VINÍCIUS FERNANDES CANASSA

RESISTÊNCIA DE GENÓTIPOS DE COUVE-DE-FOLHA *Brassica oleracea* var. *acephala* (L.) A *Brevicoryne brassicae* (L.) (HEMIPTERA: APHIDIDAE)

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RESISTANCE OF COLLARD GREEN *Brassica oleracea* var. *acephala* (L.) GENOTYPES TO *Brevicoryne brassicae* (L.) (HEMIPTERA: APHIDIDAE)

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TÍTULO DA TESE: RESISTÊNCIA DE GENÓTIPOS DE COUVE-DE-FOLHA *Brassica oleracea* var. acephala L. A *Brevicoryne brassicae* (L.) (HEMIPTERA: APHIDIDAE)

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To my parents, Mario Canassa Júnior and Maria Ivone Nunes Fernandes, who allowed my access to education and made endless efforts to make me the professional I am, giving me support, strength and love to go forward.

To my sister, Nathália Fernandes Canassa, for love and encouragement.

I Dedicate

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RESUMO

A família Brassicaceae contém aproximadamente 3700 espécies, das quais apenas vinte são consumidas e cultivadas. A couve-de-folha [*Brassica oleracea* (L.) var. *acephala*] destaca-se entre as hortaliças como alimento importante para o consumo humano, possuindo níveis significativos de vitaminas A, C, K e ácido fólico, além de ser uma boa fonte de fibra. As brassicas também são conhecidas por terem elevado conteúdo de glucosinolatos, os quais são associados com a colonização de insetos-praga, além de reduzirem os riscos de ocorrecia de vários tipos de câncer em humanos. Um dos desafios para a cultura da couve-de-folha é a alta incidência de pragas em todas as fases da planta, sendo o pulgão *Brevicoryne brassicae* (L.) (Hemiptera: Aphididade) considerado espécie-chave para várias regiões. Esse pulgão é responsável por danos diretos e indiretos às plantas, prejudicando seu desenvolvimento e reduzindo seu valor comercial. A aplicação de inseticidas sintéticos é a principal tática de controle adotada pelos produtores para o manejo do afídeo, provocando impactos ambientais e colocando em risco a saúde do ser humano. O uso de resistência varietal no controle de pragas pode ser uma ferramenta valiosa para o Manejo Integrado de Pragas (MIP) e pode ser usado junto com outros métodos de controle. Neste estudo foi caracterizada a expressão de antibiose e/ou antixenose em 37 genótipos de couve-de-folha sobre *B. brassicae* por meio de bioensaios em casa de vegetação. Tambem foi avaliado o comportamento alimentar de *B. brassicae* em genótipos de couve-de-folha com expressão de antixenose e/ou antibiose por meio de Electral Penetration Graph – EPG. Adicionalmente, verificou-se teores de glucosinolatos, cera epicuticular e dureza das folhas, a fim de estabelecer possíveis correlações com o comportamento alimentar do afídeo. Os genótipos 20T e 24X foram os menos infestados no teste de preferência. 2B, 5E, 8H, 19S, 21U, 27VA, 30OP, PE, MGH e TP impediram que *B. brassicae* completasse o período ninfal, indicando a expressão de antibiose e/ou antixenose. Os genótipos 4D, GAU, 20T, 14N e MGI impediram que o pulgão atingisse a fase reprodutiva, também indicando resistência. Com base nos ensaios de EPG, os genótipos 22V, 5E e 27VA apresentaram elevado número de ondas Pd (queda de potencial), indicando que os fatores antioxenóticos são os principais responsáveis pela resistência verificada nesses materiais. 22V e 24X, por requererem mais tempo até o início de alimentação em floema, também

indicam a antixenose como categoria de resistência presente. Os genótipos 22V e PE apresentaram elevada índice de dureza foliar, o que justifica a ocorrência de antixenose. 20T e HS apresentaram maior quantidade de cera e cera/mg. Em 20T, é possível que os fatores antioxenóticos estejam relacionados a outros aspectos físicos (cor) ou químicos (voláteis), uma vez que os parâmetros de alimentação foram semelhantes aos do genótipo HS (suscetível). Os parâmetros de alimentação de ARI e 24X também foram semelhantes aos observados com o HS, de tal forma, a antibiose é provavelmente a categoria de resistência predominante nesses materiais. 24X apresentou maior quantidade de sinigrina; ARI e HS apresentaram maior quantidade de glucobrassicina; ARI apresentou maior quantidade de glucorapharina, enquanto HS apresentou maior quantidade de gluconapina. Como o genótipo HS foi considerado padrão suscetível neste estudo, é provável que o elevado teor de gluconapina não influencia o comportamento alimentar do afídeo. Os dados obtidos neste estudo podem ser úteis para programas de melhoramento com foco no desenvolvimento de brássicas resistentes a *B. brassicae*.

Palavra-chave: Resistência de plantas a insetos; pulgão-da-couve; antibiose; antixenose; EPG; glucosinolatos

ABSTRACT

Brassicaceae family contains approximately 3700 species, however, only about 20 are usually consumed and collard green [*Brassica oleracea* (L.) var*. acephala*] stand out among vegetables as an important food for human consumption having significant levels of vitamin A, C, K and folic acid, besides being a good source of fiber. Brassicas is also known for having a high content of glucosinolates, which are associated with colonization by pests, in addition to reduction risk of several cancer types for humans. One of the challenges for collard green crops is the high incidence of some pests in all plant stages, and the cabbage aphid, *Brevicoryne brassicae* (L.) (Hemiptera: Aphididade)*,* is considered a key pest in several regions. This aphid is responsible for direct and indirect damage to plants, impairing collard green development, which reduces its commercial value. Synthetic pesticide sprays are the main control tactic adopted by growers for managing cabbage aphid, triggering environmental impacts and endangering human's' health. The use of varietal resistance on pest control might be a rich tool for the Integrated Pest Management (IPM) and can be used along to other control methods. In this study the expression of antibiosis and/or antixenosis was characterized in 37 collard green genotypes on *B. brassicae* through greenhouse bioassays. In addition, the feeding behavior of the cabbage aphid was evaluated on collard green genotypes exhibiting antixenosis and/or antibiosis resistance through Electral Penetration Graph - EPG analysis. In addition, the levels of glucosinolates, epicuticular wax and leaf hardness were verified in order to establish possible correlations with the aphid feeding behavior. The 20T and 24X genotypes were the least infested 24 h after releasing. The 2B, 5E, 8H, 19S, 21U, 27VA, 30OP, PE, MGH and TP genotypes prevent *B. brassicae* from completing nymphal period, indicating antibiosis and/or antixenosis expression. The 4D, GAU, 20T, 14N and MGI genotypes prevented the cabbage aphid from reaching the reproductive phase, also indicating resistance. The 22V, 5E and 27VA presented a large number of Pd (Potential drop) waves, indicating that antioxenotic factors are the main responsible for the resistance verified in these materials. 22V and 24X genotypes, by requiring more time until the beginning of phloem feeding, also suggesting antixenosis as category resistance. The 22V and PE genotypes had high rates of hardness leaf, which justifies the occurrence of antixenosis. 20T and HS genotypes presented higher total wax and wax/mg. The

20T genotype possibly indicate the antioxenotic factors are related to other physical (color) or chemical (volatile) aspects, as the feeding parameters were similar to the susceptible HS. The ARI and 24X feed parameters were similar to those observed with the susceptible HS, such that antibiosis is likely to be the predominant resistance category in these materials. 24X genotype had higher sinigrin amount; ARI and HS genotypes had higher glucobrassicin amount; ARI genotype had higher glucorapharin amount; HS genotype had higher gluconapin amount. As HS genotype was considered as susceptible standard in this study, it is probable that the high gluconapin amount does not influence cabbage aphid feeding behavior. The data obtained in this study may be useful for breeding programs focusing on the development of brassicas resistant to *B. brassicae*.

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SUMÁRIO

GENERAL INTRODUCTION

Brassicaceae family contains approximately 3700 species, however, only about 20 are usually consumed, and many varieties contain significant levels of the vitamins A, C and K and folic acid to meet the recommended daily intake, besides being a good source of fiber (CAMPBELL et al., 2012). Cabbage [*Brassica oleracea* (L.) var. *capitata*], collard green [*B. oleracea* (L.) var. *acephala*], cauliflower [*B. oleracea* (L.) var. *botrytis*], broccoli [*B. oleracea* (L.) var. *italica* Plenck] and Chinese cabbage [*B. pekinensis* (L.)] arugula [*Eruca versicaria* (L.)], kale-radish [*B. olaracea* (L.) var. *gongylodes*] radish [*Raphanus sativus* (L.)], mustard [*B. juncea* (L.)] are the main botanical species of great social and nutritional value (FILGUEIRA, 2008).

Collard green plants [*B. oleracea* (L.) var*. acephala*] present erect stalk, which sustains the plant. Because it does not have a "head" formation, the leaves are distributed in a rosette form, around the stem. The propagation is done through the lateral seedlings, which are emitted in large numbers in the clones. It is a rustic plant compared with other Brassicaceae species, including nutritional requirements, being able to provide leaves for consumption all year round (FILGUEIRA, 2008).

Collard green stands out among vegetables as an important food for human consumption because it is rich in vitamins and minerals, and it is widely prepared as salad or braised (FRANCO, 1992). Brassicaceae is also known for having a high content of glucosinolates, which are associated with colonization by pests (COLE, 1997) as well as with possibility reduction of several cancer types including gastric, breast, lung, prostate, bladder, colorectal, and endometrial cancers (VERERHOEVEN et al., 1996; TANG et al., 2010; SIEGEL et al., 2016).

World statistics estimate that China is the world's largest producer of collard greens and other brassicas, followed by Russia, South Korea, Ukraine, Indonesia and Japan (FAOSTAT, 2017). In São Paulo State, Brazil, five vegetables occupied total area of 23.3 thousand hectares in 2015, standing out lettuce, cabbage, cauliflower, broccoli and collard green (1.3 thousand hectares) (CARVALHO et al., 2016), concentrated in Sorocaba, Mogi das Cruzes and Campinas regions (IEA, 2017).

It is important to study vegetables of the Brassicaceae family due to their influence on human nutrition as well as their high nutritional value and yield. However, one of the important limitations in the cultivation of vegetables has been the attack of

biotic agents, such as pests and pathogens (MASKELL et al., 1999; SCHLICK-SOUZA et al., 2011; DOMINGOS et al., 2018). Among the pests, we stand out the cabbage aphid, *Brevicoryne brassicae* (L.) (Hemiptera: Aphididade), which causes direct and indirect damage to the plants, compromising yield in the field.

Brevicoryne brassicae is native to Europe and is widely spread in temperate and tropical regions of the world (ELLIS, SINGH, 1993). Cabbage aphid alate female has 1.6 to 2.2 mm in lenght, dark head and thorax and yellowish-green abdome with narrow, irregular and blackish-colored bands. Wingless female has a dark head, greenish-gray thorax and abdome, with dorsal dark spots, body covered with white-gray wax, with 1.8 to 2.1 mm in lenght. It presents shorter siphunculi than the conical tail, containing 7-8 curved arrows (MARICONI et al., 1963; BLACKMAN; EASTOP, 1984).

The life cycle is variable, with sexual and asexual reproductive period, varying according to the host plant (LIU; SPARKS JR, 2001; WILLIAMS; DIXON, 2007). Females parthenogenic reproduction may be cyclic, producing wingless or alate individuals (BLACKMAN; EASTOP, 2007), with high prolificacy and the ability to reproduce about 50 to 100 nymphs/female (LIU; SPARKS JR, 2001).

In Brazilian climatic conditions, aphids are viviparous and reproduce by thelytoky parthenogenesis, rapidly developing numerous colonies, with 15 to 20 generations per year, depending on climatic conditions (PAL; SINGH, 2013). Thus, they reach high densities, which compromise the quality and crop yield when they feed (GIROUSSE et al., 2003; AHMAD; ASLAM, 2005). Under unfavorable conditions with low food quality and high aphid density, the appearance of alate individuals is stimulated and favors the migration/dispersion of the species (LIU; SPARKS JR, 2001).

Brevicoryne brassicae is the predominant species infesting collard green, cabbage and cauliflower grown for seed production around the world (KOTWAL et al.,1984; PAL; SINGH, 2013). Major economic hosts of this aphid include: cabbage, collard green, broccoli, Brussels sprouts and cauliflower and can sporadically attack carrot, celery, Chinese broccoli, Chinese cabbage, daikon, radish, rape and most other members of the genus Brassica (PAL; SINGH, 2013). The aphid feeds on both sides of the leaves and flowers of seed plants. Infested plants have retarded growth, and flowers fall down, not forming fruits. When cabbage aphid feeds on leaves and flowers, yellow spots are observed and the Brassicaceae leaves twist and dry up. Sticky excreta

(honeydew) also pollute the leaves and can compromise photosynthesis. When there are high insect numbers, yield may decrease by 34-80 % (PAL; SINGH, 2013).

Cabbage aphid also transmits harmful virus. It has been reported that is associated with more than 20 viral diseases within the family Brassicaceae (HILL, 1983) as *Turnip mosaic virus* (CHIVASA et al., 2002); *Beet yellow closterovirus* (BYV), *Beet mild yellow virus* (BMYV) (CIONI et al., 2001) and *Brevicorine brassicae virus* (BrBV) (RYABOV, 2007).

Upon host plant selection, cabbage aphid responds to physical and chemical stimuli. Plants shape, size, and density, as well as high light intensity (especially wavelengths of 550 to 590 nm) are significant cues. It also is important the contrast between light reflected from bare soil and plants. Summer migrants do not respond to host plant volatiles from large distances; however, they do react positively to host plant volatiles in close proximity, especially the volatile products of glucosinolate breakdown (NAULT; STYERS, 1972; GABRYS, 1999).

In collard green crops, *B. brassicae* control has been performed mainly by application of synthetic insecticides, often used in higher doses than those recommended. This has accelerated the selection of insect populations that are resistant to most active ingredients, besides increasing yield costs and the risks of human and environmental contamination (NAUEN; ELBERT, 2003). This imbalance has stimulated studies with alternative methods to chemical management, and at the same time it can be efficient in regulating the aphid population. In this way, resistant genotypes stand out as a valuable control strategy (PAINTER, 1951; LARA, 1991; BALDIN et al., 2019). Varietal resistance has proven efficient, in reducing pest populations below the economic injury level and, consequently, reducing yield costs. Besides these aspects, this practice is compatible with the other control methods.

Host plant resistance to insects is classified into three categories: antixenosis, antibiosis and tolerance (SMITH, 2005). The expression of resistance may involve behavioral or biological changes of the insect and, in other cases, it is a reaction of the plant itself, but does not affect the insect at all (LARA, 1991; BALDIN et al., 2019). Antixenosis occurs when plants affect the insect's behavior during host selection. In general, it is caused by allelochemicals or by the morphological characteristics of the plant (architecture, presence of trichomes, coloring of structures), making it less used for feeding, oviposition and/or shelter by insects (PANDA, 1979; LARA, 1991; SMITH; CLEMENT, 2012).

Antibiosis is determined by a set of plant characters that negatively affect insect's biology, trying to use the plant normally as food. When feeding on plants with antibiosis, the insects are affected regarding their reproductive potential and generally present high mortality of the immature phase, low emergence of adults, reduction in the insect's size and weight, as well as changes in longevity, mortality and fertility. The causes of antibiosis are mainly related to the presence of undesirable chemical compounds in plants (PANDA, 1979; LARA, 1991; SMITH; CLEMENT, 2012).

Tolerance occurs when one genotype is less damaged than the others under similar conditions of insect infestation without any deleterious effect on the behavior or biology of the insect. Tolerant genotypes generally present regeneration or compensation capacity in areas attacked by herbivorous insects PANDA, 1979; LARA, 1991; SMITH; CLEMENT, 2012).

Antixenosis and antibiosis can be commonly confounded in resistance trials, due to the difficulties involved in experimental design, which makes it difficult to interpret these two categories of resistance alone (SMITH, 2005). Thus, some studies have used chemical analyzes of plant tissues in order to quantify levels of specific compounds and, therefore help in the differentiation of antixenosis and antibiosis.

More than 120 glucosinolates have been identified in nature (KJAER; SKRYDSTRUP, 1987; FAHEY et al., 2001) and they are the main secondary metabolites from brassica plant specie and their concentrations reachs 10 mM (HAHN et al., 2016). Among them, the most common is sinigrin, which is directly involved in plant-insect interactions, particularly those involving phytophagous insects (COLE, 1997, THULER et al., 2007). Plant tissues brokendown by herbivores provide the degradation of glucosinolates in compounds with metabolic activities (HALKIER; GERSHENZON, 2006). Their effect can or can not be stimulating to insects (THULER et al., 2007; KATISANIS et al., 2016) and interfere and modify *B. brassicae* distribution (CIVIDANES; SANTOS, 2003).

Epicuticular waxes are the major components of a plant cuticle and play an important role in protecting aerial organs from damage caused by biotic and abiotic stresses (ZHANG et al., 2007; WÓJCICKA, 2014). Plant epicuticular waxes are complex mixtures of long chain aliphatic and cyclic components including fatty acids, hydrocarbons, alcohols, aldehydes, ketones, β-iketones and esters, as well as low levels of terpenoids, sterols, flavonoids, and phenolic substances. This layer may also contain sugars, amino acids and secondary plant substances such as glucosinolates,

furanocumarins and alkaloids (EIGENBRODE; ESPELIE, 1995; SCHOONHOVEN et al., 1998; STÄDLER; REIFENRATH, 2009; SUPAPVANICH et al., 2011; HALIŃSKI et al., 2012;).

The morphology, as well as the composition of epicuticular wax vary widely between species or genotypes and are also affected by the plant age and certain environmental factors, such as heat, humidity and irradiance levels. This film gives the cuticle its hydrophobic character that determines the extent of wettability of the plant surface. Thus, the epicuticular wax layer prevents formation of stable, macroscopic water phases and, hence, germination of the spores of many plant pathogens. The external layer of epicuticular wax may have other ecological functions including UV-B shielding, protection against pathogen invasion (bacteria and fungi) and insect behavior influence by functioning as allelochemicals (MÜLLER; RIEDERER, 2005; STÄDLER; REIFENRATH, 2009; YIN et al., 2011).

In addition to chemical analysis, other techniques have been used in host plant resistance studies, such as the electrical monitoring of sucking insect feeding through Electrical Penetration Graph (EPG). Thus, it is possible to specifically study the behavior of sucking insects in the process of acceptance or rejection of the host plant as a food source (Van HELDEN, TJALLINGII, 2000; DIAZ-MONTANO et al., 2007). EPG technique is based on the principle that the insect and the plant make up an electrical circuit (TJALLINGII, 1978, DIAZ-MONTANO et al., 2007) and when the insect inserts its stylet into the plant, the electrical circuit is closed, a current is generated and signals passes through an amplifier device signals connected to a computer, where the record is displayed in the form of waves, similar to an electrocardiogram (TJALLINGII, 1978)

Considering the importance of *B. brassicae* for collard green crop and the lack of studies that focused on the search for resistant genotypes using a broader germplasm of this crop, this work aimed to characterize resistance mechanisms of 37 commercial and non-commercial genotypes of collard green on cabbage aphid.

The specific objectives were: a) to characterize the possible expression of antibiosis and/or antixenosis in 37 collard green genotypes on *B. brassicae*; b) to evaluate *B. brassicae* feeding behavior in collard green genotypes with antixenosis and/or antibiosis using EPG and c) to quantify glucosinolates and epicuticlar wax contents in order to establish possible correlations with resistance categories.

The dissertation was divided in two chapters in order to reach the objectives above mentioned. The first was entitled "Assessing the resistance of collard green genotypes to cabbage aphid (Hemiptera: Aphididae)" written according to the Journal of Economic Entomology's guidelines and the second was entitled "Feeding behavior of *Brevicoryne brassicae* in resistant and susceptible collard green genotypes: interactions among morphological and chemical factors", written according to the Entomologia Experimentalis et Applicata's guidelines.

