

### Abiotic and cultivar effects on the incidence of pest arthropods in 16 citrus scion varieties grafted on two rootstocks

Edgar Herney Varón-Devia<sup>1</sup>\* <sup>(b)</sup>, Claudia Milena Flórez-Cárdenas<sup>1</sup> <sup>(b)</sup>, Camilo Ignacio Jaramillo-Barrios<sup>1</sup> <sup>(b)</sup> & Mauricio Fernando Martínez<sup>2</sup> <sup>(b)</sup>

#### **SUMMARY**

Interactions among pests could differ among types of citrus hosts. Also, the climate factors may affect the population fluctuation of these pests. Therefore, the aim of this study was to establish the potential vulnerability of citrus cultivars to pest pressure over time as an aid to pest management. During 48 months between 2017 and 2020, pest arthropods from the families Aphididae, Liviidae, Gracillariidae, Aleyrodidae, Diaspididae and the order Acari were sampled bi-weekly in a common orchard with 32 cultivars [(16 citrus scion varieties of three citrus types (9 oranges, 4 mandarins, and 3 limes-lemons) grafted onto two rootstocks (CPB 4475 and SxE)]. Percentage of presence of each arthropod taxon was determined by visually inspecting five leaf buds or five leaves or five fruits, or combination of those, in two of six randomly sampled trees, in each one of three 210 m<sup>2</sup> plots established for each cultivar, for a total of 192 trees in each sampling. Percentages of presence of each arthropod taxon on each tree among cultivars were compared and correlations between these percentages and the climatic factors temperature, relative humidity, and rainfall recorded on a daily basis, were calculated. Among the three citrus types, mandarins had the greatest presence of aphids; oranges had the greatest presence of whiteflies, leafminers and Diaphorina citri, and limes-lemons had the greatest presence of mites. Presence of mites, aphids, D. citri and leafminer were positively correlated to relative humidity. Among mandarin varieties aphids had higher percentage of presence on Oneco and Owari Satsuma and among orange varieties Pineapple had a higher presence of it than the other varieties in study. Leaf miner had higher presence on Orange Sweet variety grafted on SxE rootstock than on any other orange cultivar.

**Index terms:** rootstock-scion variety effect, *Diaphorina citri*, *Phyllocnistis citrella*, population fluctuation.

## Efeitos abióticos e de cultivares sobre a incidência de artrópodes-praga em 16 variedades copas de citros enxertadas sobre dois porta-enxertos

#### **RESUMO**

As interações entre pragas podem diferir entre os tipos de hospedeiros cítricos. Além disso, os fatores climáticos podem afetar a flutuação populacional dessas pragas. Portanto, o objetivo

<sup>\*</sup>Corresponding author: Edgar Herney Varón-Devia, Corporación Colombiana de Investigación Agropecuaria – Agrosavia, Nataima Research Center, Km 9 Espinal-Ibagué, Postal Code: 763008, Espinal, Tolima, Colombia. E-mail: evaron@agrosavia.co



<sup>&</sup>lt;sup>1</sup> Nataima Research Center, Corporación Colombiana de Investigación Agropecuaria – Agrosavia, Espinal, Tolima, Colombia

<sup>&</sup>lt;sup>2</sup> Palmira Research Center, Corporación Colombiana de Investigación Agropecuaria – Agrosavia, Palmira, Valle del Cauca, Colombia

deste estudo foi estabelecer a vulnerabilidade potencial de cultivares de citros à pressão de pragas ao longo do tempo como auxílio ao manejo de pragas. Durante 48 meses, entre 2017 e 2020, artrópodes-praga das famílias Aphididae, Liviidae, Gracillariidae, Aleyrodidae, Diaspididae e da ordem Acari foram amostrados quinzenalmente em um pomar comum com 32 cultivares [(16 variedades de citros de três grupos diferentes (9 laranjas, 4 tangerinas e 3 limas-limões) enxertadas em dois porta-enxertos (CPB 4475 e SxE)]. A porcentagem de presença de cada táxon de artrópode foi determinada pela inspeção visual de cinco brotos de folhas ou cinco folhas ou cinco frutos, ou combinação deles, em duas de seis árvores amostradas aleatoriamente, em cada uma das três parcelas de 210 m<sup>2</sup> estabelecidas para cada cultivar, totalizando 192 árvores em cada amostra. e foram calculados os fatores climáticos temperatura, umidade relativa e precipitação pluviométrica registradas diariamente. Entre os três grupos de citros, a tangerina teve a maior presença de pulgões; nas laranjas ocorreu maior presença de ácaros, pulgões, *D. citri* e lagarta minador foi positivamente correlacionada com a umidade relativa do ar. Entre as variedades de tangerina, os pulgões tiveram maior porcentagem de presença na tangerina Oneco e Satsuma Owari e entre as variedades de laranja, Abacaxi teve maior presença do que as outras variedades em estudo. A lagarta minadora teve maior presença na variedade de laranja Orange Sweet enxertada no porta-enxerto SXE do que em qualquer outra cultivar de laranjeira.

