and 97 mm, respectively, which may have suppressed whitefly populations early in the season in those locations and years. The negative impact of rainfall events on whitefly populations has previously been reported in the southeastern US (31).

In our study, the reflective silver plastic mulching and insect row cover treatments reduced whitefly populations, maximized dry biomass accumulation, and increased total fruit yields. The reflective silver plastic mulching treatment reduced the number of whitefly adults captured on sticky traps by 87% in Georgia 2020, 33% in Georgia 2021, and 30% in Alabama 2021 compared to white plastic mulching. Previous studies have reported similar results with zucchini plants grown in a living buckwheat (*Fagopyrum esculentum*) mulch and silver reflective mulching having lower white populations than white plastic mulch. Authors also suggested that using living or reflective mulch alone or combined with imidacloprid can reduce whitefly populations by 70% on squash plants compared to non-treated plants grown on white plastic mulch (5). Studies conducted with other crops, such as watermelon (*Citrullus lanatus*) and tomato (*Solanum lycopersicum*) (11,12), have reported lower whitefly populations when using reflective silver plastic compared to black plastic mulch. Silver plastic mulching may affect the whiteflies' phototactic responses to specific wavelengths of reflected light (32). Under visible wavelengths, the phototactic behavior of the greenhouse whitefly (*Trialeurodes vaporariorum*) was studied, and the violet and orange spectra attracted the lowest and the highest number of whiteflies, respectively (33). The reflective silver plastic mulching is known to reflect light in the blue (400 to 500 nm) and the near ultraviolet (395 nm) region (13), which explains the lower number of whiteflies being attracted to plants grown in the silver reflective plastic mulching.

Insect row covers are a temporary pest exclusion system that reduced the whitefly populations on zucchini plants to zero until their removal. A previous study reported similar results, with row covers reducing the mean densities of whiteflies recorded on zucchini plants, resulting in a higher total yield per plant than uncovered pesticide-treated plants (21). Because row covers exclude all pests, total yields increased due to the exclusion of not only whitefly but also pickleworm, *Diaphania nitidalis* (Stoll), melonworm, *Diaphania hyalinata* (L.), and several colonizing aphid species from zucchini plants commonly reported in the southeastern US (20). It is still important to highlight that insects excluding row covers must be removed at anthesis to allow flower pollination; consequently, the whitefly population increased on zucchini plants after cover removal. However, due to the larger size of the zucchini plants at anthesis, the subsequent negative impact of whiteflies on zucchini yields is lessened when compared to exposure immediately after transplanting or seedling emergence.

Whitefly populations affected the dry biomass accumulation during the growing season in all locations. High whitefly populations in the early season in Georgia 2020 likely led to a lower dry biomass accumulation compared with other locations, regardless of the mulching treatment. However, the reflective silver plastic mulching and insect row cover treatments maximized the total dry biomass accumulation in Georgia 2020 and Georgia 2021. In Georgia 2020, total dry biomass accumulation was 246.3 kg ha^{-1} higher for the reflective silver plastic mulching than the white plastic mulching and 426.9 kg ha^{-1} higher for the cover treatment than the no-cover treatment. In Georgia 2021, total dry biomass accumulation was 605.6 kg ha^{-1} higher for the reflective silver plastic mulching compared to the white plastic mulching and 130.8 kg ha^{-1} comparing cover to no-cover treatments. In Alabama 2021, using reflective silver plastic and row covers resulted in a greater crop biomass accumulation rate constant and reduced the number of days to half biomass accumulation. The effect of plastic mulches and row cover treatments on zucchini plant growth may be related to several factors besides whitefly populations. Previous studies testing different color mulches against whiteflies in tomatoes indicated that the differences in plant height may be caused by the effects of blue light on plants under high and low light intensities and also by the effects of the increased far-red to red (FR/R) light ratio of the blue mulch on the seedlings, where under relatively low light intensity, the FR/R ratio increased and resulted in increased plant heights (13). Warmer temperatures and increased plant transpiration for plants grown under row covers may have also contributed to superior dry biomass accumulation (31,34).

Differences among treatments for dry biomass accumulation corresponded to differences in total yields. Plants grown under the reflective silver plastic mulching had a 17% average increase in total yield compared to white plastic mulching, while the insect row cover treatment increased zucchini total yields by 14% compared to the no-cover treatment. These results suggest that utilizing multiple cultural practices may reduce whitefly populations and increase total yields but could be an additional cost to the grower, and the economic viability should be considered in further studies. Combining the reflective silver plastic mulching and an insect row cover could be a sustainable pest management practice for controlling whiteflies in zucchini production in the southeastern US while reducing broad-spectrum pesticide use. These practices can be considered IPM practices since row covers provide a barrier to whiteflies early season while the silver plastic mulching may assist in repelling whiteflies until the expanded plant foliage covers the mulch.

Conclusions

Sweet potato whitefly is currently the main challenge of vegetable production in the southeastern US during the fall growing season due to the insect's direct damage to vegetable crops and its role as a vector of many plant viruses. This study indicated that the insect row cover and silver reflective mulch treatments decreased whitefly populations, increased dry biomass accumulation, and enhanced total yields compared to the no-cover and standard white plastic mulching treatments in all locations. Zucchini yield increased by 17% using the silver reflective mulching and by 14% with the insect row cover. Prior research emphasizes the importance of combining different management practices to reduce the whitefly populations, and the present study corroborates with the literature. Furthermore, this study introduces new integrated pest management practices for whitefly management in the southeastern US. Future studies can be conducted to evaluate the influence of cultural practices on the severity of whitefly vector viruses and whitefly's natural enemies that were not observed in this study.

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Chapter 3 - Physical and chemical characterization of wild tomato *Solanum habrochaites* accessions for resistance to the sweetpotato whitefly

Introduction

In recent years, the sweetpotato whitefly (*Bemisia tabaci*) became one of the most significant challenges of vegetable production during the fall months in the southeastern United States. This is primarily due to the direct damage of the insect to vegetable crops and the whitefly's role as a vector of many plant viruses that lead to significant yield losses (1–5). Alternative crop management practices that reduce insecticide use to control virus vectors such as aphids, thrips, and whiteflies have been continuously evaluated on specialty crops (6–10). Alternative management tools are needed because elevated insecticide exposure has increased the resistance of whiteflies to key chemical control agents such as organophosphates, carbamates, pyrethroids, neonicotinoids, diamides, and insect growth regulators (11,12). Sustainable crop management practices are required to help growers with effective Integrated Pest Management (IPM).

Incorporating host-plant resistance (HPR) within an IPM program can be considered a relatively straightforward approach focused on excluding cultivars demonstrating significant susceptibility to arthropod pests, such as whiteflies (13). Commercially adopted single-resistance genes are commonly utilized to offer a robust defense against crucial targeted pests or pathogens. Nevertheless, the long-term effectiveness of these traits is jeopardized due to the emergence of pests and pathogens that can overcome resistance mechanisms. Tomato breeding poses a challenge due to the need to manage insect vectors and the viral diseases they transmit

(14), compounded by the crop's high susceptibility to a wide range of pests and pathogens. This susceptibility is greater than in many other crops, leading to significant yield losses and requiring more comprehensive IPM strategies (15,16). Evaluating key traits that confer reduced host attractiveness in tomato plants is essential for effective whitefly management.

One promising source of pest resistance is the wild tomato accessions of *Solanum habrochaites*, which produces specialized metabolites, such as sesquiterpenes, in the leaves' glandular trichomes. Glandular trichomes produce these defensive compounds that act as chemical and physical barriers, and sesquiterpenes, such as zingiberene, curcumene, and their derivatives, have been reported to confer resistance to various insect pests (17,18). Studies have shown that natural defense mechanisms can reduce pest populations and damage (19). Type-VI glandular trichomes of wild tomato accessions of *S. habrochaites* generate high levels of 7-epizingiberene and derivatives that are repellant and toxic to several pests, insects, and pathogens (20–22). Specific sesquiterpenes have been linked to reduced oviposition and feeding by pests, providing a dual defense mode through chemical deterrence and physical obstruction (23). The biosynthetic pathways and genetic regulation of these defensive compounds in wild tomato accessions of *S. habrochaites* have been identified as responsible for the sequential oxidation of zingiberene to more toxic derivatives, which exhibit substantial bioactivity against *B. tabaci* (17).

More knowledge is needed on the role of sesquiterpenes and glandular trichomes in pest resistance and understanding terpene production and their potential in IPM strategies. Analytical tools such as Gas Chromatography-Mass Spectrometry (GC-MS) are indispensable for characterizing and identifying these terpenes due to their precision and sensitivity (24). GC-MS has been used to identify the diverse terpenoid profiles in wild tomato species, study the genetic and biochemical pathways of terpenoid biosynthesis in tomato trichomes, and reveal terpenoids' role in plant defense against herbivores. (18,25,26). Analyzing terpene abundance and diversity in wild tomato accessions of *S. habrochaites* provides insights into their genetic diversity and potential applications, aiding in selecting desirable traits for cultivation and enabling precision breeding and metabolic engineering to enhance pest resistance in tomato varieties (20,27).

The objective of this study was to characterize the resistance of wild tomato accessions of *S. habrochaites* and commercial tomato cultivars of *Solanum lycopersicum* to the sweet potato whitefly. The research focused on categorizing trichome types (i.e., glandular and non-glandular) and detecting specific terpenes in the leaves of the tomato accessions and the commercial tomato cultivars in this study.

Materials and Methods

Experimental design, field management, and data acquisition

Field experiments were conducted at the Wiregrass Research and Extension Center of Auburn University located in Headland, AL, USA (31°21′ N, 87°14′ W) to assess the impact of wild tomato accessions on the whitefly population. The experiment was conducted in a complete randomized block design with four replications. Treatments comprised eight wild tomato accessions of *S. habrochaites* obtained from the United States Department of Agriculture (USDA) - Agricultural Research Service (ARS) - Germplasm Resources

Information Network (GRIN-Global), as described in Table 1. In addition, four commercial tomato cultivars of *S. lycopersicum*, the cherry tomatoes Cherry Bomb and Apple Yellow (Jhonny Seeds, Waterville, ME, USA) and the beefsteak tomatoes Patsy (Bejo Seeds, Oceano, CA, USA) and Mountain Man (Syngenta US, Greensboro, NC, USA) were used as controls. Each experimental unit consisted of five plants in one row, and the plants evaluated were the three central plants in the plot.

Table 3. 1. Description of the wild tomato accessions of Solanum habrochaites S. Knapp & D.M. Spooner and commercial tomato cultivars of Solanum lycopersicum L. used in the experiment.

Accession	**Taxon**	**Origin**
G29258	*S. habrochaites*	United Kingdom
G29255	*S. habrochaites*	Ecuador
PI503515	*S. habrochaites*	Peru
PI134418	*S. habrochaites*	Ecuador
PI379056	*S. habrochaites*	Chimborazo, Ecuador
PI390658	*S. habrochaites*	Peru
PI127826	*S. habrochaites*	Cajamarca, Peru
PI247087	*S. habrochaites*	Ecuador
Cherry Bomb	*S. lycopersicum* cv. Cherry Bomb	Commercial cultivar
Apple Yellow	*S. lycopersicum* cv. Apple Yellow	Commercial cultivar
Patsy	*S. lycopersicum* cv. Patsy	Commercial cultivar
Mountain Man	*S. lycopersicum* cv. Mountain Man	Commercial cultivar

The seeds of wild tomato accessions of *S. habrochaites* and commercial tomato cultivars of *S. lycopersicum* were sanitized with a 10% sodium hypochlorite solution for five minutes. They were then individually planted into 36 mm peat pellets (Jiffy Group, Lorain, OH, USA) on July 19, 2022. These pellets were then placed in 28 °C growth chambers until germination; seedlings were grown until transplanting on August 23, 2022. The soil group of the area was classified as sandy soil. Tomato plants were grown in the field in 15 cm tall, raised beds set 1.8 m apart on a center-to-center basis, with plants in rows spaced 30 cm apart. Cultural practices, including fertilization, irrigation, pest, disease, and weed management, were uniformly applied across all treatments and followed recommendations outlined in the Southeastern US 2022 Vegetable Crop Handbook (28).

A natural whitefly infestation was monitored weekly, beginning when whiteflies were observed 23 days after transplant (DAT). Populations were counted in the field 30, 37, 44, and 51 DAT. These counts were performed on two leaves from three plants within each experimental unit in the lower third of each plant before 9 am. Whitefly nymphs and eggs were counted in the laboratory on an additional leaf collected at 37, 44, and 51 DAT from the lower third of each plant. Leaflets were sampled and sealed in a Ziplock bag, transported to a laboratory, and counts were performed using a Leica M165 C High-Performance Stereo Microscope (Leica Microsystems, Wetzlar, Germany) at a magnification of 5 - 20x. To standardize the area of the count, a random area of 1cm^2 in the terminal part of the leaflet was considered, and the average number of nymphs and eggs was recorded and used for data analysis.

Weather data was collected during the growing season using an on-site weather station (Vantage Pro2 Plus, Davis Instruments, Hayward, California, USA). Air temperature, relative humidity, solar radiation, wind speed, reference evapotranspiration (ETo), rainfall, and daily maximum and minimum temperatures were recorded daily.

Scanning electron microscopy of tomato leaf trichomes

One fully developed young leaf from the upper third of the plant in the pre-flowering stage of each treatment was collected at 51 DAT to identify and quantify trichomes. The leaflets were sampled before 9 am, placed in Ziplock bags, and transported to the lab, where they were stored at room temperature (25 °C) until the SEM analysis (one day after collection). The specimens analyzed with the SEM were prepared from three small fragments of paradermal sections of the abaxial face of the leaflet's upper, middle, and lower measuring approximately 10 mm^2 from parts were mounted on an aluminum support stub with double-stick carbon tape and coated with a thin layer of gold in a sputter coating machine (EMS Q150R SCD, Quorum Technologies, Calgary, AB, Canada). Photomicrographs of the coated specimens were then taken using a scanning electron microscope (EVO 50, Carl Zeiss Vision Inc., Hebron, KY, USA) at 500X magnification and 20 kV voltage. The trichomes counting and classification (i.e., glandular and non-glandular) were employed following the methodology established by Channarayappa et al. (29) and Toscano et al. (30).

