The whitefly *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) in processing tomato crop – attack differences among four cultivars

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Abstract

The Portuguese 2017 processing tomato season was one of the worst regarding the color parameter. The tomato coming from Ribatejo region fields was showing symptoms of irregular ripening disorder (IRR), probably caused by the large population of *Bemisia tabaci* present.

This pest, favoured by global warming, by its versatility and resilience, has the potential to become a major pest, hence relevant to analyse different management alternatives in the processing tomato crops, such as the development of lesser susceptible or attractive cultivars (cvs).

Several studies carried out on wild *Solanum* spp. species revealed the existence of resistance to *B. tabaci* through its trichomes. However, as far as we know, there are no studies conducted on cvs commonly used by farmers, either on susceptibility to this pest or on the type and distribution of its trichomes, which may influence it.

This study aimed to verify and compare abundance and type of trichomes, glandular and non-glandular, in four commercial processing tomato cultivars (CXD277, H1015, H3402 and KG273) and evaluate its influence on *B. tabaci* attractiveness. To achieve that, two free choice trials were performed in Kagome's greenhouse at Águas de Moura.

The greenhouse was uniformly infested with *B. tabaci* specimens.

The trichomes count was done using a stereoscopic microscope and the type recognition by scanning electronic microscopy.

The analysis of variance did not reveal significant differences in the number of either non-glandular, glandular trichomes or both combined.

The analysis of the number of nymphs on the leaves, excluding H3402, revealed a significant lower number of nymphs on KG273, but only in the 1st trial.

There were no correlations between nymphs and trichomes counts. So, this factor was considered not determinant for a different infestation and, probably, does not play a role on different attractiveness of *B. tabaci* on commercial cvs.

Other factors that can affect attractiveness are discussed.

Keywords: *Solanum lycopersicum*, whitefly, trichomes, glandular, attractiveness.

Resumo

A campanha de tomate de indústria no ano de 2017 foi uma das piores quanto ao parâmetro cor. O tomate proveniente do Ribatejo apresentava sintomas de maturação irregular, provavelmente, causada pela população elevada de *Bemisia tabaci*.

Esta praga, favorecida pelo aquecimento global, e pela sua versatilidade e resiliência, tem o potencial de se tornar uma praga relevante, tornando-se necessário analisar diferentes alternativas à sua gestão em tomate de indústria, tal como, o desenvolvimento de cultivares menos suscetíveis ou atrativas.

Vários estudos realizados em espécies selvagens de *Solanum* spp. revelaram a existência de resistência a *B. tabaci*, através dos seus tricomas. No entanto, que se saiba, não existem estudos realizados em cultivares normalmente utilizadas pelos agricultores, quer seja quanto à suscetibilidade a esta praga ou sobre o tipo e distribuição dos seus tricomas, que pode influenciá-la.

Este estudo visou verificar e comparar a abundância e o tipo de tricomas, glandulares e não glandulares, em quatro cultivares comerciais de tomate de indústria (CXD277, H1015, H3402 e KG273) e avaliar a sua influência na atratividade para *B. tabaci.* Para isso, dois ensaios de escolha livre foram realizados na estufa da Kagome em Águas de Moura.

A estufa foi, uniformemente, infestada com espécimes de *B. tabaci*.

A contagem dos tricomas foi feita através de um microscópio estereoscópico e o reconhecimento do tipo através de microscopia eletrónica.

A análise de variância não revelou diferenças significativas no número de tricomas não glandulares, glandulares ou ambos combinados.

A análise da contagem de ninfas, excluindo H3402, revelou um número significativamente inferior em KG273, mas apenas no 1º ensaio.

Não houve correlações entre o número de ninfas e tricomas. Portanto, este fator foi considerado não determinante de diferente infestação e, provavelmente, não é relevante para a atratividade para *B. tabaci* em cultivares comerciais.

Outros fatores que podem afetar a atratividade foram discutidos.

Palavras-Chave: *Solanum lycopersicum*, mosca branca, tricomas, glandular, atratividade.

Resumo alargado

A campanha de tomate de indústria no ano de 2017 foi uma das piores em termos de qualidade dos últimos anos, especialmente no parâmetro cor. O tomate que chegou às fábricas, originário da região do Ribatejo, apresentava sintomas inusuais. Era visível que em alguns dos tomates, em estado maduro, a parte exterior apresentava zonas bem delimitadas de verde e outros apresentavam uma maturação aparentemente homogénea no exterior, mas quando cortados era possível verificar que o seu interior não tinha atingido a maturação. Aquele ano coincidiu com uma das maiores infestações, senão a maior, infestação de *Bemisia tabaci*, jamais registada na região do Ribatejo, podendo ser a razão deste fenómeno.

Abordando a temática dessa mesma campanha e considerando que *B. tabaci* tem o potencial de se tornar uma das maiores pragas nos anos vindouros, favorecida pelo panorama do aquecimento global e pela sua versatilidade e resiliência, é relevante analisar diferentes alternativas à sua gestão, neste caso na cultura de tomate para indústria, sendo uma das estratégias possíveis, encontrar ou desenvolver cultivares que sejam menos suscetíveis.

Em anos recentes, foram realizados vários estudos com diferentes cultivares de tomate selvagem a demonstrar a existência de resistência para *B. tabaci*, nomeadamente, através dos seus tricomas. Porém, até ao momento, não existia nenhum estudo feito em cultivares comerciais de tomate comumente usadas pelos agricultores, quer a nível de suscetibilidade a esta praga, quer a nível do tipo e distribuição dos seus tricomas,que poderão influenciar a suscetibilidade.

Com o objetivo de determinar se, de facto, nas cultivares comerciais de tomate de indústria existem diferenças nos tricomas presentes (número, tipo) e/ou diferenças de atratividade para *B. tabaci* foi realizado um procedimento experimental numa estufa localizada em Águas de Moura, pertencente à empresa Kagome Agri-Business Research and Development Center, Lda. Este procedimento experimental, consistiu em dois ensaios de livre escolha.

No primeiro ensaio, foram selecionadas quatro cultivares de tomate de indústria, CXD277, H1015, H3402 e KG273, que foram plantadas em três grupos de três plantas cada. Na estufa onde se encontravam foram libertados espécimes de *Bemisia tabaci* de forma homogénea.

Uma folha de uma planta por grupo foi colhida e levada para a FFUL (Faculdade de Farmácia da Universidade de Lisboa), onde foi feita a preparação das amostras para posterior observação na unidade de microscopia da FCUL (Faculdade de Ciências da Universidade de Lisboa). A observação foi efetuada com recurso a lupa estereoscópica automatizada, que possibilita o controlo da profundidade de campo, e a microscópio eletrónico de varrimento, para fotografar e

proceder a análise de imagem para identificar e contabilizar os tricomas presentes. No segundo ensaio, foram usadas as mesmas cultivares, mas desta vez com quatro grupos por cultivar, cada um composto por cinco plantas cada.

Em ambos os ensaios, decorrido um mês após a largada, as folhas da planta central de cada grupo foram recolhidas e contabilizadas as ninfas.

Não se verificaram diferenças no número de tricomas glandulares, não glandulares e totais entre as cultivares estudadas. No primeiro ensaio, KG273 apresentou menos ninfas de *B. tabaci* do que H1015 e CXD277, se se excluir da análise H3402. No segundo ensaio não se verificaram diferenças significativas, o que pode indicar que as diferenças que possam existir entre as diferentes cultivares serão pouco expressivas. Não se verificou, também, correlação entre número de tricomas e o número de ninfas de *B. tabaci* presentes, pelo que os parâmetros tipo e quantidade de tricomas não parecem explicar a diferença de suscetibilidade, a existir, nas cultivares estudadas, e provavelmente não será um factor para a diferença de atractividade em cultivares de tomate .

Discutem-se outras hipóteses explicativas para diferenças de atratividade para *B. tabaci,* como a cor da folhagem e a arquitetura da vegetação.

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1. Introduction

The processing tomato is one of the most important crops in Portugal, especially in Ribatejo region. The products originated from this tomato are, quality wise, one of the best in the world, with good color, sweetness and few acidity (Stilwell 2006).

In the 2017 processing tomato crop season it was possible to verify a general decrease of quality in the tomato arriving at the factories, being color the most affected. Many factors were considered to explain this general decrease in quality, being one of them a great population level of *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) like never seen before.

Part of the harvested tomatoes were ripened in the outside but when opened showed a white tone in the inside flesh; other part was fully ripened but with green zones in the outside of the fruits. Both symptoms were described by McCollum *et al.* (2003) and by Masuda *et al.* (2016) as an effect of *B. tabaci* attack, which allow us to put the hypothesis that this pest was the origin of that season's disappointing results.

In the management of this pest there are already many strategies being applied. However, this insect seems to be evolving, easily adapting to adverse conditions (Ma *et al*., 2014) and developing resistance against insecticides (Castle *et al.*, 2009; Garcia, 2013). This is even more problematic considering the current global warming context, where the overall temperature is expected to increase up to 1 to 2 °C in the next years, and so, the insect population is expected to intensify in the areas where it is already established (Gilioli *et al*., 2014).

To avoid scenarios like the 2017 season, it is necessary to search for alternatives to control *B. tabaci* in the processing tomato crop. One of the possible alternatives is the identification and/or creation of different processing tomato cultivars that are resistant / less susceptible to its outbreak.

The objective of this study was to verify and compare the abundance and type of trichomes (glandular and non-glandular) amongst commercial cultivars of processing tomato and evaluate if the differences in this parameter affects the attractiveness to *Bemisia tabaci*.

2. Tomato

Tomato (*Solanum lycopersicum* L., formerly *Lycopersicon esculentum* L.) is nowadays one of the most economically important and widely grown plants in the Solanaceae family. Botanically, it is a fruit and horticulturally it is a vegetable (Benor *et al.*, 2008). The production worldwide in 2017 was of 182 million tons of fresh weight on 4.8 million cultivated hectares (FAO, 2017).

The popularity of tomatoes relates to the fact that it can be eaten in multiple forms, either fresh or processed. Among the processed products there are: (i) tomato preserves (e.g. whole peeled tomatoes, tomato pulp and juice, tomato puree, pickled tomato and tomato paste); (ii) dried tomatoes (tomato powder, tomato flakes, dried tomato fruits); and (iii) tomato-based foods (e.g. tomato soup, tomato sauces, chili sauce and ketchup) (Costa and Heuvelink 2018).

In terms of processed tomato industry, Portugal is one of the biggest in value worldwide, being the 5th biggest exporter of tomato paste and 8th biggest exporter of peeled tomato (FAO, 2017). The country has exceptional conditions for its production, producing sweet paste with few acidity (Stilwell 2006).

The quality attributes such as color, consistency, and flavor of the processed tomato products influence the buying behavior of the consumer, and parameters like pH, total acidity and sugar content of the raw material affect the yield and efficiency of the processing industry (Thakur *et al.*, 1996). Amongst all, color is the first quality attribute that stimulates consumer to purchase. Tomatoes are known for their vibrant red color, which indicates not only maturity and therefore level of desired flavor, but also relative content of the beneficial antioxidant lycopene. Tomatoes that are deep red in color, as compared to those that are lighter red or pink, are usually more mature fruit with desirably sweet flavor and a high content of lycopene (Barrett and Anthon 2008).