**Termos de indexação:** efeito da variedade porta-enxerto-scion, *Diaphorina citri*, *Phyllocnistis citrella*, flutuação populacional.

#### **INTRODUCTION**

Citrus is an important part of the fruit production in Colombia, but its production is constrained by several insect pests. Mainly, six arthropod taxa comprise this pest complex. Among these, the most important is Diaphorina citri Kuwayama (Hemiptera: Liviidae) (Citrus dragon). Although approximately 84.000 ha of citrus were in production and 1.290.000 t of fruit were produced in 2020, this was a 2.34% and a 0.17% decrease in production and area, respectively, from 2019 (MADR & UPRA, 2021), partly attributed to the increasing prevalence of Huanglongbing (citrus greening-HLB) (Bové, 2006) in Colombia (ICA, 2019). The causal agents of HLB, Candidatus Liberibacter asiaticus and Candidatus Liberibacter americanus (Halbert & Manjunath, 2004) require D. citri as a vector. D. citri is considered the most important citrus pest worldwide (Galdeano et al., 2020) and was first reported in Colombia in 2007 (King, 2012). In addition to D. citri, five other arthropod taxa constrain citrus production in Colombia: citrus leafminer [Phyllocnistis citrella Stainton. (Lepidoptera: Gracillariidae)], Aphids (Hemiptera: Aphididae), Mites (Acari), scales [Pinnaspis strachani (Cooley) (Hemiptera: Diaspididae)] and whiteflies [Aleurothrixus floccosus (Hemiptera: Aleyrodidae)].

Citrus leafminer, originated in the Asiatic southeast and is considered one of the most important pests of citrus worldwide (Morakote & Ujiye, 1992). In Colombia, the citrus leafminer was recorded initially in the central coffee region in 1995 (Castaño, 1996). Currently, the citrus leafminer is widespread throughout Colombia. These little moths are active during sunset, ovipositing on citrus leaves (León & Kondo, 2017). The larvae are leafminers that damage leaves and buds, injuring the cuticle and causing to detach, exposing the parenchyma and causing the tissues to desiccate (Barrientos, 2009). As a result, plant growth is decreased. This injury is more damaging to seedlings and saplings than to mature trees.

Aphids are plant sap sucking insects that feed upon the sap of new leaves, causing their deformation. Its main damage happens in young plants and heavy infestations can reduce the growth, cause flower and fruit dropping and promote the appearance of sooty molds, which grow on their excreted honeydew (León & Kondo, 2017). Among the main aphid species that attack citrus are the black aphid, *Toxoptera aurantii* (Boyer de Fonscolombe), the green or spirea aphid, *Aphis spiraecola* (Patch), the cotton aphid *Aphis gossypii* (Glover) and the brown aphid, *Toxoptera citricidus* (Kirkaldy) (León & Kondo, 2017).

Two species of mites are especially important in Colombian citrus. Red spider mites, *Tetranychus* sp. (Acari: Tetranychidae) feed by sucking the cell substances. The affected areas become reddish yellow, with concavities and bulges. High infestations produce defoliation and diffuse stains on the fruit rind, mainly closer to the peduncle (León & Kondo, 2017); citrus rust mite *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae), like other eriophyids are small mites (100-150 um). They are considered strictly phytophagous, with more than 3.500 species in 300 genera (Krantz & Walter, 2009).

*P. oleivora* is distributed worldwide affecting citrus in parts of America, Asia, Africa, Europe, and Australia (Mc Coy, 1996; Smith & Peña, 2002; Walter et al., 2009). Although this species affects both leaves and fruits, its main damage is the rough surface and bronze patches its feeding produces on citrus fruit (León & Kondo, 2017).

