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Morphological and chemical characteristics of onion plants (*Allium cepa* L.) associated with resistance to onion thrips

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ABSTRACT. *Thrips tabaci* Lindeman is the main pest of onion crops, and chemical control is the main method adopted by farmers. Alternative control methods should be prioritised to reduce the amount of insecticides used. Resistant cultivars are one efficient way to control thrips in the field. Our aim was to assess the influence of morphological and chemical characteristics of seven onion cultivars and their resistance to *T. tabaci*. The number of thrips and the morphological and chemical characteristics of the plants were assessed. Among the evaluated cultivars, Alfa São Francisco RT, BR 29 and Sirius showed resistance to *T. tabaci*, as indicated by the lower number of thrips observed during the cycle (64, 87, and 74 thrips, respectively). Morphological and chemical characteristics were associated with onion's resistance to *T. tabaci*. For the cultivar Alfa São Francisco RT, a wider central angle (16.4°), a thinner cuticle, a larger amount of epicuticular waxes, and stomata on the surface of leaves accounted for resistance. For the cultivars BR 29 and Sirius, the resistance was likely due to the presence of resistance-conferring substances or high amounts of some component in the chemical composition of plants.

Keywords: *Thrips tabaci*, Thysanoptera, Thripidae, onion, plant resistance to insects.

Características morfológicas e químicas de plantas de cebola (*Allium cepa* L.) associadas à resistência ao tripes da cebola

RESUMO. *Thrips tabaci* Lindeman é a principal praga da cultura da cebola e o controle químico é o principal método adotado pelos agricultores. Métodos alternativos de controle devem ser priorizados, visando diminuir a quantidade de inseticidas utilizada. Cultivares resistentes representam um método eficiente de controle de tripes no campo. Nosso objetivo foi verificar a influencia de características morfológicas e químicas de sete cultivares de cebola conferindo resistência a *T. tabaci*. O número de tripes, características morfológicas e químicas das plantas foram avaliadas. Dentre as cultivares avaliadas, Alfa São Francisco RT, BR 29 e Sirius mostraram-se resistentes a *T. tabaci*, uma vez que apresentaram um baixo número de tripes observados durante o ciclo (64, 87 e 74 tripes respectivamente). Características morfológicas e químicas estão associadas à resistência de plantas de cebola a *T. tabaci*. Para a cultivar Alfa São Francisco RT, um maior ângulo central (16,4°), uma menor espessura de cutícula, e uma maior concentração de ceras epicuticulares e estômatos na superfície das folhas conferem resistência. Para as cultivares BR 29 e Sirius, a resistência provavelmente esta relacionada à presença de substâncias ou de quantidades elevadas de algum componente na composição química das plantas.

Palavras-chave: *Thrips tabaci*, Thysanoptera, Thripidae, cebola, resistência de plantas a insetos.

Introduction

Onion thrips, *Thrips tabaci* Lindeman, (Thysanoptera: Thripidae), is the main pest of onion (*Allium cepa* L.). Larvae and adults live in the leaf sheath and stalk, sucking up the contents of plant cells, causing a series of damages to the crop and thus severely affecting onion production (MOUND; MARULLO, 1996; PALMER et al., 1989).

T. tabaci is controlled mainly by means of chemical products, but their efficiency in the control of thrips is low, and high amounts of insecticides are required because the insects have a high escape capacity, high reproductive rate, fast development rate and low sensitivity to the insecticides (BACCI et al., 2008; MO et al., 2008). Some populations of *T. tabaci* have acquired resistance to the insecticides used in several regions of the world due to the indiscriminate use of agrochemicals (MARTIN et al., 2003; MACINTYRE ALLEN et al., 2005).

The usage of plant varieties resistant to pests is an alternative method of control in the field; it is environmentally sustainable, inexpensive and efficient (CUARTERO et al., 1999). In onions, resistance to *T. tabaci* is related to the morphological and/or chemical characteristics of the plants. The morphological factors responsible for resistance are the thickness and the rigidity of the cellular wall, the distribution and the size of stomata, the rugosity of the leaf surface, the composition and amount of wax accumulated on the surface of the leaves, the leaf shape and, the most investigated, the central angle between the leaves, once, all these features make it difficult for pests and microorganisms to attack the plant. (CUARTERO et al., 1999; FREI et al., 2003; MARTIN; WORKMAN, 2006; MO et al., 2008; MORSELLO et al., 2008; VOORRIPS et al., 2008).