CHAPTER 1 - Assessing the Resistance of Collard Green Genotypes to the Cabbage Aphid (Hemiptera: Aphididae)

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Abstract

Severe *Brevicoryne brassicae* (Hemiptera: Aphididae) attacks on Brassica crops may lead to significant decrease in yield and, depending on the phenological stage, cause plant death. Host plant resistance is considered a valuable tool in integrated pest management and can assist in reducing the damage caused by aphids. This research evaluates the behavioral and biological parameters of *B. brassicae* in 37 *Brassica oleraceae* var. *acephala* genotypes, aiming to characterize mechanisms of antixenosis and/or antibiosis. In antixenosis bioassay, plants were arranged equidistantly in the circle inside a cylindrical arena, releasing to the center of the arena one hundred adult aphids. The number of insects in each different genotype was evaluated after 1, 2, 3, 6, and 24 h of infestation. For the antibiosis bioassay, 30 adults were confined inside each clip-cage of each genotype. One clip-cage per plant and one nymph per clip-cage was used, totaling 30 replicates per genotype. Total number of nymphs produced; number of nymphs produced; total production of nymphs until 10 d after the first nymph was produced; duration of the pre-reproductive phase; duration of the reproductive phase; nymphal survival; adult longevity; nymph-adult period and nymphal mortality at 5, 7 and 10 d were evaluated. The 20T and 24X genotypes were the least infested in the antixenosis 24 h free-choice test. The 2B, 5E, 8H, 19S, 21U, 27VA, 30OP, PE, MGH and TP genotypes did prevent *B. brassicae* from completing nymphal period, indicating antibiosis and/or antixenosis expression. The 4D, GAU, 20T, 14N and MGI genotypes prevented the cabbage aphid from reaching the reproductive phase. These genotypes may be useful for breeding programs that focus on the resistance of collard greens to insects.

The consumption of leafy vegetables in Brazil and worldwide has increased due to the adoption of healthier feed habits by the global population. However, the production and quality of these horticultures oscillate considerably due to the dependence on climatic conditions and disease and pest attacks (GMF 2018). Global data estimate that China is the world's largest producer of collard greens and other brassica species, followed by Russia, South Korea, Ukraine, Indonesia and Japan (Faostat 2017).

One of the challenges for collard green crops is the high incidence of some of these pests in all plant stages, and the cabbage aphid, *B. brassicae,* is considered a key pest in several regions (Reddy 2017, Amoabeng 2018). This aphid is responsible for direct and indirect damage to plants, impairing collard green development, which reduces its commercial value (Ma et al. 2010, Opfer and McGrath 2013). Honeydew produced from insects feeding favors the development of fungi on the leaves, decreasing the plant photosynthetic rate (Asi et al. 2009). Secondary damage is due to transmission of viruses (*Turnip mosaic virus*–TuMV) that are present in aphid's saliva (Maskell et al. 1999) and can compromise up to 80 % of brassica yield (Razaq et al. 2011).

Synthetic pesticide sprays appear to be the main control tactic adopted by growers for managing cabbage aphid, causing environmental impacts and endangering consumers' health (Ulusoy and Olmez-Bayhan 2006). To reduce the risks associated with chemical control, alternative forms of pest management have been proposed, such as the use of resistant genotypes (Anzabi et al. 2014). Host-plant resistance to insects can be divided into three categories: antibiosis, antixenosis, and tolerance (Painter 1951). Regarding antibiosis, the affected plant causes deleterious effects on insect's biology, without affecting its behavior. In antixenosis, the insect's behavior is affected by a chemical, physical and/or biological factor possessed by the plant. Tolerant plants do not cause any biological or behavioral changes in the insect, but they have the ability to withstand or recover from insect attacks while maintaining their yield production capacity (Smith 2005).

Although there are reports from studies evaluating the resistance of collard greens genotypes to attacks from other arthropods (Domingos et al. 2018, Schlick-Souza et al. 2011), there is no research that has investigated the broad germplasm of *B. oleraceae* var. *acephala* against *B. brassicae* aphids.Therefore, considering the damage potential that *B. brassicae* presents to collard green crops,

and the need to develop and improve sustainable control methods in the long term, the objective of this work was to assess 37 collard green genotypes for resistance aiming to characterize antixenosis and antibiosis expression to *B. brassicae*

Materials and Methods

This research was carried out in a greenhouse and in the Laboratory of Host Plant Resistance to Insects and Botanical Pesticides (LARESPI) of the Department of Crop Protection in the School of Agriculture – São Paulo State University - UNESP, in Botucatu, Brazil, between 2016 and 2018.

***Brassica oleraceae* var. *achephala* genotypes**. Thirty-seven collard green genotypes were assessed (Table 1) for this research. Thirty genotypes (commercial and/or resistance sources) with broad genetic variability, that were part the germplasm bank of the Agronomic Institute of Campinas (IAC), were provided by that institution. The commercial genotypes GAU, ARI and PE were supplied by the Feltrin Seeds Company (Farroupilha, Rio Grande do Sul, Brazil); genotypes HS and MGH were supplied by the Horticeres Seeds Company (Indaiatuba, São Paulo, Brazil), while TP and MGI were provided by the Isla Seeds Company (Porto Alegre, Rio Grande do Sul, Brazil). The plants were cultivated in pots containing soil, sand, manure and substrate, in a ratio of 1:1:1:1. For vegetative propagation, the shoots were removed from the plants and stored in polystyrene trays with 128 cells filled with the commercial substrate Tropstrato® (Vida Verde Indústria e Comércio de Insumos Orgânica Ltd., Mogi Mirim, São Paulo, Brazil) to produce the required number of plants of each genotype. The pots were kept in a greenhouse that was free of insect infestation. The plants received other necessary cultural treatments (irrigation, thinning, cleaning, etc.) as needed.

***Brevicoryne brassicae* rearing**. Cabbage aphid rearing was started with insect nymphs collected in collard green and broccoli plants from the municipal gardens in Botucatu (22° 55' 24.9" S and 48° 25' 30.4" W). The aphids were stored on Manteiga collard green genotype (variety not evaluated in the study) by Feltrin Seeds Company (Farroupilha, Rio Grande do Sul, Brazil), grown in pots inside metal cage (40 × 40 × 60 cm) coated with a 200 mesh screen in a greenhouse, under partially controlled conditions (Mean 24.3 ± 0.20 ºC, maximum 33.6 ±0.34 ºC e minimum 15.0 ± 0.32 ºC,

70 ± 0.64 % RH and natural light). Plants were sown in styrofoam trays (72 cells) and after 30 d of planting, they were transplanted into plastic pots (2L) containing soil that had been prepared in the same ratio as previously described. The plants were irrigated and replaced by healthy plants periodically and as needed (Fig. 1).

Antixenosis bioassay. A feeding preference test was performed using the 37 genotypes. When the seedlings had two expanded leaves, the plants, one of each genotype, were randomly arranged in a circle, inside plastic arenas (60 cm diameter × 25 cm), similar to that performed with *Aphis glycines* (Matsumura) (Hemiptera: Aphididae) in soybean (Díaz-Montano et al. 2006, Baldin et al. 2016). The plants inside each arena were spaced 5 cm apart, to avoid contact between them. One hundred adult aphid were collected by using a brush and stored in a plastic cup (200 ml) until their release. After collection, these aphids were released on the center of each arena and the number of aphid/genotypes was evaluated at 1, 2, 3, 6, and 24 h after the release of the insects. Each arena was considered a replication (total of fifteen), in a randomized block design (Fig. 2).

Antibiosis bioassay. The aphid biological performance on each of the 37 collard green genotypes was evaluated in a greenhouse using clip-cages for insect confinement (Baldin et al. 2016). Collard green plants of different genotypes were sown in plastic pots (2 L) containing 1:1:1:1 soil, sand, manure and substrate proportion, which were conditioned in a greenhouse under partially controlled conditions (Mean 24.3 ± 0.20 ºC, maximum 33.6 ± 0.34 ºC and minimum 15.0 ± 0.32 ºC, 70 ± 0.64 % RH and natural light). Only one plant per pot was maintained.

In the beginning of the bioassay, 30 adults were confined inside each clip-cage on the leaves of plants of each genotype. After 24 h, the females were removed and only one nymph within each clip-cage was left on each respective plant. One clip-cage per plant was used, which was coupled to the first true leaves, totaling 30 replicates per genotype (Fig. 3).

In this assay, each nymph comprised a replicate (thirty per genotype) in a completely randomized design. Evaluations were performed an a daily basis in the morning, until the adult's death, observing the following parameters: total number of nymphs produced; number of nymphs produced (adult/d); total production of nymphs until 10 d after the first nymph was produced; duration of the pre-reproductive phase;

duration of the reproductive phase; nymphal survival; adult longevity; nymph-adult period and nymphal mortality at 5, 7 and 10 d. After each evaluation, the nymphs produced that day were removed from the interior of the clip-cages with the aid of a brush.

Statistical analysis. Data collected from the antibiosis bioassay were submitted to ANOVA and F-tests. Normality was verified with the Shapiro-Wilk test and homogeneity was verified with the Levene test. When an F-test was significant, Tukey's test (p < 0.05) was used to compare the means.

Due to the collection of many zeros in the antixenosis bioassay evaluations, the analysis were modeled with zero-inflated generalized linear mixed models with the "glmmTMB" function (Bolker et al. 2009, Brooks et al. 2017). The arenas were considered random effects and the genotypes were considered fixed effects. The models were fit by Akaike Information Criterion, considering models including zero-inflated Poisson distributions, zero-inflated negative binomial distributions and excluding zero-inflation Poisson distributions. Significant values were calculated from type II Wald chi-square tests using the 'ANOVA' with pairwise comparisons from LSmeans with a Tukey adjustment (P≤0.05) (Winer et al. 1991). All analysis were performed using R 3.5.2 software (R Development Core Team 2018).

Results

Antixenosis bioassay. One h after the release of the aphids in the arenas, it was observed that the 20T, 18R and 15O genotypes had the lowest mean numbers of aphids per plant, differing from 4D, 5E and 8H (χ^2 = 128.99; df = 36, 504; P < 0.0001). In 2 h, 20T and 24X stood out as the least infested differing from 11K, 5E and 8H (χ^2 = 134.27; df = 36, 504; P < 0.0001). At 3 h post release 24X, 18R and 23W were significantly less infested (χ^2 = 126.40; df = 36, 504; P < 0.0001). At 6 h, 20T, 24X, 18R, 22V and 28TZ presented the lowest mean numbers of aphids on the plants (χ^2 = 128.88; df = 36, 504; P < 0.0001). Twenty-four h after aphid release, the 20T, 24X and 22V genotypes stood out with lowest mean numbers of aphids (χ^2 = 128.81; df = 36, 504; P < 0.0001). Considering the mean values for all evaluations, the 20T (0.24 aphids), 24X (0.26 aphids) and 18R (0.41 aphids) genotypes were the least infested by significant margin (χ^2 = 152.27; df = 36, 504; P < 0.0001), while genotypes 8H (3.92

aphids), 2B (3.77 aphids) and 5E (3.48 aphids) presented the highest mean numbers of aphids per plant (Table 2).

Antibiosis assay. Regarding the nymphal stages, MGH (6.66 d) prolonged the first instar, differing from the majority of the genotypes, and 6F, 5E, and 27VA genotypes presented intermediate duration means (F = 2.18; df = 36, 1073; P = 0.0001). For the second instar duration, 14N (5.12 d) and 32GUA (5.07 d) stood out with the highest means, differing from most genotypes (F = 2.63; df = 34, 1015; P < 0.0001). There was no significant difference among the genotypes in the third nymphal instar duration (F = 1.38; df = 32, 195; P = 0.0986). For the last nymphal instar, 34L1 (6.66 d) stood out with the longest period, significantly differing from most genotypes mainly from 3C (2.50 d), 1A (3.00 d), and 15O (3.00 d) (F = 1.83; df = 26, 783; P = 0.0155). The longest mean duration of the nymph to adult period was verified in 4D (30.00 d), which differed from all the genotypes (F = 3.03; df = 26, 783; P < 0.0001). The ARI (12.42 d), 29MN (12.45 d), 28TZ (12.75 d), and 1A (12.87 d) genotypes provided the shortest nymphal period. Genotypes PE, 27VA, 30OP, 5E, 21U, 19S, 8H, MGH, TP, and 2B caused mortality in all the aphids that fed on them throughout the immature phase and did not allow the emergence of adults (Table 3).

There were no significant differences among the genotypes regarding the number of nymphs/d (F = 1.43; df = 21, 76; P = 0,1305) and the number of nymphs produced until 10 d (F = 1.60; df = 21, 68; P = 0,0764). However, the 23W (1.66), 32GUA (2.00), 18R (2.00), 22V (3.00), 9I (3.00), 10J (4.00), and 3C (4.00) genotypes presented the lowest means for the total number of nymphs produced, differing from 28TZ (18.66 d) and 29MN (15.09 d) (F = 1,76; df = 21, 77; P = 0,0388) (Table 4).

In the pre-reproductive phase, the 24X genotype showed the longest duration (5.66 d), differing from 14N (2.50 d), 28TZ (2.25 d), 15O (2.20 d), HS (2.16 d), 16P (2.11 d), 9I (1.75 d), ARI (1.64 d), 1A (1.50 d), 18R (1.50 d) and MGI (1.00 d) (F = 3.03; df = 26, 783; P < 0.0001). The genotype 22V presented the shortest reproductive phase (1.00 d), followed by 18R (1.50 d), 32 GUA (1.50 d) and 23W (2.33 d) and differing from 33CPS (13.00 d), HS (12.33 d), 28TZ (11.00 d), 29MN (9.90 d), 15O (9.37 d) and ARI (8.50 d) (F = 2.13; df = 21, 638; P = 0.0089). Aphids confined in 9I (4.00 d), GAU (3.00 d), 14N (2.50 d), 2OT (2.50 d) and MGI (1.00 d) presented the shortest longevity means, differing from 33CPS (21.00 d) and HS (20.66 d) (F = 3.25; df = 26, 783; P < 0.0001). The shortest mean duration for the total cycle was observed

in MGI (15.00 d), 14N (16.25 d), 20T (18.50 d), GAU (18.50 d), 23W (19.16 d), 1A (19.25 d), 9I (19.25 d), 3C (20.00 d), 18R (20.50 d), ARI (21.21 d), 22V (21.33 d), 11K (21.37 d) and 16P (21.88 d), which significantly differed from those of 33CPS (37.00 d) and HS (35.16 d) ($F = 2.25$; df = 26, 783; $P = 0.0017$) (Table 5).

The highest mortality percentage accumulated at 5 d was observed in the 27VA genotype (90.00%), followed by PE, MGH, and 5E ($\cong$ 80.00%). After 7 d, the 27VA (96.66%), PE (93.33%) and 5E (93.33%) genotypes stood out causing mortality rates higher than 90%. After 10 d, only the PE genotype caused 100% nymphal mortality (Fig. 4). In thegenotypes 2B, 5E, 8H, 19S, 21U, 27VA, 30OP, PE, MGH, and TP, B. brassicae can not complete its nymphal phase (Fig. 5).

Discussion

Antixenosis bioassays: The aphid results presented high variability among genotypes during the evaluated periods of the preference assays. Our study is pioneering in its characterization of antixenosis expression in *B. brassicae* when assessing a broad collard green germplasm. In free-choice tests with other insects, it was observed that the 20T, 24X, 18R, 22V and 15O genotypes expressed resistance (low oviposition rates) to *B. tabaci* biotype B after 48 h of adult release (Domingos et al. 2018). In bioassays with *A. monuste orseis*, the 24X, 18R and 22V genotypes presented antixenosis against adults (Schlick-Souza et al. 2011), in addition to being less attractive to larvae in evaluations up to 12 h and exhibiting feeding and oviposition antixenosis (Nogueira et al. 2018). In the present study, the 20T and 24X genotypes also stood out as the least infested, suggesting multiple resistance expression by antixenosis to insects of the orders Hemiptera and Lepidoptera, which makes the two genotypes interesting subjects for study with other Brassica pests.

Antibiosis bioassays: Our results demonstrated that the total number of nymphs, pre-reproductive period, reproductive period, longevity, nymphal period and total cycle of *B. brassicae* were influenced by the collard green genotype. Plant characteristics, such as the chemical composition of several metabolites and morphological factors, can affect survival, reproduction and growth. Other researchers have also studied the impact of different host plants on biological parameters cabbage aphid (Mirmohamad et al. 2009, Aslam et al. 2011, Jahan et al. 2014). However, there is limited information

on the effect of the collard green genotypes on *B. brassicae*. According to previous papers on conventional nonresistant genotypes, *B. brassicae* females are able to produce 4-6 nymphs per day (Pal and Singh 2013). These results are different from those found in this study (0.38-3.00), probably because we assessed genotypes with different levels of resistance (antixenosis or antibiosis) and used clip cages to perform the bioassay.

Susceptible genotypes of *B. oleraceae* var. *acephala* (L.), *B. napus* (L.) and *Sinapis alba* (L.) usually support populations of *B. brassicae* during the entire vegetative period in which one female can produce approximately 20 nymphs in 10 d (Pal and Singh 2013). The number of nymphs produced in the first days of the adult phase is very important, because it represents the period of greatest reproductive potential of the insect, which is reduced in the subsequent days until the end of the adult phase (Awmack and Leather 2007; Maremela et al. 2013). In our study due to use of genotypes with different levels of resistance, we found a wide variability in the number of nymphs produced among genotypes.

Based on the pre-reproductive period of *B. brassicae*, it was found that not all genotypes that allowed individuals to complete the biological cycle were able to reproduce. In addition to 10 genotypes that did not support survival (19S, PE, 5E, 27VA, 30OP, MGH, 21U, 8H, TP and 2B), the 4D, GAU, 20T, 14N, and MGI genotypes prevented aphid reproduction. In other studies, carried out with four different host Brassica species to *B. brassicae,* the authors revealed a higher pre-reproductive period than those found in this study, with 7.0, 8.0 and 8.11 d for cabbage, collard greens and broccoli, respectively (Maremela et al. 2013, Aziz et al. 2016). These results indicate that aphids feeding on resistant collard green genotypes, for nutritional (Zarghami et al. 2010) and/or biochemical reasons (Cole 1997), despite anticipating reproductive maturity, have a shorter reproductive period and longevity, as well as a smaller number of offspring, which are evidence for the occurrence of antibiosis in the genotypes.

The reproductive period may vary depending on the host plant (Awmack and Leather 2007). This parameter showed a gradient with different levels of susceptibility and/or resistance among the evaluated collard green genotypes. In studies with different brassica crops there was no difference in the duration of the reproductive period among others, however, when comparing the plants within the

same species, the authors found a difference in the reproductive periods of brussels sprouts, cabbage (Ellis et al. 1996) and cauliflower (Jahan et al. 2014).

The treatments, part from presenting a great difference among *B. brassicae* longevity (1.0 to 21.0 d), also revealed that in the resistant genotypes that allowed for the completion of the nymphal phase, the resulting adult aphids presented reduced body sizes and reproduction capacity, in addition, the resistant genotypes shortened the life cycle of the insect. Reductions in body size and body weight are generally associated with resistance expression (Smith and Clement 2012). Other authors have shown that, different species and genotypes of brassica interfere in adult longevity (Maremela et al. 2013, Aziz et al. 2016).

Collard green genotypes significantly affected the nymphal parameters of *B. brassicae*, again indicating the occurrence of antibiosis and/or antixenosis. The genotypes that exhibited resistance through antibiosis and those with strong antixenotic factors may cause deleterious effects on insect biology, especially during the early stages of development (Painter 1951, Smith 2005). The increased nymphal development period of *B. brassicae* may be a probable reason for the reduction of aphid reproduction observed in the genotypes 4D and 32GUA.

The high mortality rates of the immature *B. brassicae* stages may be associated with temperature, thin nymphal wax layer (Smith 1999, Soh et al. 2018), difficulties with stylet insertion during feeding (Pal and Singh 2013), and secondary compounds that may interfere with insect biology (Cole 1997, Pal and Singh 2013, Hahn et al. 2016). Thus, in future research, the quantification of some chemical compounds as well as the feeding behavior on the most resistant collard greens genotypes should be considered.

Our study demonstrated that the use of resistant genotypes may be an effective control method for reducing the *B. brassicae* population and can be a valuable strategy for pest management in *B. oleraceae* var. *acephala.* Here, we evaluated a broad collard green germplasm, with high degrees of genetic variability, whose plants presented expressive differences regarding physical, chemical and morphological resistance factors. According to the collected data, several genotypes were shown to be less infested and more harmful to the biology of *B. brassicae* in collard green. The 20T and 24X genotypes expressed antixenosis, being less infested; in no-choice test the 2B, 5E, 8H, 19S, 21U, 27VA, 30OP, PE, MGH, and TP genotypes can be considered highly resistant or no host plant to cabbage aphid (100% nymphal

mortality); the 4D, GAU, 20T, 14N, and MGI genotypes prevented the cabbage aphid from reaching the reproductive phase with expression of high levels of antibiosis and/or antixenosis. Therefore, these genotypes can be used in pest management programs to control aphids and to reduce the consumption of synthetic pesticides on *B. oleraceae* var. *acephala* crops.