The wild relatives of the cultivated tomato are native of western South America along the coast and high Andes, from central Ecuador, through Peru, to northern Chile, and in the Galápagos Islands. The pre-domestication started in Peru or in Mexico, before it was brought to Europe in the 16th century (Peralta and Spooner 2007).

In Europe, tomatoes were initially cultivated as ornamental plants in gardens due to their similarities to poisonous plants, being only accepted for culinary purposes around the 17th and 18th centuries (Peralta and Spooner 2007). By the end of the 19th century, several cultivars of tomato were available in different colors and for different purposes, products of domestication and some early breeding. Since the 20th century, new

morphological distinct cultivars have been created from *S. lycopersicum* through hybridization (Bai and Lindhout 2007).

The domestication on the tomato in the early stages focus on its growth habit, its fruit weight, its reproduction cycle, its seeds weight and also in its fruits flavour (Yang et al. 2019). This process of domestication led the domesticated tomato to a much poorer genetic reservoir than it's wild ancestors, being estimated that the genomes of domesticated tomato cultivars contain only 5% of the genetic variation of their wild relatives (Miller and Tanksley 1990).

In more recent years, with the crop development, the breeding objectives depend also on which market the cultivar (cv) is dedicated to: the fresh or the processed market (Bergougnoux 2014).

The processing tomato cultivars have now a very define set of traits as a standard model variety such as multiple diseases resistance, jointless pedicel, high yield, concentrated fruit setting, earliness, fruit firmness, crack resistance, and heat-tolerant fruit setting. Successful cultivars require soluble solids higher than 5.4 °Brix, a pH inferior than 4.4, Botswick lower than 4.0 on paste (viscosity), puree color of < 24 in Agtron scale, fruit size of 75–80 g, and blocky fruit shape (Scott *et al.*, 2013) .

3. Bemisia tabaci

Bemisia tabaci, commonly named the tobacco whitefly or the sweet potato whitefly, first described on tobacco from Greece by Gennadius in 1889, is a phloem-feeding insect belonging to a group of insects commonly known as whiteflies. It is a highly polyphagous insect with a continuously expanding list of host plants, currently comprising more than 1 000 plant species (EFSA Panel on Plant Health (PLH), 2013). Of the six hundred host plant species described by Oliveira *et al.* (2001), 50 % to five families (Fabaceae, Asteraceae, Malvaceae, Solanaceae and Euphorbiaceae), comprising a large number of cultivated and non-cultivated annual and perennial plants, including economically important crops (i.e. beans, tomato, cucurbits, poinsettia and many more).

Nowadays, *B. tabaci* is not considered as a species. It's a cryptic complex of species (De Barro *et al.*, 2011). By comparisons against consensus sequences and delimited by 3.5% mtCO1 (*mitochondrial cytochrome oxidase 1*) sequence pairwise genetic distance divergence, it was possible to conclude that it is composed of, at least, 24 morphologically indistinguishable species (De Barro *et al*., 2011). It has been reported as present in all continents except Antarctica, and it is considered to be one of the most invasive species worldwide. Several geographic origins have been proposed for *B. tabaci*, however, recent evidence proposes sub-Saharan Africa as the most likely geographical origin (De Barro *et al.*, 2011).

Even though this whitefly is now considered as a complex of species, biotypes designation has been commonly used to distinguish within this complex. These different designations were achieved by comparing the combined use of biological data among different populations on: host range; host utilization; capacity to induce physiological changes in some hosts; insecticide resistance; capacity to disperse widely; capacity to transmit begomoviruses; and esterase and RAPD profiles. So far 36 biotypes were identified: A, AN, B, B2, BR, C, Cassava, Cv, D, E, F, G (India), G (Guatemala), H, I, J, Jatropha, K, L,M, N, NA, Okra, P, PCG-1, PCG-2, PK1, Q, R, S, Sida, SY, T, ZHJ1, ZHJ2, and ZHJ3 (De Barro et al., 2011). Among the described, B and Q biotypes are the most predominant and damaging worldwide (Mahadav *et al.*, 2009) and both were already identified as existent in Portugal (Lopes, 2003; Mateus et al. 2007), being that another biotype, probably an hybrid of B and Q, was also seen (Marques, 2002; Lopes, 2003). The latest reports by INIAV (2018) revealed only biotype Q as existent in Ribatejo although some problems occurred with the sampling for this study (E. Valério, pers,. comm.). So, probably there coexist two biotypes in this region: B and Q.

3.1. Morphology, Biology and Life Cycle of *Bemisia tabaci*

Bemisia tabaci has three developmental stages: the egg, four nymphal instars (I,II,III,IV), and the adult. At the nymphal stage morphology is very simple; however adult whiteflies have all the typical anatomical features of Sternorrhyncha adults (Walker, *et al*., 2010) (Table 1).

Table 1: Developmental stages and morphological characteristics of *Bemisia tabaci* life stages.

Stage	Characteristic
Egg	White eggs, which gradually turn brown (PLH, 2013), pear shaped with a pedicel spike at the base, approximately 0.2 mm long (CABI 2015).
Nymph (I, II, III, IV-initial instars)	Yellow-white scales, 0.3-0.6 mm long (CABI 2015). All instars are oval in shape and flattened dorso-ventrally, the first instar has well developed legs while the others have reduced (Walker *et al*., 2010).
IV-late nymphal instar or pupa	Flat, irregular oval shape, 0.7 mm long. On a smooth leaf the puparium lacks enlarged dorsal setae, but if the leaf is hairy, two to eight long dorsal setae are present (CABI 2015).
Adult	Small insect, about 1 mm long for females and 0.8 mm for males, with a white to light yellow body covered in a waxy powdery material, especially the wings (PLH, 2013)

The eggs are usually laid by the females in a semi-circular arrangement on the underside of leaves (PLH, 2013).The egg has a pedicel that is an extension of the egg chorion that is inserted directly into epidermal tissue; its apex has a porous fibrous structure which absorbs water and possibly nutrients from the leaf. The early first instar or “crawler” has well-developed legs and, after hatching, it wanders over the leaf surface in search of a suitable settling site. Once a site is found, the nymph inserts its stylets and remains at that site for the rest of its nymphal development. The dispersal ability of this “crawler” is considered limited, the host and leaf choice is still largely controlled by the oviposition location (Walker *et al.*, 2010).

The fourth instar is sometimes referred to as the “pupal” stage but during the early phase of this stage it still feeds, and thus it is not a pupa in the normal sense of holometabolous

insects. At the latter part of this stage a dramatic metamorphosis to an adult morphology takes place. The first externally observable feature in the fourth instar that signals this metamorphosis is the enlargement of the eyes from small red pinpoints to larger diffuse red oval spots. So, pupa can be correctly used for the last phase of this instar. Close to the time of adult emergence, the fourth instar cuticle is mostly transparent, and the pharate adult becomes visible underneath (Walker *et al.*, 2010).

Bemisia tabaci is an arrhenotokous being: unfertilized eggs give rise to haploid males while fertilized eggs develop into diploid females. Each female has a high reproductive potential and can lay an average of 80 to more than 300 eggs during its lifetime. The number of eggs laid depends on temperature and host plant (PLH, 2013). Fecundity of over 500 progenies in optimal conditions has already been witnessed (Naranjo *et al.*, 2010).

It is also considered as a multivoltine species (more than 1 generation per year), having multiple and overlapping generations; under very highly favorable conditions, it can lead to more than 12 generations per year (Naranjo et al. 2010). The life cycle can occur at temperatures between 10 °C and 35 °C, with an optimum between 25 °C and 27 °C. Depending on the prevailing temperatures, development from egg to adult lasts between 15 and 70 days (PLH, 2013).

3.2. Damages caused by *Bemisia tabaci*

This insect is capable of inflicting damage to plants in multiple ways. High populations may remove sufficient phloem sap to reduce plant vigor and the secretion of their honeydew results in a sooty mold that can reduce the quality and marketability of harvested products. Feeding, even by a few number of insects, may induce debilitating plant disorders or the transmission of numerous plant viruses (Walker *et. al,* 2010).

Regarding viruses, *B. tabaci,* due to its characteristics, is considered as a supervector, transmiting virus from five different genus: *Begomovirus, Ipomovirus, Crinivirus, Carlavirus* and *Torradovirus* (Gilbertson *et al.*, 2015).

3.3. Tomato Irregular Ripening Disorder

Besides, the previously mentioned problems in the previous topic "3.2. Damages caused by *Bemisia tabaci*", this whitefly has been known for causing disturbance in fruits ripening with symptoms similar to the ones observed in the 2017 processing tomato season in the Ribatejo region (fig.1), that lead to disappointing results in the color parameter.

Figure 1: Tomato abnormal ripening symptoms in 2017 season.

The tomato irregular ripening disorder (IRR) was firstly observed in southwest Florida in late 1987 and became widespread in south Florida in 1988 spring (Maynard and Cantliffe 1989) and in west central Florida in 1989 spring. The disorder was firstly characterized by an incomplete or inhibited ripening of longitudinal sections of fruit, also associated with an increased amount of internal white tissue, and its appearance was only existent in scenarios where there was large whitefly population (Schuster *et al.*, 1990). *Bemisia tabaci* responsible for this damage was identified as the B biotype (Mcauslane 2007).

After checking this phenomenon, some studies were carried on to understand the relation of the *B. tabaci* biotype B with this disorder. The correlation between both was found to be positive and significant (Schuster et al. 1990) and high densities of this pest nymphs and pupae also seem to have direct impact on the IRR, with the symptoms only revealing sometime after (Schuster 2001). The changes in the fruit can be due to a physiological response of the plant to the whitefly feeding (McKenzie et al. 2004) that affects the ethylene biosynthesis (responsible for the ripening) and therefore leads to an irregular ripening process (McCollum et al. 2004).

In 2016, an experiment was carried out to verify what was the difference among infested plants with IRR tomatoes and non-infested plant with normal tomatoes in terms of

carotenoid content, revealing that the tomatoes with symptomatic tissues revealed a 69% reduction of lycopene content and a 79% reduction of phytoene, an intermediary in the synthesis of carotenoids (Masuda *et al*., 2016). This could explain the general decrease in the color parameters, since lycopene content is very correlated with this factor (Arias *et al.*, 2000).

Considering that the symptoms identified in 2017 Portuguese season were in fact the IRR disorder, there's the possibility that the latest study lead in Portugal by INIAV (2018) failed to catch and identify biotype B, or that in fact the biotype Q is the only existent in Portugal and it caused the same symptoms. However, in the literature as far as we know, there is no evidence of IRR caused by biotype Q.

4. Trichomes in domesticated and wild tomato plants

4.1. Trichomes

Trichomes are defined as unicellular or multicellular appendages originated from epidermal cells only that develop outwards on the surface of various plant organs. Trichomes can be found on all aerial organs of plants, including vegetative (leaves, young stems and bracts) and reproductive (sepals, petals, stamens, gynoecium), as well as in fruits and seeds (Werker, 2000).

The diversity of trichomes is enormous, so their classification is done according to their morphology, origin, size, location, surface microstructure, capability to secrete, mode of secretion, timing of activity, function, being the major distinction between "glandular" and "non-glandular" trichomes (Werker, 2000).