*Pinnaspis strachani*, the minor citrus snow scale damages the host by feeding on plant sap, causing chlorosis on the leaves along with stem drop and stem death. Severe infestations can even lead to the death of the entire plant (Tavares de Castro et al., 2020).

*Aleurothrixus floccosus* immatures (nymphs) and adults of this whitefly species suck the sap of the leaves and excrete great amount of honeydew that causes the growth of sooty molds that interfere with photosynthesis, thus causing harvest losses (León & Kondo, 2017). To control these pest arthropods, different types of management practices have been implemented, including cultural and biological control (Nath & Deka, 2019).

The citrus variety of the scion (Santos et al., 2019), and the rootstock can affect the management strategies for these pests. Rootstocks are used to improve fruit quality and increase yield (Fadel, 2015; Qureshi et al., 2022; Ruiz, 2016; Tietel et al., 2020), but can also promote resistance to pests to the scion (Sy & Mauleon, 1989). Rootstock can affect aphids (Trapero et al., 2008) and mites' incidence (Bruessow et al., 2010; Andrade et al., 2013; Silva et al., 2016), but its effects on other pests has not been well determined.

Pest densities fluctuate temporally, affecting their levels of potential injury to the crop (Nawaz et al., 2021) and these fluctuations may differ among pests, introducing dynamics in their net potential injurious effects on the crop. Some of this variation is attributable to climatic variables (Rathod et al., 2020; Tandel et al., 2020; Prabhudev et al., 2021). This study was conducted to assess the prevalence of these six arthropod taxa over a four-year period on citrus with differing scions and rootstocks in a common orchard in Colombia. Climatic data were also collected to assess the effects on these dynamics. The purpose of the study was to improve understanding of the potential vulnerability of Colombian citrus to pest pressure over time as an aid to pest management.

#### **MATERIALS AND METHODS**

#### Plot and experimental design

In 2016, 32 citrus cultivars (scion variety X rootstock) were established in a planting at the Agrosavia Nataima Research Center, located at the municipality of El Espinal (Tolima, Colombia) (Table 1). The cultivars (Table 2) consisted of 16 scion varieties of citrus, belonging to the types: mandarin (*Citrus reticulata*), sweet orange (*C. sinensis*), true lemon (C. limon) and lime (C. aurantifolia), grafted onto one of two rootstocks, Sunky x English (C. sunki hort. ex Tanaka x Poncirus trifoliata) and Citrumello CPB 4475 [C. paradisi x P. trifoliata (L.) Raf.]. The 16 scion varieties were 9 sweet orange varieties, 4 mandarin varieties, 2 true lemon varieties and one lime variety (Pajarito). The cultivars (scion variety X rootstock) were planted in a split plot, Randomized Complete Block Design (RCBD) with three replications, where the main plots were the scion varieties (16) and the subplots were the rootstocks (2). The experimental unit was a plot of six trees of the same cultivar (scion variety X rootstock) within a 210  $m^2$  area.

 Table 1. Climatic, geographical and location conditions at Agrosavia, Nataima Research Center. El Espinal, Tolima, Colombia

Factor	CI Nataima
Annual rainfall (mm)	1476
Anual evapotranspiration (mm)	1766
Mean temperature (°C)	28.1
Mean relative humidity (%)	69
Mean solar brightness (hour/day)	5.8
Altitude (m.a.s.l.)	323
Geographical location	04°11′ 20.558" N, 74°57′ 46.917" W

Source: Adapted from Montes-Rodríguez et al. (2020).

#### Visual sampling for arthropods.

During 48 months, between January 2017 and December 2020, bi-weekly samplings of pest arthropods from six taxons (Aphididae, Liviidae, Gracillariidae, Aleyrodidae and Diaspididae families and the order Acari) were carried out. A visual sampling with a NO 9881 30X magnified glass was performed, depending on the sampled arthropod, either on five randomly chosen young buds, five mature leaves, five new fruits or combinations of these depending on the phenological stage of the plant. The different sampling for each taxon was selected taking into account the organ in which the arthropod is most commonly found. The sampling was carried out in two randomly selected trees, in each plot (6 trees in total) for a total of 192 sampled trees in each sampling (Table 3).

