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Dwarf cashew antibiotic and antixenotic resistance to the whitefly *Aleurodicus cocois*

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Abstract: The aim of the present study was to identify *Aleurodicus cocois* resistant genotypes among five dwarf cashew clones available in the germplasm bank of Embrapa/Centro Nacional de Pesquisa Agroindústria Tropical. Free-choice and no-choice tests were applied in order to evaluate the relative attractiveness of the clones and the oviposition preferences, egg-to-adult development times and adult emergence rates of the pest. In comparison with other clones, PRO143/7 exhibited the lowest attraction to the whitefly and was least preferred for oviposition in the free-choice test. Conversely, CCP76 attracted the highest number of *A. cocois* and was preferred for oviposition. In the no-choice test, the rates of emergence of adults from clones BRS274, CCP76 and PRO143/7 varied between 53.0 and 56.8%, values that were significantly lower ($p < 0.05$) than those of clones BRS226 and EMBRAPA51, which were 72.34 and 75.16%, respectively. Some of the cashew clones tested showed antibiotic (CCP76, PRO143/7 and BRS274) and antixenotic (PRO143/7 and BRS226) resistance to *A. cocois*. These clones are good candidates for use in breeding programs of cashew.

Key words: *Anacardium occidentale,* cashew whitefly, host plant resistance, oviposition, attractiveness.

INTRODUCTION

Cashew (*Anacardium occidentale* L.; Anacardiaceae) is native to northeastern Brazil, a region encompassing the main centers of production of cashew nuts, oil and pseudofruits (peduncles) in the country. The principal producers of cashew nuts are the State of Ceará, which accounts for more than 50% of the total national production, followed by Piauí, Rio Grande do Norte, Bahia, Maranhão and Pernambuco (Serrano & Oliveira 2013).

The establishment of extensive areas cultivated with cashew and the absence of effective control of arthropod pests and diseases, have generated imbalance of the agroecosystem and potentiated phytosanitary problems that can reduce crop productivity. In this context, intense infestations of the cashew whitefly, *Aleurodicus cocois* (Curtis, 1846) (Hemiptera: Aleyrodidae), have caused significant losses to cashew crops in recent times (Mesquita & Braga-Sobrinho 2013, Mesquita et al. 2016) and the insect is now considered an important pest.

However, *A. cocois* is not restricted to Brazil and in Chile and Peru can be found infesting avocado (*Persea americana* Mill.), mango (*Mangifera indica* L.), apricot (*Prunus armeniaca* L.), guava (*Psidium guajava* L.), pomegranate (*Punica granatum* L.), passion fruit (*Passiflora incarnata* L.), and some ornamental species (Núñez 2008, Núñez et al. 2008).

Cashew whiteflies have the potential to inflict direct damage by inoculating toxins while feeding on phloem sap, giving rise to anomalies or phytotoxic disorders characterized by wilting and yellowing of leaves and branches. In addition, *A. cocois* can cause indirect damage by releasing a sugary exudate that favors the growth of the sooty mold, *Capnodium mangiferae* (Cooke & Broome) (Liu et al. 2012).

No efficient methods to control *A. cocois* are currently available to cashew growers, and no specific insecticides have been registered. As a consequence, growers have adopted indiscriminate chemical controls that have led to increments in the cost of crop management and the emergence of resistant insect populations. Thus, to reduce the impact of agriculture on the environment, it is important to evaluate methods of pest control that are less aggressive, time-efficient, and that are aligned with the principles of Integrated Pest Management (IPM) (Mitchell et al. 2016). Host plant resistance is an important tool for IPM because of its low cost, persistence, ability to reduce the risk of pest resistance to insecticides, lack of toxic residues in fruit, and compatibility with other control methods (Smith & Clement 2012, Boiça Júnior et al. 2013).

The Cashew Genetic Improvement Program of Embrapa (Empresa Brasileira de Pesquisa Agropecuária) has achieved a number of important outcomes such as the selection and breeding of elite cashew genotypes with advantageous traits, including the highly productive premature dwarf cashew clones (Serrano & Oliveira 2013). While the improvement program has manipulated cashew variability significantly, few studies have focused on the identification of factors responsible for insect resistance or on the development of resistant varieties of cashew.