Trichomes have a range of functions: non-glandular trichomes protect the plant from extensive light, extreme temperatures, excessive water loss, allelopathy, against competitor plants, herbivores and pathogens (physical barrier). On the other hand, glandular trichomes offer chemical protection against herbivores and pathogens (Werker, 2000) and also physical by entrapping the insect upon rupture of the trichome gland (Kang *et al.*, 2010). Trichomes can also provide a positive oviposition stimuli and enhancing survival by amelioration environmental factors in some species (Peter *et al.*, 1995).

Leaf trichomes not only affect herbivores but also their natural enemies, which indirectly affect the intensity of damage caused by herbivores. In theory, the effect on the abundance and effectiveness of natural enemies may be neutral, negative or positive. Plant damage will be: (i) negatively correlated with trichome density if the effect on natural enemies is weaker than that on herbivores; (ii) uncorrelated with trichome density if the effect on the natural enemies and herbivores is equal or (iii) positively correlated with trichome density if the effect on natural enemies is stronger than that on the herbivores. From a plant protection perspective, the first scenario would be most desirable (Dalin, 2008).

The genus *Solanum* is one of reference in the field of trichomes (Tissier 2012). It has been studied since early times. In 1943, Luckwill performed a taxonomic survey, to these plants, in which were identified seven types of trichomes (fig.2):

- non-glandular:
  - II – 0,2 to 1.0 mm with multicellular base;
  - III – 0.2 to 1.0 mm with unicellular base;
  - V – 0.1–0.3 mm with a unicellular base;
- glandular:
  - I – 2.0 mm multicellular stalk, and a small glandular tip;
  - IV – 0.3 mm unicellular base, a multicellular stalk, and small glandular tip;
  - VI – 0.1 mm multicellular stalk containing a four-celled glandular head;
  - VII – 0.05 mm unicellular stalk and an irregularly shaped 4- to 8-celled gland.

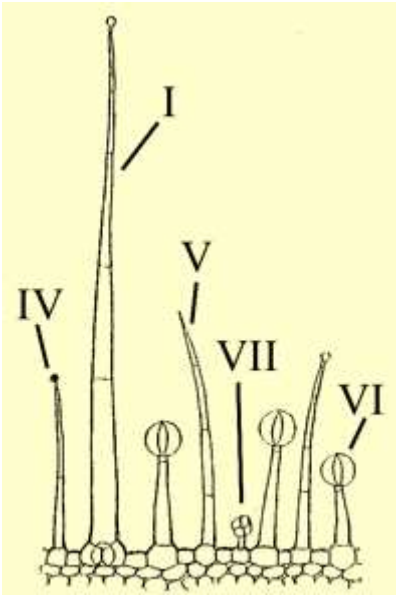

Figure 2: Morphological differences between some types of trichomes found on *Solanum* genus (Luckwill, 1943).

Trichome morphology, density and chemical composition are important mechanisms of defense to prevent or decrease herbivore damage (Tian *et al.*, 2012). The differences in these parameters can explain the higher resistance level to arthropod pests of the wild *Solanum* species compared with *S. lycopersicum*, the domesticated one (Simmons and Gurr 2005).

The presence and density of each type of trichomes change within the genus *Solanum* (table 2).

Table 2: Types and abundance of different trichomes in *Solanum* species.

Species	Former Species	Trichome type[1]							Reference
		I	II	III	IV	V	VI	VII	
Solanum lycopersicum	*Lycopersicon esculentum*	A	-	A	-	A	A	S	Luckwill, (1943)
Solanum pimpinellifolium	*Lycopersicon pimpinellifolium*	-	VS	-	-	A	A	-	Luckwill (1943)
Solanum peruvianum	*Lycopersicon peruvianum*	A	-	-	-	A	A	S	Luckwill (1943)
Solanum cheesmaniae	*Lycopersicon cheesmanii*	-	-	-	-	A	S	S	Luckwill (1943)
Solanum cheesmaniae	*Lycopersicon cheesmanii*	-	-	-	-	S	S	VS	Simmons & Gur (2004)
Solanum galapagense	*Lycopersicon cheesmanii f. minor*	VS	-	-	A	S	S	VS	Simmons & Gurr (2004)
Solanum habrochaites	*Lycopersicon hirsutum*	A	-	S	A	-	A	S	Luckwill (1943)
Solanum habrochaites	*Lycopersicon hirsutum*	VS	-	VS	A	A	A	VS	Simmons & Gurr (2004)
Solanum habrochaites	*Lycopersicon hirsutum f. glabaratum*	VS	-	VS	A	A	A	VS	Simmons & Gurr (2004)
Solanum pennellii	*Lycopersicon pennellii*	VS	-	VS	A	-	S	VS	Simmons & Gurr (2004)

[1] A-Abundant; S -sparse; VS- very sparse.

The density of trichomes is not only a characteristic of the genotype, it can also be changed according to external conditions. Water availability, light intensity, photoperiod, temperature and relative humidity greatly influence the trichome density (Nihoul, 1993; Wilkens *et al.* 1996), herbivory or wounding by pests can also increases trichomes density as a plant response (Tian et al. 2012).

The changes in density are controlled by phytohormones. The trichome density is indirectly affected by ethylene, gibberellic acid (GA) and auxins, since those hormones interfere with cell length growth and longer cells have less trichomes. Jasmonic acid (JA)

and brassinosteroids (BR) seem to have a direct impact on the trichomes formation (Campos et al. 2009).

Not only the density seems to vary among the different species of *Solanum* but also the morphology of the trichomes can have a slightly variation between the domesticated tomato and the wild species.

The IV type that was described as inexistent by Luckwill (1943), is now considered as of possible occurrence in *Solanum lycopersicum.* In a more recent experiment, it was possible to observe trichomes IV-like structures, although those can also be a developmentally immature stage of the type I trichome (Kang et al. 2010).

The external appearance of VI type was identified as clearly four glandular cells in domesticated tomato while in a identified wild species (*S. habrochaites* S. Knapp & D.M. Spooner) it was already identified externally as round (although at a further analysis it's also four cells but the contour is not so clearly divided) (Bergau *et al.*, 2015).

4.2. Exudates produced by trichomes

The secondary metabolites released by plant trichomes are derived from primary metabolites such as amino acids, fatty acids, and sugars (Wang, 2014). Some of these compounds such as acyl sugars, methyl ketones, and sesquiterpenes have shown to confer resistance against many arthropods, including whiteflies (Rakha *et al.,* 2015).

The domestication led to a limited genetic diversity among the cultivated tomato, but the wild relatives show a wide range of polymorphism and this richness of genetic polymorphism is reflected in the type and quantity of the special compounds accumulated in their trichomes such as methylketones (Ben-Israel et al. 2009) terpenoids (Besser et al. 2008) and acylsugars (Ghosh *et al.*, 2014).

Solanum habrochaites is a wild tomato species native from Southern Ecuador and Peru (Sifres *et al.*, 2011). This species is considered as a reference among this genus as the most resistant species against insects (Vosman et al. 2018) and has been subject of many scientific papers as comparison to *S. lycopersicum,* including in the existing metabolites it produces and different amounts of each.

4.2.1. Acylsugars

Acylsugars exhibit insecticidal properties against whitefly and aphids (Chortyk *et al.*, 1996). Within the tomato clade on the genus *Solanum*, acylsugars consist of a sugar backbone, either glucose or sucrose, to which two to five aliphatic acyl chains are esterified.

The acyl chains range in length from two to thirteen carbons and may be either straight or branched. The type of structure vary widely among the species of Solanaceae and can also display significant variation within accessions of a single species (Schilmiller et al. 2015).

Ghosh *et al.* (2014) compared two accessions of *S. habrochaites* and one of *S. lycopersicum*. In general, most part of the acylsugars showed a higher abundance in the wild *Solanum*, and some were only present in this one (Fig. 3; Table 3).

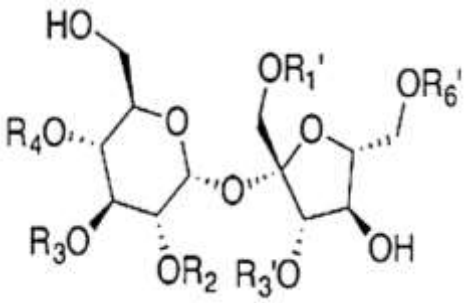

Figure 3: Aclysugars structure.

Table 3: Comparison of aclysugars type and abundance in one domesticated tomato accession and two wild tomato accessions (based on fig.3) (Gosh *et al.*, 2014).

Acylsugar	R2[1]	R3	R4	R1'	R3'	R6'	Metabolite abundance		
							S. lycopersicum M82	*S. habrochaites* LA1392	*S. habrochaites* LA1777
Triacylsucroses									
S3:19[5]	H	iC4	iC5	H	iC10	H	+	++	+++
S3:19[9]	iC4	iC10	iC5	H	H	H	ND	++	ND
S3:20[4]	H	aiC5	iC5	H	iC10	H	+	+++	ND
S3:21[1]	H	aiC5	iC5	H	aiC11	H	ND	++	+
S3:21[5]	iC5	aiC11	iC5	H	H	H	+	++	++
S3:22[4]	H	nC12	iC5	H	iC5	H	++	+++	++
S3:22[5]	aiC5	nC12	iC5	H	H	H	ND	+++	+
Tetraacylsucroses									
S4:16[3]	C2	iC4	iC5	H	iC5	H	++	++	+++
S4:17[2]	iC4	aiC5	iC5	H	iC5	H	+++	+++	+++
S4:19[7]	iC5	iC5	iC5	H	H	iC5	ND	++	++
S4:20[6]	C2	iC5	iC5	H	H	iC5	ND	+++	+
S4:20[7]	C2	iC4	iC4	H	iC10	H	++	++	+++
S4:21[2]	C2	iC4	iC5	H	iC10	H	++	+++	+++
S4:22[2]	C2	aiC5	iC5	H	iC10	H	+	+++	+++
S4:22[3]	C2	iC4	iC5	H	aiC11	H	ND	++	++
S4:22[6]	C2	iC4	iC5	H	nC12	H	+	+	+++
S4:23[3]	C2	aiC5	iC5	H	aiC11	H	ND	++	++
S4:23[5]	C2	iC4	iC5	H	iC12	H	+	++	++
S4:23[6]	C2	iC4	iC5	H	nC12	H	+	++	+++
S4:24[5]	C2	aiC5	iC5	H	iC12	H	+	++	++
S4:24[6]	C2	aiC5	iC5	H	nC12	H	+	++	++
S4:24[8]	C2	nC12	iC5	H	iC5	H	++	++	++
Pentaacylsucroses									
S5:24[3]	aiC5	iC4	iC5	iC5	H	iC5	ND	+++	++
S5:25[4]	iC5	iC5	iC5	iC5	H	iC5	ND	+++	++

[1] Radical groups: C2 = acetate, iC4 = $(CH_3)_2CHCO$, iC5 = $(CH_3)_2CHCH_2CO$, aiC5 = $CH_3CH_2(CH_3)CHCO$, iC10 = $(CH3)_2CH(CH_2)_6CO$, aiC11 = $CH_3CH_2(CH_3)CH(CH_2)_6CO$, iC12 = $(CH_3)_2CH(CH_2)_8CO$, nC12 = $CH_3(CH_2)_9CO$.

