CLEITON ANTÔNIO WARTHA

RESISTANCE MECHANISM OF SUGARCANE GENOTYPES TO THE SUGARCANE BORER

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Curso de Pós-Graduação em Fitotecnia, para obtenção do título de *Magister Scientiae*.

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APROVADA: 17 de julho de 2018.

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ABSTRACT

WARTHA, Cleiton Antônio, M.Sc., Universidade Federal de Viçosa, July, 2018. R**esistance mechanism of sugarcane genotypes to the sugarcane borer**. Advisor: Márcio Henrique Pereira Barbosa.

The sugarcane borer *Diatraea saccharalis* Fabr. (Lepidoptera: Crambidae) is the main insect pest affecting sugarcane fields in Brazil. Damage caused by borer herbivory occurs through the opening of galleries, breaking of stalks, lateral sprouts and reduction of agricultural yield. The development of borer resistant cultivars is a strategy for integrated pest management, reduction of production costs and long-lasting cultivars. Anatomical, biochemical and morphological traits are involved in resistance. However, there is a shortage of studies seeking for resistance sources at the initial feeding of the pest in younger leaves. In this sense, the objective of this study was to identify the constitutive or induced chemical components of sugarcane epicuticular wax that can be associated with resistance before infestation (BI) and after infestation (AI) with sugarcane borer. A greenhouse experiment was performed in a factorial scheme in a completely randomized design with four replicates, with and without borer infestation using 11 clones previously characterized as resistant or susceptible in field-based experiments. Sugarcane whorl of six-month-old plants were collected before and after 72 hours of sugarcane borer infestation. The chemical composition of the epicuticular wax was assessed by gas chromatography associated with mass spectrometry (GC-MS). The relative importance of each compound was analyzed by means of logistic regression, using the integrated area data of the 17 main peaks identified. Before infestation, the most important compounds in the classification of resistant and susceptible genotypes were octacosanal, triacontane, lanosterol and tetratriacontane. After the borer infestation, the compounds selected by the adjusted model were hexacosanol, octacosanol and dotriacontanol and in the combined analysis (BI and AI) were hexacosanol, hexacosanal, octacosane, lanosterol, triacontanol and dotriacontanol. The apparent error rate of the adjusted logistic models for BI, AI and combined analysis was respectively of 13.6%, 11.4% and 6.8%. Our findings have applicability in the development of a screening methodology to identify potential genotypes for resistance to sugarcane borer in early stages of sugarcane breeding programs.

RESUMO

WARTHA, Cleiton Antônio, M.Sc., Universidade Federal de Viçosa, julho de 2018. **Mecanismo de resistência de genótipos de cana-de-açúcar à broca-da-cana**. Orientador: Márcio Henrique Pereira Barbosa.

A broca-da-cana *Diatraea saccharalis* Fabr. (Lepidoptera: Crambidae) é a principal praga que ataca a cultura da cana-de-açúcar no Brasil. Os danos causados pela herbivoria da broca-da-cana ocorrem pela abertura de galerias, quebramento de colmos, brotações laterais e redução da produtividade agrícola. A obtenção de cultivares resistentes à broca-da-cana é uma estratégia de manejo integrado de pragas, redução dos custos de produção e longevidade das cultivares. Características anatômicas, bioquímicas e morfológicas estão envolvidas na resistência. Entretanto, há escassez de estudos visando a busca por fontes de resistência por ocasião da alimentação inicial da praga em folhas jovens. Nesse sentido, o objetivo da pesquisa foi identificar os compostos químicos constitutivos ou induzidos da cera epicuticular que estão associados com a resistência antes (BI) e após a infestação (AI) com a broca-da-cana. Foi conduzido um experimento em casa de vegetação em esquema fatorial no delineamento inteiramente casualizado com quatro repetições, com e sem infestação da broca utilizando 11 clones, previamente caracterizados em campo como resistentes ou suscetíveis. Os cartuchos de plantas de seis meses de idade foram coletados antes e após 72h de infestação com broca-da-cana. A composição química da cera epicuticular foi avaliada por cromatografia gasosa associada à espectrometria de massas (GC-MS). A importância relativa de cada composto foi analisada por meio de regressão logística, utilizando os dados de área integrada dos 17 principais picos identificados. Antes da infestação, os compostos de maior importância na classificação dos genótipos em resistentes e suscetíveis foram: octacosanal, triacontane, lanosterol e tetratriacontane. Após a infestação da praga, os compostos selecionados pelo modelo ajustado foram o hexacosanol, octacosanol e dotriacontanol e na análise combinada (BI e AI) foram hexacosanol, hexacosanal, octacosane, lanosterol, triacontanol e dotriacontanol. A taxa de erro aparente dos modelos logísticos ajustados para BI, AI e análise combinada foi de 13,6%, 11,4% e 6,8%, respectivamente. Os resultados obtidos possuem aplicabilidade no desenvolvimento de metodologia de *screening* para identificação de genótipos potenciais para resistência à broca-da-cana em fases iniciais de programas de melhoramento genético da cana-de-açúcar.

