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Article

Resistance of strawberry genotypes to the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae)

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ABSTRACT

Cultivating resistant genotypes can be an efficient method for keeping mite populations below levels that cause economic damage in crop plants because it is inexpensive and can be integrated with other pest control tactics. The present work evaluated the resistance of nine experimental strawberry genotypes (MCA89, MFA444, MCA93–01, MFA443–01, MFA451, MCA111, MOGSC468, MFA443, and MDA22) to the two-spotted spider mite *Tetranychus urticae* Koch compared to two commercial cultivars (Camarosa and Dover). Laboratory oviposition assays were performed using excised leaf discs. Although significant differences were observed in mite's oviposition, none of the strawberry genotypes stood out for high resistance levels. The lowest oviposition averages were found in the genotype MDA22 (abaxial 17.13 ± 4.20 and adaxial 30.63 ± 6.80), resulting from a cross between cv. Dover and cv. Aromas, genotypes considered to have intermediate resistance to *T. urticae*. In addition, MDA22, provided neutral stimulus for mite's oviposition on the abaxial and adaxial surfaces. Given the results, MDA22 genotype should be used as a parent for new crossings in breeding programs that aim to increase the genetic basis of genotypes adapted to Brazilian edaphoclimatic conditions and resistant to *T. urticae*.

KEY WORDS: *Fragaria ananassa*; genetic breeding; horticulture; plant selection; resistance.

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INTRODUCTION

Strawberry (*Fragaria ananassa* Duch.) is a crop with significant economic and social importance whose fruits have a growing demand in the consumer market. Strawberry plants are very susceptible to the incidence of diseases and arthropod pests, and some of which are difficult to diagnose and control and can cause significant losses in fruit yield and quality (Maas 1998, 2014). Among the main pests of strawberry are tetranychid mites, especially the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) due to its great potential to cause damage to agricultural crops (Nyoike and Liburd 2013).

Conventional chemical methods, namely the application of synthetic acaricides, are commonly used for *T. urticae* control. However, repeated use of these products can lead to selection of

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resistant populations of *T. urticae* or cause decreases in populations of its natural enemies (Bi *et al.* 2016). In addition, the use of some chemicals can result in outbreaks, with an exaggerated increase in the abundance of secondary pests, resulting from the phenomena hormesis and hormoligosis, which can entail additional agricultural management and production costs (Cordeiro *et al.* 2013). An alternative to conventional chemical methods is biological control with predatory mites (Castilho *et al.* 2015) and the development of resistant or tolerant strawberry genotypes (Lourenção *et al.* 2000). The use of resistant host-plant genotypes is an important component of integrated pest management programs. Host plants characteristics can affect pest population density by impairing the growth and fecundity of herbivorous arthropods, injury to the plants, efficiency of natural enemies, and amount of insecticide application in agricultural ecosystems (Fathipour and Sedaratian 2013).

The selection of strawberry plants with chemical and morphological traits that provide an efficient defense, making them capable of withstanding attacks by some arthropod pests, such as mites, is the main step in developing novel genotypes (Lourenção *et al.* 2000). However, cultivars that are adapted to specific climatic conditions and, at the same time, capable of effectively resisting the major diseases and pests in the producing regions of Brazil are scarce (Barneche and Bonow 2012).

Thus, the objective of this work was to evaluate the resistance of experimental genotypes, previously selected from the strawberry breeding program of Universidade Federal de Lavras (UFLA), to infestation of *T. urticae* in comparison with two commercial cultivars.

MATERIALS AND METHODS

The experiment was carried out with 11 strawberry genotypes, two of which are commercial cultivars: cv. Camarosa is considered susceptible to *T. urticae* (Iwassaki 2010) and cv. Dover is classified as possessing intermediate resistance (Figueiredo *et al.* 2010). Other nine genotypes, belonging to the genetic improvement program of Federal University of Lavras (UFLA), were previously selected for productive characteristics of the plants and physical-chemical quality of fruits, as follows: MCA89, MFA444, MCA93–01, MFA443–01, MFA451, MCA111, MOGSC468, MFA443, and MDA22. The genotypes were originated by crossing parentals that have been largely cultivated in Brazil and present traits of economic importance (Aromas, Camarosa, Dover, Florida Festival, Oso Grande, 'Sweet Charlie and Milsei Tudla culltivars). The hybridization method was thoroughly described in Vieira *et al.* (2017).