4.2.2. Metylketones

Methylketones are molecules with carbon chains ranging from seven to fifteen carbons formed by the hydrolysis of acyl-ACPs (intermediates of the fatty acid biosynthesis pathway), and subsequently decarboxylation of the -ketoacids in plant cells (Wang, 2014).

According to an experiment done by Antonious (2001), where levels of methylketones were measured in six different accessions of *S. habrochaites* and one of *S. lycopersicum*, the levels were up to 33.51 to 67.65 times more than on the domesticated one (except one of the wild tomato accessions that had only around 50% increase in these metabolites amount), being the 2-undecadone and the 2-tridecadone the most representative ones (table 4).

Table 4: Comparison of methylketones type and abundance in one domesticated tomato accession and six wild tomato accessions (Antonious, 2001).

		Methyl ketones (µg.g $^{-1}$)				**Total methyl ketones**
Taxon	Acession	2-undecadone	2-dodecanone	2-tridecanone	2-pentadecanone	
S. habrochaites	PI 251304	636.2	9.7	3028.2	169.8	3843.9
	PI 251305	18.3	0	0	108.6	126.9
	PI 126449	871.8	15	3330.8	176.3	4393.9
	PI 134417	971.6	16.3	4310.4	202	5500.3
	PI 134418	613.4	14.6	2479.8	148.1	3255.9
	LA407	2024.8	17.3	574	98.4	2714.5
S. lycopersicum	Fabulous	10	0	16.8	54.5	81.3

4.2.3. Terpenoids

Terpenoids (isoprenoids) are any compounds that are derived from the isomeric 5-carbon building blocks isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). These formed compounds are isoprene (C5), cyclic and acyclic monoterpene hydrocarbons and monoterpene alcohols (C10), cyclic and acyclic sesquiterpene hydrocarbons and sesquiterpene alcohols (C15) and cyclic and acyclic diterpene hydrocarbons and diterpene alcohols (C20) (Chen *et al*., 2011). In *Solanum* genus, sesquiterpenes can be divided in class I and class II (van der Hoeven *et al.*, 2000). Class I sesquiterpenes are thought to be modified, thus forming sesquiterpene carboxylic acid which have strong insecticidal properties (Coates *et al.*, 1988).

Besser *et al.* (2008) compared two accessions, one of *S. lycopersicum* and one of *S. habrochaites*, in their terpenoids metabolites production. In terms of quantity, wild *Solanum* showed a concentration more than 9 times superior to its domesticated relative. In terms of type and ratio of produced terpenoids, domesticated tomato showed a high level of monoterpenes (more than 90%), an insignificant amount of class I sesquiterpenes (below 1%) and none class II sesquiterpenes neither sesquiterpene carboxylic acid, while the wild tomato had an insignificant amount of monoterpenes, class I and class II sesquiterpenes, and very high amount of sesquiterpene carboxylic acids (table 5).

Table 5: Comparison of terpenoids type and abundance in one domesticated tomato accession and one wild tomato accession (Besser et al. 2008).

Compound	*S. habrochaites* LA1777		*S. lycopersicum* cv M82	
	Quantity (ng mm^{-2})	% of total	Quantity (ng mm^{-2})	% of total
Monoterpenes				
Limonene	2.2		ND	
α-Pinene	ND		19.8	
Verbenene	ND		20.5	
δ -2-Carene	ND		150.1	
β -Phellandrene	ND		458.4	
Total	2.2	0.04	648.7	90.48
Class I sesquiterpenes				
β-Elemene	6.4		1.1	
γ-Elemene	4.8		ND	
Germacrene D	6		ND	
Germacrene B	2.4		ND	
β-Caryophyllene	4.1		1.2	
α -Humulene	3.7		1.1	
Total	27.4	0.55	3.3	0.52
Class II sesquiterpenes				
α-cis-Bergamotene	8.6		ND	
α-Santalene	21.8		ND	
α-trans-Bergamotene	5.3		ND	
Total	35.7	0.71	0.0	
Carboxylic acids				
α-cis-Bergamotenoic acid	713.8		ND	
α-Santalenoic acid	2340.6		ND	
α-trans-Bergamotenoic acid	174.9		ND	
β-cis-Bergamotenoic acid	1968.9		ND	
Total	5198.2	93.49	0.0	
Not defined terpenoids class				
β-Cubebene	9.7		ND	
Unknown	15.2		ND	
Total	24.9	0.49	0.0	
Total terpenoids	5288.4		652.0	

ND – Not Defined

4.3. Influence of the type of *Solanum* spp. trichomes on *Bemisia tabaci*

Some studies have been done using *Solanum* spp. to know about their resistance to *Bemisia tabaci* through trichomes. The results obtained show a clear tendency, in which higher densities of non-glandular trichomes leads to higher number of eggs, nymphs and adults, while glandular trichomes have a negative correlation with the pest population on plants (Oriani and Vendramim, 2010; Firdaus *et al.*, 2012; Rakha, *et al.*, 2015). This results lead to the conclusion that non-glandular trichomes provide a more suitable microclimate for oviposition and protect the eggs and nymphs of *B. tabaci* from their enemies and glandular trichomes led to antibiosis between the plant and this pest (Firdaus et al. 2012).

Besides trichomes and their metabolites, it has already been proven there are other sources of resistance from tomatoes species such as the *Mi-1* gene (Nombela *et al.*, 2003), although its role is not yet clarified (Rodríguez-Alvarez *et al.*, 2019).

5. Materials and methods

5.1. Free choice trial

The chosen accessions were commercial cultivars (cvs) usually used by farmers and commonly used in the HIT (Holding da Indústria Transformadora de Tomate, SGPS) group factories: H1015, H3402, CXD277 and KG273 (table 6).

Table 6: Vegetative traits of the cultivars used in the free choice trials in FIT.

Cultivarr	Plant shape	Internode	Leaflet shape	Type of leaf blade	Leaf curl	Leaf color intensity	Leaf length	Leaf width	Vigor	Leaf cover	Cycle
CXD277	Middle	Middle	Normal	Bipinnate	Mid-weak	Mid-dark	Medium	Medium	Mid-Strong	Mid-strong	Mid-late
KG273	Open	Mid-Long	Normal	Bipinnate	Mid-weak	Mid-pale	Medium	Medium	Mid-Strong	Medium	Mid-late
H1015	Middle	Middle	Normal	Bipinnate	Mid-weak	Middle	Medium	Medium	Middle	Medium	Mid-early
H3402	Mid-open	Middle	Normal	Bipinnate	Mid-weak	Middle	Medium	Medium	Mid-Strong	Medium	Medium

Two free choice trials were done successively in the same section of a greenhouse owned by Kagome (Kagome Agri-Business Research & Development Center, Lda.), located inside FIT (Fomento da Indústria do Tomate) factory, in Águas de Moura.

The greenhouse (Fig. 4) with 45 x 16 x 5 m has four different sections, each section composed of four stands being each stand of 13.8 x 1 m. Both trials were done in the same section.

Figure 4: Experimental greenhouse in FIT where the free choice trials took place (Google, n.d.).

In both trials the plants were conducted in 3 L capacity black pots, with an organic substrate composed of forest humus and turf incorporated with a small amount of mineral fertilizer (20-5-11). The plants also received throughout the cycle, according to their needs, irrigation water with fertilizer solutions such as a NPK fertilizer (20-20-20) combined with other fertilizer (16-5-0) with MgO (5%), B (0.2%), Fe (2%), Mn (4%) and Zn (4%), alternated with a calcium nitrate (N (15.5%) and CaO (26.5%)) at electroconductivity between 800 to 1500 µS.

Whitefly nymphs were brought from Instituto Superior de Agronomia (ISA) facilities. The nymphs, at arrival, were firstly identified, using a key made by Hill (1969) (fig. 5) to distinguish between *Bemisia tabaci* and *Trialeurodes vaporariorum* (West.) at the fourth nymphal stage, in Kagome laboratory facilities, using a stereoscopic microscope, to confirm the species.

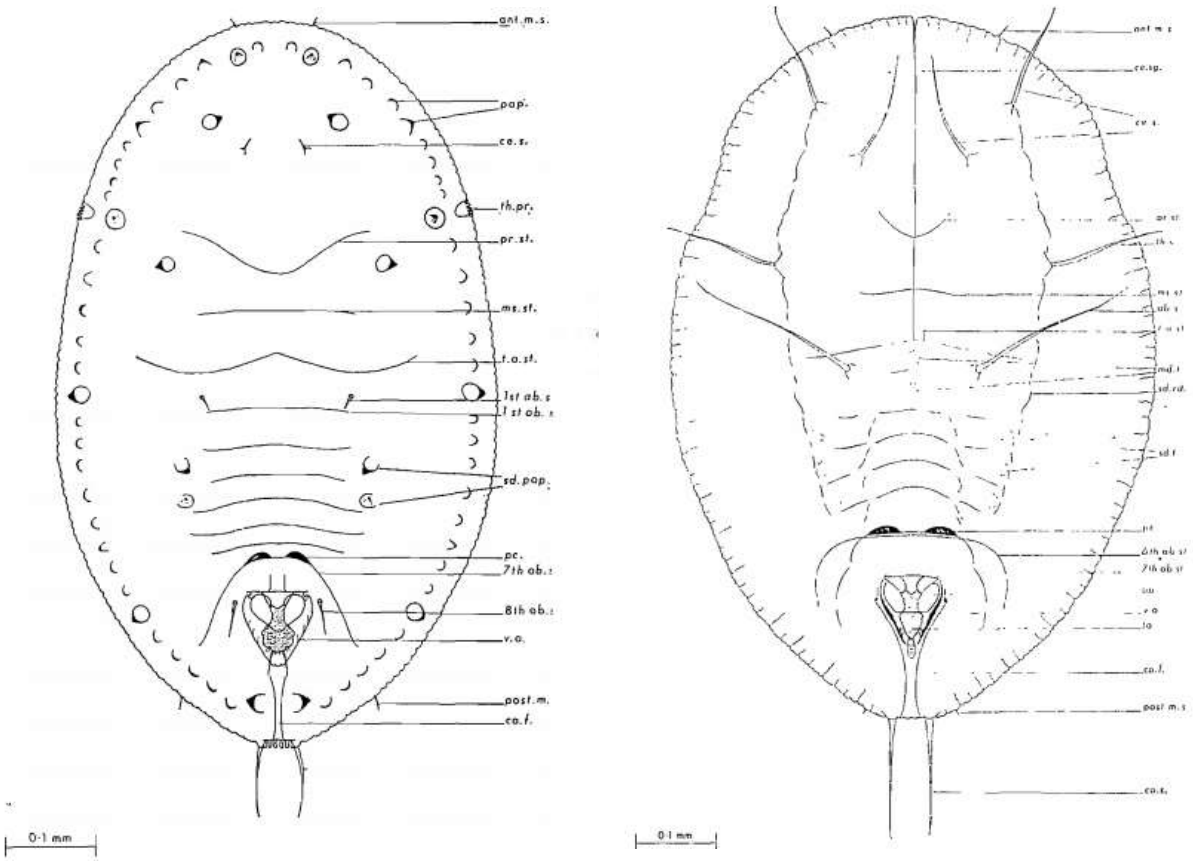

Figure 5: Morphology of the fourth nymphal stage on *Trialeroudes vaporariorum* (left) and *Bemisia tabaci* (right) – Hill (1969).