Chemicals and extraction of terpenes

Two terpenes standard mixes (Restek Corp, Bellefonte, PA, USA), namely Cannabis Terpenes standards #1 and #2, containing (-)-α-bisabolol, camphene, δ-3-carene, β-caryophyllene, geraniol, (-)-guaiol, α-humulene, p-isopropyltoluene (p-cymene), (-)-isopulegol, d-limonene, linalool, β-myrcene, nerolidol, β-ocimene, α-pinene, (-)-β-pinene, α-terpinene, γ-terpinene, terpinolene, (-)-caryophyllene oxide, 1,8-cineole (eucalyptol), each at a concentration of 2,500 µg mL^{-1}; α-zingiberene (≥ 95%) were used for the chemical profile analysis. Stock solutions of Cannabis Terpenes standards #1 and #2 and α-zingiberene standard were combined (1:1:1 v/v/v), and the working solutions were prepared by diluting stock solutions with n-hexane (VWR International LLC, Radnor, PA, USA) and stored at -18 °C.

Terpenes were extracted from intact tomato leaflet trichomes using an established leaf dip method (24). First, tomato leaflets were weighed (0.5–1.1 g), and then 5.0 mL of n-hexane was added to the plant material in a falcon tube. After shaking for 2 min at 25 ± 2 °C, the solution was centrifuged at 9,000 g for 2 min, and the extract was transferred to GC-MS glass vials.

GC-MS analysis

The GC-MS analysis was carried out using an Agilent 5977B GC/MSD (Agilent Technologies, CA, USA) according to the methodology described by Pizzo et al. (24). Terpenes were separated on an HP-5MS column (30 m × 250 µm diameter capillary, 0.25 µm film thickness). The injection volume was 1.0 µL in splitless mode at 300 °C. The solvent delay was 2 min and 48 s. The GC oven temperature program was as follows: 50 °C for 1 min, followed by temperature increasing to 300 °C at 7 °C min^{-1} and then 20 °C min^{-1} to 320 °C and held for 2 min. Helium was used as a carrier gas with a constant flow rate of 1 mL $\text{min}^{-1,}$ and terpenes were ionized using electron ionization at 70eV. The transfer line and ion source temperature were 250 °C and 230 °C, respectively. The MS data were acquired in scan mode from 50 to 550 *m/z*. All samples were injected in triplicate.

The terpenes were identified using a combination of retention time matching and mass spectral library searches, including the National Institute of Standards and Technology (NIST) MS spectral database (version 2.4, 2020). Authentic standards were used to confirm the identification of specific compounds where available. When these standards were unavailable, identification relied on achieving a NIST library matching score exceeding 80%. Compounds were categorized as "similar to" the matched compound for matching scores between 80% and 90% and identified for scores between 91% and 100%. Compounds with matching scores below 79% were deemed unidentified and excluded from the reported results. Results were expressed in a relative percentage area. Data was processed by MassHunter Qualitative Analysis software 10.0 and MassHunter Quantitative Analysis software 10.0.

Statistical analyses

The number of whitefly adults, nymphs, and eggs evaluated along the time were analyzed through repeated measures, applying restricted maximum likelihood for variance-covariance matrix modeling via the PROC-GLIMMIX procedure in SAS (Version 9.4, 2024). This approach was chosen to handle the correlation effects appropriately due to repeated sampling. The structure of the variance-covariance matrix was effectively described using an ante-dependence structure of the first order. Correlation significances were determined at a *p*-value of ≤ 0.05, with Pearson's coefficient measuring the correlation strength. In the model, the accessions were considered the fixed effects and blocks as random effects. An additional orthogonal contrast step was added to compare the wild and commercial accessions group of means. Counts of nymphs, eggs, and trichomes were modeled using a Poisson distribution, incorporating the logit function and the Laplace method. Multiple means comparisons were conducted via Tukey's test at p ≤ 0.05 upon finding significant F-values in the variance analysis.

Multivariate analyses were conducted using the number of whitefly's adults, nymphs, and eggs, the number of glandular and non-glandular trichomes, and terpene relative percentages to identify associations between wild tomato accessions of *S. habrochaites* and commercial tomato cultivars of *S. lycopersicum* and test for correlation among the measurements. A heatmap that depicted the data's patterns and correlations was generated, showcasing dissimilarities and trait variances (31,32). These differences were measured using the Euclidean distance and visualized with dendrograms created from hierarchical UPGMA cluster analysis. A Principal Component Analysis (PCA) (33,34) was also conducted to establish the level of correlation between the number of whitefly adults, nymphs, and eggs, the number of glandular and non-glandular trichomes and the type and quantity of terpenes produced by each treatment and according to their contributions toward the development of the main components. An additional Pearson's correlation matrix was also calculated to investigate the correlation between the variables measured (Appendix B).

Results

Weather conditions and whitefly infestation levels

Weather conditions varied considerably during the study (Figure 1A). In general, rainfall events were sporadic and minimal, with events rarely exceeding 2 mm, culminating in a total seasonal accumulation of 5 mm. Average daily temperature was recorded at 23.5 °C, with extremes ranging from a high of 36.2 °C to a low of 10.0 °C. Daily air temperature fluctuations became pronounced at 20 DAT. Overall, there were significant differences in the number of adult and nymph whiteflies during the growing season (Figure 1B and Figure 1D, respectively); however, there were no significant differences in the number of eggs over time (Figure 1C). The number of adult whiteflies per leaf was higher in the mid to late evaluation dates. Similarly, the number of nymphs per cm² of leaf initially recorded at one nymph on 37 DAT increased by 44 DAT before slightly decreasing at 51 DAT.

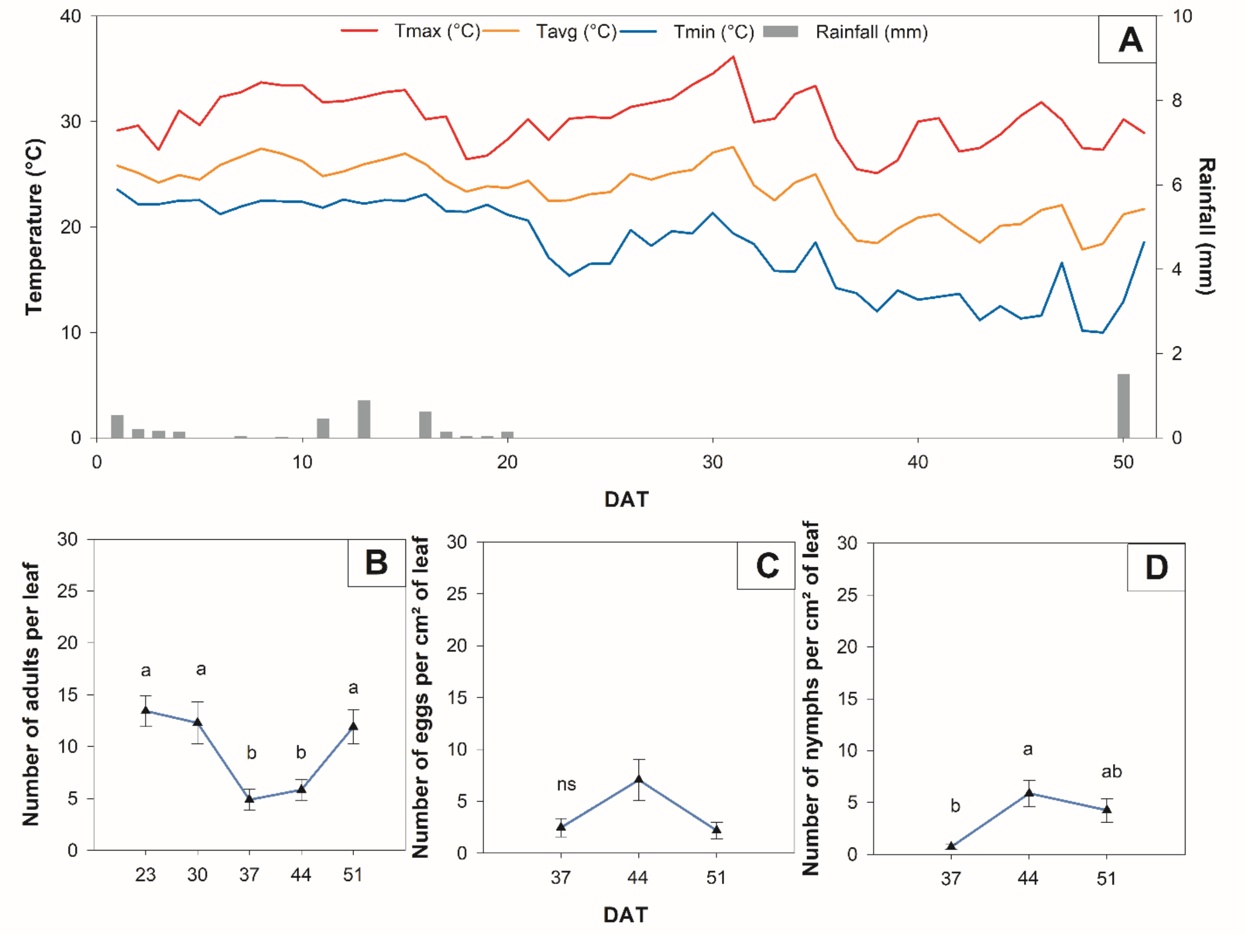

Figure 3. 1. Daily weather variation with minimum (Tmin), average (Tavg), and maximum (Tmax) temperatures in degrees Celsius (°C) and rainfall events in millimeters (mm) over days after transplant (DAT) of wild tomato accessions of *Solanum habrochaites* and commercial tomato cultivars of *Solanum lycopersicum* (A) and the impact on the number of adult whiteflies per leaf (B), the number of eggs per cm² of leaf (C), and the number of nymphs per cm² of leaf (D) in Alabama 2022. According to Tukey's mean test, different lowercase letters indicate a significant difference ($p \leq 0.05$) among accession treatments, and ns indicate no significant difference.

Although weather conditions likely influenced the whitefly population, the interaction between repeated assessments over time and tomato plant treatments was non-significant. Only the main effect of the treatments had a significant impact on the whitefly population (Figure

2). Generally, wild tomato accessions of *S. habrochaites* had significantly higher whitefly adult levels than commercial tomato cultivars of *S. lycopersicum* (Figure 2C). Commercial tomato cultivars averaged 5 adults per leaf, whereas the wild tomato accessions averaged 11 adults per leaf. Among the wild tomato accessions, the G29258, PI127826, and PI134418 accessions presented the lowest counts with 5, 7, and 5 adults per leaf, respectively. The commercial tomato cultivar Apple Yellow had the lowest infestation rate, averaging 4 adults per leaf.

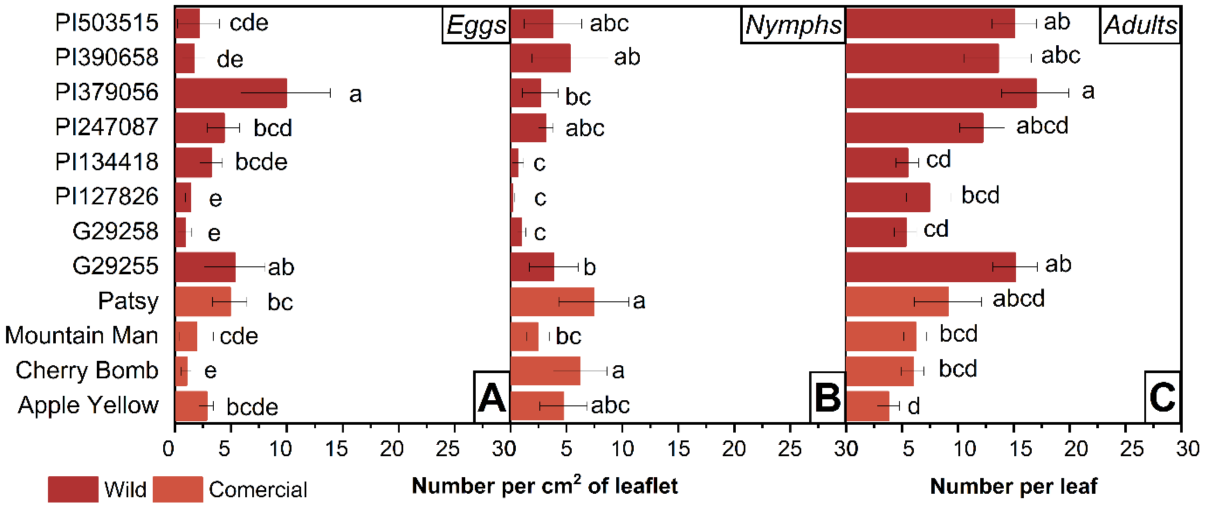

Figure 3. 2. Effect of wild tomato accessions of *Solanum habrochaites* and commercial tomato cultivars of *Solanum lycopersicum* on the number of eggs per cm^2 of leaf (A), number of nymphs per cm^2 of leaf (B), and number of adult whiteflies per leaf (C). Each bar represents the mean value plus the error bar represented by the standard error of the mean. According to Tukey's mean test, different lowercase letters indicate a significant difference ($p \leq 0.05$) among accession treatments.

There were no significant differences in egg counts between commercial and wild tomato cultivars, recording averages of 3 and 4 eggs per cm^2 (Figure 2A), respectively. However, the wild tomato accessions G29258 and PI127826, along with the commercial tomato cultivar Cherry Bomb, reported fewer eggs than other treatments, underscoring some variability within the respective groups.

The number of nymphs differed significantly among treatments (Figure 2B). Wild tomato accessions averaged 3 nymphs per cm^2, while the commercial tomato cultivars averaged 5 nymphs per cm^2. This indicates that the wild tomato accessions generally reduced the average number of nymphs compared to the commercial tomato cultivars. Within the wild tomato accessions of *S. habrochaites*, the PI127826 and G29258 accessions reduced the number of nymphs per cm^2 by 80% compared to the average of 5 nymphs per cm^2 observed in the commercial tomato cultivars. Among all the accessions studied, the PI134418 accession represented the most substantial reduction in the number of nymphs, lowering the average number per cm^2 to zero.