SUMMARY

1. INTRODUCTION

The global interest in sugarcane as a renewable biomass crop has increased significantly in recent years. The ethanol production contributes to the worldwide energy requirements, preserving the natural sources and mitigating the effects of greenhouse gas emission. The sugarcane sector holds a substantial market share of the biomass source of renewable energy supply in Brazil and Brazil is the world largest sugarcane producer. In the 2017/2018 crop season, the Brazilian sugarcane sector produced more than 633 million tons in a harvested area of 8.73 million hectares (CONAB, 2018). Consequently, there was a production of 27.6 billion liters of ethanol and 37.8 million tons of sugar.

Along with improved agronomic techniques, plant breeding contributed to increased sugarcane yields and reduced production costs. Sugarcane is a perennial crop, clonally propagated, out-crossing, polyploid species, belonging to the Andropogoneae tribe in the Poaceae family. The combination of high sugar content from *Saccharum officinarum* L. and the disease resistance from *Saccharum spontaneum* L. resulted in interspecific hybrids and modern cultivars (Cheavegatti-Gianotto et al., 2011). Steady yield increase in the last decades was supported by the modern cultivars released by the two main Brazilian sugarcane breeding programs: the Inter-University Network for the Development of Sugarcane Industry (RIDESA) and the Sugarcane Technology Center (CTC) (Barbosa et al., 2012a). According to the same authors, besides broad adaptability to different environments, the breeding programs target a range of agronomic traits including high yield, sucrose content, disease and pest tolerance, fiber content and proper milling traits.

Among several abiotic and biotic stresses limiting production, insect pests are responsible for more than 10% of sugarcane yield losses worldwide (Leslie, 2004). The sugarcane borer *Diatraea saccharalis* Fabr. (Lepidoptera: Crambidae) is the main insect pest affecting sugarcane fields in Brazil. Early-instar larvae feed on the leaf parenchyma and sheaths while older larvae bore into the stalks opening holes and galleries. The direct damages of borer herbivory are associated with stalk breakage, side shoots and roots, and reduced agricultural yield. Indirect damages occur due to the entrance through the holes of opportunistic microorganisms such as *Fusarium moniliforme* (Went) and *Colletotrichum falcatum* (Sheldon). These fungi cause red root disease of

sugarcane, with sucrose inversion and decreased sugarcane quality, reducing stalk sugar content, milling capacity and increasing fiber content.

Increased infestation levels have been observed with expansion of the acreage and proximity of areas with alternative hosts, susceptible cultivars, climate conditions and incipient sampling. Moreover, the traditional harvesting method with pre-burning has received environmental pressure and increased unburnt mechanized harvesting has been noticed (Eggleston et al., 2014), facilitating biological cycle completion and increased borer population. The occurrence of sugarcane borer in the fields can be extremely destructive, depending on the attack intensity. According to Arrigoni (2002), damages of 0.49% in sugar, 0.28% in ethanol, and 1.50% in cane yield can be observed for each 1% of infestation intensity.

The borer presents holometabolous life cycle varying from 53 to 60 days, depending on climate and host plant conditions. After mating, the female adult moths oviposit most of their eggs on the adaxial face of sugarcane leaves during up to four days. Newly hatched larvae stablish and feed on the parenchyma of younger leaves in the whorl of sugarcane plants. Approximately 15 days after hatching, the larvae are able to descend, pierce and penetrate the stalks (Gallo et al., 2002).

The main control strategy of sugarcane borer in Brazil is the mass release of egg and larval parasitoids respectively of the wasps *Trichogramma galloi* and *Cotesia flavipes* (Hymenoptera: Braconidae). Even though they are a successful example of biological control, fluctuations in control take place because of geographical and cropping season conditions. Other factors that hamper the biological control are the costs with sampling and monitoring borer populations and the requirement of larger number of wasps (Gitahy et al., 2007). Furthermore, alternative strategies such as cultural practices and mechanical methods are limited by costs and moderate efficacies. Therefore, host plant resistance is an important strategic component in the integrated pest management. It is an economically and environmentally effective approach for protecting crops against insect damage (Schoonhoven et al., 2005). Varietal resistance has simple use and reduces production costs with insecticides or other control strategies of an insect that damages millions of hectares of sugarcane fields in Brazil. Moreover, it is compatible with other control managements, being a long-lasting approach to decrease borer damage and provide substantial benefits to sugarcane production (Posey

et al., 2006; Vargas et al., 2015). However, development of host plant resistance to sugarcane borer is still incipient.