The plants' chemical and nutritional composition affects the feeding rate, development and reproduction of *T. tabaci*, resulting in resistant plants by antibiosis and antixenosis. Riefler and Koschier (2009) observed that different concentrations of primary metabolites, such as sugars, proteins and carbohydrates, influence oviposition, feeding time, plant exploitation and, consequently, the duration of each stage of life of *T. tabaci*. Plants less attractive to *T. tabaci* can yield a smaller number of larvae because they do not stimulate oviposition (DIAZ-MONTANO et al., 2010). Part of the plant chemical defence system against insects is composed of volatiles and various allelochemicals, such as monoterpenes, which have deterrent activity, and inhibit its feeding (KOSCHIER et al., 2000).

Due to the importance of plant resistance for the control of *T. tabaci*, this work was conducted to identify morphological and chemical characteristics of onion cultivars that can reduce thrips populations in the crop.

Material and methods

Field experiments were conducted in 2010 in the Center of Experimental Stations of *Canguiri* (CEEx – Canguiri) of the Federal University of Paraná (UFPR), Pinhais, Paraná State, Brazil (25° 25' S and 49° 08' W).

Seven cultivars of onions were evaluated: Alfa São Francisco, Alfa São Francisco RT, Alfa Tropical, BR 29, Sirius, Buccaneer and Vale Ouro IPA 11. Alfa São Francisco RT was the only cultivar previously known to be resistant to *T. tabaci*. The plants were sown on March 11, 2010 in expanded polystyrene trays and kept in a greenhouse until April 9, 2010, when they were transplanted to the field. The experiment was carried out in a completely randomised design, with an analysis of split-plot parcels × time. Four rows of plants were used, with 30 cm between rows and 15 cm between plants. For each cultivar, 60 plants were used. No phytosanitary treatment was carried out in the area during the experiment.

Evaluation of the number of thrips per plant

The thrips population density was evaluated in fortnightly samplings; five randomly chosen plants were collected from each cultivar, and the number of thrips (larvae and adults) was recorded. The first sampling was done on May 28, 2010 and the last one on August 20, 2010.

Comparative analysis of plant architecture

Two variables were employed to evaluate the architectonic characteristics of the plants: the angle between the two fully developed central leaves, measured with the help of a protractor using the central axis of the leaves, and the height of the plant, from the leaf sheath to the tip of the highest leaf. The measurements were taken fortnightly during the whole crop cycle in five randomly chosen plants of each cultivar.

Evaluation of cell wall and cuticle thickness in the plants

For the morpho-anatomic analysis, two fully developed central leaves were collected from 3 cm above the sheath of the leaf. Samples were taken from the internal side of the leaves, where the incidence of thrips is higher. Samples were fixed in F.A.A. 50, formalin, 50% ethanol and acetic acid (18:1:1) (JOHANSEN, 1940) and preserved in 70% ethanol. Cross-sections of leaves were made, and semi-permanent slides were mounted. Samples were sectioned by hand with a razor blade, and some of the samples were stained with Sudan III (SASS, 1951) for 20 min. The semi-permanent slides were mounted in glycerin with stained and non-stained samples and sealed with colourless nail polish. For each cultivar, 12 samples were analysed. Cell and cuticle thickness were measured with a photonic and stereoscopic microscope with a Sony Cyber-shot P200® photographic camera attached to the microscope.

Anatomical analysis under scanning electronic microscope

Leaf surface analysis was carried out under a Phenom desktop scanning electronic microscope (FEI Company). Samples were fixed in F.A.A.