Comparison of non-glandular and glandular trichome densities across accessions

The number and type of trichomes (i.e., non-glandular and glandular – Appendix A) significantly differed among treatments (Figure 3). The commercial tomato cultivar Apple Yellow had the most non-glandular trichomes, averaging 80 non-glandular trichomes μm^{-2}. In contrast, the wild tomato accession PI127826 had the lowest number of non-glandular trichomes, averaging 2 non-glandular trichomes μm^{-2}. Contrarily, the wild tomato accessions G29258 (14 glandular trichomes μm^{-2}) and PI127826 (13 glandular trichomes μm^{-2}) had the highest number of glandular trichomes μm^{-2}, followed by PI134418 (9 glandular trichomes μm^{-2}) and PI390658 (10 glandular trichomes μm^{-2}). Commercial tomato cultivars Apple Yellow, Mountain Man, Cherry Bomb, and Patsy had no glandular trichomes.

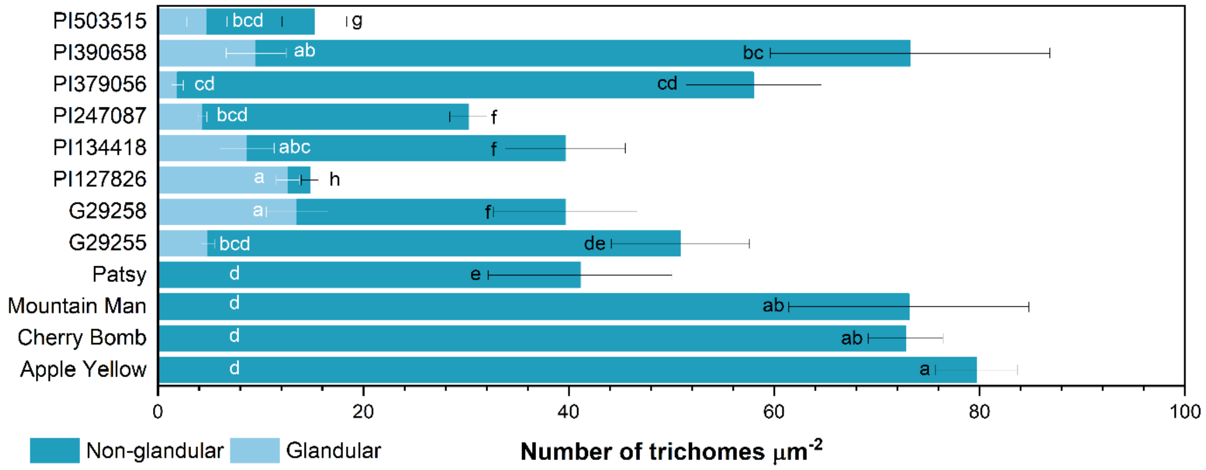

Figure 3. 3. Distribution of non-glandular and glandular trichome densities comparing wild tomato accessions of *Solanum habrochaites* and commercial tomato cultivars of *Solanum lycopersicum*. According to Tukey's mean test, different lowercase letters indicate a significant difference ($p \leq 0.05$) among accession treatments.

Relative abundance of terpenes in the tomato plants

The GC-MS analysis of leaf extracts indicated the presence of 25 terpene compounds among wild tomato accessions and commercial tomato cultivars (Figure 4). Terpenes generally differed significantly between wild tomato accessions and commercial tomato cultivars. Commercial tomato cultivars (i.e., Patsy, Apple Yellow, Cherry Bomb, and Mountain Man) had higher levels of monoterpenes than sesquiterpenes. The monoterpene D-limonene was the most prominent compound. However, smaller amounts of β-phellandrene, β-caryophyllene, α-humulene, and an unidentified compound that produced a mass spectrum similar to zingiberenol were also identified in the commercial tomato cultivars. In contrast, wild tomato accessions had higher levels of sesquiterpenes than monoterpenes. Accessions G29258, G29255, and PI134418 had the highest levels of β-caryophyllene and α-humulene, while PI127826, PI247087, PI379056, PI390658, and PI503515 had the lowest. Notably, the α-zingiberene and 9-hydroxy-zingiberene were only identified for the PI127826 accession.

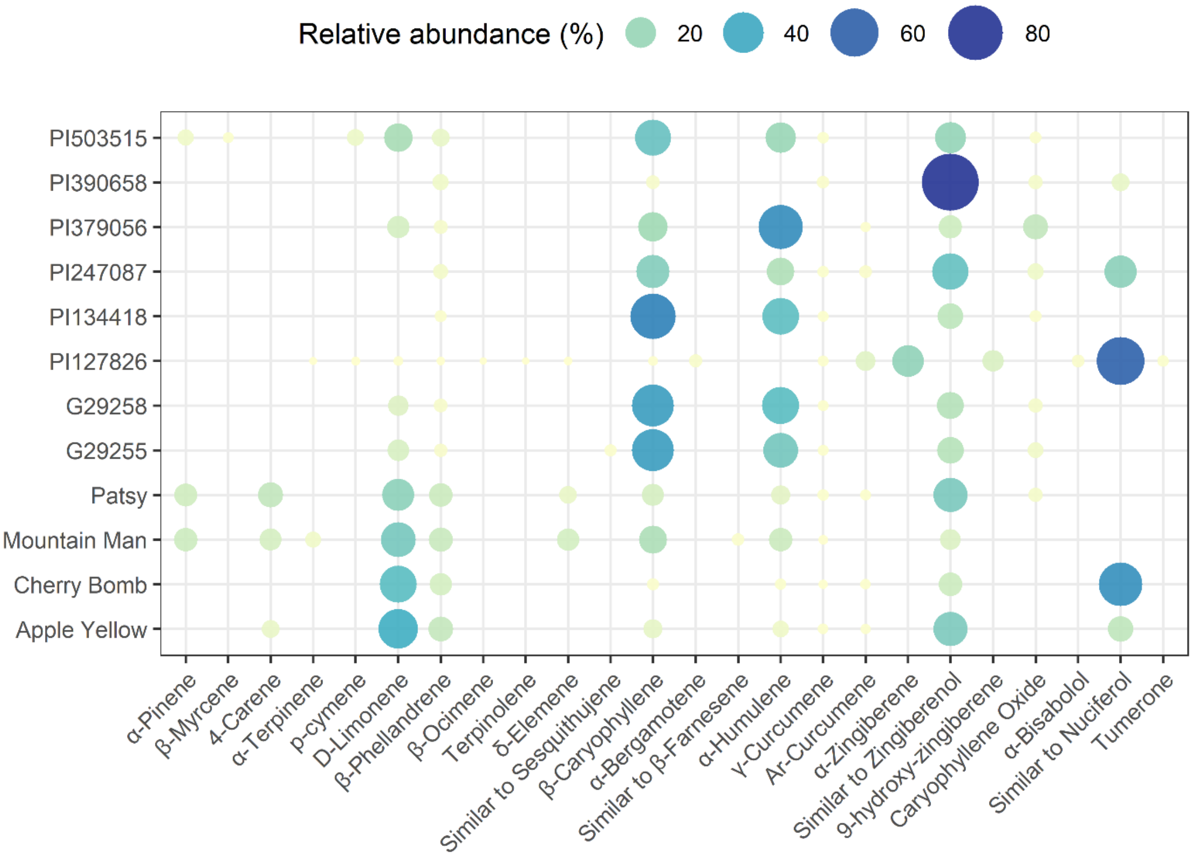

Figure 3. 4. Relative abundance of terpenes in wild tomato accessions of *Solanum habrochaites* and commercial tomato cultivars of *Solanum lycopersicum*.

Multivariate analysis among wild and commercial tomato accessions

The multivariate analysis separated treatments into three distinct groups (Figure 5). The PCA biplot (Figure 5A) illustrates the distribution of tomato accessions based on whitefly infestation levels, the number of non-glandular and glandular trichomes, and the terpene profile represented by the most important terpenes identified. The two principal components, PC1 and PC2, accounted for 53.6% and 23.5% of the variance, respectively.

Cluster 1 included all the commercial tomato cultivars of *S. lycopersicum* (i.e., Patsy, Mountain Man, Apple Yellow, and Cherry Bomb) characterized by high levels of β-phellandrene, D-limonene, 4-carene, δ-elemene, number of nymphs, and non-glandular trichomes. Cluster 2 included wild tomato accessions of *S. habrochaites* PI503515, PI390658, PI379056, PI247087, PI134418, G29255, and G29258, which presented the highest glandular trichomes and different terpenes. Cluster 3 comprised a particular wild tomato accession of *S. habrochaites* PI127826, distinguished by unique traits not strongly associated with other accessions, such as the low number of whitefly nymphs, eggs, and adults, high numbers of glandular trichomes, and terpenes, such as α-bisabolol, Ar-curcumene, β-ocimene, terpinolene, tumerone, α-bergamotene, nuciferol, α-zingiberene, and 9-hydroxy-zingiberene.

The hierarchical cluster analysis (Figure 5B) confirmed the three clusters identified by the PCA and further separated the analyzed variables. It revealed that the number of nymphs was inversely proportional to the number of glandular trichomes and the presence of terpenes. The heatmap showed common patterns shared by wild tomato accessions and commercial cultivars within the same cluster. A positive correlation was found between the number of whitefly adults and eggs, but no correlation with nymphs. Conversely, a negative correlation was observed between glandular trichomes and nymphs. Also, D-limonene and β-Phellandrene were positively correlated with the number of nymphs and glandular trichome density. The Appendix B show the complete Pearson's correlation matrix.

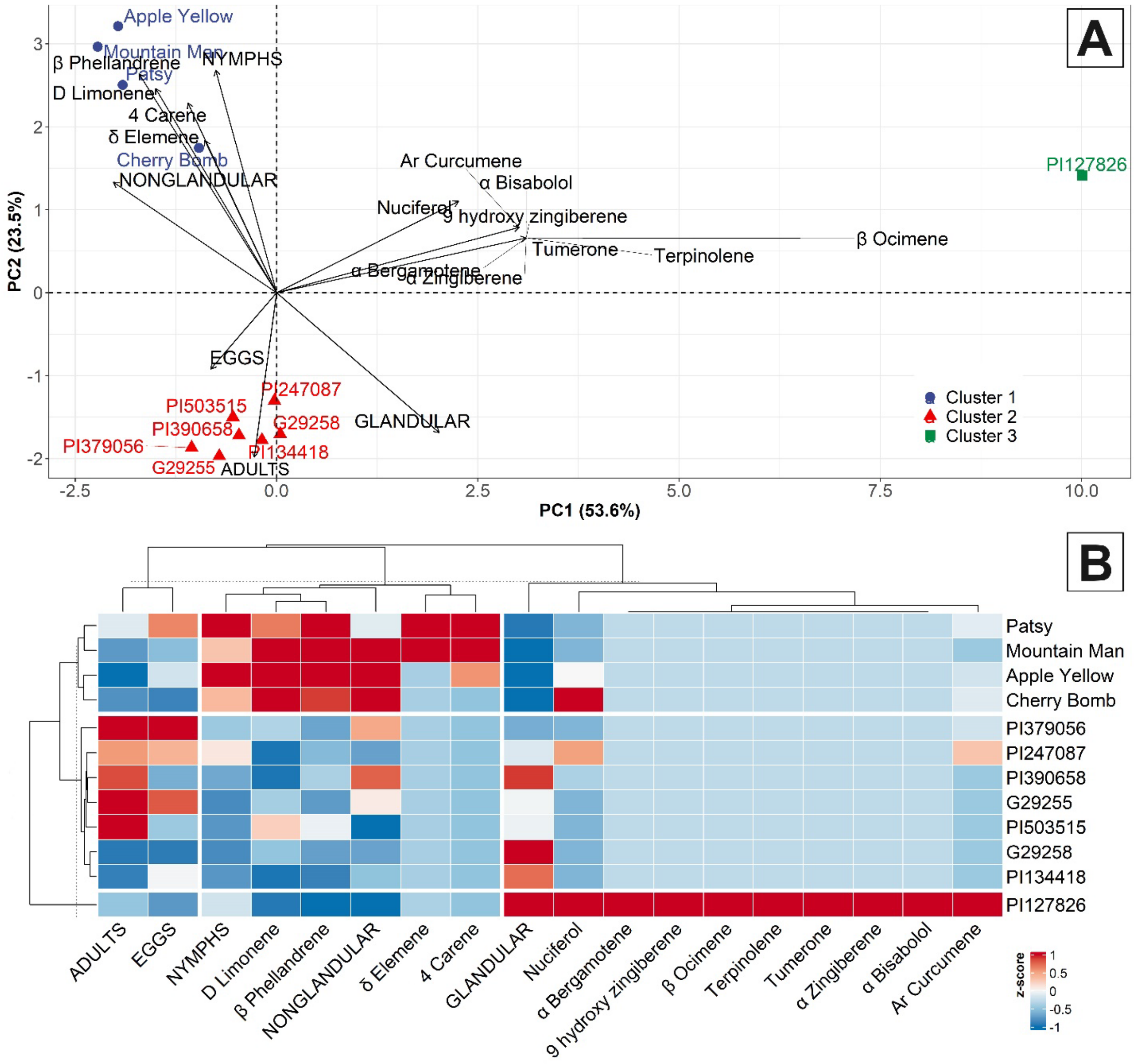

Figure 3. 5. Multivariate analysis of the wild tomato accessions of *Solanum habrochaites* and commercial tomato cultivars of *Solanum lycopersicum* based on terpene profiles and field traits response to sweet potato whitefly. The PCA biplot (A) identifies three distinct clusters of accessions, revealing significant variations in terpene abundance and trichome types. The cluster analysis (B) further delineates these relationships, showing the relative expression of traits across accessions and highlighting biochemical and biological trait interactions.

Discussion

Rainfall events were lacking and did not negatively impact whitefly populations in this study (Fig 1). Fluctuations in temperatures before, during, and after 30 to 50 DAT mirror trends in whitefly adult counts observed; numbers were higher when the average daily air temperature was higher during 23, 30, and 51 DAT insect counts. A reduction in adult whiteflies did not result in a significant decrease in the number of eggs. Nymphs increased from 37 to 44 DAT and were intermediate at 51 DAT. The number of nymphs was like the number of eggs and increased after the eggs hatched. Weather conditions, particularly temperature variations, significantly influenced whitefly population dynamics during the growing season. Warm average daily air temperatures from 20 to 30 °C without extreme fluctuations are generally favorable for whitefly reproduction and development (35–37). Additionally, rainfall events physically knock off whiteflies and disrupt their lifecycle (1,38,39).