In each evaluated plant part, the presence (1) or absence (0) of the pest arthropod was recorded (ICA, 2012; Ripa & Larral 2008; Cáceres 2006). Later, the presence/absence per plant organ data was averaged and converted to the response variable used for this study:

average percentage presence of each arthropod taxon in each sampled organ calculated as (number of organs inspected where the taxon was detected/total number of organs inspected (5) \*100. This variable is referred to throughout this paper as the "Percentage of Presence" for each taxon, and it was used for the statistical analysis.

#### **Climatic variables**

During the study, a daily record of the climatic variables: temperature, relative humidity and rainfall was carried out by means of a climatic station Watchdog® 2000 series, located at the center of the experimental plot.

#### Management and pest control

Standard agronomic tasks were carried out on a schedule: weed control once a month, pruning every 3 months and soil fertilization every 4 months. Pesticides

Table 2. Citrus cultivars (scion variety X rootstock) established at Agrosavia, Nataima Research Center and evaluate	ed
by the incidence of 6 groups of arthropods (Aphididae; Liviidae; Gracillariidae; Acari; Aleyrodidae and Diaspididae	e)
for the 2017-2020 year-period	

Scion variety <sup>*</sup>			
ORANGES	MANDARINS	LEMONS	LIMES
Sweety O	Oneco	Eureka	Pajarito or Mexicana
Washington	Arrayana	Perrine	
Frost Valencia	Owari Satsuma		
García Valencia	Fairchild		
Lannelate			
Hamlin			
Pera del río			
Parson Brown			
Pinneapple			

\*All scion varieties were grafted on Sunki x English: (*Citrus sunki* hort. ex Tanaka x *Poncirus trifoliata*) and Citrumello CPB 4475: [*C. paradisi* x *P. trifoliata* (L.) Raf.].

**Table 3.** Pest arthropods and evaluated organs in 16 citrus scion varieties grafted in two rootstocks at Agrosavia,Nataima Research Center. El Espinal, Tolima, Colombia. 2017-2020 year-period

Pest arthropod	Evaluated organ
Aphids (Aphis spiraecola)	5 buds/tree
Asian citrus psyllid (Diaphorina citri)	5 buds/tree
Citrus snow scale (Pinnaspis strachani)	5 mature leaves (underside)/tree
Leafminer (Phyllocnistis citrella)	5 buds/tree
Mites (Phyllocoptruta oleivora, Tetranychus sp.)	5 leaves and/or 5 small fruits/tree
Whiteflies (Aleurothrixus floccosus)	5 mature leaves (underside)/tree

were applied as foliar sprays to the tree canopies, biweekly. Beginning in June 2019, pesticide applications occurred weekly to control mites in those cultivars (scion variety X rootstock) that began producing fruit (i.e., limes and lemons). Different types of insecticides were applied, the main active ingredients used were: Spiromesifen (0,9 L ha<sup>-1</sup>), Thiamethoxam (100 g ha<sup>-1</sup>), Propargite (0,2 L ha<sup>-1</sup>), Abamectin (0,2 L ha<sup>-1</sup>), Thiacloprid + deltmethrin (0,2 L ha<sup>-1</sup>) Spinosad (0,2 L ha<sup>-1</sup>), Chlorfenapyr (0,18 L ha<sup>-1</sup>), propargite (2-(4-terbutylfenoxy)-ciclohexylpropenyl-sulfite) (1 L ha<sup>-1</sup>) and a product based on *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii* and *Bacillus thuringiensis* (400g ha<sup>-1</sup>).

#### **Taxonomic identification**

To identify the species of mites, aphids, whiteflies and scales in this study, the specimens were collected under the framework collection permit conferred to AGROSAVIA through the resolution number 1466 of December 3, 2014 from ANLAS (Colombia). They were specifically processed, put in a mounting slide and observed with a Nikon Eclipse Ci Model microscope at the Instituto Colombiano Agropecuario-ICA Diagnosis Center located at Ibagué (Colombia). Identification keys used were Evans (2007) for whiteflies; Ramos-Portilla & Caballero (2017) and Miller & Davidson (2005) for scale insects, Blackman & Eastop (2008), Simbaqueba & Serna (2021) and Simbaqueba et al. (2014) for aphids, and Baker & Tuttle (1994) and Vacante (2010) for acari.

#### Depository

Voucher specimens were deposited at the Insect Taxonomic National Collection of Colombia "Luis Maria Murillo" located at Agrosavia Tibaitatá Research Center (Mosquera, Cundinamarca), with the catalogue numbers No. 7645, 7646, 7647, 7648 and 7662.