After identification, similar amounts of nymphs were transferred to four different cages, with four plants each, where the population evolved during three months.

In the first free choice trial, the plants were transplanted to the pots on 10th June of 2019, being each of the four varieties divided in three groups of three plants each and placed randomly in the first three of the four existent stands of this greenhouse section (fig. 6).

Between the stands, the cages with *B. tabaci* infested tomato plants were strategically placed and opened equally distant from the groups of the pots of the different cultivars. So, the places where these cages were placed would make the distribution homogeneous throughout the section, so that the choice made by the whiteflies was not due to location but due to preference (fig. 6, fig. 7).

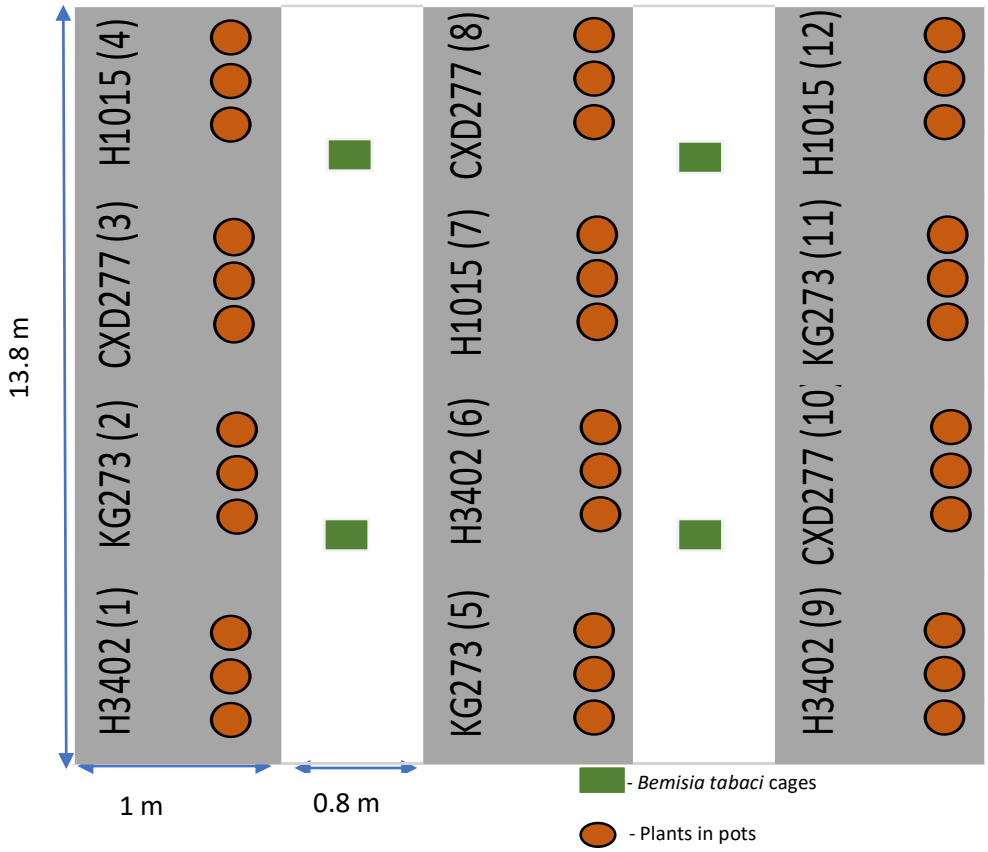

Figure 6: First free choice trial plan design, with the location of the different groups and location of the cages.

While the first trial was in place, the plants necessary for the second trial were developing in a nursing tray in another section of the greenhouse, located 15m to the right side, that was highly populated by *B. tabaci*. The plants became highly infested.

After counting the nymphs of the first trial on 19th July and remove the plants on 21th July, the second free choice trial was placed. In this trial, bigger than the first one, four stands of the same section were used, being the four cvs divided in four groups of five plants each, and randomly placed (fig. 8). The counting of nymphs, in this case, was done on 29th August, and the plants removed days after.

For the counting of the nymphs in both trials, one plant in the middle of each group was selected and nymphs were counted in every developed leaf. The leaves were removed and checked just after removal, in the lab facilities besides the greenhouse. The counting from each leaf was registered individually. In the 1st trial twelve leaves were analyzed and in the 2nd trial only eight since the hot temperatures lead to de deterioration of the leaves in the bottom layer of the plant.

Figure 7: Greenhouse trial display showing the location of the cages with the infested tomato plants.

5.2. Trichome analysis

In the first trial, on 17th July, the third leaf of the second shoot (counting from down to top), that was in a fully developed stage and healthy, in all the plants, was removed and prepared at FFUL (Faculdade de Farmácia da Universidade de Lisboa) for further observation at the FCUL (Faculdade de Ciências da Universidade de Lisboa) microscopy department. To verify the type of trichomes existent in the leaves, small leaf sections were cut from the central part of each fresh leaf, under study, and fixed in 5% glutaraldehyde buffered with 0.1 M sodium phosphate, pH 7.0, during 4 hours at 4ºC, later washed by water and then dehydrated by employing graded series of ethanol following (Hayat 1981) procedure. Samples were critical-point dried in a Critical Point Polaron BioRad E3500 and coated with gold in a Jeol JFC-1200. Observations were carried out at 20 kV on a Jeol JSM-5220 LV scanning electron microscope equipped with a direct image acquisition system. Measurements and counts were obtained by computer-assisted image analysis.SEM observations focused on trichomes distribution on the upper and lower epidermis and their characterization, following the nomenclature given by Luckwill (1943).

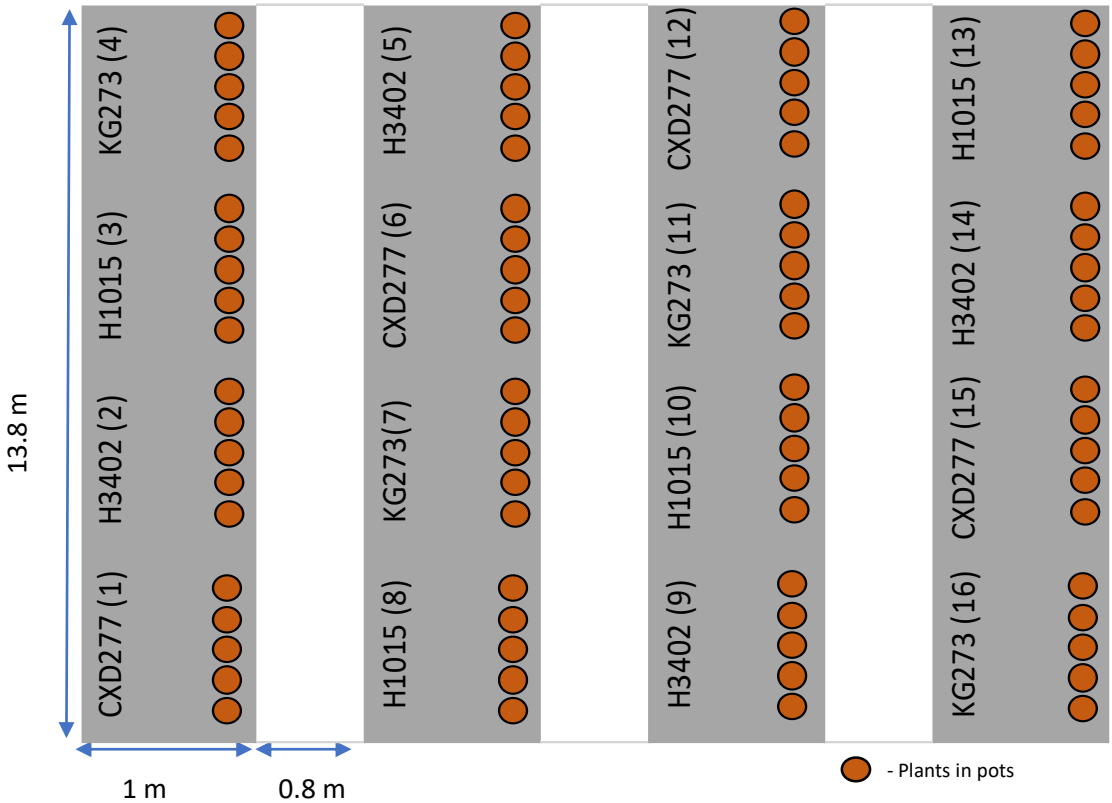

Figure 8: Second trial design.

For the counting of the trichomes, the leaflet in the fourth position counting from the leaf base was selected. From each leaflet, small squares (around 0.8mm x 0.8mm), from the middle section near the main vein were cropped (fig.9) using a scalpel.

Figure 9: Leaflet after cropping the rectangle for trichome analysis.

Afterwards the sample area was observed, covering all the surface of each square (fig. 10), using a Zeiss Lumar V1.2 stereoscopic microscope which allows the control of the field depth. A 80x magnification was used in all pictures which were captured with a the help of a software.

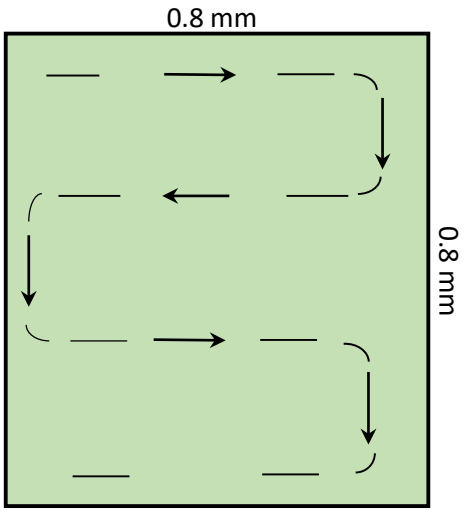

Figure 10: Picture capture method.

From each of the cropped leaves squares, from the twelve leaflets analysed (three from each cultivar) were taken around hundred pictures.

The pictures (fig. 11) were then analysed to count the glandular and non-glandular trichomes, and the results collected.

Figure 11: Image of trichomes found in the pictures for counting.

5.3. Statistical Analysis

A two-factors ANOVA was performed to compare the cultivars regarding number of nymphs of whitefly on leaves and number of glandular and non-glandular trichomes. These analyses were performed after verifying the normality (by Shapiro- Wilk test) and homoscedasticity (by Levene test).

Pearson correlation tests were performed between the number of nymphs on the leaves and the number of both (non-glandular and glandular) trichomes and total number of trichomes. For these correlations we used whitefly nymph counting on the third leaf (the same position used for trichome counting) and the total number of nymphs present in the second, third and fourth leaves. IBM SPSS vs 25 was used to perform these analyses.

6. Results

6.1. Whitefly nymph analysis

The vertical distribution of the nymphs seemed to show a bigger preference for the mid-top part of the plants, however, it seems to be very heterogenous (fig. 12). There were no statistical differences found regarding the vertical distribution of the nymphs on the plant (comparing the different leaves) among the stands (blocks) in both trials in the case of cv. KG273 and H1015, and in CDX277 in the 1st trial and H3402 in the 2nd trial. So, it seems that *B. tabaci* has not a clear preference for a particular stratum (leaf level) of the plant.