The development of resistant cultivars can be improved with insights gained on host plant resistance mechanisms along with the physiological responses of the sugarcane genotypes to the attack of different pests (Broekgaarden et al., 2011). According to Smith (2005), insect resistance accounts for the sum of constitutive, genetically inherited traits that result in a plant of one cultivar being less damaged than a susceptible one lacking these traits.

Stout (2013) reevaluated the conceptual framework for applied research on host-plant resistance and created a dichotomous scheme with a major division between resistance and tolerance. The resistance was classified as plant traits that reduce the extent of injury done to a plant by an herbivore and tolerance as plant traits or physiological processes that reduce the amount of yield loss per unit injury. Moreover, the resistance category was divided into the subcategories constitutive/inducible and direct/indirect. The constitutive plant resistance was defined as the resistance that is expressed regardless of the prior history of the plant. Meantime, inducible resistance is only expressed or expressed to a greater extent after prior injury, with defense expression being contingent on prior attack. Furthermore, direct resistance was classified as plant traits that have direct or unmediated effects on herbivore behavior or biology. Differently, indirect plant resistance do not affect herbivore fitness directly but facilitates the actions of predators and parasitoids of insect herbivores. Among the categories, only tolerance does not present selection pressure on herbivore populations.

Determining which traits to assess and how to relate these traits to genetic resistance has been difficult particularly for sugarcane. Efforts have been reported in the literature, seeking to identify differences of borer infestation under field conditions. Rutherford and Van Staden (1996) reported that variety resistance to stalk borer (*Eldana saccharina*) has been a selection criterion in the breeding program of the South African Sugarcane Association. However, breeders are reluctant to incorporate insect resistance into the breeding programs because of the undesirable large-scale insect bioassays (Smith, 2005). The development of resistance screening protocols under field conditions is hampered by notorious erratic spatial and temporal distribution of the borer that not inflict a consistent level of damage. In this way, field-based bioassays are restricted to a

few selection sites with high natural infestation and the natural infestation is limited to weather conditions such as rainfall.

The prerequisites of accurate efficient techniques for the identification of plants with insect resistance and a broad-based germplasm collection are required for the success of breeding programs focusing on insect resistance. Improved selection efficiency of pest-resistant genetic materials is an important demand of the Sugarcane Breeding Program of the Federal University of Viçosa (PMGCA/UFV). Therefore, continuous search for resistance sources and mechanisms is desirable to develop fast, inexpensive, and reliable methods for screening large sugarcane populations in early stages of selection. Thus, only the promising genotypes would reach the next phase of experimental field trials for further evaluation and selection.

Findings published in the literature indicates that sugarcane resistance is expressed based on complex contributions and interactions of several components (Tomaz et al., 2017; Pimentel et al., 2017). In this way, Dinardo-Miranda et al. (2012) observed differences among genotypes regarding stalk damage, larvae weight and length that might be related to suppressant substances or some degree of deterrence. Moreover, White (1993) attributed the higher mortality of young larvae to the presence of physical or chemical barriers deterring or delaying larvae tunneling into the internodes. Furthermore, other researches indicated that the presence of chemical compounds could be related to direct effects on borer behavior or biology when sense organs on the arthropod tarsi and mouthparts perceive negative chemical and tactile stimuli from the leaf surface (Souza et al., 2013; White et al., 2010).

First instar borer larvae spent about one week to ten days on the sugarcane plant surface before the survivors attempt to perforate the stalk (Rutherford & Van Staden, 1996; Gallo et al. 2002). Plant anti-nutritional factors can act as pre-ingestion factors, limiting food supply or as post-ingestion factors, reducing the nutritional value of plants to herbivorous insects. Increased exposure of larvae to adverse biological and environmental factors may increase mortality rate (Dinardo-Miranda et al., 2012). Therefore, the epicuticular wax components act as a barrier and can influence the behavior and survival of larvae and partially explain the observed differences in insect resistance. Both the quantity and the chemical composition of the epicuticular wax can be associated to effects on the behavior, biology and overall fitness in the early stages of

chewing insects (Keeping & Rutherford, 2004). As some insects use olfactory response and surface chemistry to identify their host plants, the chemical traits are often based on inner plant tissues components that may act as toxins, feeding deterrents, and digestibility reducers (Purcell et al., 2005).