50 formalin, 50% ethanol and acetic acid (18:1:1) (JOHANSEN, 1940), dehydrated in an increasing ethylic series until absolute ethanol and dried up to critical point with CO_2 with a BAL-TEC CPD-030 dryer. Samples were fixed in metallic supports with a double-phase copper strip and metallised with gold in SCD 030 Balzers Union FL 9496. Six samples were analysed for each cultivar. The number of stomata per square millimetre was counted, and the epicuticular waxes and cuticular striation were visually estimated.

Near-infrared spectroscopy (NIRS)

Reflectance spectra of onion leaves were obtained in a spectrometer (Excalibur Bio-Rad FTS 3500GX, Bio-Rad Laboratories, Cambridge, MA, USA) equipped with a KBr beam splitter, a deuterated triglycine sulphate detector, a radiation source of silicon carbide and an accessory of diffuse reflectance in the near-infrared range, from 7,500 to 4,000 cm^{-1} (1,428 to 2,500 nm) with a resolution of 1 cm^{-1}. Samples of ~1 cm^2 of two fully developed central leaves of each cultivar were collected at 3 cm above the leaf sheath to analyse the characteristics of the area that is most attacked by thrips and that is positioned to receive diffuse reflectance. Twelve samples were analysed for each cultivar.

Statistical analysis

The data on thrips numbers were transformed by $\sqrt{(x+0.5)}$. After verification of the homogeneity and normality of the data by the Bartlett test and Shapiro-Wilk test, a factorial analysis with split-plot parcels × time was conducted. Two parameters were analysed, as was the interaction between them: the first was the number of thrips (larvae and adults) per plant, and the second was the sampling dates. The software used was Statistica.

The data of central angle, plant height, wall thickness and cuticle thickness were submitted to the Bartlett and Shapiro-Wilk tests followed by an analysis of variance ANOVA. Differences were considered significant when $p < 0.05$, and they were classified by minimum significant differences (DMS).

To identify the variables of the chemical composition of the plants related to resistance to *T. tabaci*, a regression analysis by partial least squares (PLS) was carried out between the total number of thrips per plant and the spectra generated by NIRS.

Dendrograms of similarity were obtained through hierarchical clustering by the Average method, at first with all variables generated by NIRS and afterwards only with the variables related to the number of thrips per plant. The tests were conducted using the software The Unscrambler™ version 9.1 (Camo Software AS, Oslo, Norway) for the analyses, and NIRS data were analysed with JMP™ version 8.0.1 (SAS, 2010) to construct the dendrograms.

Results and discussion

Number of thrips in the cultivars

The factorial analyses indicated highly significant differences in the number of larvae ($F = 4.8658$, $df = 6$, $p < 0.01$) and the total number of thrips (larvae and adults) ($F = 5.0258$, $df = 6$, $p < 0.01$) sampled on the different cultivars. The lowest thrips densities were recorded on Alfa São Francisco RT, BR 29 and Sirius (Table 1), indicating that these cultivars present antibiosis and/or antixenosis as resistance mechanisms. These cultivars significantly differed from Vale Ouro IPA 11, which was the most susceptible to thrips infestation. Diaz-Montano et al. (2010) evaluated 49 onion cultivars in relation to resistance to *T. tabaci*. Of these, 11 were resistant, two of them by antixenosis and/or antibiosis because the number of insects per plant was reduced. The other nine cultivars presented a variable number of insects, resistance being expressed by a combination of antixenosis, antibiosis and tolerance. Loges et al. (2004) evaluated seven cultivars and two hybrids of onions and concluded that the cultivar Duquesa and two hybrids were moderately resistant to *T. tabaci* due to the small number of insects per plant.

Table 1. Number of larvae and adults of *Thrips tabaci* Lindeman, 1888 in different onion (*Allium cepa* L.) cultivars – Pinhais, Brazil. May – August/2010.

	Number of thrips		
Cultivar	**Larvae**	**Adults**	**Total**
Alfa São Francisco	166 bc	28 a	194 bc
Alfa São Francisco RT	57 a	7 a	64 a
Alfa Tropical	103 abc	16 a	119 abc
BR 29	51 a	23 a	74 ab
Sirius	63 ab	24 a	87 ab
Buccanner	149 abc	23 a	172 abc
Vale Ouro IPA 11	223 c	41 a	264 c

Values followed by the same letter in the columns are not significantly different ($p < 0.05$) by factorial analysis with split-plot parcels in the time [classified by minimum significant differences (DMS)]. Coefficient of Variation (CV) % larvae = 68.31; CV% total = 62.71.