The plants were grown in 5–dm^3 pots containing subsurface soil corrected to 70% of base saturation, previously fertilized with 25 g of 04–14–08 (N–P–K). Plants cultivation was carried out without phytosanitary products and maintained in the greenhouse. The indoor temperature of the greenhouse was kept at 25 ± 5 °C with 70 ± 10% RH, and natural photophase (~10 h). The plants were irrigated with micro drippers according to the plant requirements.

The mite specimens were originally collected from infested weeds at forest fragments located at Lavras Federal University (UFLA), Brazil (21° 13' 46.48" S, 44° 58' 42.98" W). Mites of *T. urticae* were reared in the Acarology Laboratory of "Centro de Pesquisa em Manejo Ecológico de Pragas e Doenças de Plantas – EcoCentro, EPAMIG", at UFLA, Lavras, Minas Gerais, under 25 ± 2 °C, 70 ± 10% RH, and a 14L:10D hour photoperiod. The mite population was multiplied in arenas made with whole leaves of jack bean (*Canavalia ensiformis* (L.) DC.) free of acaricides and placed on a nylon sponge (1-cm thick) inside a Petri dish (20–cm in diameter) moistened daily with distilled water (Reis *et al*. 1997). The mites from the laboratory colony were reared on jack beans for four generations before conducting the experiments.

The oviposition assay consisted of eleven independent replicates, and for each replicate, one leaflet of strawberry was used. The leaflets were individually placed on nylon foam in Petri dishes (9 cm in diameter) and kept moist by periodic addition of distilled water. A fine paintbrush was used to release an individual *T. urticae* female (2–3 days old specimen) collected from the breeding colony on each leaflet of the respective genotype, after which the number of eggs was recorded every 24 hours for eight days. The observation period was conducted for eight days as it is the average time that *T. urticae* females remain ovipositing without decaying in performance (Vásquez *et al.* 2018; Fahim *et al.* 2020). After performing the experiment, all mite individuals were mounted on slides in Hoyer medium (Walter and Krantz 2009) and were observed under a phase-contrast microscope to confirm the identification of the species and gender.

During this period, the eggs were counted and subsequently removed from the dishes. The oviposition assay was performed in the same way for each leaflet's abaxial and adaxial surfaces. The method of the current study was adapted from those of Luczynski *et al.* (1990), Toscano *et al.* (2002) and Karlec *et al.* (2017).

After counting the eggs for each genotype, the oviposition preference index (OPI) was calculated according to Fenemore (1980), as: $OPI = ((P - T) / (P + T)) \times 100$, where P is the mean value of eggs on the evaluated genotypes and T is the number of eggs on the susceptible standard genotype (Camarosa). This index ranges from +100 (stimulant), values next to 0 (neutral), to −100 (deterrent). The sum of all results, obtained from the 11 replications for each genotype, were used for these calculations, being separated only between abaxial and adaxial leaf surfaces.

Homogeneity of variance for oviposition rates was checked using the Levene test. The results showed that the variance values are homogeneous ($F > 0.05$), so statistical analysis can be performed without data transformation. After that, the oviposition rates were compared separately for each leaf surface (abaxial and adaxial) and were done using the total number of eggs accumulated over the eight days for each replication. Values obtained for each genotype were compared by Tukey's test with multiple comparisons, whereby samples were compared pairwise, using the significance level of $\alpha = 0.05$. The analyses were performed using R software (R Development Core Team 2021).

RESULTS AND DISCUSSION

After eight days of oviposition on the abaxial and adaxial surfaces of the strawberry leaflets, egg counting revealed significant differences (abaxial, $p = 0.002$ and $F = 3.10$, adaxial, $p < 0.001$ and $F = 4.17$) among some of the studied strawberry genotypes (Table 1).

Table 1. Mean number (± SE) of eggs of *Tetranychus urticae* on abaxial and adaxial surfaces of leaflets of strawberry genotypes after eight days of infestation under laboratory conditions.