#### **Statistical analysis**

Data were submitted to a descriptive and explanatory analysis where measures of central tendency and dispersion were identified, in addition to the detection of atypical data with boxplots. In the analysis, the response variable was defined as "the percentage of presence" of each taxon, formerly defined in this manuscript. Thus, the averages of the percentage of presence in each taxon were considered.

Subsequently, Spearman correlations were carried out between these percentages of presence of each arthropod with the climatic parameters of the study area partitioning for each citrus type (oranges, mandarins, and limes-lemons), rootstock (CPB 4475 y SXE), and scion variety of citrus. The number of organs with the presence of arthropods / total number of organs sampled was analyzed using generalized linear model (GLM) with a binomial family with logit link. Year, scion variety, rootstock factors and their interactions corresponded to the fixed effects of the model. The random effects were block and assignment of plot splits. An analysis of deviance (ANODE) was performed according to Hastie & Pregibon (1992) for generalized linear models. The percentage of presence of the arthropods was compared with LSD Fisher test at 5% significance. A cluster analysis with the ward method and the Euclidean distance was performed to observe the general relationship between the presence of arthropods and the citrus types. The resulting clusters were characterized and interpreted with v-tests, relating each original variable to clusters. V-tests are based on the difference between the mean of the variable in the cluster and the overall mean of the variable. Higher absolute values indicate better characterization of the cluster (Escofier & Pagès, 2008). Statistical analyses were performed using the statistical package R version 4.1.1. (R Core Team, 2021).

#### **RESULTS AND DISCUSSION**

Of the 1518 total sampled trees, 56.92% corresponded to sweet oranges (864), 25.30% to mandarins (384) and 17.79% to lemons-limes (270). During the four years of evaluation, the order of percentage of presence of taxons across all entries in the experiment was: leafminer > aphids > Asian citrus psyllid > mites > whitefly > citrus snow scale. In citrus type, sweet orange had the same distribution as above, while in limes-lemons the order was leafminer > mites > aphids > Asian citrus psyllid > whitefly > citrus snow scale. In mandarins, the order was leafminer > aphids > Asian citrus psyllid > whitefly > citrus snow scale > mites. In years of study, the order of percentage of presence of arthropods was: 2020 >2019 > 2017 > 2018.

#### Multivariate analysis

The relative percentages of pest arthropods in different taxa differed between the three citrus types in samples taken

over the 48 months (Figure 1). Leafminer had the highest incidence on all citrus types. Mites had a higher incidence in lemons-limes than in the other two types, while the incidence of aphids was higher in mandarins. Leaf miner, whitefly, and Asian citrus psyllid had higher incidence in sweet oranges than in mandarins and lemons-limes.



**Figure 1.** Distribution of the average relative percentage of six pest arthropods in each citrus type during 48 months in an experiment that included 16 citrus scion varieties (oranges, mandarins, and limes-lemons) grafted on two rootstocks (SxE and CPB4475) at Agrosavia, Nataima Research Center. Espinal (Tolima, Colombia). 2017-2020 year-period.

Cluster analysis: a cluster analysis based on the percentage of arthropods sampled in each of the six arthropod taxa clearly separated the three citrus types: [mandarins, (G1), sweet oranges (G2) and limes-lemons (G3). There was no clear separation based on the rootstocks, within each citrus type (Figure 2).

Relationships between citrus types and pests: significant relationships were found between arthropods and the evaluated citrus types (mandarins, sweet oranges, and lemons-limes) according to a V-test in mean comparison for cluster analysis. Orange cultivars (scion variety X rootstock) were significantly associated with the higher presence of three groups of arthropods (whitefly, leafminer and *D. citri*) (Table 4).

#### **Climate correlations**

Some significant correlations were found between the arthropods in study and climatic variables. The presence of several arthropods were correlated with relative humidity (Table 5; Figure 3). For mites, aphids, *D. citri*, and leafminer the correlations were positive, whereas in whitefly it was negative. Other variables such as temperature was negatively related to aphids and positively related to whitefly, whereas rainfall was negatively related to whitefly and positively related to lifeminer (Table 5).