According to Gniwotta et al. (2005), the chemical composition of waxes and cutins of the plant surface and underlying layers are factors involved in plant-insect and plant-pathogen interaction. The epicuticular wax is the outer layer of the cuticle with a thin layer of a complex mixture of largely hydrophobic constituents on its coverage. The primary physiological function of this layer is to seal the tissue against the dry atmosphere, avoiding desiccation and water loss by other regions than the stomata. In addition, the epicuticular wax acts protecting against insect herbivory and pathogen invasion (Rutherford, 2013). In a study conducted with pea (*Pisum sativum*), Gniwotta et al. (2005) observed that the leaf epicuticular layer contains 74% and 83% of the total wax respectively on the adaxial and abaxial surfaces. Sugarcane epicuticular wax is chemically analyzed as a complex and variable mixture of long chain alkanes, alkenes, aromatic hydrocarbons, fatty acids, ketones, aldehydes, fatty alcohols, flavonoids and triterpenoids (Lamberton, 1960; Dragota & Riederer, 2007; Kunst & Samuels, 2009).

The application of the component profiling technique on insect-plant interactions involves the chromatographic separation and quantification of the components from the plant extract (Rutherford & Van Staden, 1996). A logistic regression model can provide the relative importance of individual components in either resistance or susceptibility (Zhou, 2015). Moreover, the same model can be used to predict the proportion of resistance provided by those components.

Researchers from the South African Sugarcane Research Institute (SASRI) observed that wax components of the stalk surface such as alcohols and shorter-chain length aldehydes contribute to resistance to stalk borer in the period prior to stalk penetration: the high proportion of fatty alcohols in the stalk epicuticular wax was associated with higher larvae mortality. Meanwhile, the high aldehyde/alcohol ratio was associated to lower larvae mortality. In the same study, chemical differences were observed in the wax composition among the studied genotypes, which can be correlated with resistance or susceptibility of the genotypes (Rutherford & Van Staden, 1996).

Bischoff et al. (2009) evidenced sugarcane cultivar resistant to the borer selected through conventional breeding. However, the knowledge of the factors that confer plant-host resistance is limited and partial, with differences in the results obtained by several researchers (White et al., 2006; Dinardo-Miranda et al., 2012). Based on the research findings reviewed above, we verified that the chemical composition of the epicuticular wax of sugarcane whorl could contribute to the resistance to the sugarcane borer. Clearly, the development of more diverse, cost-effective and sustainable management programs for insect pests will be a crucial component of increasing food and energy production in the upcoming decades. In order to support the current sugarcane breeding programs, the objective of this study was to identify the constitutive or induced chemical components of sugarcane epicuticular wax that can be associated with resistance before and after infestation with sugarcane borer.

2. MATERIAL AND METHODS

An experiment was performed in the "Diogo Alves Mello" experimental field of the Federal University of Viçosa located in Viçosa (20°45' S, 42°52'W, H = 650 m), Minas Gerais state, southeastern Brazil from February/2017 to September/2017.

2.1. Plant genetic material and insect utilized in the experiment

The genetic material used in this study was composed of a panel of genotypes belonging to the germplasm bank of the Sugarcane Breeding Program at the Federal University of Viçosa (PMGCA/UFV) along with the Inter-University Network for the Development of Sugarcane Industry (RIDESA). The genotypes were selected jointly with the breeders and technicians of the PMGCA because they presented morphological and biochemical traits possibly related to sugarcane borer resistance. Selected genotypes included unreleased clones from the 04 and 05 series of the advanced stage of experimental evaluation in the PMGCA/UFV, the RB867515 cultivar and the IM76-228, an interspecific hybrid of *Saccharum robustum*. The experimental clones were extensively field-based rated at four locations and two agricultural seasons, exhibiting different levels of resistance to natural infestations of sugarcane borer (Table 1). The resistance classification was based on the infestation index (percentage of bored internodes divided by total internodes). The RB867515 cultivar is widely grown in the country (Barbosa et al., 2012a), being a resistant control for high mortality of early-stage larvae feeding on leaves (Tomaz et al., 2017). Meanwhile, the IM76-228 *S.*

robustum hybrid causes high mortality of early-stage larvae feeding in stalks and little damage on the leaves (Pimentel et al., 2017). Thus, the genotypes chosen were: RB867515, IM76-228, RB047201, RB047212, RB047055, RB047226, RB047050, RB047248, RB057231, RB047016 and RB057249.

Table 1 - Classification of ten genotypes of the studied germplasm obtained by field-based ratings for the traits infestation index by sugarcane borer (II, in %), fiber content in the stalks (Fiber, in %), soluble solids content in juice (Brix), total recoverable sugar (TRS, in kg/t of cane) and tons of cane per hectare (TCH) at four locations and two agricultural seasons with natural infestation.