Acknowledgments

References Cited

Amoabeng, B. W., P. C. Stevenson, S. Pandey, M. B. Mochiah and M. G. Gurr. 2018. Insecticidal activity of a native Australian tobacco, *Nicotiana megalosiphon* Van Heurck & Muell. Arg. (Solanales: Solanaceae) against key insect pests of brassicas. Crop Prot. 106: 6-12.

Anzabi, S. H. M., A. Eivazi, M. R. Zargaran and A. Gasemi-Kahrizeh. 2014. Effect of seven canola genotypes on cabbage aphid *Brevicoryne brassicae* growth parameters. Rom. Agric. Res. 31: 75-80.

Asi, M. R., H. B. Muhammad, H. M. Jaffar, A. Muhammad and I. Saqib. 2009. In vitro efficacy of entomopathogenic fungi against cabbage aphid, *Brevicoryne brassicae* L. Pak. Entomol. 31: 43-47.

Aslam, M., M. Razaq, S. Hussain and A. K. Pathan. 2011. Biology of cabbage aphid under laboratory conditions. Pak. J. Zool. 43: 1009–1012.

Awmack, C. S. and S. R. Leather. 2007. Growth and development, pp. 135-151. In H. F. van Emden and R. Harrington (eds.), Aphids as crop pests. Cromwell Press, Trowbridge, UK.

Aziz, M. A., M. Irfan, I. Bodlah, and M. Hanif. 2016. Effect of different brassica vegetables on biology and demographic parameters of *Brevicoryne brassicae* (Homoptera: Aphididae) under laboratory conditions. Asian J. Agric. Biol. 4: 17-24.

Baldin, E. L. L., L. Marchi-Werle, L. E. R. Pannuti, A. L. Lourenção, T. M. Heng-Moss and T. E. Hunt. 2016. Evaluating categories of resistance in soybean genotypes from the United States and Brazil to *Aphis glycines* (Hemiptera: Aphididae). Fla. Entomol. 99: 487-495.

Bolker, B. M., M. E. Brooks, C. J. Clark, S.W. Geange, J. R. Poulsen, M. H. H. Stevens and J. S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Ecol. Evol. 24: 127-135.

Brooks, M. E., K. Kriatensen, K. J. van Benthem, A. Magnusson, C. W. Berg. A. Nielsen, H. J. Skaug, A. Mächler, B. M. Bolker. 2017. Modeling zero-inflated count data with glmmTMB. R J. 9: 378-400.

Cole, R. A. 1997. The relative importance of glucosinolates and amino acids to the development of two aphid pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated brassicae species. Entomol. Exp. Appl. 85: 121- 133.

Diaz-Montano, J., J. C. Reese, W. T. Schapaugh, and L. R. Campbell. 2006. Characterization of antibiosis and antixenosis to the soybean aphid (Hemiptera: Aphididae) in several soybean genotypes. J. Econ. Entomol. 99: 1884-1889.

Domingos, G. M., E. L. L. Baldin, V. F. Canassa, I. F. Silva and A. L. Lourenção. 2018. Resistance of collard green genotypes to *Bemisia tabaci* biotype B: characterization of antixenosis. Neotrop. Entomol. 47: 560-568.

Ellis, P. R., R. Singh, D. A. C. Pink, J. R. Lynn and P. L. Saw. 1996. Resistance to *Brevicoryne brassicae* in horticultural brassicas. Euphytica. 88: 85-96.

FAO – Food and Agriculture Organization of the Unites Nations – FAOSTAT - 2017. <http://www.fao.org/faostat/en/#data/QC>. Acessed 15 Jan. 2019.

GMF - George Mateljan Foundation. 2018. The World's Healthiest Foods. http://www.whfoods.org. Accessed Feb. 2019.

Hahn, C., A. Müller, N. Kuhnert and D. Albach. 2016. Diversity of kale (*Brassica oleracea* var. *sabellica*): glucosinolate content and phylogenetic relationships. J. Agric. Food Chem. 64: 3215−3225.

Jahan, F., H. Abbasipour, A. Askarianzadeh, G. Hassanshahi and A. Saeedizadeh. 2014. Biology and life table parameters of *Brevicoryne brassicae* (Hemiptera: Aphididae) on cauliflower cultivars. J. Insect Sci. 14: 284.

Ma, J., S. M. Tong, P. Wang, H. Liao and L. Zhang. 2010. Insecticides activity of camptothecin against *Nilaparvata lugens*, *Brevicoryne brassicae* and *Chilo supressalis*. J. Econ. Entomol. 103: 492-496.

Maremela, M., B. Tiroesele, M. Obopile and A. B. Tshegofatso. 2013. Effects of *Brassica* cultivar on population growth and life table parameters of the cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae). J. Entomol. Res. 37: 95-100.

Maskell, L. C., A. F. Raybould, J. I. Cooper, M. L. Edwards and A. J. Gray. 1999. Effects of turnip mosaic virus and turnip yellow mosaic virus on the survival, growth and reproduction of wild cabbage (*Brassica oleracea*). Ann. Appl. Biol. 135: 401-407.

Mirmohamadi, S. H., H. Allahyari, M. Nematolahi, A. Sabouri, S. Zarghami and S. H. Khaghni. 2009. Effect of four oilseed rape cultivars on biological parameters and intrinsic rate of increase of cabbage aphid*, Brevicoryne brassicae* L. (Hemiptera: Aphididae). J. Agr. Sci. Nat. Res. 13: 749-755.

Nogueira, L., A. C. D. S. Almeida, M. M. D. Freitas, Z. A. Ribeiro, A. L. Boiça Junior and F. G. D. Jesus. 2018. Attractiveness, non-preference for feeding and oviposition

to *Ascia monuste orseis* Godart (Lepidoptera: Pieridae) in collard greens. Arq. Inst. Biol. 85: 1-9.

Opfer, P. and D. McGrath. 2013. Oregon vegetables, cabbage aphid and green peach aphid. Oregon State University. Corvallis, United States.

Painter, R. H. 1951. Insect resistance in crop plants. The Macmillan Company. New York, United States.

Pal, M. and R. Singh. 2013. Biology and ecology of the cabbage aphid, *Brevicoryne brassicae* (Linn.) (Homoptera: aphididae): a review. J. Aphidology. 27: 59-78.

R Core Team, R. 2017. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Razaq, M., A. Mehmood, M. Aslam, M. Ismail, M. Afzal and S. Ali Shad. 2011. Losses in yield and yield components caused by aphids to late sown *Brassica napus* L., *Brassica juncea* L. and *Brassica carrinata* A. braun at Multan, Punjab. Pak. J. Bot. 43: 319-324.

Reddy, G. V. P. 2017. Integrated management of insect pests on canola and other *Brassica* oilseed crops. CABI, Wallingford, United Kingdom

Schlick-Souza, E. C., E. L. L. Baldin and A. L. Lourenção. 2011. Variation in the host preferences and responses of *Ascia monuste orseis* Godart (Lepidoptera: Pieridae) to cultivars of collard greens *Brassica oleracea* (L.) var. *acephala*. J. Pest. Sci. 84: 429-436.

Smith, C. M. 2005. Plant resistance to arthropods. Springer Science & Business, Dordrecht, Netherlands.

Smith, C. M. and S. L. Clement. 2012. Molecular bases of plant resistance to arthropods. Annu. Rev. Entomol. 57: 309-328.

Smith, R. G. 1999. Wax glands, wax production and the functional significance of wax use in three aphid species (Homoptera: Aphididae). J. Nat. Hist. 33: 513–530.

Soh, B. S. B., S. Kekeunou, S. Nanga Nanga, M. Dongmo and R. Hannah. 2018. Effect of temperature on the biological parameters of the cabbage aphid *Brevicoryne brassicae*. Ecol. Evol. 8: 11819-11832.

Trani, P. S., S. W. Tivelli, S. F. Blat, A. Prela-Pantano, E. P. Teixeira, H. S. Araújo, J. C. Feltran, F. C. Passos, G. J. B. Figueiredo and M. C. S. S. Novo. 2015. Collard green: from planting to post-harvest. Agronomic Institute, Campinas, Brazil.

Ulusoy, M. R. and S. Olmez-Bayhan. 2006. Effect of certain *Brassica* plants on biology of the cabbage aphid *Brevicoryne brassicae*, under laboratory conditions. Phytoparasitica. 34: 133-138.

Winer, B. J., D. R. Brown and K. M. Michels. 1991. Statistical principles in experimental design. MacGraw-Hill. New York, United States.

Zarghami, S., H. Allahyari, M. R. Bagheri and A. Saboori. 2010. Effect of nitrogen fertilization on life table parameters and population growth of *Brevicoryne brassicae*. Bull. Insectology. 63: 39- 43.
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Table 1. Code, names and characteristics of collard green genotypes evaluated for resistance to *Brevicoryne brassicae*.

Code	Genotype	Technical features[1]	Code	Genotype	Technical features[1]
1 A	Manteiga de Ribeirão Pires I 2620	Margin irregularly sinuous, denticulate	21 U	Orelha de Elefante	Denticulated margin
2 B	Manteiga I 1811	Margin irregularly sinuous, denticulate	22 V	Vale das Garças	Purple ribs
3 C	Roxa I 919	Purple ribs	23 W	Arthur Nogueira 1	Oval-oblong limbus
4 D	Manteiga de São Roque I 1812	Slightly denticulated margin, sinuated	24 X	Comum	Elliptic-oblong limbus
5 E	Gigante I 915	Green petiole, purple spots	27 VA	Variegata de Andradas	Whitish edges
6 F	Manteiga I 916	Short petiole	28 TZ	Tozan	Long petiole
8 H	Manteiga de Ribeirão Pires I 2446	Green petiole	29 MN	Campinas Mendonça	Leaves soft to the touch
9 I	Crespa de Capão Bonito	Prominent ribs	30 OP	Manteiga de Osvaldo Pires	Oval limbus
10 J	Manteiga de Tupi	Asymmetric atrial base	32 GUA	Guaranésia	Serrated edges
11 K	Couve Seção de Leguminosas	Green color margin	33 CPS	Campinas	Rounded edges
12 L	Manteiga de Jundiaí	Light green coloration	34 L1	Leguminosa 1	Light green coloration
13 M	Manteiga de Mococa	Bulb limbus	ARI	Arieli	Light green coloration, smooth edges
14 N	Manteiga São José	Asymmetrical apex	GAU	Gaudina	Dark green coloration, curly edges
15 O	Manteiga Monte Alegre	Purple ribs	PE	Manteiga Pé Alto	Round and large[2]
16 P	Verde Escura	Dark green coloration	HS	Manteiga HS-20	Medium green color, smooth edges
17 Q	Pires 1 Campinas	White-greenish ribs	MGH	Manteiga - Horticeres®	Dark green coloration[2]
18 R	Pires 2 Campinas	Orbicular limbus	TP	Tronchuda Portuguesa	Dark green, ribs protruding[2]
19 S	Japonesa	Bright green coloring	MGI	Manteiga - Isla®	Dark green[2]
20 T	Hortolândia	Orbicular limbus			

[1]Description according to Trani et al. 2015; [2]Companies's information

Table 2. Mean number (± SE) of adult *Brevicoryne brassicae* infested by 37 collard green genotypes obtained in different periods of observation.

Genotype	Number of insects[1]					
	1h	2h	3h	6h	24h	Mean
20T	0.20 ± 0.14 a	0.26 ± 0.12 a	0.40 ± 0.16 ab	0.13 ± 0.09 a	0.20 ± 0.11 a	0.24 ± 0.09 a
24X	0.40 ± 0.27 ab	0.26 ± 0.27 a	0.20 ± 0.20 a	0.20 ± 0.14 ab	0.26 ± 0.15 a	0.26 ± 0.15 a
18R	0.33 ± 0.16 a	0.53 ± 0.22 abc	0.26 ± 0.15 a	0.46 ± 0.29 ab	0.46 ± 0.22 abc	0.41 ± 0.16 ab
22V	0.53 ± 0.26 ab	0.60 ± 0.24 abc	0.60 ± 0.24 ab	0.46 ± 0.19 ab	0.26 ± 0.15 a	0.49 ± 0.19 abc
15O	0.33 ± 0.16 a	0.53 ± 0.24 abc	0.60 ± 0.27 ab	0.86 ± 0.35 abc	0.66 ± 0.29 abc	0.60 ± 0.22 abcd
19S	0.53 ± 0.29 ab	0.53 ± 0.27 abc	0.73 ± 0.30 ab	0.66 ± 0.29 abc	0.80 ± 0.34 abc	0.65 ± 0.27 abcd
27VA	0.60 ± 0.21 ab	0.73 ± 0.23 abc	0.80 ± 0.24 ab	0.60 ± 0.21 abc	0.66 ± 0.29 abc	0.68 ± 0.20 abcd
17Q	0.93 ± 0.27 ab	0.46 ± 0.19 ab	0.73 ± 0.25 ab	0.66 ± 0.21 abc	0.73 ± 0.21 abc	0.70 ± 0.20 abcd
21U	0.66 ± 0.30 ab	0.66 ± 0.32 abc	0.66 ± 0.29 ab	0.66 ± 0.29 abc	0.86 ± 0.34 abc	0.70 ± 0.28 abcd
32GUA	0.73 ± 0.33 ab	0.66 ± 0.23 abc	1.00 ± 0.32 ab	0.80 ± 0.37 abc	0.60 ± 0.21 abc	0.76 ± 0.24 abcd
28TZ	0.93 ± 0.33 ab	1.13 ± 0.36 abc	0.73 ± 0.25 ab	0.66 ± 0.21 ab	0.40 ± 0.21 ab	0.77 ± 0.23 abcd
16P	0.73 ± 0.40 ab	0.66 ± 0.23 abc	0.93 ± 0.32 ab	0.93 ± 0.30 abc	0.80 ± 0.31 abc	0.81 ± 0.29 abcd
23W	0.53 ± 0.25 ab	0.53 ± 0.26 abc	0.33 ± 0.19 a	1.33 ± 0.55 abc	1.40 ± 0.59 abc	0.82 ± 0.28 abcd
14N	0.60 ± 0.19 ab	1.13 ± 0.35 abc	1.06 ± 0.34 ab	1.06 ± 0.27 abc	0.60 ± 0.16 abc	0.89 ± 0.21 abcd
PE	1.13 ± 0.40 ab	0.80 ± 0.20 abc	1.06 ± 0.27 ab	0.86 ± 0.27 abc	1.00 ± 0.24 abc	0.97 ± 0.21 abcd
MGH	1.06 ± 0.43 ab	1.00 ± 0.40 abc	1.13 ± 0.38 ab	0.93 ± 0.34 abc	1.20 ± 0.34 abc	1.06 ± 0.33 abcd
29MN	1.46 ± 0.50 ab	1.13 ± 0.48 abc	1.20 ± 0.54 ab	3.06 ± 0.40 abc	1.00 ± 0.41 abc	1.18 ± 0.44 abcd
HS	1.00 ± 0.43 ab	1.33 ± 0.56 abc	1.20 ± 0.48 ab	1.60 ± 0.50 abc	1.46 ± 0.42 abc	1.32 ± 0.46 abcd
30OP	1.33 ± 0.46 ab	1.26 ± 0.41 abc	1.40 ± 0.40 ab	1.26 ± 0.36 abc	1.33 ± 0.39 abc	1.32 ± 0.31 abcd
GAU	1.20 ± 0.44 ab	1.13 ± 0.40 abc	1.33 ± 0.32 ab	1.53 ± 0.54 abc	1.53 ± 0.51 abc	1.34 ± 0.45 abcd
ARI	1.86 ± 0.51 ab	1.80 ± 0.64 abc	1.86 ± 0.64 ab	2.06 ± 0.68 abc	1.53 ± 0.57 abc	1.82 ± 0.57 abcd
TP	2.20 ± 0.58 ab	2.33 ± 0.52 abc	2.33 ± 0.48 ab	1.93 ± 0.38 abc	1.93 ± 0.33 abc	2.14 ± 0.42 abcd
34L1	2.00 ± 0.65 ab	2.20 ± 0.74 abc	2.66 ± 0.94 ab	1.73 ± 0.64 abc	2.40 ± 0.79 abc	2.20 ± 0.71 abcd
1A	2.80 ± 0.78 ab	1.86 ± 0.55 abc	2.06 ± 0.69 ab	2.53 ± 0.80 abc	1.93 ± 0.52 abc	2.24 ± 0.61 abcd
12L	2.26 ± 0.81 ab	2.13 ± 0.85 abc	2.26 ± 0.70 ab	2.66 ± 0.85 abc	2.00 ± 0.46 abc	2.26 ± 0.64 abcd
3C	2.13 ± 0.60 ab	2.13 ± 0.78 abc	2.46 ± 0.87 ab	2.40 ± 0.73 abc	2.46 ± 0.76 abc	2.32 ± 0.72 abcd
13M	1.93 ± 0.67 ab	2.20 ± 0.57 abc	2.60 ± 0.82 ab	2.00 ± 0.75 abc	2.93 ± 1.08 abc	2.33 ± 0.72 abcd
9I	3.00 ± 0.96 ab	2.60 ± 0.73 abc	2.20 ± 0.75 ab	2.26 ± 0.67 abc	1.73 ± 0.48 abc	2.36 ± 0.61 abcd
MGI	2.53 ± 1.60 ab	2.53 ± 1.50 abc	2.13 ± 1.51 ab	2.93 ± 1.89 abc	2.26 ± 1.43 abc	2.48 ± 1.58 abcd
11K	2.66 ± 1.02 ab	3.66 ± 1.10 bc	3.20 ± 0.78 ab	2.66 ± 0.90 abc	1.86 ± 0.52 abc	2.81 ± 0.78 abcd
33CPS	2.60 ± 1.49 ab	3.20 ± 1.61 abc	2.73 ± 1.06 ab	3.06 ± 1.03 abc	2.73 ± 0.96 abc	2.86 ± 1.20 bcd
6F	2.86 ± 0.62 ab	2.46 ± 0.73 abc	2.86 ± 0.91 ab	3.40 ± 0.88 bc	3.13 ± 0.91 bc	2.94 ± 0.75 bcd
4D	3.73 ± 1.22 b	3.40 ± 1.08 abc	3.26 ± 0.95 ab	2.33 ± 0.81 abc	2.80 ± 0.81 abc	3.10 ± 0.94 bcd
10J	3.13 ± 0.91 ab	3.20 ± 0.90 abc	3.73 ± 0.95 b	2.93 ± 0.71 c	3.40 ± 0.84 bc	3.28 ± 0.80 bcd
5E	3.46 ± 0.99 b	3.53 ± 1.07 bc	3.80 ± 1.26 b	3.73 ± 1.24 abc	2.86 ± 0.98 abc	3.48 ± 1.08 cd
2B	3.40 ± 1.01 ab	3.40 ± 0.92 abc	3.93 ± 1.30 b	4.20 ± 1.41 c	3.93 ± 1.33 c	3.77 ± 1.13 d
8H	3.86 ± 1.37 b	4.33 ± 1.69 c	3.73 ± 1.66 b	4.20 ± 1.86 c	3.46 ± 1.62 bc	3.92 ± 1.60 d
x^2	128.99	134.27	126.40	128.88	128.81	152.27
df	36, 504	36, 504	36, 504	36, 504	36, 504	36, 504
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[1]Means followed by the same lower-case letter per column do not differ by LSmeans test adjusted by Tukey ($p \geq 0.05$).

Table 3. Mean duration (± SE) of each nymphal instar and nymphal period of *Brevicoryne brassicae* in 37 collard green genotypes.