**Table 4.** Relationships between six pest arthropod groups and three types of citruses (mandarins, sweet oranges, and limes-lemons) including 32 cultivars [(16 scion varieties grafted on two rootstocks (SxE and CPB4475)] planted at Agrosavia, Nataima Research Center. El Espinal (Tolima, Colombia). 2017-2020 year-period

Type of citrus	Variable	V-test*	Mean±SD in type**	Overall Mean±SD***	P-value
Mandarins (8)	Aphid' presence	4.276	5.94±10.27	4.42±8.01	<0.01
	Whitefly presence	-2.392	$0.48 \pm 1.81$	0.83±3.19	0.02
	Mites' presence	-4.922	$0.07 \pm 0.59$	$1.20\pm5.21$	0.02
<b>Sweet Oranges</b>	Whitefly presence	3.549	$1.08 \pm 3.94$	0.83±3.19	<0.01
(18)	Leafminer	3.197	$14.78 \pm 14.60$	13.75±14.08	<0.01
	presence				
	D. citri presence	2.740	3.39±6.51	3.01±6.20	<0.01
	Mites' presence	-4.483	$0.68 \pm 2.86$	$1.20\pm5.21$	< 0.01
Lemons-Limes	Mites' presence	11.401	4.48±10.59	$1.20\pm5.21$	<0.01
(6)	Leafminer	-4.483	10.27±11.70	$13.75 \pm 14.08$	< 0.01
	presence				
	D. citri presence	-4.661	$1.41 \pm 3.44$	3.00±6.19	< 0.01
	Aphid' presence	-5.178	2.13±4.99	4.42±8.01	< 0.01

\* Test-value. Comparison of means. Mean of the variable for the cluster "g" (conditional mean) vs. Overall mean of the variable. \*\*Mean and standard deviation obtained in each cluster (type). \*\*\* Overall Mean and standard deviation.



**Figure 2.** Cluster analysis based on the percentage of arthropods sampled in each of the six arthropod taxa on all trees sampled in the study. Three clusters, Clusters 1 - 3, corresponding to the three citrus scion types (lemons-limes, sweet oranges, and mandarins) are evident. Each scion type was grafted two rootstocks, SxE (triangles in each cluster) and CPB4475 (circles in each cluster). The study was conducted on 1518 trees (56.92% sweet oranges, 25.30% mandarins, and 17.79% to lemons-limes) planted at Agrosavia, Nataima Research Center. Espinal (Tolima, Colombia). 2017–2020 year-period.



**Figure 3.** Relationship between pest arthropods in citrus trees (Percentage of presence) with relative humidity within three types of citrus (oranges, mandarins and lime-lemons), including 16 scion varieties grafted on two rootstocks (SxE and CPB4475) at Agrosavia, Nataima Research Center. Espinal (Tolima, Colombia). 2017-2020 year-period.

Variable	Climatic factor	N	Spearman	P-value
	RH (%)	1518	0.10	<0.0001
Mites Presence	Temperature °C	1518	-0.02	0.5510
	Rainfall	719	-0.01	0.7016
	RH (%)	1518	0.50	<0.0001
Aphids Presence	Temperature °C	1518	-0.32	<0.0001
-	Rainfall	719	0.06	0.1096
	RH (%)	1518	0.18	<0.0001
Diaphorina Presence	Temperature °C	1518	-0.02	0.4672
*	Rainfall	719	0.06	0.0819
	RH (%)	1518	-0.21	<0.0001
Whitefly Presence	Temperature °C	1518	0.07	0.0040
	Rainfall	719	-0.29	<0.0001
	RH (%)	1518	0.29	<0.0001
Leaf miner Presence	Temperature °C	1518	-0.04	0.0807
	Rainfall	719	0.12	0.0012
	RH (%)	1518	0.01	0.7898
Snow scales	Temperature °C	1518	-0.02	0.4633
	Rainfall	719	0.01	0.8369

**Table 5.** Correlations between pest arthropods and climatic factors within three types of citrus (oranges, mandarins, and limes-lemons) including 16 scion varieties grafted onto two rootstocks (SxE and CPB4475) at Agrosavia, Nataima Research Center. Espinal (Tolima, Colombia). 2017-2020 year-period

# Differences in arthropods presence among cultivars (scion variety X rootstock) within citrus types.

Mandarin cultivars (scion variety X rootstock)

Aphids: aphids had a significantly higher percentage of presence on the Oneco and Owari Satsuma varieties than on the Fairchild and Arrayana varieties (P<0.0001) (Figure 4) and a higher presence on the SxE rootstock than on the CPB4475 rootstock (P=0.0187).