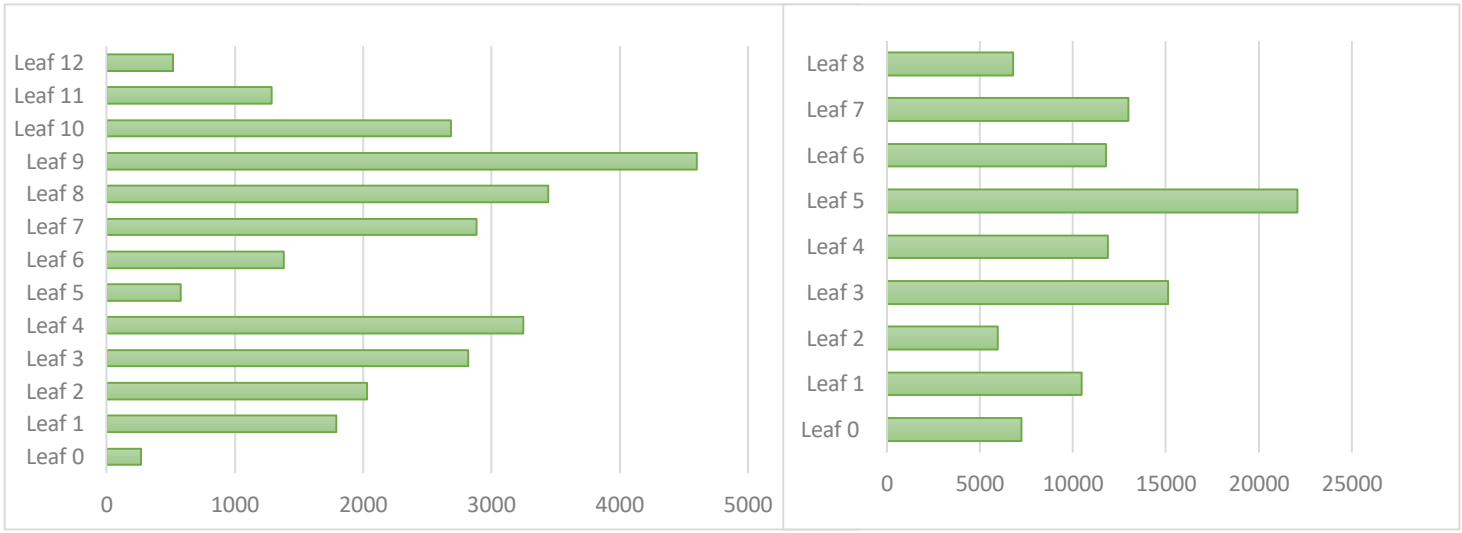

Figure 12: Vertical distribution of the nymphs of *Bemisia tabaci* in the tomato plants – total number of counted nymphs from each leaf from the bottom to the top of the plant, summing up all the numbers from the analyzed plants in the first free choice trial (left side) and second free choice trial (right side).

Regarding the number of whiteflies/plant on the scouted plants (Table 7) the analysis of variance did not detect effect of the accessions ($F_{(3,6)}$ =1.472; p = 0.314) or the stands (blocks) ($F_{(2,6)}$ =0.734; p = 0.519) on the amount of nymphs on plant. As blocks did not show to influence, an ANOVA without blocks was performed and it did not show effect of the accessions ($F_{(3,8)}$ =1.577; p = 0.269) either.

Table 7: Results of nymphs counting on first free choice trial (total number /plant).

cv	Mean	Standard error of the mean
H3402	1756.3	847.9
KG273	1934.3	84.0
CXD277	2536.3	135.8
H1015	2949.3	148.2

Seeing the very high standard error of the mean of the number of nymphs on H3402 (table 7; Annex I), a second ANOVA was made without this considering cv. This ANOVA revealed significant differences between KG273 and the other two varieties ($F_{(2,6)}$=16.461; p =0.040), presenting KG273 a lower number of whiteflies than the remaining cultivars.

The second free choice trial showed a higher number of nymphs than the first on all cvs (table 8) and the distribution between the cvs seemed also more even (Table 8; Annex VII).

Table 8: Results of nymphs counting on second free choice trial.

cv	Mean	Standard error of the mean
H3402	5378.3	1011.5
KG273	6929.3	1560.4
CXD277	4892.0	1157.3
H1015	8878.3	1816.4

The analysis of variance considering the stands as blocks, in this case, did not show effect of accessions ($F_{(3,9)}$= 1.371; p = 0.313) or blocks ($F_{(3,9)}$= 0.440; p = 0.730) on the number of nymphs. The analysis without the blocks (considering as replications) did not reveal effect of the accessions ($F_{(3,12)}$= 1,595 ; p = 0.242) either.

6.2. Trichome analysis

In the trichomes observation, it was possible to observe the existence of many more trichomes in the lower surface of the leaves than in the upper surface of the leaves.

In both surfaces, the non-glandular trichomes observed were of the V type (fig. 13 - A and B). The glandular trichomes were mostly located nearby the leaf veins and were identified mostly as the VI type (fig. 13 - A and C), although it also appeared few identified as the IV type (fig13 - D). In the stereoscopic microscope the distribution of the trichomes was possible to verify (Fig 13 - E) being common the observation of some *B. tabaci* nymphs (Fig 13 - F). The number of trichomes were collected and divided per the mm^2 of the cropped square, being the results presented in table 9.

Table 9: Combined trichome data of the different groups divided by each cultivar - means and standard error.

Cultivar	Non – glandular trichomes (per mm^2 of leaf)		Glandular trichomes (per mm^2 of leaf)		Total trichomes (per mm^2 of leaf)	
	Mean	Standard error of the mean	Mean	Standard error of the mean	Mean	Standard error of the mean
H3402	14.9	0.4	0.1	0.05	15.0	0.43
KG273	14.2	3.2	0.1	0.04	14.4	3.14
CXD277	13.8	1.5	0.2	0.05	14.0	1.55
H1015	13.2	1.0	0.2	0.00	13.4	1.00

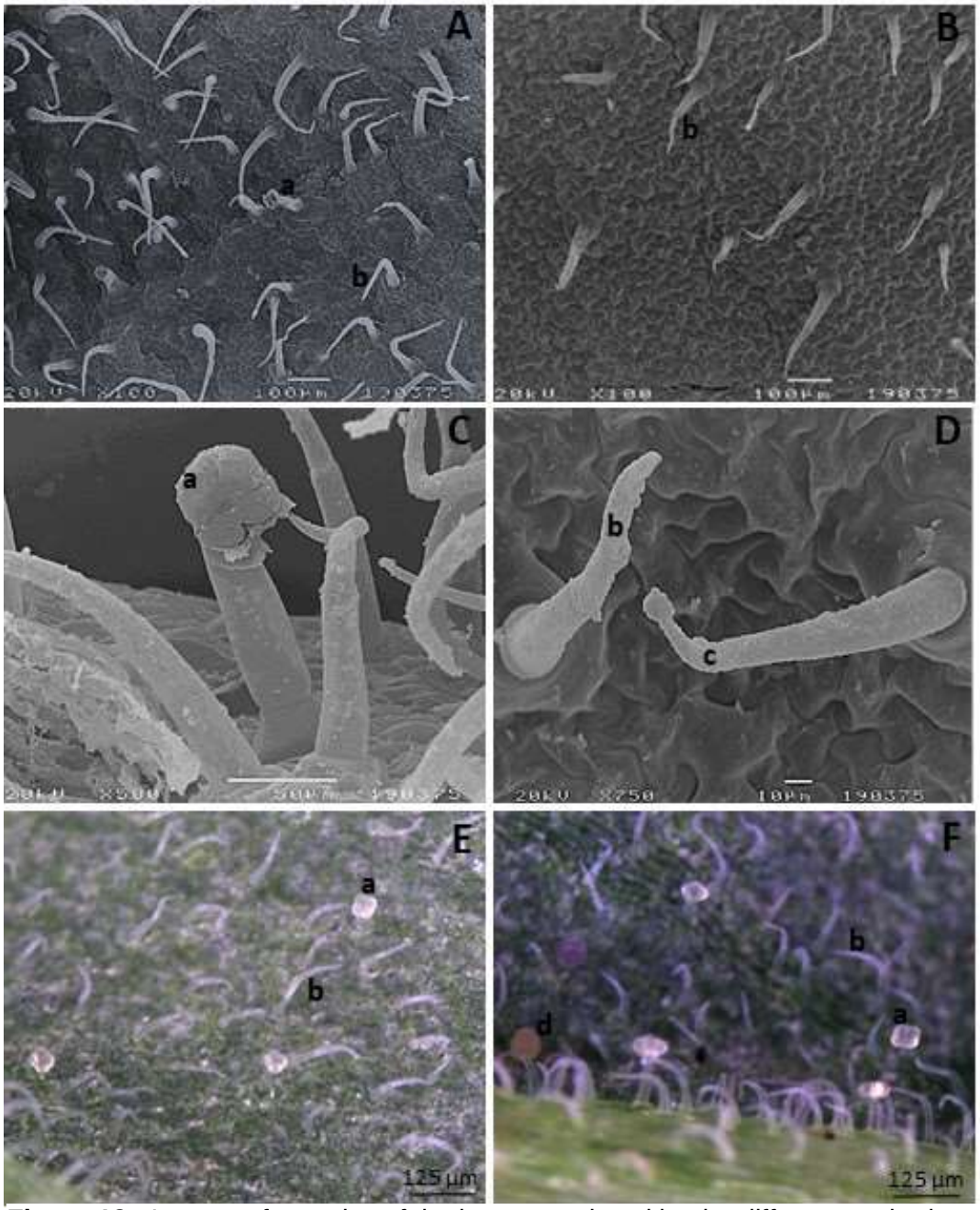

Figure 13: Images of samples of the leaves analyzed by the different methods and equipments. A - lower surface on H1015 (SEM); B – upper surface on CXD277 (SEM); C - glandular trichome (a) on the lower surface on CXD277 (SEM); D - non-glandular trichome, type V (b) and glandular trichome, type IV (c) on the lower surface on H3402 (SEM); E - lower surface H1015 (stereoscopic microscope); F - *Bemisia tabaci* nymph (d) (stereoscopic microscope).

There was no significant difference among the cvs regarding the number of trichomes/mm^2 of non-glandular trichomes ($F_{(3,8)} = 0,15$; $p = 0.927$), glandular trichomes ($F_{(3,8)} = 0,56$; $p = 0.656$) or all the trichomes combined ($F_{(3,8)} = 0,138$; $p = 0.934$).

6.3. Trichomes and nymphs correlation

The correlation between the number of non-glandular trichomes and the number of whitefly nymphs on all leaves of the plant showed a non-significant and very weak negative correlation (Pearson correlation: n=12; r= −0.036; *p* = 0.911). The same correlation but with the number of glandular trichomes showed a non-significant and moderate positive correlation (Pearson correlation: n=12; r= 0.412; *p* = 0.183). In the case of the total number of trichomes and the number the correlation was found to be non-significant, negative and very weak (Pearson correlation: n=12; r= –0.026; *p* = 0.937).

Considering the number of nymphs only on the leaf where the trichomes were counted, the correlation was also found negative and weak for non-glandular (Pearson correlation: n=12; r= –0.360; *p*= 0.250), positive and moderate for glandular trichomes (Pearson correlation: n=12; r= 0.495; *p*= 0.102) and negative and weak for both combined (Pearson correlation: n=12, total: r= –0.349, *p*= 0.266), but not significant in all cases.