Genotype	Class†	II	Fiber	Brix	TRS	TCH
RB047055	Resistant	3.489	12.649	20.364	146.967	138.650
RB047201	Resistant	3.700	12.314	18.636	134.462	164.099
RB047212	Resistant	4.429	12.306	17.858	128.469	150.635
RB047050	Resistant	4.948	11.825	19.345	141.678	137.446
RB047226	Resistant	5.033	12.422	18.939	134.043	146.827
RB867515	Resistant	6.926	12.081	19.871	145.263	168.195
RB057231	Susceptible	7.824	12.055	18.783	135.442	140.989
RB047016	Susceptible	7.826	12.600	20.445	146.977	149.212
RB047248	Susceptible	8.293	13.117	19.292	138.301	145.851
RB057249	Susceptible	8.973	12.382	19.379	143.365	149.695

† Resistance classes were defined based on the infestation index by sugarcane borer (II, number of bored internodes divided by total number of internodes in percentage).

The first-instar larvae of sugarcane borer were obtained from a laboratory mass rearing kept on artificial diet as described by the methodology of Hensley and Hammond (1968), with slight modifications (Araújo et al., 1985). The larvae were kept in the artificial diet until infestation in sugarcane plants.

2.2. Planting and plant development

Experimental plants were obtained from single-node stem cuttings containing one lateral bud. They were placed in plastic trays with the proper commercial substrate based on pine bark and coconut fiber (Tropstrato, Vida Verde Indústria e Comércio de Insumos Orgânicos Ltda, Mogi Mirim, SP, Brazil). Trays were daily irrigated. After 45 days, the seedlings were transplanted to 7.5 liter plastic pots containing a mixture of clay soil and washed sand in the 1:1 ratio in order to provide proper aeration and root development. Each seedling was transplanted to an individual plastic pot. The plants were maintained in greenhouse benches with controlled environment [26 ± 10 °C; 75 ± 20% relative humidity (RH); 12 ± 2 h photoperiod] to avoid natural insect infestation. Limestone and fertilizers were applied to adjust soil pH according to the soil chemical

analysis, aiming to achieve suitable growth conditions (Korndorfer et al., 1999). Plants were irrigated by an automatic dripping system throughout the crop development period at a rate of 0.5 to 2.0 L pot day^{-1}.

2.3. Plant collection and infestation with sugarcane borer

The experiment was performed in a completely randomized design arranged in a 11 × 2 factorial (genotypes × before and after infestation) scheme. Four replicates per treatment were used. The basic experimental unit was one pot containing a single plant. Six-month-old single plants (no tillers or secondary stalks) of all genotypes were utilized for the chemical characterization of the constitutive epicuticular wax of sugarcane leaves. The sugarcane whorl of each plant was collected for epicuticular wax extraction. Furthermore, six-month-old single plants (no tillers or secondary stalks) of all genotypes were artificially infested with 20 larvae of first-instar (five to seven days), according to the methodology described by Tomaz et al. (2017). The larvae maintained on artificial diet were gently transferred to the whorl region with the aid of a brush. The success of the infestation was visually verified by the presence of scraping, holes and sawdust in the whorl region. After three days (72h) of infestation, the sugarcane whorl of each plant was also collected for chemical characterization of the induced epicuticular wax of sugarcane leaves.

2.4. Sample preparation and wax extraction

Epicuticular wax extraction from the sugarcane leaves was performed based on the methodology developed by Ferreira et al. (2005), with modifications. Bands of epicuticular wax were exposed by carefully removing and individualizing the leaves from the whorl and placed into a test tube with a cap. Crude wax was extracted with 50 mL of spectroscopic grade chloroform (Sigma-Aldrich, Missouri, United States) in two extractions of 25mL. The test tube was gently shaken during one minute for each extraction, avoiding cell disruption and intending to minimize extraction of internal plant constituents. The extract was filtered with qualitative filter paper and transferred to an Erlenmeyer flask and evaporated on a heating plate at 130°C until the solution volume was reduced to approximately 2 ml. The remaining hot suspension was transferred to a preweighed vial and the solution was evaporated to dryness in a 70°C water bath. Epicuticular wax samples were stored at -4°C.

After, a silylation procedure was performed following Purcell et al. (2005), with modifications. A 1 mL ampoule of trimethylchlorosilane: hexamethyldisilane: pyridine in the ratio (1: 3: 9) (Supleco) was added to the vial containing the refined wax. The derivatization was carried out for peak identification of fatty alcohols. The vial was kept in a water bath at 70°C for 20 minutes to perform the reaction. Samples were stocked at 4°C for 24 hours. Subsequently, the vial was kept for two minutes in a water bath and then filtered to another vial using a 0.2 μm PTFE syringe filter. Samples were hot injected in the gas chromatography-mass spectrometry (GC-MS) instrument.

2.5. Data acquisition

The GC-MS analysis was carried out in a Shimadzu PQ2010 Plus Gas Chromatograph-Mass Spectrometer (GC-MS). Separations were performed in a SH-Rtx-5MS column with 30 m, 0.25 mm of intern diameter and a film thickness of 0.25 μm. The injector worked in a split mode of 20:1 and the carrier gas was helium. The injector temperature was 320°C and the temperature of the detector was 350°C. The temperature program was 200°C for two minutes, ramping at 2°C/min to 300°C and holding for 15 minutes. The total running time was 67 minutes.