Our results identified different impacts of wild tomato accessions of *S. habrochaites* and commercial tomato cultivars of *S. lycopersicum* on whitefly populations under field conditions. Fewer differences in the number of eggs were observed among wild tomato accessions and commercial tomato cultivars. More differences were observed in the number of adults and nymphs among the wild tomato accessions of *S. habrochaites* and the commercial tomato cultivars of *S. lycopersicum*. When averaged across treatments, the wild tomato accessions had five more adults per leaf than the commercial tomato cultivars. The wild tomato accessions PI134418, PI127826, and G29258 did not differ from Apple Yellow, which had the fewest adults per leaf, but differed from PI379056, which had the most adults. However, the most notable reduction was observed in the number of eggs and nymphs for accessions PI134418, PI127826, and G29258 compared to the other accessions tested. These results elucidate findings of previous studies suggesting that some wild tomato accessions of *S.*

habrochaites may deleteriously affect whitefly adults' oviposition and nymphs' development (18,40–42).

In our study, it was possible to identify that the wild tomato accessions PI127826 and G29258 had more glandular trichomes type IV than other treatments. Also, PI134418 and PI390658 presented a high amount of glandular trichomes but did not differ from the other wild accessions. The presence of type IV trichomes in tomato genotypes from the wild tomato accession PI127826 correlated with higher resistance to arthropods, suggesting that trichome density and type play an essential role in plant defense mechanisms (40,41). These trichomes act as a physical barrier, but their primary role is the release of toxic compounds such as the 7-epi-zingiberene and its derivatives that provide a chemical defense mechanism against whiteflies (17,18). Glandular trichomes secrete various secondary metabolites such as terpenes, acylsugars, phenylpropanoids, and flavonoids, which are involved in plant defense by acting as repellents against pests and inhibiting pathogens' growth (43,44).

The GC-MS analysis of leaf extracts was a valid quantitative method to quantify zingiberenoids in tomato leaflet washes (45). It was possible to identify 25 terpenes and calculate the relative abundance of those in the samples extracted from the tomato leaflets. The terpene profile showed that the wild tomato accession PI127826 was the only one to produce Ar-curcumene, α-zingiberene, and 9-hydroxy-zingiberene, a derivative of 7-epi-zingiberene, compounds that are known to confer the resistance to whitefly in tomato plants from *S. habrochaites*, corroborating previous studies (17,18). Other terpenes, such as D-limonene, were identified in high proportion in all commercial tomato cultivars of *S. lycopersicum* and the wild tomato accessions PI503515 and PI379056. Similar results were found when testing essential oils of two varieties of rosemary (*Rosmarinus officinialis*) associated with the limonene, one of the volatiles responsible for attracting whiteflies at moderate concentrations in the bioassays (46).

The multivariate analysis revealed a strong correlation between glandular trichomes, specific terpene profiles, and reduced whitefly populations in tomato plants. Commercial tomato cultivars of *S. lycopersicum* in Cluster 1 had the highest number of non-glandular trichomes and four specific terpenes (i.e., β-phellandrene, D-limonene, 4-carene, and δ-elemene), which increased the number of nymphs. In contrast, wild tomato accessions of *S. habrochaites* within Cluster 2 had more glandular trichomes and distinct terpene profiles. Individually, wild tomato accession PI127826 had the highest number of glandular trichomes and a unique terpene profile (α-bisabol, Ar-curcumene, β-ocimene, terpinolene, tumerone, α-bergamotene, nuciferol, α-zingiberene, and 9-hydroxy-zingiberene) leading to significant reductions in the number of whitefly adults, eggs, and nymphs. Previous studies reported that wild tomato accessions of *S. habrochaites*, PI127826, and PI134418 had glandular trichomes that negatively impacted pests (47,48); however, these studies had not assessed the chemical profile of tomato leaves.

Our study is the first to report the impact of wild tomato accession of *S. habrochaites* G29258 on whiteflies, indicating the significant potential this accession offers breeding programs for commercial tomatoes focused on whitefly resistance. The other wild tomato accessions of *S. habrochaites* evaluated, PI503515, PI390658, PI379056, PI247087, and

G29255, had fewer glandular trichomes than PI134418 and G29258. The negative correlation between glandular trichomes and whitefly nymphs suggests that a higher density of glandular trichomes effectively reduces the number of nymphs, reinforcing the importance of both the presence and density of glandular trichomes for pest resistance (49).

Whitefly resistance is linked to allelochemicals in glandular trichomes, which are natural chemicals antagonistic to pests (50,51). Overall, terpene profiles differed among the wild tomato accessions of *S. habrochaites* and between the wild tomato accessions and commercial tomato cultivars of *S. lycopersicum*. Previously, the 9-hydroxy zingiberene and 9-hydroxy,10,11-epoxy-zingiberene were reported to be more effective repellents against mites than 7-epi-zingiberene (52). In the present study, high levels of α-zingiberene and 9-hydroxy-zingiberene, along with Ar-curcumene and other terpenes, explain the adequate capacity of wild tomato accessions of *S. habrochaites*: PI127826 in reducing the number of whiteflies. Also, this accession can be utilized in breeding programs aimed at enhancing whitefly resistance by serving as a donor parent for its valuable allelochemical profile. These terpenes, identified using GC-MS, exhibit strong repellent and deterrent properties against whiteflies. The beneficial alleles associated with these terpenes can be introgressed into commercial tomato cultivars through conventional breeding or marker-assisted selection, thus enhancing their resistance to whitefly infestation. Overall, the GC-MS method's ability to identify and quantify terpene profiles was crucial for selecting donor and recurrent parents in breeding programs, influencing the identity and abundance of these compounds (45).

Conclusions

Our research demonstrates that specific wild tomato accessions of *S. habrochaites*, particularly PI127826, G29258, and PI134418, exhibit significant resistance to whiteflies compared to four commercial tomato cultivars of *S. lycopersicum*. This resistance is likely due to glandular trichomes, which secrete secondary metabolites such as Ar-curcumene, α-zingiberene, and 9-hydroxy-zingiberene. These compounds, identified through GC-MS analysis, act as strong deterrents against whiteflies, providing physical and chemical defenses. The unique terpene profiles of these wild accessions, confirmed by multivariate analysis, further support their effectiveness in reducing whitefly populations. Our findings highlight the potential of incorporating wild *S. habrochaites* accessions into breeding programs to enhance whitefly resistance in commercial tomatoes, contributing to more sustainable integrated pest management (IPM) strategies. The analysis of terpene abundance offers valuable insights into the genetic diversity and potential applications of these wild tomatoes for breeding purposes. However, future research should explore the genetic mechanisms behind trichome development and terpene biosynthesis to facilitate the transfer of these traits into commercial cultivars, with field trials under diverse environmental conditions to validate their efficacy.

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Chapter 4 – Role of glandular trichomes and terpene diversity on tomato resistance against the sweetpotato whitefly

Introduction

Sweetpotato whiteflies *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) represent a significant global agricultural pest, particularly impacting vegetable crop production. This pest directly damages plants by feeding on their sap and serves as a vector for plant viruses, resulting in considerable yield losses and increased production costs (1,2). Recent studies highlight whitefly infestations' severe impact on crop efficiency, with losses correlating to a drop in technical efficiency in affected farms (3). Managing whitefly populations is challenging due to the rapid development of resistance to chemical pesticides, raising concerns over ecological impacts and human health risks from pesticide overuse (4–7). Consequently, there is an urgent need for Integrated Pest Management (IPM) strategies that emphasize sustainable pest control methods and reduce reliance on chemicals while maintaining crop productivity.

To reduce dependence on synthetic pesticides, researchers are increasingly exploring plant-based defense mechanisms as an additional strategy for pest management (8). Plants have developed complex chemical defenses, such as the synthesis of terpenes, which play a crucial role in preventing herbivores by interfering with feeding behaviors and triggering defense signaling pathways (9). Terpenes are a diverse group of organic compounds primarily produced by plants, derived from isoprene units. They can range from simple monoterpenes to more complex sesquiterpenes, diterpenes, sesterpenes, triterpenes, tetraterpene, and polyterpenes (10,11). Given their versatility properties, terpenes represent a promising approach for developing pest-resistant crops that rely on intrinsic chemical defenses.

Wild tomato species from *Solanum* sp. lycopersicon section exhibit broader pest resistance traits than cultivated varieties from *Solanum lycopersicum*, often attributed to their dense glandular trichome coverage and presence of secondary metabolites (12–14). Glandular trichomes can produce, store, or secrete a variety of non-volatile and volatile specialized metabolites, providing physical and chemical barriers against different insect herbivores (15–19). The type-IV and type-VI glandular trichomes present in wild tomato species such as *Solanum habrochaites* (20–22) and *Solanum galapagense* (23–25) are associated with the presence of specific sesquiterpenes such as zingiberene and their derivatives, which have shown significant resistance against whiteflies as demonstrated by various research studies (26–29).

Type-IV and type-VI glandular trichomes in *Solanum* species contain distinct yet overlapping specialized metabolites. Although some metabolite classes are shared between trichome types, quantitative differences in these profiles can distinguish individual *Solanum* species (30). In this context, analyzing terpene diversity and abundance in wild tomato accessions offers valuable insights into phenotypic variation and aids in identifying desirable traits for tomato cultivation (31). This knowledge supports precision breeding and metabolic engineering efforts, significantly enhancing pest resistance in cultivated varieties. Exploring these wild relatives deepens our understanding of natural pest resistance and presents promising avenues for breeding durable, pest-resistant tomato crops (32).

Therefore, the objective of this study was to investigate whitefly resistance in wild tomato accessions of *Solanum habrochaites*, *Solanum galapagense*, *Solanum chilense*, and cultivars of *Solanum lycopersicum*. The research focused on characterizing both glandular and non-glandular trichomes, identifying and quantifying terpenes, and correlating these factors with reductions in whitefly populations.

Results

Whitefly population and trichome density (non-glandular vs. glandular)

The interaction between repeated assessments over time and tomato plant treatments was not statistically significant. However, a significant main effect of wild tomato accessions and commercial tomato cultivars on whitefly populations was observed, specifically in the density of nymphs per cm^2 (Table 1). While no significant differences were found for adults and egg densities, wild tomato accessions, including *S. habrochaites* (G29258, PI126449, PI127826, PI134418, PI209978), *S. galapagense* (LA1401), and *S. chilense* (LA1932), exhibited significantly lower nymph densities than the cultivars of *S. lycopersicum* (LA3475, Cherry Bomb, Apple Yellow, Patsy, and Mountain Man). On average, commercial cultivars hosted around 5 nymphs per cm^2, while wild accessions reduced this number to 0 (Figure 1A). Among the commercial cultivars, Apple Yellow and Cherry Bomb were the most susceptible, showing infestation rates of 8 and 7 nymphs per cm^2, respectively.

Table 4. 1. Effect of wild tomato accessions and commercial tomato cultivars on key parameters associated with whitefly resistance and trichome distribution. Values represent the mean and standard error of the mean number of adult whiteflies per leaf, number of eggs and nymphs per cm^2 of leaf, and the distribution of non-glandular and glandular trichome densities from wild tomato accessions of *S. habrochaites* (G29258, PI126449, PI127826, PI134418, PI209978), *S. galapagense* (LA1401), and *S. chilense* (LA1932), and cultivars of *S. lycopersicum* (LA3475, Cherry Bomb, Apple Yellow, Patsy, and Mountain Man).

Accession	**Number of adults per leaf**	**Number of eggs per cm^2**	**Number of nymphs per cm^2**		**Number of non-glandular trichomes µm^{-2}**		**Number of glandular trichomes µm^{-2}**	
Apple Yellow	2 ± 0	2 ± 1	8 ± 2	a †	73 ± 6	a	0 ± 0	e
Cherry Bomb	2 ± 0	1 ± 1	7 ± 1	a	49 ± 4	b	0 ± 0	e
Mountain Man	2 ± 0	2 ± 1	4 ± 1	ab	53 ± 2	b	0 ± 0	e
Patsy	2 ± 0	1 ± 1	4 ± 2	ab	48 ± 2	b	0 ± 0	e
LA1401	1 ± 0	1 ± 1	0 ± 0	b	0 ± 0	e	27 ± 2	a
LA1932	1 ± 0	2 ± 1	1 ± 0	b	48 ± 6	b	3 ± 1	de
LA3475	1 ± 0	3 ± 1	5 ± 2	ab	51 ± 5	b	0 ± 0	e
G29258	2 ± 0	0 ± 0	0 ± 0	b	23 ± 3	d	6 ± 1	d
PI126449	1 ± 0	0 ± 0	0 ± 0	b	40 ± 3	bc	7 ± 1	d
PI127826	1 ± 0	0 ± 0	1 ± 1	b	0 ± 0	e	20 ± 2	b
PI134418	2 ± 1	0 ± 0	0 ± 0	b	30 ± 3	cd	14 ± 1	c
PI209978	2 ± 0	2 ± 1	0 ± 0	b	0 ± 0	e	26 ± 2	a
p-value	ns	ns	*		*		*	

ns and *: nonsignificant or significant at $p \leq 0.01$, respectively. † According to Tukey's mean test, values (mean ± SE) followed by different letters indicate a significant difference ($p \leq 0.05$) among treatments.

Significant differences were also observed in trichome numbers and types across the accessions (Table 1). *S. lycopersicum* cultivars displayed a higher density of non-glandular trichomes, averaging 55 non-glandular trichomes per µm², compared to an average of 20 non-glandular trichomes per µm² in wild accessions. In contrast, glandular trichomes were present in wild accessions, averaging 15 glandular trichomes per µm², but were generally absent in *S. lycopersicum* cultivars. Apple Yellow had the highest density of non-glandular trichomes among the cultivars, averaging 73 non-glandular trichomes per µm². At the same time, wild accessions, such as LA1401, PI127826, and PI209978, exhibited the lowest densities, with 0 non-glandular trichomes recorded. Wild accessions such as LA1401 (27 glandular trichomes per µm²) and PI209978 (26 glandular trichomes per µm²) demonstrated the highest densities of glandular trichomes. *S. lycopersicum* cultivars, including LA3475, Cherry Bomb, Apple Yellow, Patsy, and Mountain Man, did not exhibit any glandular trichomes. Detailed density and types of trichomes are illustrated in Figure 1B.