Comparative analysis of plant architecture

There were significant differences in the central angle among the cultivars ($F = 3.4320$, $df = 6$, $p < 0.05$). Alfa São Francisco and Sirius showed a significantly smaller angle between the central leaves than Alfa São Francisco RT (Table 2). Cultivars with a wider central angle and more round leaves in horizontal cuts suffer fewer infestations by *T. tabaci* due to the non-preference of the insects for these leaves due to the smaller contact surface of the leaves with one another compared to more flattened leaves (FREI et al., 2003; MARTIN; WORKMAN, 2006; MO et al., 2008; MORSELLO et al., 2008). Loges et al. (2004) observed a relationship between the plant height and thrips density because taller pants have heavier leaves and therefore a wider angle between the central leaves. In this work the cultivar Alfa São Francisco RT showed a clear relationship between the central angle of the leaves and the number of thrips: a larger angle had a negative effect on thrips infestation. The opposite was observed for the cultivar Alfa São Francisco, which presented one of the smallest central angles and a high population of thrips. Sirius showed a small central angle and a low number of thrips, but this can be explained by the presence of other factors conferring resistance to *T. tabaci*. No differences in plant height were observed among the cultivars evaluated in our study.

Table 2. Central angle (°) and plant height (cm) in onion (*Allium cepa* L.) cultivars.

Cultivar	Central angle	Plant height
Alfa São Francisco	11.74±4.85 a	48.80±13.01 a
Alfa São Francisco RT	16.40±5.87 b	44.42±12.60 a
Alfa Tropical	14.85±5.73 ab	45.00±14.13 a
BR 29	13.20±4.86 ab	45.50±15.64 a
Sirius	11.71±4.83 a	46.28±16.69 a
Buccanner	13.40±5.36 ab	47.60±14.18 a
Vale Ouro IPA 11	14.62±5.66 ab	45.38±12.13 a

Values followed by the same letter within columns are not significantly different ($p < 0.05$) by factorial analysis with split-plot parcels in the time [classified by minimum significant differences (DMS)]. CV% central angle = 33.19; CV% plant height = 12.54.

Highly significant differences were found in cuticle thickness among the cultivars ($F = 7.1692$, $df = 6$, $p < 0.01$). Structural traits on the surface of the leaves, such as cuticle and cell wall thickness, accumulation of cuticular waxes, roughness, and the number of stomata, can be related to the resistance of onion plants to *T. tabaci* because these can function as physical barriers to walking, feeding and egg laying activities. Alfa São Francisco RT had the thinnest cuticle among the cultivars. No significant differences were recorded in cell wall thickness among the treatments (Table 3).

Table 3. Cell wall and cuticle thickness (μm) in different onion (*Allium cepa* L.) cultivars.

Cultivar	Cell wall thickness	Cuticle thickness
Alfa São Francisco	8.11±1.80 a	0.85±0.00 b
Alfa São Francisco RT	7.01±1.36 a	0.64±0.22 a
Alfa Tropical	7.40±1.38 a	0.85±0.00 b
BR 29	6.80+0.60 a	0.85±0.00 b
Sirius	8.04±0.81 a	0.82±0.16 b
Buccanner	7.58±0.89 a	0.85±0.00 b
Vale Ouro IPA 11	7.54±0.68 a	0.81±0.12 b

Values followed by the same letter within columns are not significantly different ($p < 0.05$) by ANOVA (classified by minimum significant differences DMS). CV% cell wall thickness = 15.68; CV% cuticle thickness = 13.87.

The leaf surfaces of Alfa São Francisco RT, Alfa Tropical and BR 29, had the highest concentrations of epicuticular wax. Sirius and Buccaneer had intermediate concentrations, while Alfa São Francisco and Vale Ouro IPA 11 contained the lowest concentrations of wax on the surfaces of leaves (Figures 1 and 2). No difference in cuticular striation was observed among the cultivars (Figure 1).