Leafminer: in mandarins, there was a higher incidence of leafminer on the SxE rootstock than on the CPB4475 rootstock (P<0.0001) (Figure 5).

Orange cultivars (scion variety X rootstock).

Aphids: there was a difference for the aphid's percentage of presence in the orange varieties (P<0.0001), having a higher presence on the Pinneaple variety over the Lannelate, Parson Brown, Hamlin, V. Washington and Pera del Rio, but not being different to the percentage of presence found on Frost Valencia and García Valencia varieties (Figure 6).



**Figure 4.** Percentage of presence of aphids (*Aphis spiraecola*) in four mandarin varieties in a plot with 16 scion varieties grafted on two rootstocks (CPB4475 and SxE) at Agrosavia, Nataima Research Center. 2017-2020 year-period.

Leafminer: there was a significant difference for the incidence of the leafminer among the orange varieties (P<0.0001), presenting a higher percentage of presence on the SweetO variety than on the Lannelate, V. Washington, Garcia Valencia, Hamlin, Pinneapple, Parson Brown and Pera del Rio varieties, but not being different to the incidence found on the Frost Valencia variety.



**Figure 5.** Percentage of presence of the leafminer (*Phyllocnystis citrella*) on two rootstocks (CPB4475 and SxE), within four mandarin scion varieties (Oneco, Owari sastsuma, Fairchild and Arrayana) in an experiment including 16 citrus scion varieties grafted on two rootstocks at Agrosavia, Nataima Research Center. 2017-2020 year-period.



**Figure 6.** Percentage of presence of aphids (*Aphis spiraecola*) on nine orange varieties within an experiment of 16 scion varieties grafted on two rootstocks (CPB4475 and SxE) at Agrosavia, Nataima Research Center. 2017-2020 year-period.

Besides, a higher incidence on the SxE than CPB4475 rootstock was found (P<0.0001). Finally, a higher percentage of presence was found in the Sweet O variety grafted on the SxE rootstock (P=0.0002), compared with the same variety grafted on the CPB4475 rootstock and all the other varieties grafted in both rootstocks, excepting Frost Valencia grafted on SxE (Figure 7).

#### DISCUSSION

In this study, it was found that the percentage of presence of key arthropod pests differed among types



**Figure 7.** Percentage of presence of the leafminer (*Phyllocnystis citrella*) on nine sweet orange varieties grafted on two rootstocks, within an experiment including 16 citrus varieties grafted on two rootstocks at Agrosavia, Nataima Research Center. 2017-2020-year period.

of citrus (mandarins, sweet oranges, and lime-lemons) and among cultivars (scion variety X rootstock) within each of these types, based on sampling for four years in a common garden with different rootstocks and scions. In general, among the arthropods sampled, the incidence of aphids was higher on mandarins than on oranges or lemons, which agrees with the result of Satar et al. (2020), who found that mandarins had higher percentage rate than oranges, lemons, and grapefruits for a group of aphids (A. gossypii, A. spiraecola, A. craccivora, T. aurantii and Myzus persicae) (Hemiptera: Aphididae). On the other hand, Arshad et al. (2020) found that mandarin and grapefruits were more susceptible to citrus leafminer infestation than the lemon varieties in their study (seedless lemon and Musambi), a result similar to that found in the present study.

Other differences found in this study, were the higher percentage of presence of mites on lemons than on other citrus types, and the higher percentage of presence of whiteflies, leafminers and *D. citri* on oranges than on the other citrus types in study. Although we did not separate mite species, one species, *P. oleivora*, which infests twigs, leaves, and fruits of all citrus species and varieties, has been reported to have the following relative prevalence ranking: lemons> grapefruits> oranges> mandarins (Knapp, 1994), in general agreement with our result.

At the scion variety level, for oranges, leafminer had a higher percentage of presence on Sweet O and Frost Valencia in the present study. Other studies (e.g. El-Afify et al., 2018) found that the leafminer significantly attacked navel orange and Eureka lemon, more than Valencia orange, Baladi and sour oranges, and mandarin varieties.

We found that *P. citrella* prevalence was greater on some varieties of citrus than others. This is consistent with Santos et al. (2020) who found differences in attack by *P. citrella* among citrus genotypes, although not the same ones as included in this study.