2.6. Data analysis

The obtained chromatograms from the sugarcane epicuticular wax were organized in a matrix, where the rows and the columns of the matrix were respectively the samples and the components. Because wax loads differed among the genotypes, the relative composition of the wax components was obtained. Peak area data of the principal GC peaks were integrated and manually collected with GCMSsolution version 4.20 (Shimadzu Corporation). Components were identified by their mass spectral fragmentation patterns and by comparison of their gas chromatographic retention times with synthetic samples. Components were confirmed with the aid of the reference library of spectral mass NIST14 from the National Institute of Technology (NIST).

Logistic regression was applied with the aim of determining the relative importance of each epicuticular wax component in the genotype classification for each condition: before infestation (BI), after infestation (AI) and combined analysis (BI+AI). Genotypes were grouped according to their resistance field-based ratings in resistant or susceptible. The impact of each explanatory variable (components) on the binomial

categorical response variable (susceptible = 0 and resistant = 1) was evaluated. Logistic regression is a statistical decision support tool from generalized linear models that can be used to predict the probability of occurrence of binary events by fitting the data of input variables to a logistic curve (Agresti, 2007). Adjusted models were selected based on the Akaike information criterion (AIC) value, which is an estimator of the relative quality of statistical models for a given dataset (Akaike, 1974). Therefore, smaller AIC values are associated with better overall model fitness and provide a means for model selection.

In model prediction, a cutoff of 0.5 was adopted, i.e., individuals i, (i = 1,2,3,...,n) with a resistance probability above 0.5 were classified as resistant. The resistance probability was estimated using the following logistic regression model: $\hat{p}_i = \exp(\sum_{j=0}^{k} \hat{\beta}_j x_{ij})/(1 + \exp(\sum_{j=0}^{k} \hat{\beta}_j x_{ij}))$, where: $\hat{\beta}_j$ = regression coefficients; x_{ij} = values of the explanatory variables (sugarcane epicuticular wax components) j in each individual i, and k = the number of variables.

Moreover, the apparent error rate (AER) indicates the number of individual that were classified incorrectly by the logistic model utilized in relation to the total number of individuals evaluated in the experiment. The AER of the regression models was calculated by the formula: $AER = \frac{1}{N} \sum_{j=1}^{r} m_j$, where N = total number of observations, r = number of responses (Resistant = 1 or Susceptible = 0), and m_j = number of incorrect classifications in each response.

In order to evaluate the prediction, a confusion matrix was generated. A confusion matrix is a tool used for the effective measurement of a classifier by exhibiting the number of correct classifications using the selected variables (sugarcane epicuticular wax components) versus the number of classifications predicted by the classifier considered for each class based on the test set. A general diagram of the confusion matrix is displayed in Table 2.

Table 2 - General diagram of the confusion matrix and some derived measures of interest.

	Predicted			
Real	S*	R	Total	**Measures of interest**
S	TN	FP	N_2	TPR = TP/P_2
R	FN	TP	P_2	AER = (FN/FP)/N
Total	N_1	P_1	N	

*S = Susceptible; R = Resistant; TN = True Negative; FP = False Positive; FN = False negative; TP = True Positive; FPR = False Positive Rate, TPR = True Positive Rate and AER = Apparent Error Rate. Real indicates the field-based ratings and predicted exhibits the classification by the adjusted logistic regression model.

From the several measures that can be obtained from the confusion matrix, the AER indicates the number of samples that were classified incorrectly by the classification model utilized. Moreover, the true positive rate (TPR) is a measure of interest that reveals the percentage of correctly classified samples by the utilized classification method. Statistical analyzes were performed using R software version 3.5.1 (R Development Core Team, 2018).

3. RESULTS AND DISCUSSION

3.1. Identification of sugarcane epicuticular wax components

The sugarcane epicuticular wax compounds identified and confirmed are exhibited in Table 3. The major components observed where the C32, C30 and C28 fatty alcohols, the C30, C28 and C26 aldehydes and the C28 fatty acid. In total, 18 components were identified from five different functional groups, namely fatty alcohols, aldehydes, fatty acids, alkanes, and triterpenes. The identification of the C26 (hexacosanol), C28 (octacosanol), C30 (triacontanol) and C32 (dotriacontanol) fatty alcohols was performed by means of derivatization of fatty alcohol standards. Aldehydes identified were C23 (tricosanal), C26 (hexacosanal), C28 (octacosanal) and C30 (triacontanal). Moreover, alkanes identified comprised C20 (eicosane), C21 (heneicosane), C28 (octacosane), C30 (triacontane) and C34 (tetratriacontane). Furthermore, the remaining fatty acids C24 (lignoceric acid), C28 (octacosanoic acid) and C30 (triacontanoic acid), the C30 pentacyclic triterpene (friedelin) and the C30 tetracyclic triterpene (lanosterol) were confirmed. Fatty acids, alkanes, aldehydes, and triterpenes identification was based on the comparison of the acquired spectral mass data with the data available in the reference library of spectral mass NIST14 from the National Institute of Technology (NIST).