Thus, we test the hypotheses that: there is genetic variability among dwarf-cashew genotypes that was associated with resistance to *A. cocois*. Considering the need to intensify studies involving varietal resistance, the aim of the present study was to select *A. cocois* resistant genotypes among five dwarf cashew clones available in the germplasm bank of Embrapa/CNPAT (Centro Nacional de Pesquisa Agroindústria Tropical) through the evaluation of attractiveness, oviposition preference, and egg-to-adult development using free-choice and no-choice tests

MATERIALS AND METHODS

Host plants

Five dwarf cashew clones (CCP76, BRS226, EMBRAPA51, BRS274, and PRO143/7) were used as host plants for the whitefly. The clones were supplied by the Embrapa/CNPAT germplasm bank. The cashew genotype selection was based on preliminary field assays of plant resistance to *A. cocois*. All plants were propagated by means of grafting, planted in seedling tubes containing carbonized rice husk substrate (*Oryza sativa* L.), ground carnauba bagasse (*Copernicia prunifera* L.), and hydromorphic soil (3:2:2). The plants were maintained in a growth chamber at 28±1 °C; 70±10% R.H., and 14:10 L:D photoperiod.

Rearing of *A. cocois*

Whiteflies were collected from an Embrapa's experimental station (4°10'35"S; 38°28'19"W; 79m elevation) and multiplied on the dwarf cashew plants (BRS189) planted in tubes. The insects were maintained screen cages (60 × 60 × 60 cm), under controlled conditions (28±1 °C, 70±10% R.H., and 14:10 L:D photoperiod). These environmental conditions were maintained in all tests. Only newly-emerged (24-48 h) whiteflies were used in the bioassays.

Attraction and oviposition preference of *A. cocois*: free-choice test

In the free-choice assay we tested the attractiveness of adult insects and oviposition preference to five cashew clones in a completely randomized design, with five replicates. The five clones were distributed randomly in a cubic screen cage (1.0 x 1.0 x 1.0 m) with plants spaced at least 15 cm from one another in order to prevent contact between the leaves. Twenty adult *A. cocois* per plant were released into the cage and the attraction of insects to plants was evaluated after 24 and 48 h by counting the number of insects present on the abaxial surfaces of the leaves with the help of a handheld mirror. Following the second evaluation, the insects were removed from the cage and eggs that had been laid on the leaves were counted. Data were analyzed by analysis of variance (One-Way ANOVA) and the mean values were compared using Scott-Knott test ($p \leq 0.05$).

Oviposition preference and egg-to-adult development of *A. cocois*: no-choice test

In order to determine whether the lowest oviposition preference detected in the free-choice test was a stable characteristic, a no-choice test was performed in which insects were forced to subsist and lay eggs on seedlings of a single clone type. The experiment was of a completely randomized design with five repetitions of each of the clones studied. Each cashew seedling was protected by an individual cage (40 cm high x 17 cm diameter) made of wire mesh and voile cloth, and 20 adult female whiteflies were released into each cage. The numbers of eggs laid on each seedling were determined after 24 h. Subsequently, the viabilities of eggs and nymphs were evaluated daily, and the duration of each developmental phase and the number of emerged adults established. Based on these data, the mean number of days required for *A. cocois* to complete development from egg-to-adult and the percentage of emerged adults were ascertained for each cashew clone, and the possible occurrence of antibiosis established. Data were analyzed by analysis of variance (ANOVA; *F* test) and the mean values were compared using Scott-Knott test ($p \leq 0.05$).

RESULTS

Attraction and oviposition preference of *A. cocois*: free-choice test

The results of the free-choice test (Table I) revealed that from the five tested clones, PRO143/7 was the least attractive to *A. cocois*, as shown by the significantly lower ($p< 0.05$) mean number of adults found on leaves after 24 h of assay, and the very low number of insects remaining after 48 h.

The oviposition preference of *A. cocois* was significantly different amongst the tested clones ($p < 0.05$) (Table II). The highest egg count was found in the CCP76 clone, whereas PRO143/7 showed the lowest number, which did not significantly differ from BRS226. BRS274 and EMBRAPA51 showed intermediary levels of oviposition preference.

Oviposition preference of *A. cocois* and egg-to-adult development: no-choice test

Although preference for oviposition was less evident in the no-choice compared with the free-choice test, PRO143/7 emerged as the clone least preferred by *A. cocois*, as indicated by the significantly lower ($p < 0.05$) number of eggs laid (Table III).

The no-choice test revealed that the viabilities of *A. cocois* eggs laid on the five cashew clones were not significantly different

Table I. Number (mean ± standard error) of *Aleurodicus cocois* adults present on leaves of different cashew genotypes distributed as a function of time in a free-choice test.