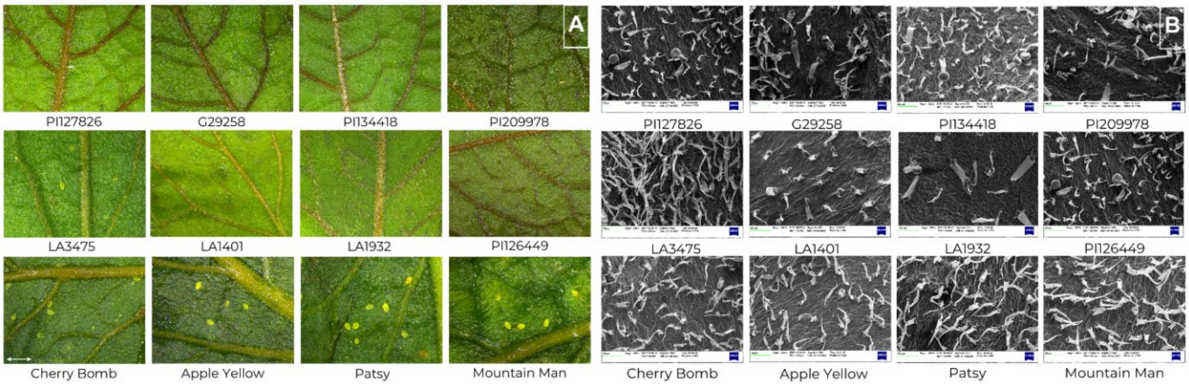

Figure 4. 1. (A) Microscope images of the underside of leaflets from tomato varieties, and (B) scanning electron micrographs showing the abaxial surface of leaflets from wild tomato accessions of *Solanum habrochaites* (G29258, PI126449, PI127826, PI134418, PI209978), *Solanum galapagense* (LA1401), and *Solanum chilense* (LA1932), and cultivars of *Solanum lycopersicum* (LA3475, Cherry Bomb, Apple Yellow, Patsy, and Mountain Man). These images highlight the absence of nymphs on the leaflets of wild accessions and emphasize the morphological differences in trichome structures between wild tomato accessions and cultivars. Scale bars: 1 cm and 100 µm.

Terpenes profile and content in tomato plants

The external calibration curves for the 22 terpene standards demonstrated high linearity within the concentration range of 0.2 to 10 µg mL^{-1}, with r^2 values ranging from 0.9936 to

0.9994 (Table 2). This strong correlation between analyte concentration and detector response indicates that the method can accurately quantify terpenes across various concentrations.

Table 4. 2. Linear range, analytical curve equation, coefficient of determination, limit of detection, and limit of quantification of terpenes using gas chromatography-mass spectrometry (GC-MS) analysis.

Compound	***m/z***	**Linear range (µg mL^{-1})**	**Equation**[a]	**r^2**[b]	**LOD**[c] (µg mL^{-1})	**LOQ**[d]
α-pinene	136.13	0.2 – 10	y = 444197.97x - 5770.52	0.9994	0.04	0.20
camphene	136.13	0.2 – 10	y = 58906.73x - 6150.07	0.9973	0.02	0.20
β-pinene	136.13	0.2 – 10	y = 466560.03x - 33510.13	0.9994	0.08	0.20
β-myrcene	136.13	0.8 – 10	y = 268988.61x + 20149.47	0.9945	0.30	0.80
3-carene	136.13	0.2 – 10	y = 80430.35x - 8495.88	0.9973	0.03	0.20
α -terpinene	136.13	0.3 – 10	y = 406526.62x - 14314.80	0.9991	0.08	0.30
p-cymene	134.11	0.2 – 10	y = 1008313.29x + 327727.73	0.9985	0.03	0.20
d-limonene	136.13	0.2 – 10	y = 315219.90x - 7767.59	0.9988	0.06	0.20
eucalyptol	154.14	0.4 – 10	y = 79675.49x + 1919.79	0.9974	0.10	0.40
β-ocimene	136.13	0.2 – 10	y = 19761.08x - 1463.91	0.9982	0.06	0.20
γ-terpinene	136.13	0.6 – 10	y = 342875.25x + 35305.80	0.9936	0.20	0.60
terpinolene	136.13	0.2 – 10	y = 266143.69x - 21650.75	0.9985	0.07	0.20
linalool	154.14	0.9 – 10	y = 168475.87x - 18808.94	0.9979	0.30	0.90
isopulegol	154.14	0.3 – 10	y = 14947.22x - 4364.33	0.9968	0.09	0.30
geraniol	154.14	0.4 – 10	y = 39554.53x - 18295.90	0.9937	0.10	0.40
β-caryophyllene	204.19	0.2 – 10	y = 149241.79x - 14478.84	0.9993	0.07	0.20
humulene	204.19	0.2 – 10	y = 439459.88x - 47020.59	0.9993	0.06	0.20
α-zingiberene	204.19	0.2 – 10	y = 96782.78x - 26039.41	0.9969	0.06	0.20
nerolidol	222.20	0.3 – 10	y = 96964.72x - 40147.24	0.9954	0.09	0.30
caryophyllene oxide	219.17	0.2 – 10	y = 35558.48x - 5201.49	0.9988	0.05	0.20
guaiol	222.20	0.3 – 10	y = 18640.30x - 5078.66	0.9978	0.10	0.30
α-bisabolol	222.20	0.2 – 10	y = 170420.91x - 49991.29	0.9983	0.06	0.20

[a] y = bx + a; [b] r^2: Coefficient of determination; [c] LOD: Limit of detection; [d] LOQ: Limit of quantification.

The limits of detection (LOD) for the various terpenes ranged from 0.02 to 0.30 µg mL^{-1}, while the limits of quantification (LOQ) varied between 0.2 and 0.9 µg mL^{-1} (Table 2). These values demonstrate the method's sensitivity for detecting and quantifying terpenes in extracts from tomato leaflet trichomes. A hexane blank was randomly included in each run to assess potential carryover. No detectable analyte peaks were observed in the blank runs, indicating minimal carryover.

The field-grown wild tomato accessions and commercial tomato cultivars were subjected to leaf dip extractions and were analyzed by GC-MS. The analysis identified 24 terpenes based on their retention times (RT) and mass spectral patterns. Table 3 presents the identified compounds, including their RT, qualitative and quantitative ions (*m/z*), molecular formula, terpene content (µg g^{-1}), and classification. The classifications include acyclic monoterpenes, bicyclic monoterpenes, monocyclic monoterpenes, acyclic sesquiterpenes, monocyclic sesquiterpenes, and bicyclic sesquiterpenes. The variability of terpenes among wild accessions and cultivars highlighted substantial differences. Among the terpenes identified, α-pinene (<LOD – 15.99 µg g^{-1}), 4-carene (0.29 – 23.04 µg g^{-1}), α-phellandrene (<LOD – 3.46 µg g^{-1}), β-phellandrene (<LOD – 10.11 µg g^{-1}), β-caryophyllene (<LOQ – 23.51 µg g^{-1}), δ-elemene (<LOQ – 2.41), humulene (<LOQ – 7.81 µg g^{-1}), and α-zingiberene (1.27 – 27.77 µg g^{-1}) are common. However, their concentrations varied significantly across tomato accessions and cultivars.

PI209978 and PI127826 exhibited notably high concentrations of zingiberene-related sesquiterpenes, including 9-hydroxy-10,11-epoxy-zingiberene (28.93 µg g^{-1} and 46.82 µg g^{-1}, respectively) and 9-hydroxy-zingiberene (30.19 µg g^{-1} and 24.06 µg g^{-1}, respectively), which were absent in other tomato plants. Similarly, α-zingiberene was abundant in PI209978 and PI127826, with 27.77 µg g^{-1} and 27.07 µg g^{-1}, respectively. In contrast, it was present at lower levels in other wild tomato accessions, including G29258 (2.96 µg g^{-1}), LA1401 (2.89 µg g^{-1}), LA1932 (1.44 µg g^{-1}), and in the commercial cultivar Mountain Man (1.27 µg g^{-1}).

Table 4. 3. Terpene content in wild tomato accessions (*Solanum habrochaites*: G29258, PI126449, PI127826, PI134418, PI209978; *Solanum galapagense*: LA1401; *Solanum chilense*: LA1932) and tomato cultivars (*Solanum lycopersicum*: LA3475, Cherry Bomb, Apple Yellow, Patsy, Mountain Man) extracted from intact tomato leaflet trichomes.

Compound	**Terpene content ($\mu g\ g^{-1}$)[a]**											
	LA1401	**LA3475**	**LA1932**	**G29258**	**PI209978**	**PI127826**	**PI126449**	**PI134418**	**Cherry Bomb**	**Apple Yellow**	**Patsy**	**Mountain Man**
α-pinene	3.40 ± 0.73	<LOQ	0.64 ± 0.96	N.D.	15.99 ± 24.29	N.D.	N.D.	N.D.	0.59 ± 0.89	<LOD	0.96 ± 0.75	0.81 ± 0.21
camphene	N.D.[c]	N.D.	N.D.	N.D.	0.65 ± 0.98	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
similar to p-cymene[b]	<LOQ	<LOQ	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	<LOQ	<LOQ	2.05 ± 0.27	2.23 ± 0.57
β-pinene	<LOQ	N.D.	<LOD	N.D.	4.08 ± 6.21	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
4-carene[b]	2.85 ± 0.36	11.18 ± 4.99	1.28 ± 0.12	0.29 ± 0.44	1.85 ± 1.54	N.D.	N.D.	0.31 ± 0.46	12.34 ± 7.79	2.17 ± 0.90	23.04 ± 10.59	14.69 ± 4.45
α-phellandrene[b]	<LOD	<LOQ	<LOD	N.D.	1.90 ± 0.06	0.53 ± 0.10	N.D.	<LOD	1.51 ± 2.48	<LOQ	3.46 ± 1.53	2.47 ± 0.70
3-carene	N.D.	N.D.	N.D.	N.D.	<LOQ	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
α-terpinene	<LOQ	<LOQ	N.D.	N.D.	<LOQ	<LOQ	N.D.	N.D.	<LOQ	<LOQ	0.86 ± 0.70	<LOQ
p-cymene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
d-limonene	N.D.	N.D.	N.D.	N.D.	N.D.	<LOQ	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
β-phellandrene[b]	<LOQ	5.67 ± 2.63	<LOD	<LOD	1.21 ± 1.83	<LOQ	N.D.	<LOD	3.81 ± 3.45	0.87 ± 0.76	10.11 ± 4.59	7.08 ± 2.15
β-Ocimene	N.D.	N.D.	N.D.	N.D.	0.44 ± 0.67	N.D.	N.D.	N.D.	N.D.	N.D.	<LOQ	N.D.
γ-terpinene	<LOD	<LOD	N.D.	N.D.	<LOQ	<LOD	N.D.	N.D.	N.D.	N.D.	<LOD	<LOD
terpinolene	0.15 ± 0.42	<LOQ	N.D.	<LOQ	0.69 ± 0.91	<LOQ	N.D.	N.D.	N.D.	N.D.	<LOQ	<LOQ
linalool	N.D.	N.D.	N.D.	N.D.	<LOD	N.D.	N.D.	<LOD	N.D.	N.D.	N.D.	N.D.
δ-elemene[b]	2.42 ± 0.62	0.75 ± 0.56	N.D.	N.D.	<LOQ	<LOQ	N.D.	N.D.	<LOQ	N.D.	0.68 ± 0.56	1.45 ± 0.24
γ-elemene[b]	<LOQ	<LOQ	N.D.	N.D.	0.90 ± 1.36	3.25 ± 1.41	N.D.	N.D.	N.D.	N.D.	<LOQ	<LOQ
β-caryophyllene	3.42 ± 1.02	1.07 ± 0.80	0.97 ± 1.46	10.24 ± 5.99	5.25 ± 4.34	N.D.	23.51 ± 10.24	9.11 ± 1.99	<LOQ	<LOQ	0.96 ± 0.81	1.57 ± 0.53
humulene	<LOQ	<LOQ	0.96 ± 1.44	3.70 ± 2.06	1.19 ± 0.92	<LOQ	7.81 ± 0.96	2.85 ± 0.42	<LOQ	<LOQ	<LOQ	<LOQ
α-zingiberene	2.89 ± 0.27	N.D.	1.44 ± 2.16	2.96 ± 4.44	27.77 ± 16.64	27.07 ± 0.94	N.D.	N.D.	N.D.	N.D.	N.D.	1.27 ± 1.01
β-sesquiphellandrene[b]	N.D.	N.D.	N.D.	N.D.	1.20 ± 1.80	<LOQ	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
nerolidol	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	12.07 ± 079	N.D.	N.D.	N.D.	N.D.	N.D.
caryophyllene oxide	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2.86 ± 0.56	N.D.	N.D.	N.D.	N.D.
9-hydroxy-zingiberene[b]	N.D.	N.D.	N.D.	N.D.	30.19 ± 32.40	24.06 ± 13.00	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
9-hydroxy-10,11-epoxy-zingiberene[b]	N.D.	N.D.	N.D.	N.D.	28.93 ± 37.83	46.82 ± 19.72	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

[a] Data is presented as the mean concentration ± the standard deviation, with sample leaf of n=3 and sample injection n=3. [b] Putative identification. [c] N.D.: not identified.

Multivariate analysis

Pearson's correlation matrix (Appendix E) highlighted key relationships among tomato plant traits, particularly the interplay between terpene content, trichome types, and pest resistance indicators, such as nymph density per cm^2. The Correlation-Based Network Analysis (CNA) established significant correlations among terpenes associated with resistance traits (Figure 2A). In this network, positively correlated compounds were indicated by green lines, demonstrating strong associations, particularly among 9-hydroxy-10,11-epoxy-zingiberene, 9-hydroxy-zingiberene, and α-zingiberene, as well as terpinolene, 9-hydroxy-zingiberene, and α-zingiberene, and between the number of nymphs per cm^2 and the number of non-glandular trichomes per μm^2. Conversely, negative correlations, shown with red lines, linked specific terpenes to non-glandular trichomes, highlighting an inverse relationship: tomato plants with higher concentrations of certain terpenes generally had fewer non-glandular trichomes and more glandular trichomes. Also, the number of glandular trichomes negatively correlates with the number of nymphs and non-glandular trichomes per μm^2.