The majority of the identified compounds were also observed in sugarcane wax in previous researches. In early studies of sugarcane wax composition, Lamberton and Redcliffe (1960) concluded that the sugarcane stalk cuticle wax presented a predominance of long-chain aldehydes, free alcohols and acids, differing from other plant species. Meanwhile, Rutherford and Van Staden (1996) found that the major

components of the sugarcane stalk surface wax were the C26, C28, C30 aldehydes and the C28 fatty alcohol. Nevertheless, the presence of triterpenes is not surprising as these compounds are usually found in leaf tissues of sugarcane plants (Jäger et al., 2009; Attard et al., 2015).

Table 3 - Identified components of the sugarcane epicuticular wax collected from the whorl of 11 genotypes and analyzed by GC-MS.

Peak number	RT (min)	Wax Components	Molecular Formula	Functional Group
1	26.145	Eicosane	$C_{20}H_{42}$	Alkane
2	28.990	Heneicosane	$C_{21}H_{44}$	Alkane
3	30.811	Hexacosanol	$C_{26}H_{54}O$	Fatty Alcohol
4	33.704	Hexacosanal	$C_{26}H_{52}O$	Aldehyde
5	35.464	Octacosane	$C_{28}H_{58}$	Alkane
6	36.943	Octacosanol	$C_{28}H_{58}O$	Fatty Alcohol
7	39.748	Octacosanoic acid	$C_{28}H_{56}O_2$	Fatty Acid
8	39.906	Octacosanal	$C_{28}H_{56}O$	Aldehyde
9	41.479	Triacontane	$C_{30}H_{62}$	Alkane
10	42.105	Lanosterol	$C_{30}H_{50}O$	Triterpene
11	42.822	Triacontanol	$C_{30}H_{62}O$	Fatty Alcohol
12	45.432	Triacontanoic acid	$C_{30}H_{60}O_2$	Fatty Acid
13	45.739	Triacontanal	$C_{30}H_{60}O$	Aldehyde
14	47.715	Friedelin	$C_{30}H_{50}O$	Triterpene
15	48.294	Dotriacontanol	$C_{32}H_{66}O$	Fatty Alcohol
16	50.944	Lignoceric acid	$C_{24}H_{48}O_2$	Fatty Acid
17	51.456	Tricosanal	$C_{23}H_{46}O$	Aldehyde
18	53.848	Tetratriacontane	$C_{34}H_{70}$	Alkane

Gas chromatography profiles of the epicuticular wax of all sugarcane genotypes exhibited nearly all the same components, with variation in the relative quantities. Comparisons of the relative abundance in the GC-MS chromatograms clearly demonstrates different epicuticular wax profiles and highlights the variability among genotypes. For instance, one gas chromatogram from the RB867515 genotype sample in the AI condition was randomly selected from the available GC-MS profiles with the purpose of component identification (Figure 1). The chromatogram running time goes from zero to 67 min but it was enlarged for the retention times from 20 to 55 minutes, once the identified components were in this interval.