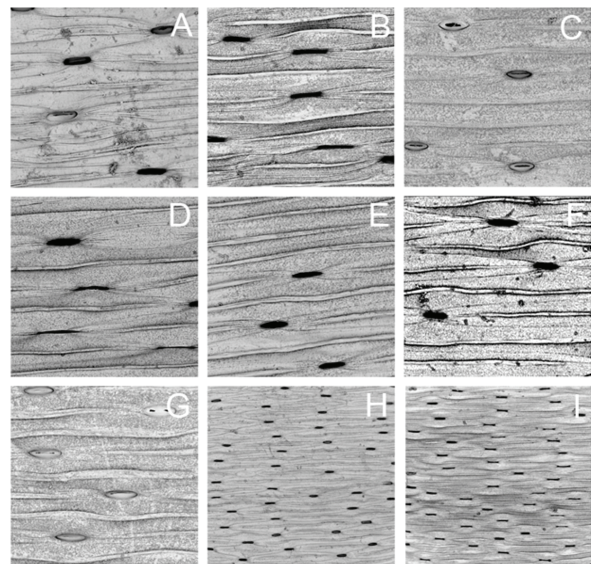

Figure 1. Scanning electromicrographs of the surfaces of onion (*Allium cepa* L.) leaves. (A) Alfa São Francisco; (B) Alfa São Francisco RT; (C) Alfa Tropical; (D) BR 29; (E) Sirius; (F) Buccaneer; (G) Vale Ouro IPA 11; (H) Alfa São Francisco (I) Alfa São Francisco RT (magnification: A-G: 1500x; H-I: 500x).

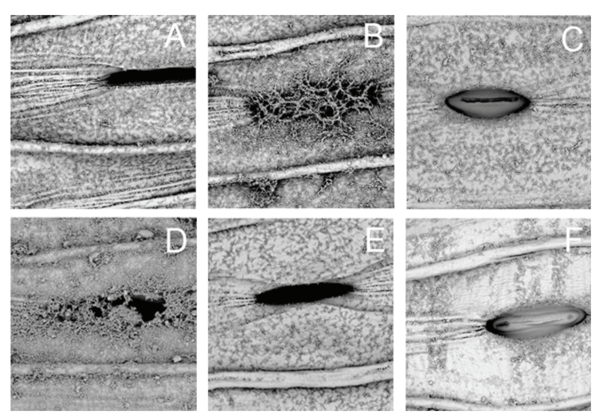

Figure 2. Scanning electromicrographs of the surfaces of onion (*Allium cepa* L.) leaves. (A) Alfa São Francisco RT; (B) Alfa São Francisco RT; (C) Alfa Tropical; (D) BR 29; (E) Sirius; (F) Vale Ouro IPA 11 (magnification: 500x). Panels B and D show detail of wax deposition in stomata openings.

The quantity of stomata was highest in Alfa São Francisco RT, with a mean of 203.4 stomata mm^{-2}. Alfa Tropical, Sirius and Vale Ouro IPA 11 showed the lowest quantities of stomata mm^{-2} (Table 4).

Table 4. Mean number of stomata mm^{-2} in the different onion (*Allium cepa* L) cultivars.

Cultivar	Stomata/mm²
Alfa São Francisco	168.8 ab
Alfa São Francisco RT	203.4 a
Alfa Tropical	129.7 b
BR 29	145.4 ab
Sirius	128.3 b
Buccanner	137.0 ab
Vale Ouro IPA 11	118.1 b

Values followed by the same letter within columns are not significantly different ($p < 0.05$) by ANOVA (classified by minimum significant differences DMS). CV% = 14.69.