The cluster analysis and heatmap (Figure 2B) categorized wild tomato accessions and commercial tomato cultivars based on terpene content, revealing distinct clustering patterns that highlight biochemical diversity. Wild accessions PI209978 and PI127826 formed a unique cluster characterized by higher levels of specific terpenes and a greater density of glandular trichomes, as well as a reduced number of non-glandular trichomes and lower nymph density—traits associated with enhanced pest resistance.

The Principal Component Analysis (PCA) biplot (Figure 2C) further delineated two primary clusters, with Principal Components 1 (PC1) and 2 (PC2) accounting for 88.1% of the observed variance. Cluster 1, comprising wild accessions, such as PI209978 and PI127826, presented a high concentration of terpenes (9-hydroxy-10,11-epoxy-zingiberene, 9-hydroxy-zingiberene, and terpinolene) and a strong association with glandular over non-glandular trichomes. In contrast, Cluster 2 included other wild accessions and commercial cultivars with lower concentrations of these resistance-related terpenes, indicating that wild accessions in Cluster 1 have distinct biochemical traits that may contribute to their enhanced resistance.

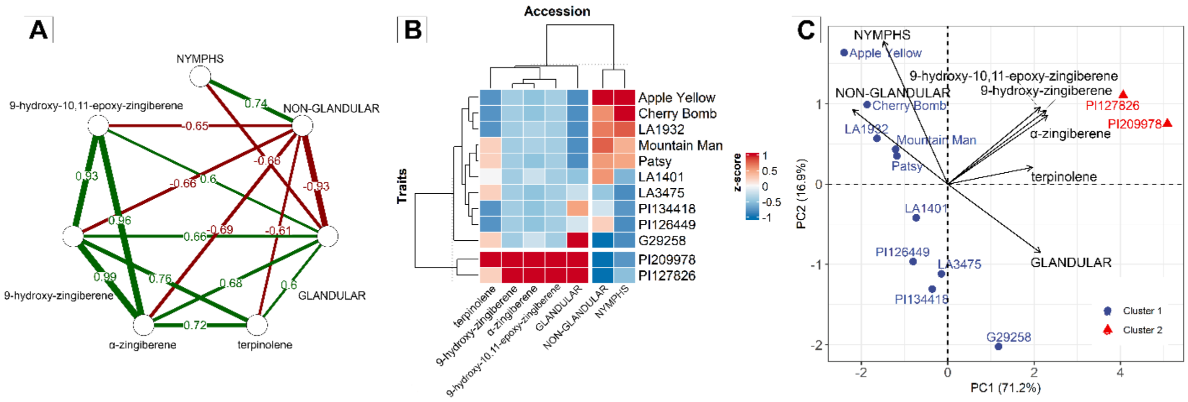

Figure 4. 2. Multivariate analysis of wild tomato accessions (*Solanum habrochaites*: G29258, PI126449, PI127826, PI134418, PI209978; *Solanum galapagense*: LA1401; *Solanum chilense*: LA1932) and commercial tomato cultivars (*Solanum lycopersicum*: LA3475, Cherry Bomb, Apple Yellow, Patsy, Mountain Man) based on terpene profiles and field responses to sweetpotato whitefly. (A) The correlation-based network analysis (CNA) summarizes the significant correlations among whitefly resistance traits and terpene profiles across the tested tomato plants. (B) Cluster analysis reveals the relative expression of these traits, highlighting the biochemical and biological interactions. (C) The PCA biplot identifies two distinct clusters, showing notable variations in terpene abundance and trichome types.

Discussion

The findings in this study demonstrate significant differences between wild tomato accessions and tomato cultivars in their whitefly resistance traits, trichome morphology, and terpene profile. The variation in nymph density, trichome density and type, and terpene content across wild accessions and commercial cultivars suggests that selective pressures on wild accessions may have promoted traits advantageous for pest resistance, which appear diminished in cultivars. The lower number of nymphs per cm^2 observed in wild accessions of *S. habrochaites*, *S. galapagense,* and *S. chilense* species, compared to commercial cultivars of *S. lycopersicum*, suggest intrinsic resistance mechanisms within these wild genotypes.

Environmental factors, such as rainfall and temperature fluctuations during the growing season, likely influenced whitefly population dynamics (Appendix D). Warm daily temperatures between 20 and 30 °C, without extreme variations, favor whitefly reproduction and development, while periodic rainfall can physically dislodge whiteflies, disrupting their lifecycle (6,33–35). The proximity of cotton and squash crops, which are preferred hosts for whitefly oviposition (36,37), adjacent to the experimental field, may partly explain the relatively low whitefly populations observed in this study. However, this study did not formally assess these environmental and crop adjacency factors.

Although no significant differences were observed in adult and egg densities, the pronounced reduction in nymph density among wild tomato accessions represents a valuable trait for pest management. Previous studies indicate that specific wild accessions of *S. habrochaites*, *S. galapagense*, and *S. chilense* negatively influence whitefly oviposition and nymph development (26,38,39). In contrast, commercial tomato cultivars, particularly Apple Yellow and Cherry Bomb, exhibited significantly higher nymph densities (Figure 1A), consistent with prior findings that cultivated tomatoes generally have lower inherent resistance to whiteflies. Since nymphs are sessile, they are especially susceptible to targeted control measures; reducing their population helps prevent development into adults, disrupting the whitefly lifecycle and reducing future infestations (40,41).

The trichome analysis revealed that wild tomato accessions exhibit a distinctive morphology, with a higher density of glandular trichomes and fewer non-glandular trichomes on the abaxial leaf surface than commercial cultivars (Figure 1B). Specifically, Type IV and VI glandular trichomes in *S. habrochaites* and Type VI in *S. galapagense* and *S. chilense* are associated with whitefly resistance, providing defensive mechanisms for the plant (30,42–44). These glandular trichomes not only form a physical barrier but also secrete defensive compounds, including terpenes, flavonoids, and acylsugars, which reduce whitefly infestations by making plants less attractive to whiteflies and reducing feeding activity, thus minimizing damage (45–48). In contrast, tomato cultivars demonstrated higher densities of non-glandular trichomes (up to 73 trichomes/μm^2 in Apple Yellow) but lacked glandular trichomes entirely. This pattern suggests that domestication may have inadvertently favored non-glandular trichomes for structural purposes while diminishing the presence of glandular trichomes essential for chemical defense (49–51).

GC-MS analysis revealed a unique terpene profile in wild tomato accessions, particularly *S. habrochaites* PI209978 and PI127826, characterized by high concentrations of sesquiterpenes such as terpinolene, α-zingiberene, and its derivatives (9-hydroxy-10,11-epoxy-zingiberene and 9-hydroxy-zingiberene). In contrast, these sesquiterpenes were absent or present at minimal levels in commercial tomato cultivars. In previous studies, α-zingiberene and its derivatives have been linked to pest resistance in S. habrochaites accessions. These findings suggest that specific sesquiterpenes may deter whiteflies by reducing their attraction to the host plant or negatively impacting their survival. High terpene concentrations on the leaf surface can inhibit whiteflies from feeding or ovipositing (52–54), disrupting their host selection process and encouraging them to avoid terpene-coated leaves, ultimately reducing infestation levels. The volatile nature of these terpenes provides antixenosis, a resistance mechanism that interferes with whiteflies' behavior and may indirectly attract their natural predators (55).

Multivariate analyses, including CNA, cluster analysis, and PCA, demonstrated strong associations among glandular trichome density, terpene concentration, and whitefly resistance in tomato accessions (Figure 2). High densities of glandular trichomes correlated with elevated levels of specific sesquiterpenes and reduced whitefly nymph counts, particularly in *S. habrochaites* accessions PI209978 and PI127826. These accessions formed distinct clusters due to their high levels of terpinolene, α-zingiberene, 9-hydroxy-zingiberene, and 9-hydroxy-10,11-epoxy-zingiberene. This clustering pattern indicates a robust resistance phenotype differentiating these accessions from commercial cultivars and other wild types. Although previous studies have highlighted the impact of glandular trichomes in reducing whitefly populations in *S. habrochaites* PI127826 (26,49,56,57), they did not investigate terpene profiles or their correlations with trichome traits. Our previous studies identified *S. habrochaites* PI209978 (58) and PI127826 as having high α-zingiberene content, corroborating the results presented in this study. Then, the glandular trichomes on these accessions effectively store and release terpenes, repelling pests and maintaining low nymph densities.

The distinctive traits observed in wild accessions PI127826 and PI209978, especially the elevated glandular trichome density and specific sesquiterpenes, highlight their potential utility in breeding programs to enhance pest resistance in cultivated tomatoes. By incorporating terpene-producing glandular trichome traits from *S. habrochaites* and related wild accessions into cultivated tomato varieties, breeders can pursue ecologically sustainable alternatives to chemical pest control. Future research should explore *S. galapagense* and *S. chilense* wild accessions that also demonstrated potential resistance to whiteflies in this research. The diversity of compounds found in the glandular trichomes of *S. habrochaites*, including acylsugars, fatty acids, alkaloids, and unique terpenoid classes such as sesterterpenes (30), suggests a broad spectrum of bioactive metabolites that warrant further investigation. In-depth studies on this metabolic diversity across different accessions could help fully leverage wild tomatoes' genetic and biochemical potential for pest-resistant cultivar development.

Conclusions

This study demonstrated that wild tomato accessions, particularly *Solanum habrochaites* PI127826 and PI209978, exhibit significant resistance to whiteflies compared to cultivars of *Solanum lycopersicum*. This resistance is primarily attributed to the high density of glandular trichomes and specific sesquiterpenes, such as terpinolene, α-zingiberene, and its derivatives, which act as natural deterrents. The correlation between glandular-type trichome traits and terpene profiles with whitefly resistance reveals a strong association between increased glandular trichome abundance, elevated levels of specific sesquiterpenes, and reduced whitefly nymph density. These findings emphasize the potential of utilizing wild tomato genetic resources in breeding programs to enhance pest resistance in commercial tomato varieties, offering an environmentally sustainable alternative to chemical whitefly control.

Materials and Methods

Experimental design and field management

A study using a randomized complete block design (RCBD) with three replications was conducted at the Wiregrass Research and Extension Center, Auburn University, Headland, AL, USA (31°21′ N, 87°14′ W) to evaluate the effects of different wild tomato accessions on whitefly populations. The treatments included seven wild tomato accessions and five cultivars as controls, which are more detailed and described in Appendix C. Five wild accessions—G29258, PI127826, PI134418, PI209978, and PI126449 from *Solanum habrochaites*—were sourced from the USDA-ARS GRIN-Global. The wild accessions LA1401 (*Solanum galapagense*), LA1932 (*Solanum chilense*), and the cultivar LA3475 (*Solanum lycopersicum*) were obtained from the C.M. Rick Tomato Genetics Resource Center, University of California, Davis, USA. Four commercial *S. lycopersicum* cultivars were also included: the beefsteak tomatoes 'Patsy' (Bejo Seeds, Oceano, CA, USA) and 'Mountain Man' (Syngenta US, Greensboro, NC, USA), and the cherry tomatoes 'Cherry Bomb' and 'Apple Yellow' (Jhonny Seeds, Waterville, ME, USA). Each experimental unit was a row with five plants, with data collected from the central three plants to reduce edge effects.

Tomato seeds, both wild accessions and commercial cultivars, were sanitized in a 10% sodium hypochlorite solution for five minutes before sowing. On July 21, 2023, seeds were planted into 36 mm peat pellets (Jiffy Group, Lorain, OH, USA) and placed in 28 °C growth chambers for germination. Seedlings were transplanted into the field on August 23, 2023. The field, with sandy soil, featured 15 cm tall, raised beds spaced 1.8 m apart, with 30 cm between plants. Uniform cultural practices, including irrigation, fertilization, pest, disease, and weed management, were applied according to the Southeastern US 2022 Vegetable Crop Handbook (59).

Data collection

Whitefly populations were monitored weekly, starting 30 days after transplanting (DAT), when whiteflies were first detected. Counts were taken at 30, 37, 44, 51, and 58 DAT by inspecting two leaves from the lower third of three plants in each plot before 9 am to minimize temperature or insect movement effects.

Additionally, detailed counts of whitefly nymphs and eggs were conducted in the lab at 44, 51, and 58 DAT to verify the effect of the treatment on immature phases. One leaf per treatment was collected on each date, placed in Ziplock bags, and examined under a Leica M165 C Stereo Microscope (Leica Microsystems, Wetzlar, Germany) with a 5-20x magnification. A 1 cm^2 area from each leaflet's terminal part was randomly selected, and the average number of nymphs and eggs in that area was recorded for statistical analysis.

Environmental data (air temperature, humidity, solar radiation, wind speed, evapotranspiration, rainfall, and daily temperature extremes) were recorded using an on-site

Vantage Pro2 Plus weather station to contextualize fluctuations in whitefly populations and assess the impact of environmental factors on plant performance and whitefly infestations across treatments (Appendix D).

Tomato leaf trichome characterization

At 62 DAT, one fully developed young leaf from the upper third of each plant in the pre-flowering stage was collected to identify and quantify trichomes. The leaflets were placed in Ziplock bags and transported to the lab, where they were stored at room temperature (25 °C) until scanning electron microscope (SEM) analysis, which took place the following day. For SEM preparation, three small paradermal fragments (10 mm^2 each) from the upper, middle, and lower abaxial surfaces of the leaflets were mounted on aluminum stubs with carbon tape and coated with gold using a sputter coating machine (EMS Q150R SCD, Quorum Technologies, Calgary, AB, Canada). Photomicrographs were captured with a scanning electron microscope (EVO 50, Carl Zeiss Vision Inc., Hebron, KY, USA) at 500x magnification and 20 kV voltage. Trichomes were counted and classified as glandular or non-glandular, following the established methodology (60,61).