Genotype	Days				
	1st instar	2nd instar	3rd instar	4th instar	Nymphal period
4D	3.75 ± 0.30 b	4.85 ± 0.31 ab	5.00 ± 0.33	5.00 ± 0.00[2] abc	30.00 ± 0.00[2] a
34L1	4.31 ± 0.34 b	4.16 ± 0.28 ab	4.50 ± 0.38	6.66 ± 0.70 a	19.66 ± 0.82 b
32GUA	3.84 ± 0.14 b	5.07 ± 0.40 a	4.66 ± 0.30	4.00 ± 0.00 bc	18.00 ± 0.00 bc
6F	4.60 ± 0.20 ab	4.15 ± 0.28 ab	4.16 ± 0.42	5.16 ± 0.48 ab	18.00 ± 1.24 b
22V	4.11 ± 0.30 b	3.54 ± 0.19 b	4.42 ± 0.30	4.16 ± 0.18 bc	16.66 ± 0.27 bc
20T	4.11 ± 0.21 b	4.40 ± 0.36 ab	4.50 ± 0.65	4.00 ± 0.00 bc	16.00 ± 0.77 bcd
33CPS	3.92 ± 0.23 b	4.00 ± 0.18 ab	3.66 ± 0.11	5.00 ± 0.00[2] bc	16.00 ± 0.00[2] bcd
24X	3.66 ± 0.19 b	4.10 ± 0.27 ab	3.50 ± 0.24	4.66 ± 0.11 abc	16.00 ± 0.18 bcd
18R	4.40 ± 0.26 b	3.66 ± 0.15 b	6.66 ± 0.28	3.50 ± 0.13 bc	16.00 ± 0.52 bcd
10J	3.64 ± 0.22 b	3.71 ± 0.14 b	4.00 ± 0.26	4.00 ± 0.18 bc	15.66 ± 0.28 bcd
GAU	3.53 ± 0.18 b	4.00 ± 0.14 ab	6.00 ± 0.18	3.00 ± 0.26 bc	15.50 ± 0.65 bcd
9I	3.66 ± 0.28 b	3.37 ± 0.31 b	3.42 ± 0.18	5.00 ± 0.26 abc	15.25 ± 0.62 bcd
13M	3.95 ± 0.24 b	3.37 ± 0.19 b	3.40 ± 0.23	5.16 ± 0.29 ab	15.14 ± 0.44 bcd
12L	3.30 ± 0.27 b	3.00 ± 0.20 b	3.55 ± 0.21	4.20 ± 0.20 bc	14.60 ± 0.78 bcd
16P	3.29 ± 0.23 b	3.68 ± 0.22 b	3.72 ± 0.26	4.00 ± 0.24 bc	14.77 ± 0.46 bcd
HS	4.00 ± 0.25 b	3.62 ± 0.17 b	4.14 ± 0.27	3.16 ± 0.27 bc	14.50 ± 0.25 bcd
11K	3.50 ± 0.26 b	3.88 ± 0.20 b	4.30 ± 0.27	3.25 ± 0.19 bc	14. 50 ± 0.26 bcd
23W	3.25 ± 0.22 b	3.43 ± 0.21 b	3.09 ± 0.28	4.50 ± 0.34 bc	14.16 ± 0.51 bcd
17Q	3.03 ± 0.19 b	3.52 ± 0.17 b	4.23 ± 0.48	4.07 ± 0.32 bc	14.15 ± 0.73 bcd
MGI	4.29 ± 0.24 b	3.66 ± 0.21 b	3.50 ± 0.24	5.00 ± 0.00[2] abc	14.00 ± 0.00[2] bcd
14N	3.50 ± 0.27 b	5.12 ± 0.27 a	2.80 ± 0.15	3.50 ± 0.11 bc	13.75 ± 0.17 cd
15O	3.82 ± 0.22 b	3.50 ± 0.18 b	3.36 ± 0.19	3.00 ± 0.26 c	13.40 ± 0.37 cd
3C	4.21 ± 0.24 b	3.75 ± 0.23 b	3.00 ± 0.18	2.50 ± 0.13 c	13.00 ± 0.00 cd
1A	3.78 ± 0.22 b	2.76 ± 0.13 b	3.50 ± 0.28	3.00 ± 0.22 c	12. 87 ± 0.30 d
28TZ	4.14 ± 0.33 b	2.72 ± 0.17 b	3.87 ± 0.46	3.75 ± 0.17 bc	12.75 ± 0.79 d
29MN	3.24 ± 0.18 b	2.68 ± 0.23 b	2.93 ± 0.24	3.27 ± 0.20 bc	12.45 ± 0.61 d
ARI	3.50 ± 0.15 b	2.90 ± 0.18 b	3.15 ± 0.16	3.21 ± 0.22 bc	12.42 ± 0.21 d
2B	4.40 ± 0.27 b	4.60 ± 0.20 ab	4.66 ± 0.11	-	-
TP	4.14 ± 0.21 b	3.00 ± 0.24 b	4.50 ± 0.11	-	-
MGH	6.66 ± 0.53 a	4.00 ± 0.00[2] ab	4.00 ± 0.00	-	-
8H	4.17 ± 0.37 b	3.87 ± 0.46 b	3.25 ± 0.17	-	-
19S	3.25 ± 0.13 b	3.33 ± 0.11 b	3.00 ± 0.00	-	-
21U	3.76 ± 0.27 b	4.25 ± 0.23 ab	2.00 ± 0.00[2]	-	-
5E	4.50 ± 0.39 ab	5.00 ± 0.00[2] ab	-	-	-
30OP	4.12 ± 0.15 b	4.10 ± 0.00 ab	-	-	-
27VA	4.66 ± 0.38 ab	-	-	-	-
PE	2.80 ± 0.33 b	-	-	-	-
F	2.18	2.63	1.38	1.83	3.03
df	36, 1073	34, 1015	32, 195	26, 783	26, 783
P	0.0001	< 0.0001	0.0986	0.0155	< 0.0001

[1]Means followed by the same lower-case letter per column do not differ by Tukey test (p ≥ 0.05). [2]Only one insect was obtained.

Table 4. Mean number of (± SE) nymphs/day, total nymphs' production in 10 days and total nymphs produced by *Brevicoryne brassicae* in 22 collard green genotypes.

Genotype[2]	Nymphs/day[1]	10 days fecundity[1]	Total nymphs[1]
23W	0.77 ± 0.04	1.00 ± 0.00	1.66 ± 0.11 d
32GUA	1.25 ± 0.06	1.50 ± 0.13	2.00 ± 0.26 cd
18R	1.25 ± 0.06	2.00 ± 0.00[2]	2.00 ± 0.26 cd
22V	3.00 ± 0.52	4.00 ± 0.00[2]	3.00 ± 0.52 bcd
9I	0.42 ± 0.00[2]	2.00 ± 0.00[2]	3.00 ± 0.00[2] bcd
10J	1.50 ± 0.13	3.00 ± 0.00	4.00 ± 0.00 bcd
3C	1.37 ± 0.03	3.00 ± 0.26	4.00 ± 0.26 bcd
33CPS	0.38 ± 0.00[2]	3.00 ± 0.00[2]	5.00 ± 0.00[2] abcd
6F	1.84 ± 0.19	4.00 ± 0.32	5.25 ± 0.38 abcd
24X	2.00 ± 0.00[2]	5.00 ± 0.00[2]	6.00 ± 0.00[2] abcd
11K	1.19 ± 0.10	5.20 ± 0.55	6.20 ± 0.55 abcd
16P	1.18 ± 0.08	8.00 ± 0.88	6.71 ± 1.01 abcd
1A	1.41 ± 0.07	5.00 ± 0.67	7.33 ± 0.75 abcd
13M	1.03 ± 0.06	6.33 ± 0.61	7.83 ± 0.74 abcd
34L1	1.22 ± 0.06	7.00 ± 1.29	8.00 ± 1.29 abcd
ARI	1.28 ± 0.09	6.89 ± 0.74	8.40 ± 0.92 abcd
12L	1.10 ± 0.08	8.40 ± 0.68	9.40 ± 0.68 abcd
17Q	1.38 ± 0.11	9.60 ± 1.56	10.90 ± 1.72 abc
HS	1.10 ± 0.07	11.50 ± 0.89	13.33 ± 0.94 ab
15O	1.50 ± 0.15	12.13 ± 1.71	14.12 ± 1.92 ab
29MN	1.38 ± 0.09	11.64 ± 1.44	15.09 ± 2.08 a
28TZ	1.72 ± 0.05	17.67 ± 0.11	18.66 ± 0.11 a
F	1.43	1.60	1,76
df	21, 76	21, 68	21, 77
P	0.1305	0.0764	0.0388

[1]Means followed by the same lower-case letter per column do not differ by Tukey test (p ≥ 0.05). [2]Only one insect was obtained.

[2]2B, 4D, 5E, 8H, 14N, 19S, 20T, 21U, 27VA, 30OP, GAU, PE, MGH, TP, MGI genotypes were excluded due to the total aphid mortality or did not produce nymphs.

Table 5. Mean number (± SE) pre-reproductive and reproductive period, longevity and total cycle by *Brevicoryne brassicae* in 27 collard green genotypes.

Genotype	Pre-reproductive (days)	Reproductive (days)[1]	Longevity (days)[1]	Total cycle (days)[1]
33CPS	4.00 ± 0.00[2] ab	13.00 ± 0.00[2] a	21.00 ± 0.00[2] a	37.00 ± 0.00[2] a
HS	2.16 ± 0.21 b	12.33 ± 0.74 a	20.66 ± 1.42 a	35.16 ± 1.21 a
4D	4.00 ± 0.00[2] ab	-	4.00 ± 0.00[2] bc	30.00 ± 0.00[2] ab
13M	4.71 ± 0.29 ab	7.50 ± 0.51 abc	12.85 ± 0.97 abc	28.00 ± 1.23 ab
12L	2.80 ± 0.30 ab	8.40 ± 0.31 ab	12.80 ± 0.30 abc	27.40 ± 1.00 ab
29MN	2.72 ± 0.36 ab	9.90 ± 1.11 a	15.54 ± 1.67 ab	26.75 ± 1.76 ab
17Q	3.66 ± 0.41 ab	7.60 ± 0.61 abc	11.84 ± 1.22 abc	26.00 ± 1.35 ab
28TZ	2.25 ± 0.17 b	11.00 ± 0.37 a	13.25 ± 1.37 abc	26.00 ± 1.11 ab
34L1	3.50 ± 0.54 ab	6.00 ± 0.77 abc	5.50 ± 0.54 bc	25.16 ± 0.84 ab
6F	3.66 ± 0.22 ab	4.25 ± 0.72 abc	7.00 ± 0.77 bc	25.00 ± 1.51 ab
32GUA	3.50 ± 0.13 ab	1.50 ± 0.13 bc	6.50 ± 0.65 bc	24.50 ± 0.65 ab
15O	2.20 ± 0.50 b	9.37 ± 0.85 a	11.00 ± 1.27 abc	24.40 ± 1.27 ab
24X	5.66 ± 0.74 a	3.00 ± 0.00[2] abc	8.33 ± 0.53 abc	24.33 ± 0.69 ab
10J	2.66 ± 0.21 ab	3.00 ± 0.26 abc	6.66 ± 0.69 bc	22.33 ± 0.90 ab
16P	2.11 ± 0.32 b	5.85 ± 0.78 abc	7.11 ± 0.79 abc	21.88 ± 0.89 b
11K	3.33 ± 0.39 ab	5.40 ± 0.31 abc	7.00 ± 0.67 bc	21.37 ± 0.70 b
22V	4.16 ± 0.35 ab	1.00 ± 0.00 c	4.66 ± 0.34 bc	21.33 ± 0.36 b
ARI	1.64 ± 0.15 b	8.50 ± 1.25 a	8.78 ± 1.41 abc	21.21 ± 1.41 b
18R	1.50 ± 0.39 b	1.50 ± 0.13 bc	4.50 ± 0.65 bc	20.50 ± 0.13 b
3C	4.00 ± 0.26 ab	3.00 ± 0.26 abc	7.00 ± 0.00 abc	20.00 ± 0.00 b
9I	1.75 ± 0.17 b	7.00 ± 0.00[2] abc	4.00 ± 0.98 c	19.25 ± 1.12 b
1A	1.50 ± 0.22 b	5.50 ± 0.59 abc	6.37 ± 0.84 bc	19.25 ± 0.98 b
23W	3.66 ± 0.36 ab	2.33 ± 0.21 bc	5.00 ± 0.42 bc	19.16 ± 0.84 b
GAU	3.00 ± 0.26 ab	-	3.00 ± 0.26 c	18.50 ± 0.39 b
20T	2.50 ± 0.39 ab	-	2.50 ± 0.39 c	18.50 ± 0.39 b
14N	2.50 ± 0.32 b	-	2.50 ± 0.32 c	16.25 ± 0.40 b
MGI	1.00 ± 0.00[2] b	-	1.00 ± 0.00[2] c	15.00 ± 0.00[2] b
F	3.03	2.13	3.25	2.25
df	26, 783	21, 638	26, 783	26, 783
P	< 0.0001	0.0089	< 0.0001	0.0017

[1]Means followed by the same lower-case letter per column do not differ by Tukey test (p ≥ 0.05). [2]Only one insect was obtained.

Fig. 1. Plant used for *Brevicoryne brassicae* stock rearing in a metal cage in greenhouse.

Fig. 2. Overview arena used in antixenosis test (left) and aphid counts on collard green seedlings (right).
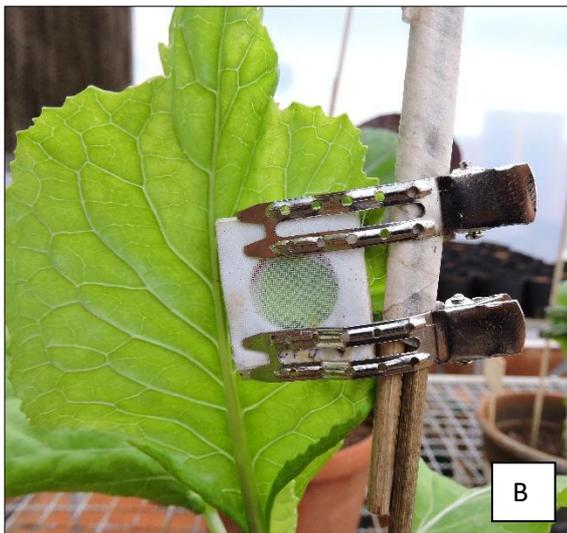
Fig. 3. A. Antibiosis bioassay overview; **B.** Leaf detail of collard green with clip cage; **C.** Opened clip cage with *Brevicoryne brassicae* adult and nymphs.
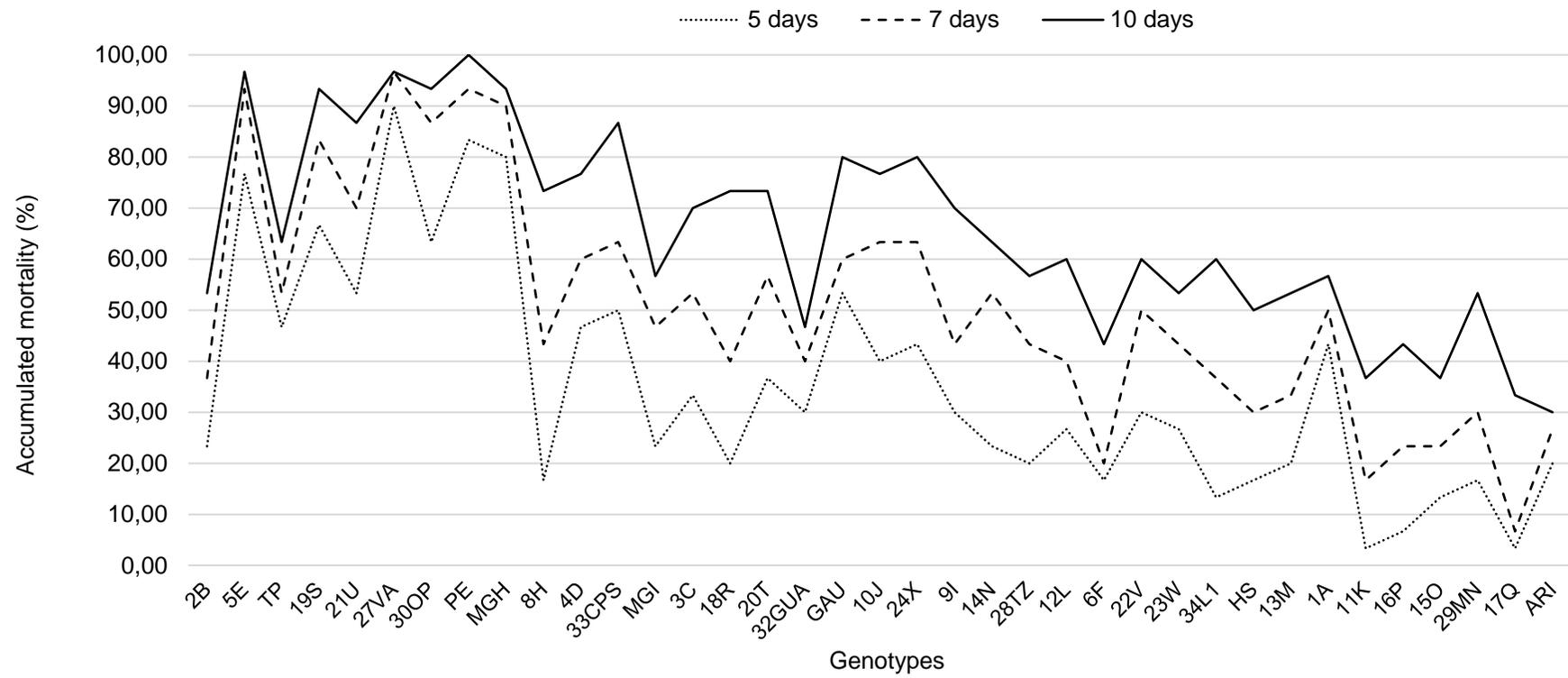
Fig. 4. Percentage (%) of accumulated mortality of *Brevicoryne brassicae* at 5, 7 and 10 days in 37 collard green.
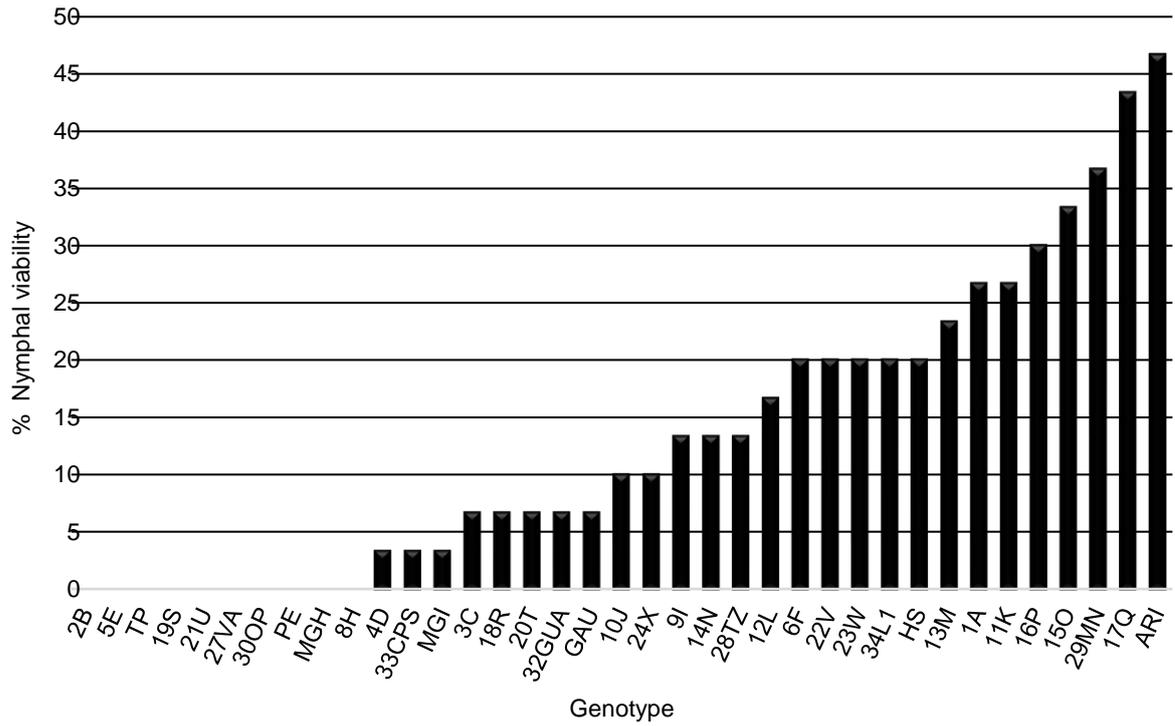
Fig. 5. Percentage (%) of nymphal viability of *Brevicoryne brassicae* in 37 collard green genotypes.