One of the functions of the leaf cuticle is protection against microorganisms and insects, which can be related to the degrees of resistance of plants to pests (LICHSTON; GODOY, 2006). The influence of epicuticular waxes on the attack and development of *T. tabaci* in onions is not clearly established. Some studies show that shiny plants with a lower content of wax are more resistant than plants with high levels of wax on the leaf surface (BOCAK, 1995; MARTIN; WORKMAN, 2006; OLIVEIRA; CASTELLANE, 1996). Eigenbrode et al. (1996) found that on cabbage leaves, a high quantity of wax hampers the movement of small insects. Voorrips et al. (2008) found that a high amount of wax resulted in low densities of *T. tabaci* in cabbage plants. A negative relationship between the amount of epicuticular waxes and damage of *T. tabaci* was described by Trdan et al. (2004) in cabbage plants.

In our work, Alfa São Francisco RT had the least cuticle thickness. Alfa São Francisco RT and BR 29 contained high concentrations of epicuticular waxes on the surface of the leaves. These conditions may be associated to *T. tabaci* resistance. No relationship was observed between the number of thrips and the variables associated with the plants' cuticle in the other cultivars evaluated. Jager et al. (1995) evaluated various morphological characteristics of chrysanthemum plants (*Dendranthema grandiflora* Tzvelev), such as the presence and quantity of trichomes, cell wall resistance, plant height, and number and area of leaves, and did not observe any relationship between attack by *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) and the variables evaluated. Similarly, Mirnezhad et al. (2010) did not find any relationship between the number of trichomes or cell wall resistance and tomato plant resistance to *F. occidentalis.* The influence of cuticle thickness and the epicuticular waxes conferring resistance to onion plants against *T. tabaci* should be further investigated. The majority of studies assume that a thicker cuticle confers resistance to onion plants, but in unique cultivars previously known to be resistant, we observed the opposite. We can assume that the cuticle of onion plants may acts as an attractant for feeding, and therefore, a smaller thickness of the cuticle can decrease the damage caused by *T. tabaci*. The epicuticular wax concentration may be more important in resistance to thrips than the cuticle thickness.

Near infrared spectroscopy

A dendrogram of similarity containing all chemical and micro-structural variables was generated after the spectroscopic analysis of the onion leaves (Figure 3). All cultivars were placed within the same group; cultivars BR 29 and Alfa São Francisco RT were similar and closer to Sirius and Alfa Tropical. Vale Ouro IPA 11 was the most differentiated of all cultivars.

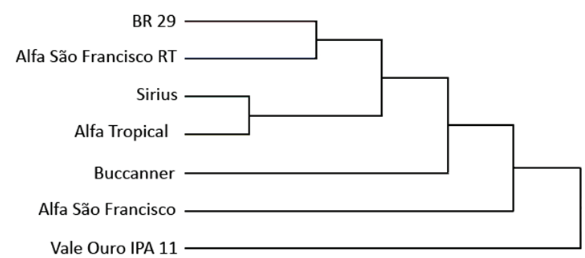

Figure 3. Dendrogram of similarity of onion (*Allium cepa* L.) cultivars by means of near infrared spectroscopy, based on chemical and micro structural composition of the plants.

A high correlation (0.99) was obtained by partial minimum squares PLS regression analysis between the number of thrips per plant and some of the spectroscopic variables (Figure 4).

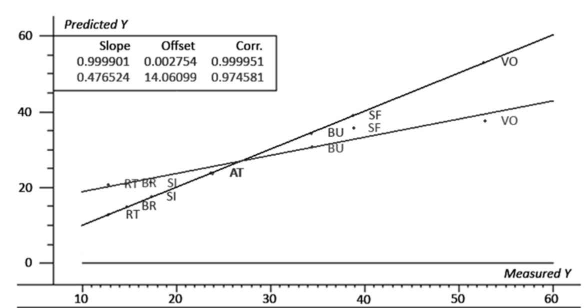

Figure 4. Correlation between density *Thrips tabaci* Lindeman, 1888 and chemical and micro structural composition of onion (*Allium cepa* L.) cultivars by the method of near infrared spectroscopy. Alfa São Francisco RT (RT); BR 29 (BR); Sirius (SI); Alfa Tropical (AT); Buccanner (BU); Alfa São Francisco (SF); Vale Ouro IPA 11 (VO).

After selecting the variables correlated to the number of thrips per plant, a second, different dendrogram of similarity was generated. Cultivars BR 29 and Sirius maintained their high degree of similarity and became closer to Vale Ouro IPA 11 and Alfa São Francisco. The least similar cultivar was Alfa São Francisco RT (Figure 5).