Genotype	Abaxial	Adaxial
Dover	18.38 ± 3.02 ^{bc}	38.63 ± 6.18 ^{bc}
Camarosa	20.88 ± 5.82 ^{bc}	39.38 ± 8.77 ^{bc}
MDA22	17.13 ± 4.20 ^{c}	30.63 ± 6.80 ^{c}
MFA443	22.13 ± 3.40 ^{abc}	42.13 ± 6.76 ^{abc}
MOGSC468	23.88 ± 4.90 ^{abc}	44.5 ± 3.65 ^{abc}
MCA111	27.88 ± 6.43 ^{abc}	46.25 ± 6.99 ^{abc}
MFA451	29.25 ± 6.14 ^{abc}	49.13 ± 11.05 ^{abc}
MFA 443-01	32.25 ± 4.59 ^{abc}	59.63 ± 5.29 ^{abc}
MCA93-01	33.63 ± 5.90 ^{abc}	35.38 ± 3.56 ^{abc}
MFA444	35.13 ± 5.23 ^{abc}	60.88 ± 5.31 ^{abc}
MCA89	40.75 ± 4.50 ^{ab}	66.88 ± 5.34 ^{ab}
F	3.10	4.17
p	0.002*	< 0.001*

* Significant by Tukey's test ($P < 0.05$). Means followed by the same letters in each column do not differ significantly.

The oviposition preference index indicated that four genotypes (MDA22, MFA443, MOGSC468, MCA111 and Dover, showed promising results that can assist in substantial control of *T. urticae*, with both surfaces, abaxial and adaxial, providing a neutral stimulus to mite's oviposition (Table 2).

Table 2. Index and classification of oviposition preference by *Tetranychus urticae* for the abaxial and adaxial leaf surfaces of 10 strawberry genotypes eight days after infestation under laboratory conditions (Camarosa was used as the standard susceptible genotype).

Genotype	Abaxial	Classification	Adaxial	Classification
Dover	−6.37	neutral	−0.96	neutral
MDA22	−9.87	neutral	−12.50	neutral
MFA443	2.91	neutral	3.37	neutral
MOGSC468	6.70	neutral	6.10	neutral
MCA111	14.36	neutral	8.02	neutral
MFA451	16.70	stimulant	11.02	neutral
MFA 443-01	21.40	stimulant	20.45	stimulant
MCA93-01	23.39	stimulant	−5.35	neutral
MFA444	25.44	stimulant	21.44	stimulant
MCA89	32.24	stimulant	25.88	stimulant

These obtained results could be useful to assist genetic plant breeding programs to select the best crosses and concentrate their efforts on the most promising genotypes of strawberry to *T. urticae* infestation (Morales *et al.* 2011). In strawberry, for example, various morphological modifications can occur, such as glandular and non-glandular trichomes on the leaves. These characteristics can confer resistance to some pests, such as mites, through constitutive mechanisms (Luczynski *et al.* 1990). The selection of parental plants for new crossings should be constantly and persistently, with crosses that can enhance the desired plant traits.

Although the oviposition rates of *T. urticae* varied among the studied strawberry genotypes, statistical analysis revealed that the differences were subtle. None of the genotypes stood out as expressing high levels of resistance to mite's oviposition. Similar results were found by Karlec *et al.* (2017) and Lourenção *et al.* (2000). MOGSC468, MFA443, MDA22, MCA111, and Dover showed satisfactory results in the oviposition experiment as their characteristics negatively affected mites oviposition, indicating that these genotypes possess genes coding for chemical or morphological traits of resistance, making them potential progenitors for new crossings. However, it is worth mentioning that crossbreeding must be a constant process in these programs, and therefore, it is important to use genotypes providing lower oviposition stimulus in an attempt to obtain better results in the future.

Genotype MDA22 was obtained from a cross between Dover and Aromas cultivars (Vieira *et al.* 2019). Thus, taking into account the results of the two tests, it can be inferred that MDA22 stands out among the evaluated genotypes with the lowest accumulated average of *T. urticae* eggs, providing a neutral stimulus for the abaxial surface and oviposition deterrence on the adaxial surface. Thus, MMDA22 is considered the most promising genotype among the studied genotypes.

Cultivar Dover is considered intermediate for resistance to *T. urticae* (Figueiredo *et al.* 2010); cv. Aromas has a high trichome density compared to other widely used commercial cultivars, such as Camarosa and San Andreas (Benatto *et al.* 2018). These results can be useful to plant breeders, in addition to helping select the most promising strawberry genotypes for suppressing *T. urticae* infestations (Morales *et al.* 2011).