7. Discussion

As far as we know, this experiment is the first study, assessing and comparing trichomes densities, and how it affects the preference on *B. tabaci* using commercial cultivars of tomato commonly used by farmers. There have been some studies, similar to this, but comparing between *Solanum* species, especially wild species (Oriani and Vendramim, 2010; Firdaus *et al.*, 2012; Rakha *et al.*, 2015) .

Some months before the start of this experiment, the greenhouse where the experiments took part was highly infested with *B. tabaci* and assessing by visual observation it seemed to exist differences among these cultivars. Therefore, the expectation while starting this study was that it could follow the trend of results achieved in the previous mentioned studies, where there were differences among cvs in number of whitefly nymphs and also differences in type and densities of the non-glandular and glandular trichomes that affected those numbers. In concrete, it would have been expected to observe a negative correlation between number of nymphs and number of glandular trichomes and/or a positive correlation between number of nymphs and number of non-glandular trichomes (Oriani and Vendramim, 2010; Firdaus *et al.*, 2012; Rakha *et al.*, 2015).

After data analysis, the first free choice trial didn't reveal any differences on *B. tabaci* infestation among cvs. The cv H3402 due to its high value dispersion could have had an impact on the results. Performing the analysis without H3402, the values revealed significant differences between the KG273 and the other two cultivars, showing a less attractiveness for the whiteflies to oviposit than in the other two cvs. This result was not confirmed by the 2nd trial.

The possibilities for the differences on the results of the 1st and 2nd trials, is that the differences in preference were not that strong or that the broader possibilities of plants choosing, between and within groups in the 2nd trial, made the oviposition more disperse; or even the environment conditions of temperatures, humidity and solar exposition affected the pest behavior. Nevertheless, it would be important to repeat these trials to check if the differences were consistent or not.

The trichome visualization and identification revealed that not all the trichomes identified in *Solanum* spp. can be found in these cvs and that the distribution of the glandular trichomes of the type V were close to the ribs, nearby the transport tissues, where the attack of this insects usually occurs.

The trichome analysis revealed that the number of trichomes were not statistically different on the studied cvs, whether non-glandular, glandular or both combined. This outcome was different than other experiments carried out by Rakha *et al.* (2015), using different species of wild *Solanum* where differences were verified. This result might indicate that commercial cultivars throughout years of breeding, that neglected trichomes as an important trait, ended up having small diversity and inferior count to its wild relatives.

Since the number of trichomes was not considered to be different among cultivars, it's not surprising that correlations between nymphs and trichomes were not significant.

Since there were no differences on the trichomes, the tendency of lower number of nymphs on KG273 in relation to CDX277 and H1015, if consistent, can be due to other characteristics of the plants.

This experiment analysed trichome differences among the different cultivars but it lacked the analysis of the exudates released by them, which was already proven to vary among *Solanum* species and could translate in a different antibiosis level.

The genetic analysis was not done either, so there's no certainty if the gene Mi2, known for its resistance against *B. tabaci*, might present in the KG273 cultivar (and not in the other cvs) and was a possible reason for lower infestation tendency.

Another possible reason for the eventual different attack level would be the plant characteristics such as plant architecture or differences in its leaves (thickness, size and color). Comparing the plants traits, it's possible to verify some differences between KG273 and the others cultivars, CDX277 and H1015. According to a visual observation and evaluation of its traits, the cv KG273 is considered to be a plant of a lighter and paler color in terms of leaf color intensity and its plants have an opener architecture due to its bigger internodes.

Bemisia tabaci is known to select the green color above other colors (Jahan *et al*., 2014). In tomato, there are studies that it's mentioned a preference for the green-yellowish foliage by *B. tabaci* (Mound 1962) although there are others that mention that color is not relevant for the choosing (Tsueda *et al.*, 2014).

Considering color as a preference parameter, KG273 has paler and lighter green color foliage than the other studied cultivars, as referred; however, it's difficult to identify how this difference in color could have affected this whitefly choice. If color, in fact, had an effect on the *B. tabaci* preference, there is the possibility that the paler color of KG273

leaves, lead to a smaller emission of the green color wave-length, making this cultivar less desirable than the others.

As far as we notice, there's no study on the choosing behavior of whiteflies regarding the architecture of the tomato plants, but it's possible to speculate that it might have had an impact on the choice, since plants that have a more open shape (cv KG273 case) can leave the whiteflies more exposed and unprotected from the surrounding environment.

According to the results here obtained, extrapolating them to other cultivars, and looking at the 2017 season, and further assuming that the observed symptoms of the external green parts and the interior white flesh of the tomatoes were in fact the Irregular Ripening Disorder identified by Schuster *et al.* (1990), and therefore caused by *B. tabaci*, the differences of quality on the harvested fruits from some fields and different cultivars might not be due to differences in plant trichomes.

For further investigation on this subject, it would be important to repeat the trials with these cvs, maybe including in the test more of the existing commercial cvs that show some tolerance to whitefly infestations and, if possible, include wild type plants as one of the control groups for an easier comparison. To include in the experiment an evaluation of the different exudates that each cv has in terms of amount and type would be also important. An easier method to count trichomes should also be considered that would act as an aid. Something similar to the hematocytometers used in hematology in counting the different types of globules.

To complement with this trichome analysis, it could also be added other factors that might have an impact on the *B. tabaci* choice such as the leaf color, that could have been measured by a chlorophyll meter, like the SPAD502 used by Tsueda *et al.* (2014).

8. Conclusions

In the first trial, the number of *Bemisia tabaci* nymphs on tomato plants were significantly inferior in cv KG273 when compared with cvs H1015 and CXD277. In the second trial, no differences were found among the same cultivars.

The trichome analysis showed that the plants had a far greater number of non-glandular trichomes (type V), with means varying from 13.2 to 14.9 per mm^2 of leaf, than glandular trichomes (mainly type VI), with means varying from 0.1 and 0.2 per mm^2 of leaf.

The comparison on the number of trichomes did not reveal differences among the studied cultivars, whether regarding the non-glandulars, glandulars or total. Consequently, we did not find correlation between the number of whitefly nymphs on leaves and the number of trichomes.

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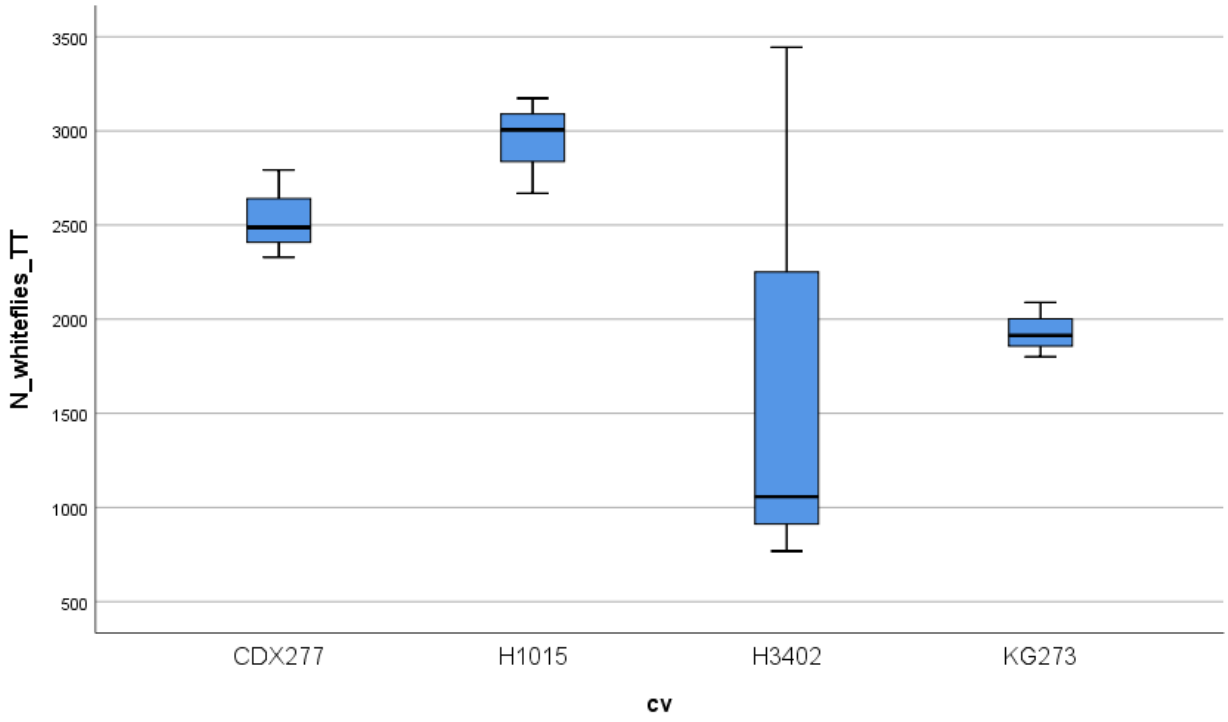
9. Annexes

Annex I - Boxplot of the First Choice Trial



Annex II – Tests of Normality of the First Choice Trial

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	cv	Statistic	df	Sig.	Statistic	df	Sig.
N_whiteflies_TT	CDX277	0.248	3	.	0.968	3	0.658
	H1015	0.254	3	.	0.963	3	0.633
	H3402	0.350	3	.	0.830	3	0.188
	KG273	0.222	3	.	0.985	3	0.768

a. Lilliefors Significance Correction

Annex III – Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
N_whiteflies_TT	Based on Mean	9.863	3	8	0.005
	Based on Median	0.974	3	8	0.452
	Based on Median and with adjusted df	0.974	3	2.141	0.537
	Based on trimmed mean	8.273	3	8	0.008

Annex IV - ANOVA of the nymphs in the first free choice trial

N_whiteflies_TT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2719898.250	3	906632.750	1.577	0.269
Within Groups	4598982.667	8	574872.833		
Total	7318880.917	11			

Annex V – Test of Homogeneity of Variances on the first free choice trial after removing H3402