Spectroscopy allows for chemical and physical analysis of the samples by using the wavelengths of the obtained spectra through electromagnetic radiation. One of the methods of spectroscopic analysis is near-infrared spectroscopy which provides information on the chemical composition of the samples (LAZZARI et al., 2010). Spectroscopy has been used as a tool to measure plant resistance to thrips, and various substances have been identified and correlated with damage caused by the insects (LEISS et al., 2009a and b; MIRNEZHAD et al., 2010).

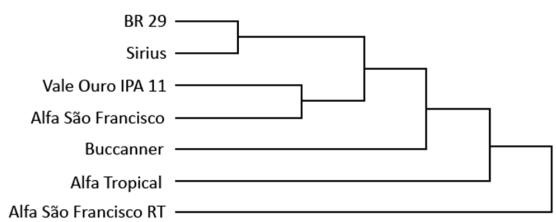

Figure 5. Dendrogram of similarity of onion (*Allium cepa* L.) cultivars by the near infrared spectroscopy method, based on chemical and micro structural composition of the leaves that influence density of *Thrips tabaci* Lindeman, 1888.

Differences were observed between the two dendrograms generated by spectroscopy, which suggests that either some substances or some structural conformations of the surface layers of the epidermis are similar among cultivars BR 29 and Sirius and are different from the cultivar Alfa São Francisco RT. Little is known about plant resistance mechanisms and their interaction with thysanopterans (LEISS et al., 2009b). Moreover, few studies have been conducted to understand the resistance of onion cultivars against *T. tabaci*. The type of resistance (antixenosis or antibiosis) or variations in plant metabolite concentrations could have contributed to the differences observed in the dendrograms. The near-infrared spectroscopy did not reveal which substances were present, but it did show differences in the concentrations and types of compounds present in the samples. According to the dendrograms, we can conclude that BR 29 and Sirius cultivars either have different metabolites or quantities of metabolites or they present a type of resistance different from that shown by cultivar Alfa São Francisco RT. The degree of resistance to *F. occidentalis* is associated with a low concentration of total amino acids compared to the total amount of protein in the leaves, thus influencing longevity, fertility and survival of the insects (OHTA, 2002; FREI et al., 2003). Tomato plants' resistance to *F. occidentalis* is mainly due to the chemical composition of the plants: the presence of acyl sugars and malic acid is related to thrips resistance (MIRNEZHAD et al., 2010). Leiss et al. (2009a) concluded that the presence of the alkaloids pyrrolizidine, jacobine and jaconine, as well as the flavonoid kaempferol-glucoside, is related to the attack by *F. occidentalis* of *Senecio* spp. L. plants. In chrysanthemum plants, resistance to *F. occidentalis* is affected by the presence of phenylpropanoids (LEISS et al., 2009b). A series of essential oils, alomones, benzenoids, monoterpenes, sesquiterpenes and other components exert a repellent effect on *T. tabaci*, both for feeding and for oviposition (KOSCHIER; SEDY, 2003; KOSCHIER et al., 2000). Frei et al. (2003) evaluated the preferences of *Thrips palmi* Karny (Thysanoptera: Thripidae) for bean (*Phaseolus vulgaris* L.) cultivars and observed that some cultivars had a negative effect on the development of the insects. Arif et al. (2004) observed a 75.6% correlation between the density and length of trichomes, the quantity of secretory glands of gossypol and the number of *T. tabaci* in cotton plants.

Conclusion

For 'Alfa São Francisco RT', morphological traits have been associated with resistance, conferring to the plants resistance by antixenosis. For 'BR 29' and 'Sirius', resistance was most likely due to the presence of certain substances or high quantities of some chemical component in both plants, given that they were chemically similar and no relationship was found between morphological characteristics and resistance in these two cultivars. To understand the mechanisms of resistance of onion cultivars to thrips, further studies should be carried out to identify the chemical components involved in onion resistance, and to determine the roles of morphological characteristics in the bioecology of the insect.

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