*Diaphrina citri* prevalence differed among sweet orange citrus scion varieties and rootstocks (Table 4). This agrees with Alves et al. (2014), who found that Valencia orange and orange jasmine were the most suitable hosts among sweet orange scion varieties for the development of *D. citri*. On the other hand, Alves et al. (2018) found that survival rates for the egg stage of this insect were highest on the Valencia orange and Sicilian lemon varieties, both grafted on Sunki mandarin, with means of 87.99 and 87.98%, respectively; and lowest (67.63%) on Hamlin X Rangpur lime.

This study detected an effect of rootstock on aphid prevalence, which was greater on SxE rootstock than CPB 4475 rootstock in mandarins. Trapero et al. (2008) found an effect of the rootstock on the populations of the aphid *A. gossypii*, which was present exclusively on leaves of Clementine de Nules grafted on Cleopatra mandarin rootstock (*C. reshni* Hort. ex Tan.) as compared with CA, (Citrange Carrizo) (*C. sinensis* L. Osb. X *P. trifoliata* L. Raf.); MP, *C. macrophylla* Wester; (*C. reshni* X *P. trifoliata*); FA13, FornerAlcaide 13 (*C. reshni* X *P. trifoliata*]; FA418, FornerAlcaide 418, ([*C. sinensis* X *P. trifoliata*] X *C. deliciosa* Ten.).

A rootstock effect was also found for leafminers on mandarins and oranges, in both cases with leafminers more prevalent on SxE rootstock. A rootstock preference in citrus has been found for other arthropods such as mites. For instance, Bruessow et al. (2010) found the best population parameters for *T. urticae* on sour orange compared to Toyer citrange, trifoliate orange, Alemow, Volkamer and Cleopatra. Likewise, Andrade et al. (2013) found higher populations of Brevipalpus phoenicis grafted on Rangpur lime and Cleopatra compared to Sunki as a rootstock. Silva et al. (2016) found that the densities of the mites P. oliveira and T. mexicanus in the sweet oranges Pera CNPMF D-6 and Valencia Tuxpan were affected by some rootstocks, with lower densities of P. oleivora on the sweet orange Pera CNPMF D-6 grafted in the hybrid rootstock TSKC  $\times$  CTTR - 002 and in the citrumelo Swingle variety, compared to the same sweet orange variety grafted on the rootstocks: hybrid LVK  $\times$ 

LCR - 010, rough lime Red and the rangpur lime Santa Cruz.

Similarly, lower densities of *T. mexicanus* were found in the sweet orange Valencia Tuxpan grafted on the hybrid HTR-051 compared to the ones that used cintrange Indio, and mandarin Sunki Tropical and LVK  $\times$  LCR - 010 as rootstocks. This means that the rootstock selection could play an important role on the pest management of citrus crops.

Notheless, rootstock has not consistently been found to affect insects of citrus. Montes-Rodríguez et al. (2020) did not find an effect of the rootstock over the presence of pest arthropods in a study that included Kryder 15-3, Citrange Carrizo, Sunki x English, Cleopatra mandarin and Volkamer lemon rootstocks.

Regarding to climatic factors, similar to Tandel et al. (2020) results, who found that populations of *D. citri* were positively correlated with temperature, relative humidity, rainfall, and wind speed in a lime crop, in the present study a positive relationship between *D. citri* populations and relative humidity was found.

Like the results of Prabhudev et al. (2021), who found that the maximum relative humidity was positively correlated with the incidence of the citrus leafminer (*P. citrella*) in mandarins, a positive relationship was found here between relative humidity and the population of this insect. This result differs from Rathod et al. (2020) who found positive relationship between the populations of *P. citrella* and the climatic variables of maximum temperature and wind speed in a sweet orange crop. On the other hand, for mites, a positive relationship between mean relative humidity was found here, contrasting to the result found by Ferraz et al. (2015), who found a negative relationship.

These climate related results, show that the weather monitoring becomes an important tool to devise potential pest problems in citrus crops, taking into account that pest arthropods respond in different ways to climate variables.

#### **CONCLUSIONS**

The findings in this study show that the incidence of pest arthropods on citrus crops, are mediated by climatic and genotype variables. These relationships are even affected by the rootstocks in some cases. Therefore, the adequate selection of cultivars (rootstock X scion variety) and the monitoring of climatic variables become important tools to the establishment of Integrated Pest Management (IPM) programs.

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