CHAPTER 2 – Feeding Behavior of *Brevicoryne brassicae* in Resistant and Susceptible Collard Green Genotypes: Interactions among Morphological and Chemical Factors

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Abstract

Brevicoryne brassicae (L.) (Hemiptera: Aphididae) is a species distributed throughout the tropical and subtropical areas of the world. The main crops attacked by the cabbage aphid are cabbage, collard green, broccoli, brussels sprouts and cauliflower. To survive the attack of pest insects, plants present different categories of resistance with factors that can affect pest feeding behavior. The use of electronic monitoring through EPG (*Electrical Penetration Graph*) can help characterize and distinguish the resistance mechanisms involved. This study evaluated the feeding behavior of *B. brassicae* in eight collard green genotypes exhibiting antixenosis and/or antibiosis resistance to this insect. The glucosinolates levels, hardness and epicuticular wax on the leaves were quantified to establish possible correlations with the aphid feeding behavior. In the EPG bioassay, the waves were recorded for 12 hours per aphid, evaluating 10 feeding parameters. The 22V, 5E, and 27VA genotypes, for which many potential drop (Pd) waves were performed, spent more time developing these characters, indicating antixenosis as the resistance category. Aphids in 22V and 24X genotypes require more time until the beginning of the phlem phase, also suggesting antixenosis as the category of resistance. The 22V and PE genotypes had high rates of leaf hardness, which can explain the occurrence of the same category of resistance. The 20T and HS genotypes presented higher total wax and wax/mg. The ARI and 24X feeding parameters also were similar to those observed with the HS; antibiosis is likely to be the predominant resistance category of these genotypes. Because the HS genotype was considered as a susceptible standard in this study, a higher gluconapin amount indicates that this compound does not influence cabbage aphid feeding behavior. The present study showed that analysis of the physical and chemical aspects of genotypes by the EPG technique can provide a useful approach for studies of plant resistance to insects.

Key words: *Brassicae oleraceae* var. *acephala,* cabbage aphid, host plant resistance, EPG, antixenosis, antibiosis.

Introduction

Brevicoryne brassicae (L.) (Hemiptera: Aphididae) is a cosmopolitan species widely distributed throughout tropical and subtropical regions of the world, being found in Europe, Asia, North America, South America, Africa, Australia and New Zealand (Carvalho et al., 2002; Carter & Sorensen, 2013; Pal & Singh, 2013; Reddy, 2017; Amoabeng, 2018). The main crops attacked by the cabbage aphid are cabbage, collard green, broccoli, Brussels sprouts and cauliflower, however this species can sporadically attack carrot, celery, Chinese broccoli, Chinese cabbage, daikon, radish, rape and most other members of the genus brassica (Pal & Singh, 2013). When cabbage aphids feed on leaves and flowers, the infested plants have reduced growth, and the flowers are dropped (Razaq et al., 2011). In high insect populations, plant yields may decrease by 34-80 % (Pal & Singh, 2013).

Considering the potential damage of *B. brassicae* to brassica crops, different control methods may be adopted to reduce the insect population density. Among these, the use of resistant genotypes stands out as a strategy for integrated pest management (IPM) (Painter, 1951; Smith, 2005; Baldin et al., 2019). In plant resistance studies, antixenosis (genotypes that negatively affect the behavior of the insect as it attempts to colonize the plant) and antibiosis (genotypes that negatively affect the biology of the insect that feeds on it) often overlap, due to the specificities of experimental designs, making it difficult to interpret these two categories of resistance independently (Smith, 2005; Baldin et al., 2019). Therefore, the identification of chemical resistance traits such as secondary compounds and/or morphological factors (wax content, hardness and the density of trichomes) may help in the differentiation between these two resistance categories (Smith, 2005).

More than 120 glucosinolates have been identified in nature (Kjaer & Skrydstrup, 1987; Fahey et al., 2001; Hahn et al., 2016). and they are the main secondary metabolites from brassica plant specie and their concentrations reachs 10 mM (Hahn et al., 2016). Among them, the most common is the sinigrin that is directly involved in plant-insect interactions, particularly those involving phytophagous insects (Cole, 1997; Thuler et al., 2007). Plant tissue breakdown that occurs with feeding by herbivores provides degraded glucosinolates in

compounds with metabolic activities (Bones & Rossiter 2006; Halkier & Gershenzon, 2006). Their effect can be stimulating to insects or not (Thuler et al., 2007; Katisanis et al., 2016) and can interfere and modify *B. brassicae* distribution (Cividanes & Santos, 2003).

Knowledge of the feeding behavior of these aphids is valuable to determine with precision the plant tissues explored by the insect stylet during feeding activities and to determine how long the stylet remains in each of these tissues, as well as the activities at each site (van Helden & Tjallingii, 2000). The Electrical Penetration Graph (EPG) technique is based on the principle that the insect and the plant make up an electrical circuit (Tjallingii, 1978; Diaz-Montano et al., 2007), and when the insect inserts its stylet into the plant, the electric circuit is closed, a current is generated, and this current passes through a signal amplifier device connected to a computer where the record is displayed in the form of waves similar to an electrocardiogram (Tjallingii, 1978, 1990). Thus, recognition of the different waveforms during insect feeding activity it is possible so that the stylet position to the respective plant tissue can be determined (van Helden & Tjallingii, 2000).

This method has been widely used to study the feeding behavior of aphids (Gabrys & Tjallingii, 2002; Baldin et al., 2018), the whiteflies (Cilovani et al., 2014), the stink bugs (Lucini & Panizzi, 2017, 2018), and the psyllids (George et al., 2017) in several crops. The analysis of morphological and chemical factors of the plants associated with the use of EPG can help in the interpretation of the feeding behavior presented by sucking insects in genotypes with different levels of resistance. Therefore, the objectives of this work were to evaluate the *B. brassicae* feeding behavior in collard green genotypes that exhibit antixenosis and/or antibiosis resistance and to determine the leaf hardness, glucosinolate and wax levels, to establish possible correlations with the resistance categories.

Materials and methods

Brassica oleraceae var. *achephala* genotypes

Based on studies carried out by Canassa et al. 2019, eight collard green genotypes were selected for evaluation; one is, susceptible (HS), and seven

showed resistance potencial (5E, 20T, 22V, 24X, 27VA, ARI and PE) (Table 1). The plants were cultivated in pots containing soil composed of sand, manure and substrate, in a ratio of 1: 1: 1. For vegetative propagation, the shoots were removed from the plants and stored in polystyrene tray with 128 cells filled with the commercial substrate Tropstrato® (Vida Verde Indústria e Comércio de Insumos Orgânica Ltda, Mogi Mirim-SP, Brazil), for production of the required number of plants of each genotype. In the rooting phase of the seedlings, special attention was gave to irrigation to ensure good root formation. The pots (0.5 L) were kept in a greenhouse free of insect infestation and were fertilized according to recommendation for the crop. The plants received the other necessary cultural treatments (irrigation, thinning, cleaning, etc.) as needed.

Brevicoryne brassicae rearing

Cabbage aphid rearing began with insects collected in collard green and broccoli plants from the municipal gardens in Botucatu, SP, Brazil (22° 55' 24.9" S and 48° 25' 30.4" W). The aphids were maintained on the Manteiga collard green genotype by Feltrin Seeds Company (Farroupilha, Rio Grande do Sul, Brazil) (variety not evaluated in the study), grown in pots inside a metal cage (40 × 40 × 60 cm) in a greenhouse, under partially controlled conditions (Mean 24.3 ± 0.20 ºC, maximum 33.6 ± 0.34 ºC e minimum 15.0 ± 0.32 ºC, 70 ± 0.64 % RH and natural light). Plants were sown in Styrofoam trays (72 cells) and 30 d after being planted, they were transplanted into plastic pots (2 L) containing soil in the same proportions as previously described. The plants were irrigated and replaced by healthy ones periodically and as needed.

EPG analysis

The stylet penetration assessment of *B. brassicae* with EPG was performed in the laboratory (T= 25 ± 2 ºC, RH = 65 ± 10 % and photoperiod = 14 h L:10 h D), using a Giga-8 system, under continuous electrical current (DC) (Tjallingii, 1978). Collard green plants infested with wingless adults (48 h) of *B. brassicae* from the rearing stock were taken to the EPG laboratory for bioassay. Under a stereoscopic microscope (40 X), the wax present on the aphid's body

was removed with the aid of a brush, to reduce the possible interference in collecting EPG wave data. Aphids starved for 1 h were connected to a gold wire electrode (≈ 18 μm diameter × 1-2 cm length), which was attached to the pronotum with water-based silver glue. Another copper wire (2 mm diameter × 10 cm long), which was used as the electrode for the plant, was inserted into the soil of the pot with the test plant (Figure 1).

Both electrodes were connected to a signal amplifier (Giga-8 DC EPG), with a resistance of 109 Ω and an adjustable voltage unit. To avoid interferences and better resolution in the records during the monitoring process, the insects, plants and probes remained inside a Faraday cage (Tjallingii, 2006). The records were performed at the same time on susceptible and resistant plants.

Data were analyzed using Stylet + software for Windows (EPG Systems, Wageningen, The Netherlands) (Tjallingii, 1978, 1988). EPG waveforms were identified based on previous work (Gabrys & Pawluk, 1999; Hao et al., 2017) and represented, non-probing (Np), potential drop (Pd), phloem salivation (E1), passive uptake or sap ingestion of the phloem sieved elements (E2) and active ingestion of xylem (G).

The records were analyzed from start to finish and included the last wave not interrupted naturally by the end of the 10 h recording. The behavioral data were compiled using an automated MS Excel file developed to work with EPG data (Sarria et al., 2009). Calculation of the observed wave parameters included the PPW (proportion of individuals that produced a specific waveform type), NWEI (number of waveform events per insect), WDI (waveform duration (min) per insect) and WDE [(waveform duration (min) per event)].

Leaf hardness

Analysis of leaf hardness was performed using a CT3 Texture Analyzer (Brookfield; Middleboro, Massachusetts, USA), calibrated for a penetration depth of 3 mm at a speed of 2.0 mm s^{-1}, with a TA 9/1000 point (Figure 2). Measurement results are expressed in grams-force per centimeter (gf/cm) and represent the maximum force required for the point to enter collard green leaf, simulating the process by which insects insert their mouthparts into a leaf. The evaluations were standardized using leaves from the central point of plants.

Wax

To characterize the adaxial and abaxial epicuticular wax layer of the collard green leaves, five leaf discs from the central point of the collard green plants of each treatment were used (Figure 3A). Three replicates were used, totaling 15 leaf discs in total. The collected samples were submerged separately in becker (200 mL) with 50 mL of chloroform, which had been previously weighed, for 20 seconds, and they were gently shaken. The solutions obtained (wax + chloroform) were taken for evaporation in an exhaust hood to obtain the solid residue (wax) (Figure 3B). After complete evaporation, the beckers were reweighed and the wax content was determined as a function of the mass difference between the two measurements (Furtado et al., 2009; Viana et al., 2010).

Glucosinolate analysis

These analyzes were conducted with the School of Pharmaceutical Sciences, Department of Physics and Chemistry, Ribeirão Preto, SP. For each collard green genotype, all leaves of the three different plants (60 d) of each treatment were collected. The harvested collard green leaves were frozen (excluding the thick midnerves) and ground in liquid nitrogen using gral and pistil. Then, 1.25 g of each sample (n = 3) was weighed, placed in an oven and treated at 120 °C for 2 h to inactivate the enzyme myrosinase, responsible for hydrolyzing glucosinolates (Song et al., 2005; Hahn et al., 2016). After inactivation, the dried plant material was stored in glass bottles, properly sealed and stored in a freezer at -20 °C.

For extraction, 2 mL of 70 % (v/v) methanol-water solution was added. The contents were homogenized by vortexing for 1 min, followed by immerson in an ultrasound bath for 10 min. Finally, 1.0 mL of the supernatant was withdrawn with a syringe and filtered using a 0.20 µm FTPE filter directly to a 1.5 mL glass vial.

The MS/MS and MRM analysis were performed on a UPLC-ESI-MS/MS (Triple Quadrupole) (ACQUITY, Waters®) using a Phenomenex Luna

Phenyl-Hexyl column (250 × 4.60 mm, 5 µm, 97 Å). The mobile phase consisted of water and acetonitrile, both of which were acidified with 0.1 % formic acid under the following gradient: 10-100 % over 13 min, followed by 100 % for 2 min, 100-10 % for 2 min and 10 % for 3 min. The flow rate was set at 1.0 mL/min, and the injection volume was 5 µL.

The mass spectrometer was operated under the following established conditions: voltage of the capillary, 2.5 kV; voltage of the cone, 20.0 kV; capillary temperature, 450 °C and *m/z* range of 50 to 600 in the negative ionization mode. The MS/MS and MRM analysis were performed for sinigrin, gluconapin, glucoraphanin and glucobrassicin using 20 kV of cone energy. The acquisition of exact mass was performed by direct injection ESI-MS-micrOTOF-QII under the following established conditions: the voltage of the capillary and the cone was 3.5 kV and 500 V, respectively; with the nebulizer at 0.4 Bar; nitrogen gas flow at 4.0 L/min; temperature at 180 °C and the mass detection range at 50 to 1300 m/z. To calculate the amount of each glucosinolate in the samples, the mean of the corresponding peak area was calculated (n = 3) for each glucosinolate (Hahn et al., 2016).

Statistical analysis

Normality of the data was verified by the Shapiro-Wilk test and the homogeneity through the Levene test. The data regarding the wax, hardness leaves, glucosinolates and EPG, which fulfilled the assumptions described previously, were submitted to analysis of variance with the F-Test. When the F-Test was significant, the Tukey test ($p < 0.05$) was used to compare the means. When the assumptions above were not met, the Kruskal-Wallis test ($p < 0.05$), which incorporate the Dunn test "Bonferroni" method, was used to compare the medians. The different EPG waveform, wax, hardness leaves and glucosinolates levels were submitted to Pearson correlation between each of the variables. All analysis were performed using software R 3.5.2 (R Development Core Team, 2018).

Results

EPG analysis

The number of waveform events per insect (NWEI) for all waveforms types (Pd, C, E1, E2, G and Np) did not show significant differences among the genotypes (Table 2). For Pd duration per insect (WDI), 22V genotype (51.96 min) presented the longest time spent in this event, differing from the ARI (23.73 min), PE (24.11 min), HS (24.13 min), 20T (25.03 min) and 24X genotypes (33.91 min) (χ^2 = 18.15; df = 7, 112; P = 0.0098). For the other waveform types (C, E1, E2, G and Np), no significant differences were observed among the genotypes (Table 2). Regarding the duration per E1 (WDE), the 22V (2.49 min) and 24X genotypes (2.74 min) presented the longest values, differing from the 27VA (1.75 min), PE (1.90 min) and ARI genotypes (2.01 min) (χ^2 = 14.22; df = 7, 112; P = 0.0472) (Table 2). For the other waveforms (Pd, C, E2, G and Np), no significant differences were observed among the genotypes (Table 2). No significant difference was observed in the time to the first probe, duration of the longest E2, total duration of the E1 that followed E2, and time to first E2 (Table 3).

Leaf hardness analysis

The 22V (0.0280 gf/cm) and PE genotypes (0.0270 gf/cm) presented the highest means for leaf hardness, differing from 20T (0.0150 gf/cm) and 27VA (0.0150gf/cm) (F = 3.87; df = 7, 72; P = 0.0012) (Table 4).

Wax analysis

The 20T (8.13 mg) and HS genotypes (7.83 mg) presented the highest wax amounts in five leaf disks, differing from 24X (5.80 mg), ARI (5.63 mg), 5E (4.90 mg), PE (4.86 mg), and 27VA genotypes (4.13 mg) (F = 15.62, df = 7, 16; P < 0.0001) (Table 4). The 20T (0.0048 mg) and HS genotypes (0.0051 mg) presented the highest wax amounts per milligram (mg), differing from all genotypes (0,0025 – 0,0033 mg), except for the 22V genotype (0,0042 mg) (F = 14.13; 7, 16; P < 0.0001) (Table 4).

Glucosinolates analysis

Among the eight collard green genotypes, four glucosinolates were found: sinigrin, glucobrassicin, glucoraphanin, and gluconapin (Figure 4). The highest sinigrin amount was observed in the 24X genotype (1.6 x 10^6) (F = 12.34; df = 7,

16; $P < 0.0001$). The ARI and HS genotypes presented the highest amount of glucobrassicin (2.9 x 10^5 and 1.3 x 10^5, respectively) ($\chi^2 = 22.01$; df = 7, 16; $P = 0,0025$). For glucoraphanin, the highest amount was observed in the ARI genotype (9.1 x 10^5) ($\chi^2 = 22.25$; df = 7, 16; $P = 0,0022$). For gluconapin, the highest amount was observed in the HS genotype (1.0 x 10^5) ($\chi^2 = 21.96$; df = 7, 16; $P = 0,0025$) (Figure 5).

Coefficients correlations

Based on the calculated coefficients (r), the correlations were not highly significant among the evaluated parameters (Table 5). However, negative correlations were found with respect to the number of Pd $\times$ wax ($r = -0.22$; $P = 0.0172$), number of C $\times$ sinigrin ($r = -0.21$; $P = 0.0212$) and hardness $\times$ wax ($r = -0.28$; $P = 0.0021$). Conversely, the number of G $\times$ hardness ($r = 0.18$; $P = 0.0470$), duration in E2 $\times$ gluconapin ($r = 0.23$; $P = 0.0100$), wax $\times$ sinigrin ($r = 0.34$; $P < 0.0001$), wax $\times$ glucobrassicin ($r = 0.45$; $P < 0.0001$) and wax $\times$ glucoraphanin ($r = 0.45$; $P < 0.0001$) were positively correlated.

Discussion

Understanding the resistance factors of collard green genotypes against the cabbage aphid is crucial in host plant resistance. Field and laboratory screening (Singh et al., 1994; Ellis et al., 1996) with behavioral or biological assays lack detailed information on how brassicas defend themselves against aphid attacks (Singh et al., 1994; Ellis et al., 1996; Maremela et al., 2013; Aziz et al., 2016). The techniques used in the mentioned studies were not enough to identify the resistance factors. The Electrical Penetration Graph (EPG) method can be used to obtain detailed information on this issue (van Helden et al., 2000; Le Roux et al., 2008), allowing studies on sucking insect behavior during the stylet penetration in plant tissues and, based on the different waveform pattern generated, to establish correlations with physical and/or chemical resistance related traits of the plants (Tjallingii, 1988; Hardie & Powell 2000; van Helden et al., 2000). In addition to helping to identify resistance factors, the EPG technique also allows to distinguish antixenosis and antibiosis (Baldin et al., 2018).

Xylem sap ingestion is an efficient method for restoring and maintaining the water balance of the insect and could have immediate value after a period of flight or after moulting, during which the insect may be very susceptible to water loss (Spiller et al., 1990; Pompon et al., 2010). The low number of aphids that feed this vascular vessel may be related to our use of only wingless adults in this study. Thus, these aphids already presented proper water balance, and they did not need to be hydrated. It is possible that those insects that used the xylem sap had to rehydrate due to the energy expenditure required to insert their mouthparts into the harder collard green leaves.

Brassicaceae plants contain specific secondary metabolites known as glucosinolates, which may stimulate host acceptance and feeding by the cabbage aphid (Nault & Styer, 1972; Gabrys et al., 1997). These glucosinolates occur predominantly in the vacuoles of mesophyll cells (Matile, 1984; Gabrys et al., 1997) and in the phloem (Weber et al., 1986; Gabrys et al., 1997). The data for the 22V genotype suggest that low concentrations of four glucosinolates (sinigrin, gluconapin, glucoraphanin, and glucobrassicin) are present, in addition to leaf hardness, which prevents penetration of the stylet below the level of the epidermis to access the parenchyma tissues and the phloem vessels (Gabrys & Pawluk, 1999). This condition explain a high number of potential attempts and, consequently a longer time spent in this activity by the aphid.

Many EPG studies on host plant resistance have indicated that phloem is involved in resistance expression (van Helden & Tjallingii, 1993; Klingler et al., 1998; Wilkinson & Douglas, 1998; Garzo et al., 2002; Tjallingii, 2006). Thus, when E1 salivation occurs in a resistant plant, we observe more frequent and/or longer durations of this event than on other plants (those that are susceptible), suggesting initial difficulties when beginning phloem sap ingestion (Tjallingii, 2006), as we observed in the 22V and 24X genotypes. The delay in finding and accepting the phloem can be explained as the presence of fagodeterrent factor in the phloem elements of these plants (Tjallingii, 1994), which may prevent aphids from establishing (Gadomski, 1992). Moreover, aphid saliva might be used for neutralizing the plant defense mechanisms located in the sieve elements (Miles, 1990), and therefore may be involved in detoxification of the plant phenolic compounds (Urbanska & Leszczynski, 1992).