The difficulty in finding strawberry genotypes that are resistant or tolerant to *T. urticae* may be related to the characteristics of the pest itself, and not only of the host plant. Being a polyphagous pest, *T. urticae* is able to establish and develop on different types of cultivars and plant species and can quickly become resistant to different control methods (Van Leeuwen *et al.* 2010; Attia *et al.* 2013).

The control of *T. urticae* on different strawberry varieties has proven to be a difficult task (Lourenção *et al.* 2000; Karlec *et al.* 2017). For this reason, the best strategy for controlling this pest may be attempting specific techniques and searching for more resistant and tolerant genotypes and integrating different actions in a pest management strategy, including attempts to determine which approaches could be combined to obtain the best results. Integrating biological control, chemical control, and the choice of genotypes providing resistance and tolerance to the main crop pests seems to be the most effective strategy for managing crops that suffer from biotic stress, such as the attack of *T. urticae*.

CONCLUSION

In addition to the promising results obtained by MDA-22, which showed resistance to spider mites, other information presented by Souza *et al.* (2019) and Vieira *et al.* (2017) corroborates and highlights the productive characters of this genotype. However, existing commercial cultivars (Albion, San Andrean, Dover and Percinque) have the greatest stability and adaptability to different Brazilian edaphoclimatic conditions (Vieira *et al.* 2017; Souza *et al.* 2019). Given these observations, at the moment, we recommend the use of MDA22 genotype as a parent for new crossings in the next stages of strawberry breeding programs to develop novel genotypes that are adapted to specific climatic conditions, better productive, and also resistant to *T. urticae*.

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مقاومت ژنوتیپ‌های توت فرنگی به کنهٔ تارتن دولکه‌ای، *Tetranychus urticae* (Acari: Tetranychidae)

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چکیده

کشت ژنوتیپ‌های مقاوم می‌تواند روشی کارآمد برای نگه‌داشتن جمعیت کنه‌ها در زیر سطوحی باشد که باعث آسیب اقتصادی در گیاهان می‌شود، زیرا ارزان است و می‌توان آن را با سایر روش‌های مهار آفات تلفیق کرد. پژوهش حاضر مقاومت ۹ ژنوتیپ آزمایشگاهی توت‌فرنگی (MCA89، MFA444، MCA93–01، MFA443–01، MCA111، MOGSC468، MFA443، و MDA22) را در برابر کنهٔ تارتن دولکه‌ای *Tetranychus urticae* Koch با دو رقم تجاری (کاماروسا و دوور) ارزیابی کرد. سنجش تخم‌گذاری آزمایشگاهی با استفاده از دیسک برگ‌های بریده شده انجام شد. اگرچه تفاوت معنی‌داری در تخم‌گذاری کنه مشاهده شد، اما هیچیک از ژنوتیپ‌های توت‌فرنگی از نظر سطوح زیاد مقاومت متمایز نشدند. کمترین میانگین تخم‌گذاری در ژنوتیپ MDA22 (سطح زیرین ۴/۲۰ ± ۱۷/۱۳ و سطح رویی ۳۰/۶۳ ± ۶/۸۰ مشاهده شد که حاصل تلاقی بین دو رقم دوور و آروماس بود، ژنوتیپ‌هایی که دارای مقاومت متوسط به *T. urticae* در نظر گرفته می‌شوند. افزون بر این، MDA22، محرک خنثی برای تخمگذاری کنه بر روی سطح‌های زیرین و رویی داشت. با توجه به نتایج، ژنوتیپ MDA22 باید به عنوان والد برای تلاقی‌های جدید در برنامه‌های اصلاحی استفاده شود که هدف آن افزایش پایه ژنتیکی ژنوتیپ‌های سازگار با شرایط ادافوکلیماتیک برزیل و مقاوم به *T. urticae* است.

واژگان کلیدی: *Fragaria ananassa*؛ اصلاح ژنتیکی؛ باغبانی؛ انتخاب گیاه؛ مقاومت.

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RESISTANCE OF STRAWBERRY GENOTYPES TO THE TWO-SPOTTED SPIDER MITE