Cashew clone	Number of adult insects	
	After 24 h *	After 48 h ns
CCP76	11.0 ± 1.41 aA	1.6 ± 0.93 aB
EMBRAPA51	8.6 ± 1.75 aA	1.0 ± 0.63 aB
BRS274	8.6 ± 1.63 aA	1.4 ± 1.17 aB
BRS226	7.6 ± 1.43 aA	0.4 ± 0.25 aB
PRO143/7	3.4 ± 0.67 bA	0.2 ± 0.20 aB

Values with different lowercase letters (within columns) or uppercase letters (within rows) are significantly different according to the Scott-Knott test ($p \leq 0.05$). *Significant difference; ns=Non-significant difference.

Table II. Number (mean ± standard error) and viability of *Aleurodicus cocois* eggs laid on leaves of different cashew genotypes in a free-choice test.

Cashew clone	Number of eggs*	Viability (%)ns
CCP76	137.10 ± 12.06 a	70.05 ± 4.26 a
BRS274	88.90 ± 6.71 b	68.71 ± 2.98 a
EMBRAPA51	76.32 ± 9.97 b	81.51 ± 2.97 a
BRS226	45.80 ± 6.44 c	72.70 ± 2.06 a
PRO143/7	32.64 ± 3.70 c	65.76 ± 2.50 a

Values with different lowercase letters within columns are significantly different according to the Scott-Knott test ($p \leq 0.05$). *Significant difference; ns=Non-significant difference.

with successful hatching in the range 74 to 88% (Table III). As shown in Table IV, the nymphal phase lasted 26 to 27 days independent of clone with around 70% of the nymphs evolving to adult insects. In addition, no significant differences were detected among cashew genotypes regarding time required for the cashew whitefly to complete development from eggs to adults, the duration of which was typically 33 to 34 days (28 ± 2ºC). On the other hand, emergence rates of adult insects differed significantly ($p < 0.05$) between the clones with more than 70% of adult whiteflies emerging from eggs laid on EMBRAPA51 and BRS226 but only 53 to 56% emerging from clones PRO143/7, BRS274 and CCP76.

Table III. Number (mean ± standard error) and viability of *Aleurodicus cocois* eggs laid on leaves of different cashew genotypes in a free-choice test.

Cashew clone	Number of eggs*	Viability (%)^{ns}
BRS226	90.21 ± 9.52 ^{a}	88.08 ± 4.33 ^{a}
CCP76	88.40 ± 6.13 ^{a}	81.57 ± 2.50 ^{a}
EMBRAPA51	84.54 ± 7.37 ^{a}	81.51 ± 5.50 ^{a}
BRS274	78.47 ± 8.58 ^{a}	77.89 ± 3.76 ^{a}
PRO143/7	55.00 ± 5.37 ^{b}	74.16 ± 2.96 ^{a}

Values with different lowercase letters within columns are significantly different according to the Scott-Knott test ($p \leq 0.05$).
*Significant difference; ^{ns}=Non-significant difference.

Table IV. Mean value (± standard error) of the duration of the nymphal phase and egg-to-adult development, nymph viability and adult emergence rate of *Aleurodicus cocois* on leaves of different cashew genotypes in a no-choice (isolation) test.

Cashew clone	Nymphal phase (days)^{ns}	Nymph viability (%)^{ns}	Egg-to-adult (days)^{ns}	Adult emergence rate (%)*
EMBRAPA51	27.65 ± 6.87 ^{a}	70.19 ± 4.94 ^{a}	34.20 ± 1.97 ^{a}	75.16 ± 4.82 ^{a}
PRO143/7	27.12 ± 10.11 ^{a}	70.87 ± 8.20 ^{a}	34.09 ± 2.71 ^{a}	56.80 ± 1.94 ^{b}
BRS274	26.51 ± 8.92 ^{a}	69.19 ± 4.85 ^{a}	33.56 ± 3.09 ^{a}	53.01 ± 5.22 ^{b}
CCP76	26.44 ± 4.98 ^{a}	68.45 ± 5.60 ^{a}	33.52 ± 1.13 ^{a}	56.60 ±1.17 ^{b}
BRS226	26.00 ± 7.66 ^{a}	72.33 ± 3.87 ^{a}	33.10 ± 2.74 ^{a}	72.34 ± 2.77 ^{a}

Values with different lowercase letters within columns are significantly different according to the Scott-Knott test ($p \leq 0.05$).
*Significant difference; ^{ns}=Non-significant difference.