Epicuticular wax is a complex mixture of different compounds (Khattab et al., 2007; Costa et al., 2014). These compounds contain a series of alkanes, alcohols (primary and secondary), aldehydes, acids, ketones, and esters, as well as secondary metabolites such as glucosinolates, which can negatively influence polyphagous insects (Costa et al., 2014). Beyond the action against pathogens, the wax presence protects against solar radiation, water losses by transpiration and the entry of chemical pesticides (Lichston & Godoy, 2006). Based on studies with different orders of polyphagous insects, such as *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), *Phyllotreta* spp. (Coleoptera: Chrysomelidae), *Eurydema ventrale* Kolenati (Hemiptera: Pentatomidae) (Znidarcic et al., 2008), and *Phaedon cochleariae* Fabricius (Coleoptera: Chrysomelidae) (Stok, 1980), the greater wax amount found in brassica leaves and plants, in addition to the lower colonization and damage caused by them, may be related to the wax contents, which represent a defense barrier against feeding of these insects. The wax layer in brassica plants has an impressive number of different compounds, among them glucosinolates (Khattab et al., 2007; Costa et al., 2014), and these also act as resistance factors to arthropods. However, some brassica specific insects (monophagous) such as *B. brassicae* and *Plutella xylostella* L. (Lepidoptera: Plutellidae), have a positive relationship with glucosinolate levels (Bodnaryk, 1997; Cole, 1997; Thuler et al., 2007).

In bioassays with adult diamondback moths, *P. xylostella*, glucosinolates (sinigrin) were present in high concentrations in the different treatments, together with waxy compounds (alkanes), which significantly increased the preference for oviposition of this pest (Spencer, 1996). In another bioassay, different brassica cultivars were correlated with the population growth rate of *B. brassicae* (monophagous aphid) and *Myzus persicae* Sultzer (Hemiptera: Aphididae) (polyphagous aphid), and the population growth of the first species increased with increasing amounts of glucosinolates in the cultivars, whereas for the second species, no change occurred as a consequence of these compounds (Cole, 1997), serving as stimulants to monophagous pests and deterrent to polyphagous pests.

The presence of an epicuticular wax layer in collard green leaves, negatively affected the numbers of Pd waves performed by the aphids. On the other hand, the glucosinolates (except for gluconapin) were at higher levels when

the wax was higher among the genotypes. Although gluconapin was not associated with the low number of Pd waves, this compound allows aphids to feed longer on the phloem cells. Regardless of their concentrations, amount and the site where found, (vacuoles of mesophyll cells and/or phloem), the different glucosinolates act as phagostimulants for the cabbage aphid (Matile, 1984; Weber et al., 1986; Gabrys et al., 1997).

Regarding the collard green leaf hardness, our results suggest that the wax presence does not increase the difficulty of the aphid in inserting the mouth paths in the collard green leaves, as observed in the 22V genotype. Conversely, leaves of the PE genotype exhibit a high hardness index; however, the wax amount found in this genotype was low. In this genotype, the high hardness index may be related to the collard green leaves thickness, a parameter that was not evaluated in this study.

Great variability occurred in the amount of the different glucosinolates among the eight collard green genotypes. Sinigrin was the compound that most stood out among the glucosinolates due to the high index found in the 24X genotype. This value was 10 times higher than the highest values of gluconapin, glucoraphanin, and glucobrassicin in all genotypes. This result opens an important line of questioning for future studies on insect specialists and their choice of host plants with varying levels of secondary compounds.

An overall analysis of the results revealed that the 22V, 5E and 27VA genotypes presented many Pd waves; therefore, the aphid spent more time involved in this activity, suggesting that low glucosinolate levels are associated with antixenotic resistance factors. In the 22V and 24X genotypes take more time for the aphids to access the phloem phase, also suggesting that the antixenosis category of resistance is involved in these genotypes against cabbage aphid. The 22V and PE genotypes also had greater leaf hardness, which indicates the occurrence of the same category of resistance. The 20T genotype was considered as having antixenosis and/or antibioses resistance in the multi-choice tests, so, the antixenotic factors are related to other physical (color) or chemical (volatile) aspects, as the feeding parameters were similar to those of the susceptible HS. The ARI and 24X feeding parameters were similar to those observed in the HS (susceptible standard), and antibiosis is likely to be the

predominant resistance category in these materials. The deleterious effects (antibiotics) of these genotypes probably would affect the feeding behavior and insect performance if monitoring was performed for a longer time. For the HS genotype, the high glucosinolate content (except sinigrin) does not seem to affect the insect feeding behavior, suggesting an association of these compounds with the susceptibility of the material. The present study showed that physical and chemical analysis of the genotypes and application of the EPG technique can be useful in studies of plant resistance to insects.

Acknowlegment

References

Amoabeng BW, Stevenson PC, Pandey S, Mochiah MB & Gurr MG (2018) Insecticidal activity of a native Australian tobacco, *Nicotiana megalosiphon* Van Heurck & Muell. Arg.(Solanales: Solanaceae) against key insect pests of brassicas. Crop Protection 106: 6-12.

Aziz MA, Irfan M, Bodlah I & Hanif M (2016) Effect of different brassica vegetables on biology and demographic parameters of *Brevicoryne brassicae* (Homoptera: Aphididae) under laboratory conditions. Asian Journal of Agriculture and Biology 4: 17-24.

Baldin ELL, Vendramin JD & Lourenção AL (2019) Resistência de plantas a insetos: Fundamentos e aplicações. Fealq, Piracicaba, SP.

Baldin ELL, Stamm MD, Bentivenha JP, Koch KG, Heng-Moss TM & Hunt TE (2018) Feeding behavior of *Aphis glycines* (Hemiptera: Aphididae) on soybeans

exhibiting antibiosis, antixenosis, and tolerance resistance. Florida Entomologist 101: 223-229.

Bodnaryk RP (1997) Will low glucosinolate cultivars of the mustards *Brassica juncea* and *Sinapis alba* be vulnerable to insect pests? Canadian Journal of Plant Science 77: 283-287.

Canassa VF, Baldin ELL, Sacilotto MG, Lourenção AL & Fanela TLM (2019) Assessing the resistance of collard green genotypes to the cabbage aphid (Hemiptera: Aphididae). Journal of Economic Entomology submitted

Carter CC & Sorensen KA (2013) Insect and related pests of vegetables. Cabbage and turnip aphid. Center for Integrated Pest Management. North Carolina State University, Raleigh, NC

Carvalho LM, Bueno VHP & Martinez RP (2002). Alate aphids survey on vegetable crops in Lavras-MG. Ciência e Agrotecnologia 26: 523- 532

Cividanes FJ & Santos DM (2003) Populational fluctuation and vertical (within-plant) distribution of *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) on kale. Bragantia 62: 61-67.

Civolani S, Cassanelli S, Chicca M, Rison JL, Bassi A, Alvarez JM, Annan IB, Parella G, Giorgini M & Fano EA (2014). An EPG study of the probing behavior of adult *Bemisia tabaci* biotype Q (Hemiptera: Aleyrodidae) following exposure to cyantraniliprole. Journal of Economic Entomology 107: 910-919.

Cole RA (1997) The relative importance of glucosinolates and amino acids to the development of two aphid pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated brassicae species. Entomologia Experimentalis et Applicata 85: 121- 133.

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Costa EMR, Marchese A, Maluf WR & Silva AA (2014) Resistance of kale genotypes to the green peach aphid and its relation to leaf wax. Revista Ciência Agronômica 45: 146-154.

Diaz-Montano J, Reese JC, Louis J, Campbell LR & Schapaugh WT (2007) Feeding behavior by the soybean aphid (Hemiptera: Aphididae) on resistant and susceptible soybean genotypes. Journal of Economic Entomology. 100: 984-989.

Domingos GM, Baldin ELL, Canassa VF, Silva I. F & Lourenção AL (2018). Resistance of collard green genotypes to *Bemisia tabaci* biotype B: characterization of antixenosis. Neotropical Entomology 47: 560-568.

Ellis PR, Singh R, Pink DAC, Lynn JR & Saw PL (1996). Resistance to *Brevicoryne brassicae* in horticultural brassicas. Euphytica. 88: 85-96.

Fahey JW, Zalcmann AT & Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry. 56: 5-51.

Furtado GQ, Alves SA, Godoy CV, Salatino ML & Massola Júnior NS (2009). Influence of light and leaf epicuticular wax layer on *Phakopsora pachyrhizi* infection in soybean. Tropical Plant Pathology 34: 306-312.

Gabrys B & Pawluk M (1999) Acceptability of different species of Brassicaceae as hosts for the cabbage aphid. Entomologia Experimentalis et Applicata 91: 105-109.

Gabrys B, Tjallingii WF & Van Beek TA (1997) Analysis of EPG recorded probing by cabbage aphid on host plant parts with different glucosinolate contents. Journal of Chemical Ecology 23: 1661-1673.

Gadomski H (1992) Oviposition of *Brevicoryne brassicae* (L.) (Homoptera: Aphididae) on cruciferous plants. Aphids and Other Homopterous Insects. 3: 33–38.

Garzo E, Soria C, Gomez-Guillamon ML & Fereres A (2002) Feeding behavior of *Aphis gossypii* on resistant accessions of different melon genotypes (*Cucumis melo*). Phytoparasitica 30: 129–140.

George J, Ammar ED, Hall DG & Lapointe SL (2017) Sclerenchymatous ring as a barrier to phloem feeding by Asian citrus psyllid: Evidence from electrical penetration graph and visualization of stylet pathways. PLoS One. 12: e0173520.

Hahn C, Müller A, Kuhnert N & Albach D (2016) Diversity of kale (*Brassica oleracea* var. *sabellica*): glucosinolate content and phylogenetic relationships. Journal of Agricultural Food Chemistry 64: 3215-3225.

Halkier BA & Gershenzon J (2006) Biology and biochemistry of glucosinolates. Annual Review of Plant Biology 57: 303-333.

Hao ZP, Hou SM, Hu BC, Huang F & Dang XL (2017) Assessment of probing behavior of the cabbage aphid, *Brevicoryne brassicae* (Hemiptera: Aphididae), on three *Brassica napus* cultivars at three developmental stages using Electro penetration graph (EPG). Journal of the Kansas Entomological Society 90: 11-24.

Hardie J & Powell G (2000) Close-up video combined with electronic monitoring of plant penetration and behavioral effects of an aphid (Homoptera: Aphididae) antifeedant. Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior (ed. by GP Walker & EA Backus) Thomas Say Publications in Entomology, Entomological Society of America, Lanham, USA, pp. 201-211

Khattab H (2007) The defense mechanism of cabbage plant against phloem-stucking aphid (*Brevicoryne brassicae* L.). Australian Journal of Basic and Applied Scieces 1: 56-62.

Katsanis A, Rasmann S & Mooney KA (2016) Herbivore diet breadth and host plant defense mediate the tri-trophic effects of plant toxins on multiple coccinellid predators. PloS one. 11: e0155716.

Kjaer A & Skrydstrup T (1987) Selenoglucosinolates: synthesis and enzymatic hydrolysis. Acta Chemica Scandinavica 41: 29-33.

Klingler J, Powell G, Thompson GA & Isaacs R (1998) Phloem specific aphid resistance in *Cucumis melo* line AR 5: effects on feeding behavior and performance of *Aphis gossypii*. Entomologia Experimentalis et Applicata 86: 79–88.

Le Roux V, Dugravot S, Campan E, Dubois F, Vincent C & Giordanengo P (2008) Wild solanum resistance to aphids: antixenosis or antibiosis? Journal of Economic Entomology 101: 584-591.

Lichston E & Godoy SAP (2006) Morphology and epicuticular wax content of coffee leaves after fungicide application. Pesquisa Agropecuária Brasileira 41: 919-926.

Lucini T & Panizzi AR (2017) Probing behavior of *Dichelops furcatus* (F.) (Heteroptera: Pentatomidae) on wheat plants characterized by electropenetrography (EPG) and histological studies. Journal of Insect Science 17: 1-15

Lucini T & Panizzi AR (2018) Electropenetrography (EPG): a breakthrough tool unveiling stink bug (Pentatomidae) feeding on plants. Neotropical Entomology 47: 6-18.

Maremela M, Tiroesele B, Obopile M & Tshegofatso AB (2013) Effects of *Brassica* cultivar on population growth and life table parameters of the cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae). Journal of Entomological Research 37: 95-100.

Matile P (1984) Das toxische kompartiment der pflanzenzelle. Naturwissenschaften. 71: 18-24.

Miles P (1990) Aphid salivary secretions and their involvement in plant toxicosis. Aphid-Plant Genotype Interactions (ed. by RK Campbell & RD Eikenbary). Elsevier Science Publishers, Amsterdam, pp. 131–147

Nault LR & Styer WE (1972) Effects of sinigrin on host selection by aphids. Entomologia Experimentalis et Applicata 15: 423-437.

Nogueira L, Paiva LA, Almeida ACS, Ribeiro ZA, Junior AB & Jesus FG (2015) Antibiosis in *Ascia monuste orseis* Godart (Lepidoptera: Pieridae) caused by kale genotypes. African Journal of Biotechnology 14: 2876-2882.

Painter RH (1951) Insect resistance in crop plants. The Macmillan Company. New York, United States.

Pal M. & Singh R (2013) Biology and ecology of the cabbage aphid, *Brevicoryne brassicae* (linn.) (Homoptera: aphididae): a review. Journal of Aphidology. 27: 59-78.

Pompon J, Quiring D, Giordanengo P, Pelletier Y (2010) Role of xylem consumption on osmoregulation in *Macrosiphum euphorbiae* (Thomas). Journal of Insect Physiology 56: 610-615.

R Core Team (2018) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Razaq M, Mehmood A, Aslam M, Ismail M, Afzal M & Ali Shad S (2011) Losses in yield and yield components caused by aphids to late sown *Brassica napus* L., *Brassica juncea* L. and *Brassica carrinata* A. braun at multan. Pakistan Journal of Botany 43: 319-324.

Reddy GVP (2017) Integrated management of insect pests on canola and other brassica oilseed crops. CABI, Wallingford, UK

Sarria E, Cid M, Garzo E & Fereres A (2009) Excel workbook for automatic parameter calculation of EPG data. Compututers and Electronics in Agriculture 67: 35-42.

Schlick-Souza EC, Baldin ELL & Lourenção AL (2011) Variation in the host preferences and responses of *Ascia monuste orseis* Godart (Lepidoptera: Pieridae) to cultivars of collard greens *Brassica oleracea* (L.) var. *acephala*. Journal of Pest Science. 84: 429-436.

Singh R, Ellis PR, Pink DAC & Phelps K (1994) An investigation of the resistance to cabbage aphid in brassica species. Annals of Applied Biology. 125: 457-465.

Smith CM (2005) Plant resistance to arthropods. Springer Science & Business, Dordrecht, Netherlands.

Song L, Morrison JJ, Botting NP & Thornalley PJ (2005) Analysis of glucosinolates, isothiocyanates, and amine degradation products in vegetable extracts and blood plasma by LC-MS/MS. Analytical Biochemistry 347: 234-243.

Spencer JL (1996) Waxes enhance *Plutella xylostella* oviposition in response to sinigrin and cabbage homogenates. Entomologia Experimentalis et Applicata 81: 165-173.

Stok NE (1980) Role of wax blooms in preventing attachment to brassicas by the mustard beetle, *Phaedon cochleariae*. Entomologia Experimentalis et Applicata. 28: 100–107.

Thuler RT, Bortoli SA & Hoffmann-Campo CB (2007) Brassicas cultivars classification in relation to resistance to diamondback moth and to the presence of glucosinolates. Pesquisa Agropecuária Brasileira 42: 467-474.

Tjallingii WF (1978). Electronic recording of penetration behavior by aphids. Entomologia Experimentalis et Applicata 24: 521-530.

Tjallingii WF (1988) Electrical recording of stylet penetration activities. Aphids: their biology, natural enemies and control (ed. by AK Minks & P Harrewijn) Elsevier, Amsterdam, Netherlands, pp. 95-108

Tjallingii WF (1994). Sieve element acceptance by aphids. European Journal of Entomology 91: 47–52.

Tjallingii WF (2006) Salivary secretions by aphids interacting with proteins of phloem wound responses. Journal of Experimental Botay 57: 739-745.

Trani PS, Tivelli SW, Blat SF, Prela-Pantano A, Teixeira EP, Araújo HS, Feltran JC, Passos FC, Figueiredo GJB & Novo MCSS (2015). Collard green: from planting to post-harvest. Agronomic Institute, Campinas, Brazil.

Urbanska A & Leszczynski B (1992) Biochemical adaptations of cereal aphids to host plants. Proc. 8th Int. Symposium Insect-Plant Relationships (ed. by SBJ Menken, JH Visser & P Harrewijn) Kluwer Academic Publishers, Dordrecht, pp. 277-279.

van Helden M & Tjallingii WF (1993) Tissue localization of lettuce resistance to the aphid *N. ribisnigri* using electrical penetration graphs. Entomologia Experimentalis et Applicata 68: 269-278.

van Helden M, TJallingii WF, Walker GP & Backus EA (2000) Experimental design and analysis in EPG experiments with emphasis on plant resistance research. Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior (ed. by GP Walker & EA Backus). Entomological Society of America, Lanham, pp. 144-171

Viana RG, Tuffi Santos LD, Demuner AJ, Ferreira FA, Ferreira LR, Ferreira EA & Santos MV (2010) Quantification and chemical composition of epicuticular wax of eucalyptus leaves. Planta Daninha. 28: 753-758.

Weber G, Oswald S & Zollner U (1986) Suitability of rape cultivars with a different glucosinolate content for *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulzer) (Hemiptera, Aphididae). Journal of Plant Diseases and Protetection 93: 113-124.

Wilkinson TL & Douglas AE (1998) Plant penetration by pea aphids (*Acyrthosiphon pisum*) of different plant range. Entomologia Experimentalis et Applicata 87: 43–50.

Žnidarčič D, Valič N & Trdan S (2008) Epicuticular wax content in the leaves of cabbage (*Brassica oleracea* L. var. *capitata*) as a mechanical barrier against three insect pests. Acta Agriculturae Slovenica 91: 361-37.

Table 1 Code, names, characteristics, origin and resistance history of collard green genotypes evaluated for resistance to *B. brassicae*.

Genotype	Code	Technical features[1]	Origin	Resistance history
Gigante I 915	5 E	Green petiole, purple spots	IAC[3]	Antibiosis to *Ascia monuste* (Nogueira et al., 2015); Antixenosis and/or antibiosis to *B. brassicae* (Canassa et al., 2019 submitted)
Hortolândia	20 T	Orbicular limbus	IAC[3]	Antixenosis and/or antibiosis to *B. brassicae* (Canassa et al., 2019 submitted)
Vale das Garças	22 V	Purple ribs	IAC[3]	Antixenosis and/or antibiosis to *B. brassicae* (Canassa et al., 2019 submitted)
Comum	24 X	Elliptic-oblong limbus	IAC[3]	Antixenosis to *A. monuste* (Schlick-Souza et al., 2011); Antixenosis to *B. brassicae* (Canassa et al., 2019 submitted)
Variegata de Andradas	27 VA	Whitish edges	IAC[3]	Antixenosis to *B. tabaci* biotype B (Domingos et al., 2018); Antixenosis and/or antibiosis to *B. brassicae* (Canassa et al., 2019 submitted)
Arieli	ARI	Light green coloration, smooth edges[2]	Feltrin Seeds Company®	Antixenosis and/or antibiosis to *B. brassicae* (Canassa et al., 2019 submitted)
Manteiga Pé Alto	PE	Round and large[2]	Feltrin Seeds Company®	Antixenosis and/or antibiosis to *B. brassicae* (Canassa et al., 2019 submitted)
Manteiga HS-20	HS	Medium green color, smooth edges[2]	Horticeres Seeds Company®	Antixenosis to *B. tabaci* biotype B (Domingos et al., 2018); Susceptible to *B. brassicae* (Canassa et al., 2019 submitted)

[1]Description according to Trani et al., 2015; [2]Company's information; [3]IAC – Agronomic Institute, Campinas, SP

Table 2 Mean (± SE) EPG non-sequential variables used to study the feeding behavior of *B. brassicae* on eight different collard green genotypes.