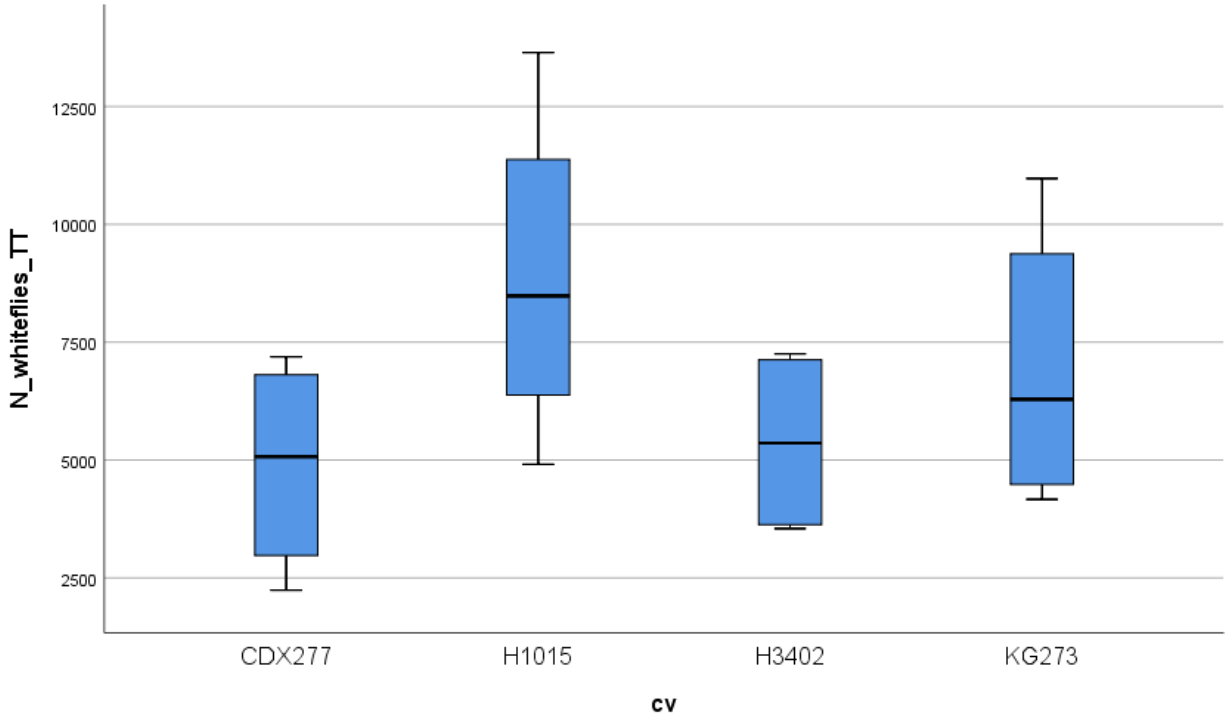
		Levene Statistic	df1	df2	Sig.
N_whiteflies_TT	Based on Mean	0.581	2	6	0.588
	Based on Median	0.219	2	6	0.809
	Based on Median and with adjusted df	0.219	2	5.030	0.810
	Based on trimmed mean	0.550	2	6	0.603

Annex VI - ANOVA of the nymphs on the first free choice trial after removing H3402

N_whiteflies_TT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1563198.000	2	781599.000	16.461	0.004
Within Groups	284894.000	6	47482.333		
Total	1848092.000	8			

Annex VII – Boxplot of the second free choice trial



Annex VIII – Tests of Normality of the second free choice trial

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	cv	Statistic	df	Sig.	Statistic	df	Sig.
N_whiteflies_TT	CDX277	0.246	4	.	0.924	4	0.562
	H1015	0.225	4	.	0.975	4	0.874
	H3402	0.295	4	.	0.780	4	0.070
	KG273	0.252	4	.	0.916	4	0.513

a. Lilliefors Significance Correction

Annex IX – Test of Homogeneity of Variances of the second free choice trial

		Levene Statistic	df1	df2	Sig.
N_whiteflies_TT	Based on Mean	0.318	3	12	0.812
	Based on Median	0.286	3	12	0.835
	Based on Median and with adjusted df	0.286	3	5.972	0.834
	Based on trimmed mean	0.317	3	12	0.813

Annex X – Tests of Normality of the trichomes

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Cv	Statistic	df	Sig.	Statistic	df	Sig.
Tric_NG_mm	CXD277	0.234	3	.	0.979	3	0.719
	H1015	0.278	3	.	0.940	3	0.528
	H3402	0.334	3	.	0.860	3	0.266
	KG273	0.319	3	.	0,885	3	0.340
Tric_G_mm	CXD277	0.376	3	.	0.772	3	0.048
	H1015	0.324	3	.	0.877	3	0.315
	H3402	0.274	3	.	0.944	3	0.543
	KG273	0.330	3	.	0.867	3	0.287
Tric_TT_mm	CXD277	0.249	3	.	0.968	3	0.656
	H1015	0.279	3	.	0.939	3	0.525
	H3402	0.327	3	.	0.872	3	0.301
	KG273	0.314	3	.	0.892	3	0.362
N_ninfas_TT	CXD277	0.248	3	.	0.968	3	0.658
	H1015	0.254	3	.	0.963	3	0.633
	H3402	0.350	3	.	0.830	3	0.188
	KG273	0.222	3	.	0.985	3	0.768

a. Lilliefors Significance Correction

Annex XI- ANOVA of the non-glandular trichomes

Tric_NG_mm

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.538	3	1.513	0.150	0.927
Within Groups	80.904	8	10.113		
Total	85.442	11			

Annex XII – ANOVA of the glandular trichomes

Tric_G_mm

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.009	3	0.003	0.560	0.656
Within Groups	0.045	8	0.006		
Total	0.054	11			

Annex XIII - ANOVA of the total of the trichomes

Tric_TT_mm

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.178	3	1.393	0.138	0.934
Within Groups	80.664	8	10.083		
Total	84.842	11			

Annex XIV -Pearson Correlation between non-glandular and nymphs on all leaves

		Tric_NG_mm	N_ninfas_TT
Tric_NG_mm	Pearson Correlation	1	-0.036
	Sig. (2-tailed)		0.911
	N	12	12
N_ninfas_TT	Pearson Correlation	-0.036	1
	Sig. (2-tailed)	0.911	
	N	12	12

Annex XV – Pearson Correlation between glandular and nymphs on all leaves

		Tric_G_mm	N_ninfas_TT
Tric_G_mm	Pearson Correlation	1	0.412
	Sig. (2-tailed)		0.183
	N	12	12
N_ninfas_TT	Pearson Correlation	0.412	1
	Sig. (2-tailed)	0.183	
	N	12	12

Annex XVI – Pearson Correlation between all trichomes and nymphs on all leaves

		Tric_TT_mm	N_ninfas_TT
Tric_TT_mm	Pearson Correlation	1	-0.026
	Sig. (2-tailed)		0.937
	N	12	12
N_ninfas_TT	Pearson Correlation	-0.026	1
	Sig. (2-tailed)	0.937	
	N	12	12

Annex XVII - Pearson Correlation between non-glandular and nymphs on the third leaf

		N_ninfas_F3	Tric_NG_mm
N_ninfas_F3	Pearson Correlation	1	-0.360
	Sig. (2-tailed)		0.250
	N	12	12
Tric_NG_mm	Pearson Correlation	-0.360	1
	Sig. (2-tailed)	0.250	
	N	12	12

Annex XVIII - Pearson Correlation between glandular and nymphs on the third leaf

		N_ninfas_F3	Tric_G_mm
N_ninfas_F3	Pearson Correlation	1	0.495
	Sig. (2-tailed)		0.102
	N	12	12
Tric_G_mm	Pearson Correlation	0.495	1
	Sig. (2-tailed)	0.102	
	N	12	12

Annex XIX – Pearson Correlation between all trichomes and nymphs on the third leaf

		N_ninfas_F3	Tric_TT_mm
N_ninfas_F3	Pearson Correlation	1	-0.349
	Sig. (2-tailed)		0.266
	N	12	12
Tric_TT_mm	Pearson Correlation	-0.349	1
	Sig. (2-tailed)	0.266	
	N	12	12

Annex XX- Pearson Correlation between non-glandular and nymphs on the second, third and fourth leaves

		N_ninfas_F2_4	Tric_NG_mm
N_ninfas_F2_4	Pearson Correlation	1	-0.212
	Sig. (2-tailed)		0.509
	N	12	12
Tric_NG_mm	Pearson Correlation	-0.212	1
	Sig. (2-tailed)	0.509	
	N	12	12

Annex XXI - Pearson Correlation between glandular and nymphs on the second, third and fourth leaves

		N_ninfas_F2_4	Tric_G_mm
N_ninfas_F2_4	Pearson Correlation	1	0.444
	Sig. (2-tailed)		0.148
	N	12	12
Tric_G_mm	Pearson Correlation	0.444	1
	Sig. (2-tailed)	0.148	
	N	12	12

Annex XXII – Pearson Correlation between all trichomes and nymphs on the second, third and fourth leaves

		N_ninfas_F2_4	Tric_TT_mm
N_ninfas_F2_4	Pearson Correlation	1	-0.201
	Sig. (2-tailed)		0.530
	N	12	12
Tric_TT_mm	Pearson Correlation	-0.201	1
	Sig. (2-tailed)	0.530	
	N	12	12

Annex XXIII – Vertical Distribution in the plant in the different blocks on the 1st trial

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks Summary

Total N	13
Test Statistic	10.080
Degree Of Freedom	2
Asymptotic Sig. (2-sided test)	0.006

Annex XXIV - CDX277 nymphs distribution on the different leaves on the 1st trial

Null Hypothesis	Test	Sig.	Decision
The distributions of CDX277_1, CDX277_2 and CDX277_3 are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	0,584	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is 0.050.

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks Summary

Total N	13
Test Statistic	1.077[a]
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	0.584

a. Multiple comparisons are not performed because the overall test retained the null hypothesis of no differences.

Annex XXV – KG273 nymphs distribution on the different leaves on the 1st trial

Null Hypothesis	Test	Sig.	Decision
The distributions of KG273_1, KG273_2 and KG273_3 are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	0.926	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is 0.050.

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks Summary

Total N	13
Test Statistic	0.154[a]
Degree Of Freedom	2
Asymptotic Sig. (2-sided test)	0.926

a. Multiple comparisons are not performed because the overall test retained the null hypothesis of no differences.

Annex XXVI – H1015 nymphs distribution on the different leaves on the 1st trial

Null Hypothesis	Test	Sig.	Decision
The distributions of H1015_1, H1015_2 and H1015_3 are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	0.794	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is 0.050.

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks Summary

Total N	13
Test Statistic	0.462[a]
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	0.794

a. Multiple comparisons are not performed because the overall test retained the null hypothesis of no differences.

Annex XXVII – H3402 nymphs distribution on the different leaves on the 2nd trial

Null Hypothesis	Test	Sig.	Decision
The distributions of H3402_1, H3402_2, H3402_3 and H3402_4 are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	0.050	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is 0.050.

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks Summary

Total N	9
Test Statistic	7.800[a]
Degree Of Freedom	3
Asymptotic Sig.(2-sided test)	0.050

a. Multiple comparisons are not performed because the overall test retained the null hypothesis of no differences.

Annex XXVIII – CXD277 nymphs distribution on the different leaves on the 2nd trial

Null Hypothesis	Test	Sig.	Decision
The distributions of CDX277_1, CDX277_2, CDX277_3 and CDX277_4 are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	0.040	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is 0.050.

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks Summary

Total N	9
Test Statistic	8.333
Degree Of Freedom	3
Asymptotic Sig.(2-sided test)	0.040

Annex XXIX– KG273 nymphs distribution on the different leaves on the 2nd trial

Null Hypothesis	Test	Sig.	Decision
The distributions of KG273_1, KG273_2, KG273_3 and KG273_4 are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	0.615	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is 0.050.

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks Summary

Total N	9
Test Statistic	1.800[a]
Degree Of Freedom	3
Asymptotic Sig.(2-sided test)	0.615

a. Multiple comparisons are not performed because the overall test retained the null hypothesis of no differences.

Annex XXX– H1015 nymphs distribution on the different leaves on the 2nd trial

Null Hypothesis	Test	Sig.	Decision
The distributions of H1015_1, H1015_2, H1015_3 and H1015_4 are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	0.019	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is 0.050.

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks Summary

Total N	9
Test Statistic	9.933
Degree Of Freedom	3
Asymptotic Sig.(2-sided test)	0.019