Reagents, solvents, and terpenes extraction

Certified cannabis terpenes mixture (2500 μg mL^{-1} in isopropanol of eucalyptol, (-)-α-bisabolol, camphene, δ-3-carene, β-caryophyllene, geraniol, (-)-guaiol, α-humulene, p-cymene, (-)-isopulegol, D-limonene, linalool, β-myrcene, nerolidol, β-ocimene, α-pinene, (-)-β-pinene, α-terpinene, γ-terpinene, terpinolene, and (-)-caryophyllene oxide) were purchased from Restek Corp (Bellefonte, PA, USA). α-zingiberene (≥ 95 %) was obtained from Alfachemistry (Ronkonkoma, NY, USA). A stock standard solution (125 µg mL^{-1}) of terpenes was prepared by appropriately diluting the original standard in hexane and stored at −18 °C. Working standard solutions at different concentration levels were prepared by appropriately diluting the stock solutions in n-hexane (VWR International LLC, Radnor, PA, USA) and stored at -18 °C. Terpenes were extracted from intact tomato leaflet trichomes using a standardized leaf dip method (58). Tomato leaflets weighing 0.1 and 0.5 g were placed in centrifuge tubes with 5.0 mL of n-hexane. The mixture was shaken for 2 minutes at a controlled temperature of 25 ± 2 °C. The solution was centrifuged at 9000 g for 2 minutes, and the supernatant was transferred into GC-MS glass vials.

GC-MS analysis

The GC–MS analysis was performed using an Agilent 5977B GC/MSD (Agilent Technologies, CA, USA) following the established methodology (58). Terpenes were separated on an HP-5MS column (30 m × 250 µm diameter capillary, 0.25 µm film thickness). A 1.0 µL injection was made in splitless mode at 300 °C, with a solvent delay of 4 min. The temperature program for the GC oven was as follows: 50 °C for 1 min, then increased to 300 °C at a rate of 7 °C min^{-1}, followed by an increase to 320 °C at 20 °C min^{-1}, where it was held for 2 min. Helium was used as a carrier gas at a constant flow rate of 1 mL min^{-1}, and terpenes were ionized using electron ionization at 70 eV. The transfer line and ion source temperatures were maintained at 250 °C and 230 °C, respectively. Mass spectrometry data were acquired in scan mode over 50 to 550 *m/z*. All samples and standards were injected into triplicate.

Terpenes were identified through RT matching and mass spectral library searches, including the National Institute of Standards and Technology (NIST) MS spectral database (version 2.4, 2020). Authentic standards were utilized to confirm the identity of specific compounds when available. In cases where standards were unavailable, identification was based on achieving a NIST library matching score greater than 80 %. Compounds were categorized as "similar to" the matched compound scores between 80 % and 90 % and definitively identified for scores between 91 % and 100 %. Compounds with matching scores below 79 % were classified as unidentified and excluded from the reported results. Identification was performed using MassHunter Qualitative Analysis software (version 10.0).

Quantitative analysis was performed using the external calibration curves created with a terpene standard mix. Calibration curves were constructed at seven concentrations ranging from 0.2 to 10 μg mL^{-1}. The method's linearity, limits of detection (LOD), and limits of quantification (LOQ) were evaluated. LOD values were calculated as 3.3 times the standard deviation (SD) divided by the slope (b) of the calibration curve, while LOQ was determined as 10 x SD/b. Quantification was conducted with Agilent MassHunter Quantitative Analysis software (version 10.2). A qualifier ion was employed to identify each analyte, and a quantifier ion was used to determine the analyte response via peak calculation from the Extracted Ion Chromatogram (EIC). Specific standards were utilized to semi-quantify the leaf extract components for components not included in the terpene standard mix. D-limonene, 3-carene, and α-humulene from the terpene standard mix were used as external standards for monocyclic monoterpenes, bicyclic monoterpenes, and monocyclic sesquiterpenes, respectively. The terpene content was expressed as μg g^{-1} fresh weight (fr. wt).

Statistical analysis

Repeated measures analysis evaluated temporal changes in whitefly adults, nymphs, and eggs, modeling the variance-covariance structure with PROC-GLIMMIX in SAS (Version 9.4, 2024) using restricted maximum likelihood (REML). This approach accounted for correlations from repeated sampling, employing a heterogeneous compound symmetry structure for the variance-covariance matrix. The significance of correlations was assessed at $p \leq 0.05$ with Pearson's coefficient. Accessions were considered fixed effects, while blocks were included as random effects. Orthogonal contrasts facilitated comparisons between wild accessions and commercial cultivars. Nymph, egg, and trichome counts were analyzed under a Negative Binomial distribution with a logit link function using the Laplace approximation. Tukey's test was applied for post-hoc mean comparisons with a significance threshold of $p \leq 0.05$.

Multivariate analysis included whitefly nymph counts, glandular and non-glandular trichome densities, and terpene content detected via GC-MS to identify associations between wild tomato accessions and commercial tomato cultivars and test for correlation among the measurements. Pearson's correlation matrix (Appendix E) significant correlations ($p \leq 0.05$ or $p \leq 0.01$) were submitted to correlation-based network analysis (CNA) to investigate and graphically represent the correlation among variables analyzed (62,63). A heatmap displayed trait patterns, while Euclidean distance and Unweighted Pair Group Method with Arithmetic

(UPGMA) hierarchical clustering produced dendrograms grouping accessions by variable similarity (64). Principal Component Analysis (PCA) further evaluated relationships among whitefly nymphs, trichome densities, and terpene profiles based on their contributions to principal components (65,66).

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Chapter 5 – Final conclusions and future directions

The results presented in this study provide comprehensive insights into sustainable management strategies for the sweetpotato whitefly (*Bemisia tabaci*) in the southeastern United States. Through a multifaceted investigation, the study assessed the effectiveness of insect exclusion row covers and silver reflective plastic mulching in reducing whitefly populations and enhancing crop yields. The findings indicated significant reductions in whitefly densities, which led to increased plant biomass and improved crop productivity, particularly in zucchini cultivation. Both silver reflective mulching and row covers demonstrated notable efficacy in managing whitefly infestations.

In addition to field-based management strategies, the study expanded its focus to investigate wild tomato accessions' physical and chemical characteristics with potential whitefly-resistant traits. The results showed that wild tomato accessions, particularly those with a higher density of glandular trichomes, exhibited increased resistance to whiteflies. These accessions also displayed elevated levels of specific terpenes, which are known to deter whiteflies. Chemical analysis underscored the potential of specific wild accessions for inclusion in breeding programs to develop whitefly-resistant tomato cultivars. This research provides valuable contributions to the long-term management of whiteflies by identifying genetic resistance traits that could be integrated into commercial crop varieties.

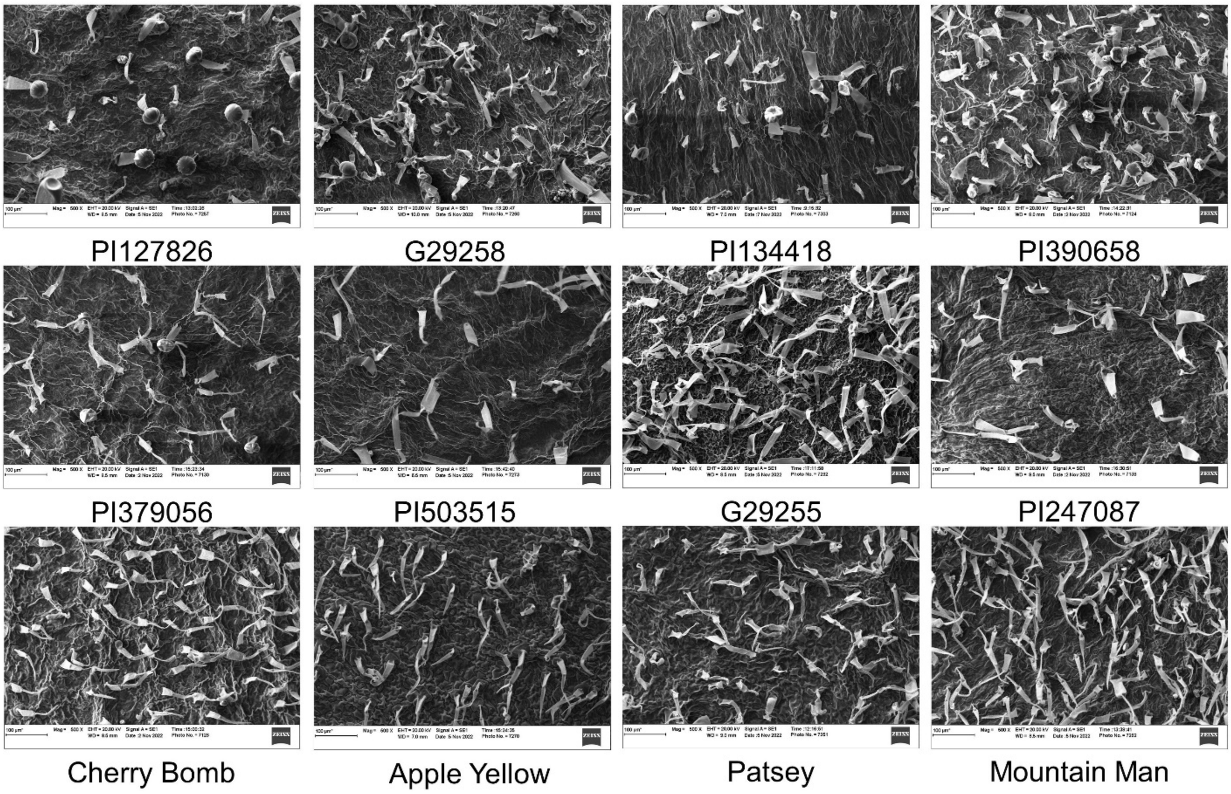
This work emphasizes the importance of integrating short-term field-based cultural control methods with long-term genetic resistance strategies to effectively manage whitefly populations and mitigate their economic impact on agriculture in the southeastern United

States. Future research should focus on several key areas to advance sustainable whitefly management strategies further. First, the long-term effects of cultural practices such as row covers and reflective mulches on the transmission of whitefly-borne viruses require further investigation. Understanding how these techniques influence viral disease incidence across various crops will help optimize their use within Integrated Pest Management (IPM) systems.

Moreover, future research should prioritize breeding programs incorporating whitefly resistance traits identified in wild tomato accessions. Developing commercial tomato cultivars with enhanced resistance based on traits such as trichome density and terpene production would provide a sustainable solution to pest management. Expanding the characterization of other wild tomato species and accessions may reveal additional resistance mechanisms that could be utilized in breeding programs for multiple crops. Also, addressing economic feasibility and broader adoption of these strategies across different crops and regions is critical to ensuring their practical implementation on a large scale. This will help build resilient agricultural systems that reduce dependency on chemical insecticides, manage pest populations effectively, and mitigate the impact of whiteflies on crop yields.

Appendix A

Scanning electron micrographs of the abaxial sides of the leaflets from tomato plants of *Solanum habrochaites* wild accessions (PI127826, G29258, PI134418, PI390658, PI379056, PI503515, G29255, PI247087) and *Solanum lycopersicum* commercial cultivars (Cherry Bomb, Apple Yellow, Patsy, Mountain Man). These images highlight the morphological differences in trichome structure between the wild and commercial tomato accessions. Scale bar = 100 µm.



PI127826 G29258 PI134418 PI390658

PI379056 PI503515 G29255 PI247087

Cherry Bomb Apple Yellow Patsey Mountain Man

Appendix B

Means, Standard Deviation, and Person's correlation matrix of the variables analyzed in chapter 3 (n = 12). Values followed by blue color indicate a significant correlation at 0.05 level. Values followed by red color indicate a significant correlation at 0.01 level.

Variables	Mean	SD	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1 Adults per leaf	9.67	4.63																													
2 Nymphs per cm^2 leaf	5.48	5.21	-0.47																												
3 Eggs per cm^2 leaf	3.28	2.57	**0.61**	-0.01																											
4 Number of non-gladular trichomes	44.00	25.57	-0.18	0.48	0.10																										
5 Number of gladular trichomes	5.06	4.98	-0.04	**-0.63**	-0.36	**-0.65**																									
6 α-Pinene	1.82	3.64	-0.12	0.34	-0.02	0.14	-0.46																								
7 β-Myrcene	0.05	0.18	0.37	-0.24	-0.14	-0.41	-0.02	0.11																							
8 4-Carene	1.98	3.91	-0.30	**0.67**	0.05	0.32	-0.54	**0.89**	-0.16																						
9 α-Terpinene	0.25	0.85	-0.24	0.10	-0.17	0.35	-0.32	**0.67**	-0.09	0.48																					
10 p-cymene	0.30	1.03	0.36	-0.25	-0.15	-0.43	0.00	0.11	**1.00**	-0.16	-0.09																				
11 D-Limonene	13.16	13.55	-0.41	**0.75**	-0.15	**0.63**	**-0.77**	0.42	0.08	0.55	0.32	0.07																			
12 β-Phellandrene	4.63	4.10	-0.42	**0.82**	-0.17	**0.66**	**-0.78**	**0.62**	-0.02	**0.76**	0.44	-0.03	**0.92**																		
13 β-Ocimene	0.00	0.00	-0.15	-0.06	-0.24	-0.52	0.48	-0.16	-0.09	-0.16	-0.08	-0.06	-0.30	-0.35																	
14 Terpinolene	0.00	0.00	-0.15	-0.06	-0.24	-0.52	0.48	-0.16	-0.09	-0.16	-0.08	-0.06	-0.30	-0.35	**1.00**																
15 δ-Elemene	1.05	2.61	-0.24	0.30	-0.07	0.31	-0.43	**0.92**	-0.13	**0.81**	**0.89**	-0.13	0.40	**0.59**	-0.12	-0.12															
16 Sesquithujene	0.08	0.29	0.37	-0.26	0.25	0.02	-0.01	-0.16	-0.09	-0.16	-0.09	-0.09	-0.13	-0.23	-0.09	-0.09	-0.13														
17 β-Caryophyllene	20.17	18.33	0.10	-0.57	0.14	-0.38	0.33	-0.17	0.16	-0.31	-0.08	0.16	-0.41	-0.49	-0.34	-0.34	-0.17	0.40													
18 α-Bergamotene	0.12	0.43	-0.15	-0.06	-0.24	-0.52	0.48	-0.16	-0.09	-0.16	-0.08	-0.06	-0.30	-0.35	**1.00**	1.00	-0.12	-0.09	-0.34												
19 β-Farnesene	0.09	0.32	-0.24	0.10	-0.17	0.36	-0.32	**0.67**	-0.09	0.49	**1.00**	-0.09	0.32	0.44	-0.09	-0.09	**0.89**	-0.09	-0.08	-0.09											
20 α-Humulene	16.08	15.78	0.39	-0.52	**0.64**	-0.19	0.13	-0.23	0.07	-0.33	-0.13	0.06	-0.37	-0.51	-0.32	-0.32	-0.22	0.23	**0.73**	-0.32	-0.12										
21 γ-Curcumene	0.58	0.33	0.17	-0.08	-0.32	-0.32	0.42	-0.06	0.20	-0.02	-0.35	0.20	-0.40	-0.18	0.00	0.00	-0.23	0.02	0.01	0.00	-0.35	-0.38									
22 Ar-Curcumene	0.78	1.68	-0.14	0.05	-0.17	-0.50	0.38	-0.17	-0.15	-0.13	-0.14	-0.12	-0.28	-0.31	**0.97**	**0.97**	-0.14	-0.15	-0.41	**0.97**	-0.15	-0.36	0.00								
23 α-Zingiberene	1.79	6.21	-0.15	-0.06	-0.24	-0.52	0.48	-0.16	-0.09	-0.16	-0.08	-0.06	-0.30	-0.35	**1.00**	**1.00**	-0.12	-0.09	-0.34	**1.00**	-0.09	-0.32	0.00	0.97							
24 Zingiberenol	21.37	22.67	0.29	-0.02	-0.12	0.24	0.13	-0.11	-0.01	-0.04	-0.21	-0.02	-0.21	0.01	-0.30	-0.30	-0.16	-0.10	-0.26	-0.30	-0.20	-0.33	**0.75**	-0.29	-0.30						
25 Caryophyllene Oxide	2.13	3.19	**0.66**	-0.25	**0.88**	0.05	-0.11	-0.20	-0.12	-0.20	-0.21	-0.12	-0.31	-0.35	-0.21	-0.21	-0.20	0.06	0.11	-0.21	-0.21	**0.72**	-0.32	-0.16	-0.21	-0.01					
26 9-hydroxy-zingiberene	0.61	2.11	-0.15	-0.06	-0.24	-0.52	0.48	-0.16	-0.09	-0.16	-0.08	-0.06	-0.30	-0.35	**1.00**	**1.00**	-0.12	-0.09	-0.34	**1.00**	-0.09	-0.32	0.00	**0.97**	**1.00**	-0.30	-0.21				
27 α-Bisabolol	0.11	0.36	-0.15	-0.06	-0.24	-0.52	0.48	-0.16	-0.09	-0.16	-0.08	-0.06	-0.30	-0.35	**1.00**	**1.00**	-0.12	-0.09	-0.34	**1.00**	-0.09	-0.32	0.00	**0.97**	**1.00**	-0.30	-0.21	**1.00**			
28 Nuciferol	12.15	20.60	-0.29	0.15	-0.36	-0.17	0.14	-0.32	-0.19	-0.27	-0.18	-0.17	0.02	-0.08	**0.72**	**0.72**	-0.25	-0.19	-0.54	**0.72**	-0.19	-0.54	-0.15	**0.79**	**0.72**	-0.25	-0.32	**0.72**	**0.72**		
29 Tumerone	0.07	0.23	-0.15	-0.06	-0.24	-0.52	0.48	-0.16	-0.09	-0.16	-0.08	-0.06	-0.30	-0.35	**1.00**	**1.00**	-0.12	-0.09	-0.34	**1.00**	-0.09	-0.32	0.00	**0.97**	**1.00**	-0.30	-0.21	**1.00**	**1.00**	**0.72**	