Waveform	Genotype	PPW	NWEI[1]	WDI[1]	WDE[1]
Pd	5E	15/15	267.80 ± 25.34	33.93 ± 3.94 cd	0.129 ± 0.009
	20T	15/15	211.40 ± 19.73	25.03 ± 2.61 abc	0.119 ± 0.004
	22V	15/15	288.33 ± 24.76	51.96 ± 13.52 d	0.223 ± 0.092
	24X	15/15	194.46 ± 20.27	33.91 ± 10.27 a	0.169 ± 0.045
	27VA	15/15	244.80 ± 24.65	39.87 ± 8.43 bcd	0.152 ± 0.016
	ARI	15/15	210.93 ± 21.27	23.73 ± 2.08 a	0.118 ± 0.005
	HS	15/15	220.80 ± 25.60	24.13 ± 2.51 ab	0.113 ± 0.004
	PE	15/15	205.86 ± 29.05	24.11 ± 3.85 abc	0.118 ± 0.007
P			0.1030	0.0098	0.0574
C	5E	15/15	34.74 ± 4.80	326.27 ± 23.15	11.79 ± 1.40
	20T	15/15	32.53 ± 3.46	284.75 ± 18.21	10.05 ± 1.10
	22V	15/15	26.00 ± 2.96	338.13 ± 19.32	15.79 ± 2.07
	24X	15/15	18.40 ± 2.00	301.89 ± 24.99	19.30 ± 3.17
	27VA	15/15	28.46 ± 6.03	304.03 ± 23.08	21.16 ± 5.69
	ARI	15/15	28.93 ± 4.10	304.87 ± 21.78	13.15 ± 1.43
	HS	15/15	26.93 ± 3.17	277.74 ± 25.55	12.21 ± 1.64
	PE	15/15	28.33 ± 5.39	267.96 ± 26.87	21.17 ± 7.81
P			0.1806	0.3568	0.0792
E1	5E	14/15	4.80 ± 0.83	15.73 ± 6.27	9.46 ± 7.06 ab
	20T	14/15	9.86 ± 1.86	21.92 ± 5.23	2.42 ± 0.55 ab
	22V	14/15	6.00 ± 0.86	13.37 ± 1.57	2.49 ± 0.21 b
	24X	14/15	6.40 ± 1.06	16.10 ± 2.69	2.74 ± 0.40 b
	27VA	15/15	8.40 ± 1.29	16.13 ± 3.39	1.75 ± 0.24 a
	ARI	15/15	6.86 ± 15.81	15.81 ± 3.50	2.01 ± 0.36 a
	HS	14/15	8.13 ± 1.53	16.48 ± 2.91	2.18 ± 0.26 ab
	PE	12/15	6.20 ± 1.29	11.60 ± 3.03	1.90 ± 0.40 a
P			0.3861	0.6331	0.0472
E2	5E	8/15	1.20 ± 0.28	54.35 ± 28.24	45.53 ± 21.46

	20T	14/15	5.00 ± 1.20	59.82 ± 15.50	16.19 ± 4.74
	22V	8/15	1.86 ± 0.54	37.99 ± 14.99	18.67 ± 3.95
	24X	13/15	2.80 ± 0.65	93.30 ± 29.58	43.01 ± 16.26
	27VA	12/15	3.60 ± 0.74	93.19 ± 28.55	28.56 ± 10.53
	ARI	9/15	2.60 ± 0.79	76.14 ± 27.51	28.73 ± 7.02
	HS	12/15	3.53 ± 0.81	138.97 ± 29.01	54.57 ± 12.99
	PE	12/15	3.53 ± 0.73	94.83 ± 31.22	25.56 ± 7.51
P			0.0614	0.1882	0.2255
	5E	0/15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	20T	2/15	0.13 ± 0.08	4.04 ± 2.73	30.34 ± 4.53
	22V	6/15	0.73 ± 0.27	20.05 ± 15.20	25.78 ± 17.21
	24X	2/15	0.26 ± 0.17	0.42 ± 0.35	1.59 ± 0.82
G	27VA	3/15	0.26 ± 0.14	7.45 ± 4.77	36.23 ± 14.86
	ARI	2/15	0.13 ± 0.08	0.07 ± 0.06	0.55 ± 0.28
	HS	2/15	0.40 ± 0.27	5.90 ± 3.91	15.92 ± 2.47
	PE	0/15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
P			0.0571	0.0571	0.0981
	5E	15/15	30.40 ± 4.91	80.48 ± 15.28	3.24 ± 0.72
	20T	15/15	23.73 ± 3.08	110.66 ± 20.71	7.90 ± 3.48
	22V	15/15	19.66 ± 2.60	72.05 ± 11.71	4.36 ± 0.94
	24X	15/15	13.26 ± 1.89	68.26 ± 15.00	5.16 ± 0.94
Np	27VA	15/15	21.06 ± 5.55	59.15 ± 13.10	3.17 ± 0.85
	ARI	15/15	23.00 ± 3.68	79.79 ± 14.95	6.37 ± 2.22
	HS	15/15	19.53 ± 3.04	40.89 ± 7.19	2.31 ± 0.28
	PE	15/15	22.66 ± 5.38	105.60 ± 17.69	9.44 ± 3.37
P			0.1591	0.1106	0.0963

C: pathway; Pd: intracellular puncture (potential drop); G: xylem feeding; E1: phloem salivation; E2: phloem ingestion; Np: non-probing; PPW: proportion of individuals that produced a specific waveform type; NWEI: number of waveform events per insect; WDI: waveform duration per insect; WDE: waveform duration per insect. Waveform values are in minutes. [1]Means followed by the same lowercase letter per column do not differ by Kruskal-Wallis test (p ≥ 0.05).

Table 3 Mean (± SE) EPG sequential variables used to study the feeding behavior of *B. brassicae* on eight different collard green genotypes.

Waveform	Genotype	PPW	WDI[1]	*P*
Time to first probe	5E	15/15	5.67 ± 2.55	
	20T	15/15	6.15 ± 2.49	
	22V	15/15	4.80 ± 2.23	
	24X	15/15	2.65 ± 1.04	
	27VA	15/15	0.73 ± 0.39	0.2029
	ARI	15/15	6.50 ± 2.47	
	HS	15/15	0.56 ± 0.23	
	PE	15/15	3.10 ± 1.58	
Duration of the longest E2	5E	9/15	84.50 ± 40.81	
	20T	14/15	33.99 ± 9.73	
	22V	9/15	46.00 ± 15.13	
	24X	13/15	86.37 ± 31.71	
	27VA	12/15	67.62 ± 22.55	0.1856
	ARI	9/15	88.78 ± 35.18	
	HS	12/15	116.86 ± 23.25	
	PE	12/15	55.88 ± 15.06	
Total duration of E1 followed by E2	5E	9/15	4.78 ± 0.47	
	20T	14/15	7.98 ± 1.72	
	22V	9/15	6.45 ± 1.11	
	24X	13/15	8.08 ± 1.05	
	27VA	12/15	8.67 ± 1.97	0.5709
	ARI	9/15	9.92 ± 2.35	
	HS	12/15	8.82 ± 1.39	
	PE	12/15	6.63 ± 1.10	
Time to first E2	5E	8/15	296.93 ± 43.49	
	20T	14/15	211.08 ± 36.08	
	22V	8/15	334.78 ± 37.33	0.2848
	24X	13/15	229.83 ± 36.02	

27VA	12/15	249.87 ± 42.20
ARI	9/15	323.47 ± 43.79
HS	12/15	214.28 ± 38.89
PE	12/15	240.15 ± 42.98

PPW: proportion of individuals that produced a specific waveform type; WDI: waveform duration per insect; Waveform values are in minutes. [1]Means followed by the same lowercase letter per column do not differ by Kruskal-Wallis test (p ≥ 0.05).

Table 4 Mean (± SE) leaf hardness, total wax and wax/mg obtained from apical leaves of eight collard green genotypes

Genotype	Hardness of leaves (gf/cm)	Total wax (mg)	Wax/mg
22V	0.0280 ± 0.0023 a	7.26 ± 0.14 ab	0.0042 ± 0.00017 ab
PE	0.0270 ± 0.0020 a	4.86 ± 0.35 c	0.0032 ± 0.00031 bc
HS	0.0250 ± 0.0038 ab	7.83 ± 0.41 a	0.0051 ± 0.00026 a
5E	0.0250 ± 0.0015 ab	4.90 ± 0.29 c	0.0025 ± 0.00016 c
ARI	0.0240 ± 0.0032 ab	5.63 ± 0.34 bc	0.0030 ± 0.00024 c
24X	0.0220 ± 0.0019 ab	5.80 ± 0.32 bc	0.0033 ± 0.00014 bc
20T	0.0150 ± 0.0021 b	8.13 ± 0.15 a	0.0048 ± 0.00016 a
27VA	0.0150 ± 0.0015 b	4.13 ± 0.33 c	0.0029 ± 0.00017 c
P	0.0012	< 0.0001	< 0.0001

[1]Means followed by the same lowercase letter per column do not differ by Tukey test ($p \geq 0.05$).

Table 5 Correlation coefficients (*r*) and respective probabilities (*P*) among the different EPG wave types and physical and chemical characteristics of collard green genotypes.

Parameters	Coefficients	Wax	Hardness	Sinigrin	Glucobrassicin	Glucoraphanin	Gluconapin
Number of Pd	*r*	-0.22	0.06	-0.15	-0.11	-0.09	-0.09
	P	0.0172	0.5160	0.1141	0.2182	0.3574	0.3442
Number of C	*r*	-0.08	0.03	-0.21	-0.03	0.01	-0.03
	P	0.3871	0.7560	0.0212	0.7553	0.9291	0.7531
Number of G	*r*	-0.10	0.18	0.01	-0.01	-0.03	0.05
	P	0.2901	0.0470	0.8761	0.9444	0.7193	0.5760
Number of E1	*r*	0.11	0.01	-0.03	-0.05	-0.01	0.14
	P	0.2282	0.9673	0.7325	0.6137	0.9281	0.1282
Number of E2	*r*	0.13	-0.05	-0.01	-0.08	-0.04	0.15
	P	0.1480	0.5640	0.9702	0.3836	0.6403	0.0941
Duration of C	*r*	-0.06	0.10	0.01	-0.01	-0.02	-0.13
	P	0.7024	0.2934	0.9893	0.9616	0.8457	0.1464
Duration in G	*r*	-0.15	0.09	-0.07	-0.08	-0.06	0.01
	P	0.0961	0.9291	0.4502	0.4113	0.5045	0.9646
Duration in E1	*r*	0.13	0.02	0.02	-0.03	-0.02	0.03

|						
	P	0.1470	0.8450	0.8702	0.7863	0.8011	0.7540
Duration in E2	r	-0.01	-0.03	0.05	0.06	0.06	0.23
	P	0.9510	0.7833	0.5931	0.4902	0.5200	0.0100
Time from the beginning of the 1st probe to 1st pd	r	0.08	0.19	-0.11	0.15	0.12	0.24
	P	0.3681	0.0421	0.2232	0.1043	0.1813	0.0084
Wax	r	-	-0.28	0.34	0.45	0.39	0.13
	P	-	0.0021	< 0.0001	< 0.0001	< 0.0001	0.1555
Hardness	r	-0.28	-	-0.05	-0.14	0.02	0.03
	P	0.0021	-	0.6074	0.1251	0.8241	0.7083
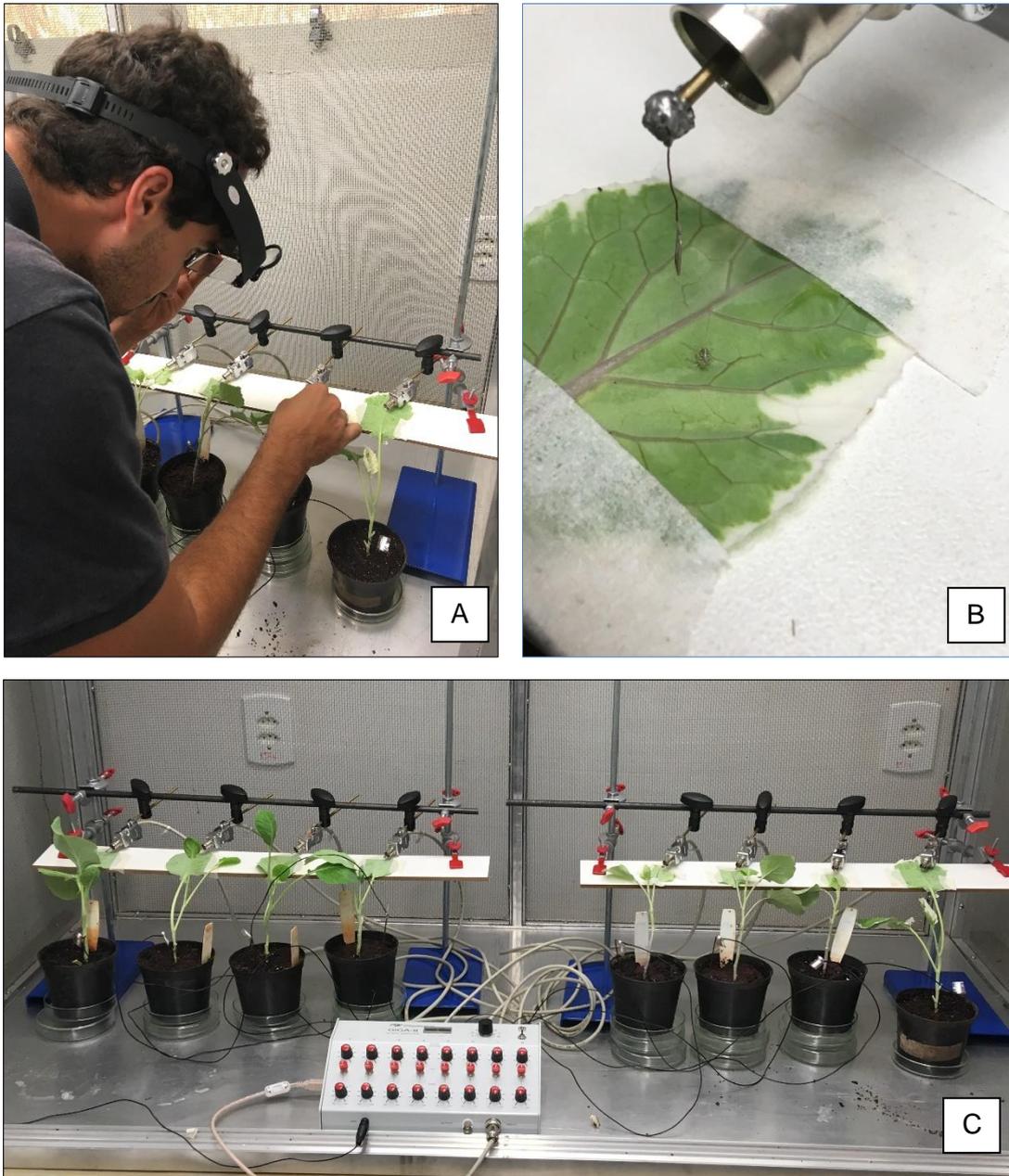
Fig. 1. A. Pinning of aphids in collard green plants. **B.** Correct position of the aphid on the leaf. **C.** View of all EPG channels with aphids on the plants.

Fig. 2. CT3 Texture Analyzer used to punch the collard green leaf.
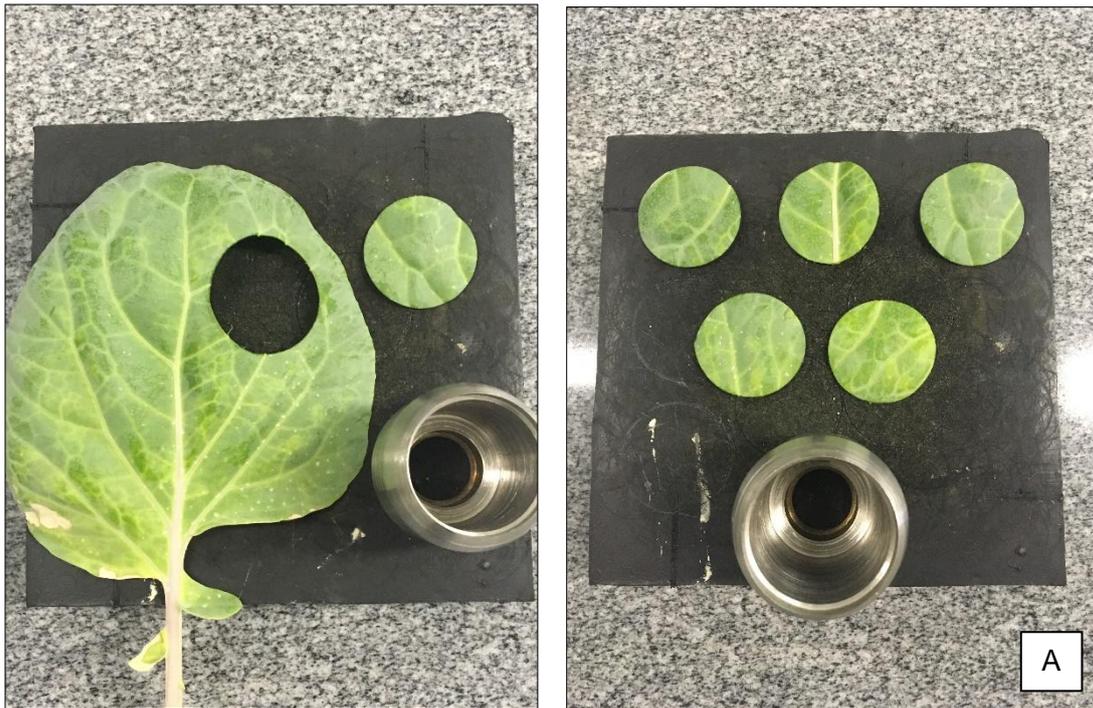
Fig. 3. A. Collard green leaf discs to obtain epicuticuluar wax. **B.** Chloroform + epicuticular wax solution for evaporation in the Chapel.
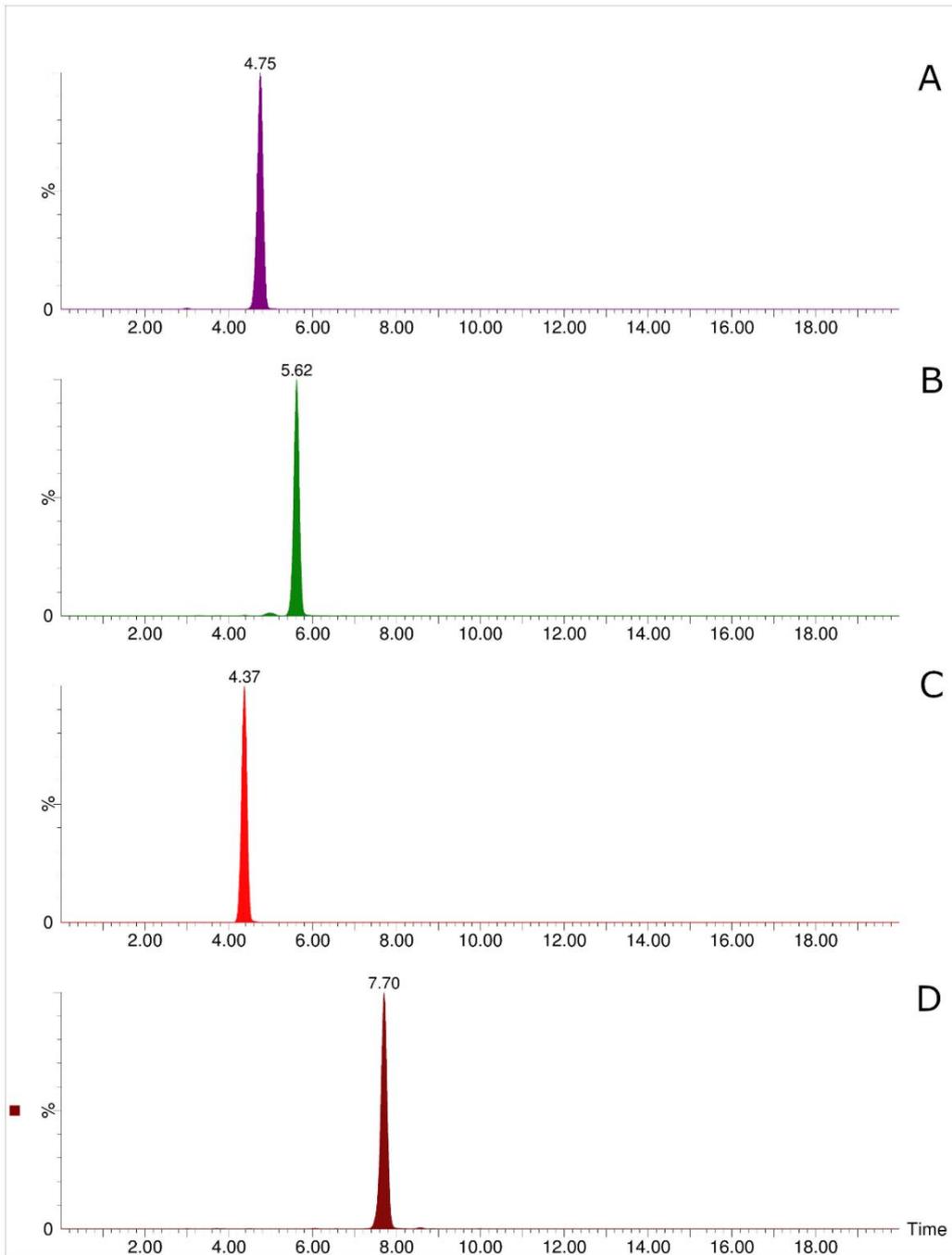
Fig. 4. Ilustration of the signals obtained for the MRM experiment and the retention time for **A**: sinigrin; **B**: gluconapin; **C**: glucoraphanin and **D**: glucobrassicin.
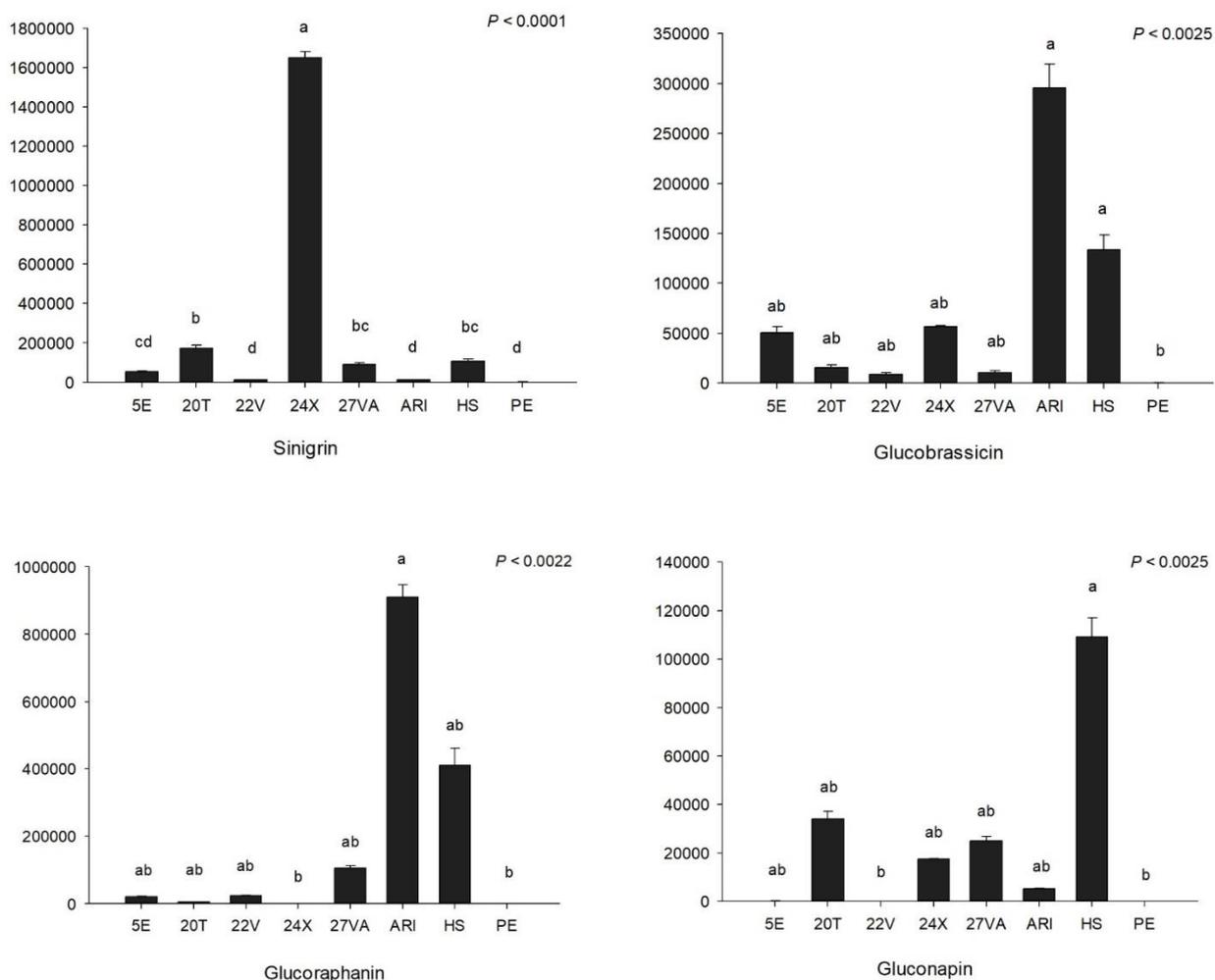
Fig. 5. Area of glucosinolates on eight different collard green genotypes obtained through means of the area of each peak of the replicates.