DISCUSSION

Previous studies with other Aleyrodidae species (silverleaf whitefly, *Bemisia tabaci* biotype B) have shown that the attraction of adult insects to plants varies according to genotype in cultures of *Solanum melongena* L. (eggplant; Hasanuzzaman et al. 2016), *Gossypium hirsutum* L. (cotton; Prado et al. 2015), *Glycine max* L. (soybean; Silva et al. 2012) and *Solanum lycopersicum* L. (tomato; Oriani et al. 2011). In addition, Cruz et al. (2012) observed that the attraction of *B. tabaci* biotype B adults to different genotypes of *Vigna unguiculata* (L.) Walp. (cowpea) varied significantly and, on this basis, these researchers were able to select genotypes that were less susceptible to the pest.

The attractiveness of adult insects towards a particular plant is determined by physical and chemical factors, or a combination of both, that normally alter at various stages over a period of time the olfactory and gustatory stimuli that generate a response by the insect. The intensities of such stimuli can vary, even in a single plant, and may be favorable or detrimental to the insect, a factor that can eventually determine the level of colonization (War et al. 2012, Mitchell et al. 2016).

As shown in Table I, individuals of *A. cocois* visited cashew plants to feed and lay eggs within 24 h following their release on the seedlings, but few insects were found on the plants after that period. This finding may be the result of the death, escape or dispersal of adults, after exploring them for the first 24 hours.

The lowest egg laying was observed in BRS226 and PRO143/7, and it is reasonable to assume that both clones expressed resistance to *A. cocois* through non-preference for oviposition. It is known that a range of factors, particularly those associated with the chemical and morphological defense systems of the potential host, can induce non-preference for oviposition and feeding (Sharma et al. 2009, Webster et al. 2010). The resistance of PRO143/7 to *A. cocois* was a stable character because the clone was not favored by insects even when they were forced to subsist on it. In an earlier study, Rodrigues et al. (2012) also employed free-choice and no-choice tests to identify a cowpea genotype that was less favored by *B. tabaci* biotype B for egg-laying. Therefore, growing a cashew genotype that is less preferred for oviposition may engender a significant reduction of the *A. cocois* population in the field.

Alterations in the insect life cycle, including prolongation of egg and nymphal development phases or reduction in adult emergence rates, indicate the possibility of antibiosis resistance (Sandoya et al. 2010). For example, Peixoto & Boiça-Filho (2014) described a common bean genotype that presented antibiosis resistance against *B. tabaci* biotype B through elongation of the nymphal phase, thereby delaying the development of eggs to adults. In addition, the morphological and chemical characteristics of a plant may influence the biology, physiology and behavior of the insect, especially with respect to host selection for feeding and oviposition (Vieira et al. 2016), leading to the non-preference of the resistant plant as a host, a phenomenon referred to as antixenosis resistance. In the present study, interactions between *A. cocois* and some of the plant genotypes were detrimental to the insect and resulted in reduced attractiveness (PRO143/7 and BRS274) and decreased survival (CCP76, PRO143/7 and BRS274) indicating the expression of antibiosis.

Chemical analyzes of hydroethanolic extracts of CCP76 cashew revealed phenolic molecules as the main compounds, especially the flavonoids quercetin glycosides (Konan & Bachi 2007). Higher amounts of total phenolics is related to lower fecundity and longevity of *B. tabaci* biotype B in eggplant (Hasanuzzaman et al. 2018). The role of flavonoids is already known in insect-plant interactions and in plant defense against herbivory (Treutter 2006). Quercetin was related to the larval mortality of *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) in peanuts (*Arachis hypogaea* L.), conferring plants with resistance of the antibiosis type (Mallikarjuna et al. 2004).

The clones CCP76, PRO143/7 and BRS274 showed antibiotic resistance and must be considered as candidates for further investigation within our genetic improvement program, whose main goal is to enhance the resistance of cashew to biotic stress. From those 3 clones, PRO143/7 was the least preferred for feeding and oviposition by *A. cocois*, and it yielded the lowest rates of adult emergence. However, clone PRO143/7 is not currently commercially available because its agronomical and industrial attributes still require improvement. The mechanisms of antibiotic and antixenotic resistance of the clones tested here to *A. cocois* require further investigation. Future studies should focus on the presence of physical barriers, such as leaves with thickened epidermis, waxy deposit sand high trichome density, and the accumulation of phytochemicals that repel or deter arthropods from feeding or ovipositing.

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Elaine S.S. Goiana, Nivia da S. Dias-Pini and Francisco das C. Vidal-Neto conceived and designed research. Elaine S.S. Goiana and Antônio Abelardo G. Filho conducted experiments. Elaine S.S. Goiana analyzed data. Elaine S.S. Goiana, Nivia da S. Dias-Pini, Cherre S.B. da Silva and Wenner Vinicius A. Saraiva wrote the manuscript. All authors read and approved the manuscript.