*** $p \leq 0.05$**

**** $p \leq 0.01$**

Appendix C

Description of the tomatoes accessions and commercial cultivars from the species *Solanum habrochaites* S. Knapp & D.M. Spooner, *Solanum galapagense* S. C. Darwin & Peralta, *Solanum chilense* (Dunal) Reiche, and *Solanum lycopersicum* L. used in the experiment.

Accession	**Taxon**	**Origin**
G29258	*S. habrochaites*	United Kingdom
PI126449	*S. habrochaites*	Ecuador
PI127826	*S. habrochaites*	Peru
PI134418	*S. habrochaites*	Ecuador
PI209978	*S. habrochaites*	Venezuela
LA1401	*S. galapagense*	Ecuador
LA1932	*S. chilense*	Peru
LA3475	*S. lycopersicum* cv. M-82	USA
Cherry Bomb	*S. lycopersicum* cv. Cherry Bomb	Commercial cultivar
Apple Yellow	*S. lycopersicum* cv. Apple Yellow	Commercial cultivar
Patsy	*S. lycopersicum* cv. Patsy	Commercial cultivar
Mountain Man	*S. lycopersicum* cv. Mountain Man	Commercial cultivar

Appendix D

Daily weather variation, including minimum (Tmin), average (Tavg), and maximum (Tmax) temperatures (°C) and rainfall events (mm), recorded over days after transplant (DAT) for the wild tomato accessions and commercial tomato cultivars tested.

Date	DAT	Tmin (°C)	Tmax (°C)	Tavg (°C)	Precipitation (mm)
8/23/2023	0	24.8	36.8	30.7	0.0
8/24/2023	1	24.4	34.6	28.9	0.0
8/25/2023	2	22.4	37.5	29.9	0.0
8/26/2023	3	25.1	37.6	30.8	0.0
8/27/2023	4	25.0	38.0	28.8	1.8
8/28/2023	5	22.9	36.5	27.8	0.0
8/29/2023	6	22.8	33.9	25.5	15.2
8/30/2023	7	23.4	31.6	26.2	0.0
8/31/2023	8	22.8	33.8	27.2	0.0
9/1/2023	9	23.2	28.6	25.1	73.4
9/2/2023	10	21.2	27.7	23.9	3.3
9/3/2023	11	22.2	31.3	25.9	0.0
9/4/2023	12	20.0	32.3	26.0	0.0
9/5/2023	13	21.8	32.6	26.6	0.0
9/6/2023	14	21.3	33.2	27.1	0.0
9/7/2023	15	23.4	34.9	28.4	0.0
9/8/2023	16	21.0	32.7	26.4	0.0
9/9/2023	17	18.2	31.7	25.4	0.0
9/10/2023	18	21.6	32.9	26.7	0.0
9/11/2023	19	21.3	33.6	27.1	0.0
9/12/2023	20	21.3	33.1	25.4	1.3
9/13/2023	21	21.5	33.5	25.5	0.3
9/14/2023	22	21.0	32.4	25.0	0.0
9/15/2023	23	20.9	30.1	24.2	0.0
9/16/2023	24	20.6	30.9	24.2	13.2
9/17/2023	25	20.3	30.8	24.7	0.0
9/18/2023	26	17.3	29.3	22.9	0.0
9/19/2023	27	17.1	29.6	23.0	0.0
9/20/2023	28	17.7	29.0	23.0	0.0
9/21/2023	29	18.8	30.8	24.3	0.0
9/22/2023	30	19.2	29.7	23.4	0.0
9/23/2023	31	16.0	31.1	23.2	0.0
9/24/2023	32	16.7	32.5	24.9	0.0
9/25/2023	33	17.9	32.6	25.1	0.0
9/26/2023	34	19.7	29.6	23.4	0.0
9/27/2023	35	20.8	24.6	22.2	0.0
9/28/2023	36	18.7	27.9	22.1	0.0
9/29/2023	37	16.2	30.7	22.8	0.0

9/30/2023	38	18.0	31.5	24.2	0.0
10/1/2023	39	17.6	30.6	23.2	0.0
10/2/2023	40	15.7	30.3	22.8	0.0
10/3/2023	41	14.4	30.8	22.4	0.0
10/4/2023	42	16.3	31.4	23.5	0.0
10/5/2023	43	17.7	30.7	24.0	0.0
10/6/2023	44	19.9	30.2	24.0	0.5
10/7/2023	45	13.2	27.1	20.9	0.0
10/8/2023	46	8.4	23.4	15.3	0.0
10/9/2023	47	8.1	26.4	17.5	0.0
10/10/2023	48	10.6	29.6	20.6	0.0
10/11/2023	49	14.9	21.4	18.1	12.2
10/12/2023	50	14.9	19.1	17.1	13.5
10/13/2023	51	17.2	21.3	19.0	0.5
10/14/2023	52	16.2	26.4	20.6	0.0
10/15/2023	53	11.3	18.7	15.0	0.0
10/16/2023	54	8.3	19.1	13.7	0.0
10/17/2023	55	8.8	20.8	14.4	0.0
10/18/2023	56	7.9	23.1	15.4	0.0
10/19/2023	57	9.8	24.9	17.2	0.0
10/20/2023	58	15.7	28.8	21.7	0.0
10/21/2023	59	11.6	27.1	19.6	0.0
10/22/2023	60	15.8	29.6	21.0	0.0

Appendix E

Means, Standard Deviation, and Person's correlation matrix of the variables analyzed in the study (n = 12). Values followed by blue color indicate a significant correlation at 0.05 level. Values followed by red color indicate a significant correlation at 0.01 level.

Variables	Mean	SD	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1 Nymphs per cm^2 of leaflet	2.31	2.77																										
2 Number of non-gladular trichomes	34.63	24.01	**0.74**																									
3 Number of gladular trichomes	8.66	10.57	**-0.66**	**-0.93**																								
4 α-pinene	1.88	4.55	-0.24	-0.37	0.45																							
5 camphene	0.05	0.19	-0.24	-0.44	0.53	**0.98**																						
6 similar to p-cymene	0.42	0.81	0.27	0.37	-0.45	-0.12	-0.16																					
7 β-pinene	0.36	1.17	-0.25	-0.43	0.52	**0.99**	**1.00**	-0.17																				
8 4-carene	5.83	7.59	0.34	0.34	-0.53	-0.13	-0.17	**0.83**	-0.18																			
9 α-phellandrene	0.87	1.18	0.27	0.12	-0.20	0.29	0.28	**0.82**	0.26	**0.82**																		
10 3-carene	0.01	0.03	-0.24	-0.44	0.53	**0.98**	**1.00**	-0.16	1.00	-0.17	0.28																	
11 α-terpinene	0.25	0.24	0.29	0.20	-0.31	0.13	0.07	0.66	0.07	**0.80**	**0.79**	0.07																
12 d-limonene	0.02	0.06	-0.17	-0.45	0.34	-0.13	-0.09	-0.16	-0.10	-0.24	-0.09	-0.09	0.07															
13 β-phellandrene	2.44	3.43	0.25	0.26	-0.45	-0.10	-0.11	**0.86**	-0.13	**0.98**	**0.82**	-0.11	**0.79**	-0.21														
14 β-Ocimene	0.05	0.13	-0.16	-0.35	0.39	**0.89**	**0.90**	0.12	**0.90**	0.15	0.56	**0.90**	0.41	-0.12	0.19													
15 γ-terpinene	0.13	0.18	-0.34	-0.44	0.39	**0.86**	**0.83**	0.16	**0.83**	0.14	0.47	**0.83**	0.42	0.12	0.22	**0.83**												
16 terpinolene	0.15	0.19	-0.41	**-0.61**	**0.60**	**0.87**	**0.87**	0.09	**0.87**	0.05	0.42	**0.87**	0.29	0.08	0.13	**0.85**	**0.94**											
17 linalool	0.05	0.12	-0.36	-0.38	0.51	**0.63**	**0.67**	-0.24	**0.67**	-0.29	0.04	**0.67**	-0.19	-0.13	-0.25	**0.58**	0.44	0.46										
18 δ-elemene	0.49	0.75	-0.13	0.25	-0.33	0.06	-0.12	0.42	-0.09	0.33	0.19	-0.12	0.35	-0.12	0.30	-0.08	0.30	0.13	-0.24									
19 γ-elemene	0.41	0.93	-0.25	-0.56	0.45	0.13	0.17	-0.14	0.16	-0.22	0.03	0.17	0.15	**0.96**	-0.16	0.13	0.38	0.33	0.02	-0.07								
20 β-caryophyllene	4.71	6.87	-0.52	-0.19	0.29	-0.02	0.02	-0.29	0.02	-0.42	-0.34	0.02	-0.53	-0.22	-0.39	-0.05	-0.22	-0.12	0.17	-0.26	-0.24							
21 humulene	1.49	2.31	-0.45	-0.18	0.28	-0.11	-0.04	-0.32	-0.05	-0.45	-0.38	-0.04	-0.58	-0.18	-0.42	-0.11	-0.33	-0.20	0.11	-0.39	-0.23	**0.98**						
22 α-zingiberene	5.28	10.40	-0.33	**-0.69**	**0.68**	**0.65**	**0.68**	-0.25	**0.68**	-0.33	0.11	**0.68**	0.07	0.66	-0.27	0.57	**0.71**	**0.72**	0.39	-0.13	**0.82**	-0.14	-0.16					
23 β-sesquiphellandrene	0.12	0.35	-0.27	-0.52	0.59	**0.96**	**0.99**	-0.19	**0.98**	-0.21	0.26	**0.99**	0.08	0.08	-0.15	**0.88**	**0.85**	**0.88**	**0.65**	-0.14	0.33	-0.01	-0.07	**0.79**				
24 caryophyllene oxide	0.24	0.83	-0.24	-0.06	0.16	-0.13	-0.09	-0.16	-0.10	-0.23	-0.22	-0.09	-0.32	-0.09	-0.22	-0.12	-0.24	-0.25	**0.67**	-0.21	-0.14	0.20	0.19	-0.16	-0.11			
25 9-hydroxy-zingiberene	4.52	10.64	-0.31	**-0.66**	**0.66**	**0.72**	**0.76**	-0.24	**0.75**	-0.29	0.17	**0.76**	0.10	0.58	-0.23	**0.66**	**0.76**	**0.76**	0.46	-0.18	**0.76**	-0.12	-0.15	**0.99**	**0.86**	-0.13		
26 9-hydroxy-10,11-epoxy-zingiberene	6.31	15.23	-0.28	**-0.65**	**0.60**	0.42	0.47	-0.24	0.46	-0.31	0.07	0.47	0.10	0.84	-0.24	0.39	0.56	0.54	0.25	-0.18	**0.94**	-0.18	-0.18	**0.96**	**0.61**	-0.13	**0.93**	
27 nerolidol	1.01	3.48	-0.26	0.08	-0.06	-0.13	-0.09	-0.16	-0.10	-0.24	-0.23	-0.09	-0.32	-0.09	-0.22	-0.12	-0.24	-0.25	-0.13	-0.21	-0.14	**0.86**	**0.86**	-0.16	-0.11	-0.09	-0.13	-0.13

*$p \le 0.05$

**$p \le 0.01$

n=12