FINAL CONSIDERATIONS

In an attempt to reduce the use of synthetic insecticides applied to collard green crops, the interest in resistant genotypes has been increasing among farmers. The adoption of resistant genotypes stands out as an important tactic to be implemented in the management program of this crop, since it reduces the pest population level and can be used in association with other IPM tools, such as chemical, biological control, cultural, among others. The increasing availability of improved techniques such as chemical analysis of secondary metabolites and electronic monitoring through Electrical Penetration Graph (EPG) has helped to understand the interactions involving aphids and plants of economic interest, allowing the correct distinction among the resistance categories.

The data obtained in the preliminary test of Chapter 1 with 37 collard green genotypes allowed to the selection of materials with resistance potential on *B. brassicae;* 20T and 24X genotypes were the least infested in antixenosis in 24h free-choice test. 2B, 5E, 8H, 19S, 21U, 27VA, 30OP, PE, MGH and TP genotypes prevented *B. brassicae* to complete the nymphal period, indicating antibiosis and/or antixenosis expression. 4D, GAU, 20T, 14N and MGI genotypes prevented the reproductive phase of the cabbage aphid also suggesting antibiosis.

An overall analysis of the results obtained through the EPG analysis indicates that the 22V, 5E, and 27VA genotypes, for having performed a large number of Pd waves, spent more time in this event, indicating that antioxenotic factors are the main responsible for the resistance verified in these materials in previous tests. Aphids in 22V and 24X genotypes required more time until the beginning of the phloeming phase, also suggesting that the antixenosis category resistance is involved in these genotypes against cabbage aphid. The 22V and PE genotypes had high rates of hardness leaf, which justifies the occurrence of the same category of resistance. The 20T genotype, considered as having antixenosis and/or antibiosis resistance in the previous tests, it is possible that the antioxenotic factors are related to other physical (color) or chemical (volatile) aspects, as the feeding parameters were similar to the susceptible HS. The ARI and 24X feed parameters were similar to those observed with HS, antibiosis is likely to be the predominant resistance category in these materials. As HS genotype was considered as susceptible standard in this study, a higher gluconapin amount

indicates that this compound in not influence cabbage aphid feeding behavior. The present study showed that analysis physical and chemical from genotypes associated with the EPG technique can be useful tool in studies of plant resistance to insects.

CONCLUSIONS

Chapter 1

-The 20T and 24X genotypes expressed antixenosis, being less infested;

-In no-choice test the 2B, 5E, 8H, 19S, 21U, 27VA, 30OP, PE, MGH and TP genotypes can be considered highly resistant or no host plant to cabbage aphid (100% nymphal mortality);

-The 4D, GAU, 20T, 14N and MGI genotypes prevented the cabbage aphid from reaching the reproductive phase with expression of high levels of antibiosis and/or antixenosis.

Chapter 2

-The 22V, 5E and 27VA genotypes presented many Pd waves and the aphid spent more time in this activity, suggesting that low glucosinolate levels are associated with antixenotic resistance factors;

-In the 22V and 24X genotypes take more time for the aphids to access the phloem phase, also suggesting antixenosis as category of resistance;

-The 22V and PE genotypes had greater leaf hardness, which indicates the occurrence of antixenosis resistance;

-The ARI and 24X feeding parameters were like those observed in the HS (susceptible standard), and antibiosis is likely to be the predominant resistance category in these materials;

-The HS genotype, the high glucosinolate content (except sinigrin) does not seem to affect the insect feeding behavior, suggesting an association of these compounds with the susceptibility of the material.

REFERENCES

AHMAD, M.; ASLAM, M. Resistance of cabbage aphid, *Brevicoryne brassicae* (Linnaeus) to endosulfan, organophosphates and synthetic pyrethroids. **Pakistan Journal of Zoology,** v. 37, n. 4, p. 293-295, 2005.

BALDIN, E. L. L.; VENDRAMIN, J. D.; LOURENÇÃO, A. L. **Resistência de plantas a insetos: Fundamentos e aplicações**. Piracicaba. FEALQ, 2019. 493 p.

BLACKMAN, R.; EASTOP, V. **Aphids on the world's crops. An identification and information guide.** 2. ed. New York: Wiley & Sons, 2000. 476 p.

BLACKMAN, R. L.; EASTOP, V. F. Taxonomic issues. In: VAN EMDEN, H. F.; HARRINGTON, R. **Aphids as Cop Pests**. 1. ed. Wallingford, CAB International, 2007, 745 p.

CAMPBELL, B.; HAN, D. Y.; TRIGGS, C. M.; FRASER, A. G.; FERGUSON, L. R. Brassicaceae: nutrient analysis and investigation of tolerability in people with Crohn's disease in a New Zealand study. **Functional Foods in Health and Disease**, v. 1, n. 2, p. 460-486, 2012.

CARVALHO, C.; KIST, B. B.; TREICHEL, M. **Brazilian vegetal yearbook**. Santa Cruz do Sul: Editora Gazeta, 2016. 64 p.

CIONI, F.; TUGNOLI, V.; GIUNCHEDI, L.; POLLINI, A. Activity of soil disinfectants on sugarbeet. **Informatore Agrario**, v. 57, n. 4, p. 57-59, 2001

CIVIDANES, F. J.; SANTOS, D. M. Populational fluctuation and vertical (within-plant) distribution of *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) on kale. **Bragantia**, v. 62, n. 1, p. 61-67, 2003.

COLE, R. A. The relative importance of glucosinolates and amino acids to the development of two aphid pests *Brevicoryne brassicae* and *Myzus persicae* on wild

and cultivated brassicae species. **Entomologia Experimentalis et Applicata**, v. 85, n. 2, p. 121-133, 1997.

DIAZ-MONTANO, J.; REESE, J. C.; LOUIS, J.; CAMPBELL, L. R.; SCHAPAUGH, W. T. Feeding behavior by the soybean aphid (Hemiptera: Aphididae) on resistant and susceptible soybean genotypes. **Journal of Economic Entomology**, v. 100, n. 3, p. 984-989, 2007.

DOMINGOS, G. M; BALDIN, E. L. L.; CANASSA, V. F.; SILVA, I. F.; LOURENÇÃO, A. L. Resistance of collard green genotypes to *Bemisia tabaci* biotype B: characterization of antixenosis. **Neotropical Entomology,** v. 47, n. 4, p. 560-568, 2018.

EIGENBRODE, S. D.; ESPELIE, K. E. Effects of plant epicuticular lipids on insect herbivores. **Annual Review of Entomology,** v. 40, n. 1, p. 171-194, 1995.

ELLIS, P.; SINGH, R. A review of the host plants of the cabbage aphid, *Brevicoryne brassicae* (Homoptera, Aphididae). **Bulletin OILB/SROP,** v. 16, n. 5, p. 192-201, 1993.

FAO – Food and Agriculture Organization of the Unites Nations – **FAOSTAT** - 2017. Available in: < http://www.fao.org/faostat/en/#data/QC>. Acess in 23 feb. 2019.

FAHEY, J. W.; ZALCMANN, A. T.; TALALAY, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. **Phytochemistry**, v. 56, n. 1, p. 5-51, 2001.

FILGUEIRA, F. A. R. **Novo manual de olericultura: Agrotecnologia moderna na produção e comercialização de hortaliças**. 3. ed. Viçosa: Editora UFV, 2008. 421 p.

FRANCO, G. **Tabela de composição química de alimentos**. 3. ed. São Paulo: Atheneu, 1992. 194 p.

GABRYS, B. Semiochemicals in the biology and ecology of the cabbage aphid *Brevicoryne brassicae* (L.). **Zeszyty Naukowe Akademii Rolniczej we Wroclawiu. Rozprawy**, v. 164, n. 356, p. 84, 1999.

GIROUSSE, C.; FAUCHER, M.; KLEINPETER, C.; BONNEMAIN, J. L. Dissection of the effects of the aphid *Acyrthosiphon pisum* feeding on assimilate partitioning in *Medicago sativa*. **New Phytologist**, v. 157, n. 1, p. 83-92, 2003.

HAHN, C.; MÜLLER, A.; KUHNERT, N.; ALBACH, D. Diversity of kale (*Brassica oleracea* var. *sabellica*): glucosinolate content and phylogenetic relationships. **Journal of agricultural and food chemistry**, v. 64, n. 16, p. 3215-3225, 2016.

HALIŃSKI, Ł. P.; PASZKIEWICZ, M.; GOŁĘBIOWSKI, M.; STEPNOWSKI, P. The chemical composition of cuticular waxes from leaves of the gboma eggplant (*Solanum macrocarpon* L.). **Journal of Food Composition and Analysis**, v. 25, n. 1, p. 74-78, 2012.

HALKIER, B. A.; GERSHENZON, J. Biology and biochemistry of glucosinolates. **Annual Review of Plant Biology**, v. 57, n. 57, p. 303-333, 2006.

Hill, D. S. **Agricultural insect pests of the tropics and their control.** Cambridge: Cambridge University press, 1983. 746 p.

IEA – Instituto de Economia Agricola. **Estatísticas da Produção Paulista**. 2017. Available in:< http://ciagri.iea.sp.gov.br/nia1/subjetiva.aspx?cod_sis=1&idioma=1>. Acess in 23 feb. 2019.

KATSANIS, A.; RASMANN, S.; MOONEY, K. A. Herbivore diet breadth and host plant defense mediate the tri-trophic effects of plant toxins on multiple coccinellid predators. **PloS one**, v. 11, n. 5, p. e0155716, 2016.

KJAER, A.; SKRYDSTRUP, T. Selenoglucosinolates: synthesis and enzymatic hydrolysis. **Acta Chemica Scandinavica**, v. 41, n. 1, p. 29−33, 1987

KOTWAL, D. R.; BHALLA, O. P.; VERMA, A. K. Natural enemies of the cabbage aphid, *Brevicoryne brassicae* (Linn.) in the mid hill region of Himanchal Pradesh. **Indian Journal Agricultural Sciences**, v. 54, n. 1, p. 1011-1012, 1984.

LARA, F. M. **Princípios de resistência de plantas a insetos**. 2. ed. São Paulo: Ícone, 1991. 336 p.

LIU, T. X.; SPARKS JR, A. N**. Aphids on cruciferous crops: identification and management**, Texas Farm Collection, 2001, 12 p.

MARICONI, F. A. M.; ZAMITH, A. P. L.; MENEZES, M. Pulgão das brássicas *Brevicoryne brassicae* (L., 1758): estudo descritivo, bionômico e de combate. **Revista de Olericultura**, v. 3, n. 1, p. 195-201, 1963.

MASKELL, L. C.; RAYBOULD, A. F.; COOPER, J. I.; EDWARDS, M. L.; GRAY, A. J. Effects of turnip mosaic virus and turnip yellow mosaic virus on the survival, growth and reproduction of wild cabbage (*Brassica oleracea*). **Annals of Applied Biology**, v. 135, n. 1, p. 401-407, 1999.

MULLER, C.; RIEDERER, M. Plant surface properties in chemical ecology. **Journal of Chemical Ecology**, v. 31, n. 11, p. 2621-2651, 2005.

NAUEN, R.; ELBERT, A. European monitoring of resistance to insecticides in *Myzus persicae* and *Aphis gossypii* (Hemiptera: Aphididae) with special reference to imidacloprid. **Bulletin of Entomological Research**, v. 93, n. 1, p. 47-54, 2003.

NAULT, L. R.; STYER, W. E. Effect of sinigrin on host selection by aphids. **Entomologia Experimentalis et Applicata**, v. 15, n. 4, p. 423-437, 1972.

PAINTER, R. H. **Insect resistance in crop plants.** New York: McMillan, 1951. 520 p.

PANDA, N. **Principles of host-plant resistance to insect pests**. New York: Osmun, 1979. 386 p.

RYABOV, E. V. A novel virus isolated from the aphid *Brevicoryne brassicae* with similarity to Hymenoptera picornalike viruses. **Journal of General Virology**, v. 88, n. 9, p. 2590-2595, 2007.

SIEGEL, R. L.; MILLER, K. D.; JEMAL, A. Cancer statistics, 2016. **CA: A Cancer Journal for Clinicians**, v. 66, n. 1, p. 7-30, 2016.

SCHLICK-SOUZA, E. C.; BALDIN, E. L. L.; LOURENÇÃO, A. L. Variation in the host preferences and responses of *Ascia monuste orseis* Godart (Lepidoptera: Pieridae) to cultivars of collard greens *Brassica oleracea* (L.) var. *acephala*. **Journal of Pest Science**, v. 84, n. 4, p. 429-436, 2011.

SCHOONHOVEN, L. M.; VAN LOON, J. J. A.; DICKE, M. **Insect-Plant Biology**. London: Chapman & Hall, 1998. 409 p.

SMITH, C. M. **Plant resistance to arthropods**: **molecular and conventional approaches**. Berlin: Springer, 2005. 243 p.

SMITH, C. M.; CLEMENT, S. L. Molecular bases of plant resistance to arthropods. **Annual Review of Entomology**, v. 57, n. 1, p. 309-328, 2012.

STADLER, E.; REIFENRATH, K. Glucosinolates on the leaf surface perceived by insect herbivores: review of ambiguous results and new investigations. **Phytochemistry Reviews**, v. 8, n. 1, p. 207-225, 2009.

SUPAPVANICH, S.; PIMSAGA, J.; SRISUJAN, P. Physicochemical changes in fresh-cut wax apple (*Syzygium samarangenese* [Blume] Merrill & L. M. Perry) during storage. **Food Chemistry**, v. 127, n. 3, p. 912-917, 2011. 2011.

TJALLINGII, W. F. Electronic recording of penetration behaviour by aphids. **Entomologia Experimentalis et Applicata**, v. 24, n. 3, p. 721-730, 1978.

TANG, L.; ZIRPOLI, G. R.; JAYAPRAKASH, V.; REID, M. E.; MCCANN, S. E.; NWOGU, C. E.; ZHANG, Y.; AMBROSONE, C. B.; MOYSICH, K. B. Cruciferous vegetable intake is inversely associated with lung cancer risk among smokers: a case-control study. **BMC cancer**, v. 10, n. 1, p. 162-171, 2010.

THULER, R. T.; DE BORTOLI, S. A.; HOFFMANN-CAMPO, C. B. Brassicas cultivars classification in relation to resistance to diamondback moth and to the presence of glucosinolates. Pesquisa Agropecuária Brasileira, v. 42, n. 4, p. 467-474, 2007.

VAN HELDEN, M.; TJALLINGII, W. F. Experimental design and analysis in EPG experiments with emphasis on plant resistance research. In: Walker, G. P.; Backus, E. A. (Eds.). **Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior**. Entomological Society of America, Lanham: Thomas Say Publications in Entomology, 2000. p. 144-171.

VERHOEVEN, D. T.; GOLDBOHM, R. A.; VAN POPPEL, G.; VERHAGEN, H.; VAN DEN BRANDT, P. A. Epidemiological studies on brassica vegetables and cancer risk. **Cancer Epidemiology and Prevention Biomarkers**, v. 5, n. 9, p. 733-748, 1996.

WILLIAMS, I. S.; DIXON, A. F. G. Life cycles and polymorphism. In: VAN EMDEN, H.; HARRINGTON, R. (eds.), **Aphids as crop pests**. Wallingford, CAB International, 2007 745 p.

WOJCICKA, A. Changes in pigment content of triticale genotypes infested with grain aphid *Sitobion avenae* (Fabricius) (Homoptera: Aphididae). **Acta Biologica Cracoviensia Botanica**, v. 56, n. 1, p. 121-127, 2014

YIN, Y.; BI, Y.; CHEN, S.; LI, Y.; WANG, Y.; GE, Y.; DING, B.; LI, Y.; ZHANG, Z. Chemical composition and antifungal activity of cuticular wax isolated from Asian pear fruit (cv. *Pingguoli*). **Scientia Horticulturae**, v. 129, n. 4, p. 577-582, 2011.

ZHANG, J. Y.; BROECKLING, C. D.; SUMNER, L.W.; WANG, Z. Y. Heterologous expression of two *Medicago truncatula* putative ERF transcription factor genes, WXP1 and WXP2, in Arabidopsis led to increased leaf wax accumulation and improved drought tolerance, but differential response in freezing tolerance. **Plant molecular biology**, v. 64, n. 3, p. 265-278, 2007.