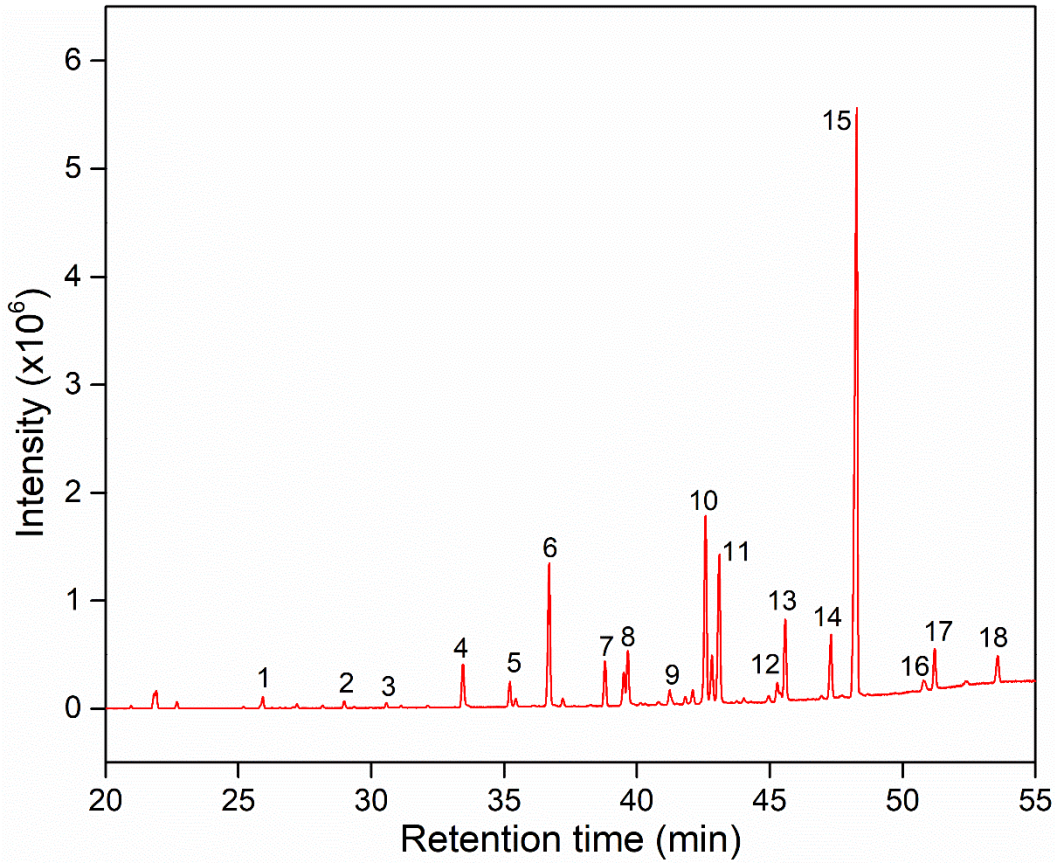

Figure 1 – GC-MS chromatogram of the refined epicuticular wax from the whorl of the RB867515 genotype after infestation, displaying the 18 identified and preselected components.

The peak number 14 associated with C30 pentacyclic triterpene (friedelin) presented a specific peak pattern variation across the different genotypes. The particular compound was absent in both conditions BI and AI with sugarcane borer in all samples of the genotypes classified as susceptible (RB047016, RB057231, RB047248, and RB057249). Meanwhile, the presence of friedelin peaks was observed in both conditions BI and AI in all the resistant genotypes, with the exception of the RB047050 genotype. Friedelin was surely produced by sugarcane plants and not deposited on the whorl by fungi or bacteria production because the chair-boat-chair conformation used by them in sterol biosynthesis only allows the production of lanosterol and not friedelin. Meanwhile, plants use the chair-chair-chair conformation and are allowed to synthetize friedelin (Alves et al., 2018).

Triterpenes are produced by plant secondary metabolism and play an important role in membrane structural and hormonal functions and plant defense (Alves et al., 2018). Moreover, terpenoids can target the arthropod nervous system acting on inhibition of acetylcholine esterase, deterrent action on feeding owing to physical

barrier and bitterness, and growth and development inhibition acting as a pheromone analog (Després et al., 2007). Moiteiro et al. (2006) studied the insecticidal potential of friedelin and derivatives by choice feeding bioassays and oral cannulation. Friedelin was known to have negative postingestive effects on sixth-instar *Spodoptera littoralis* larvae. Orally injected *S. littoralis* larvae with friedelin exhibited reduced biomass gains and food consumption. Thereby, friedelin acted as postingestive antifeedant and the lack of short-term feeding behavior modulating its effects on insects of the same order (Lepidoptera) as sugarcane borer indicated that it could be a digestive toxin. To the best of our knowledge, nothing is known about friedelin action on sugarcane borer larvae, leading to further studies in order to grow the understanding of the complex metabolic pathways of this compound.

Although many triterpene synthases have been described, the friedelin synthase genes from only two species were characterized and have been functionally described: *Kalanchoe daigremontiana* (Crassulaceae) (Wang et al., 2010) and *Maytenus ilicifolia* (Celastraceae) (Souza-Moreira et al., 2016). There is no recorded information about friedelin synthase genes, genomic features and alleles in the sugarcane transcriptome and neither in the genomes or transcriptomes of panicoids species related to sugarcane. However, gene coding for cycloartenol synthase was found in the sugarcane genome, being an orthologous gene of the friedelin synthase gene, which are genes related by vertical descent from a common ancestor and encode proteins with the same function in different species. The friedelin synthase catalyzes the following reaction: (S)-2,3-Epoxysqualene <=> Friedelin (R09910 Kegg reaction). Meanwhile, cycloarternol synthase catalyzes the following reaction: (S)-2,3-Epoxysqualene <=> Cycloartenol (R03200 Kegg reaction) and does not catalyze the biosynthesis of friedelin. Analyzing the information regarding cycloartenol synthase in *Arabidopsis thaliana* in the Swiss-Prot database of UniProtKB-P38605, we found mutations of specific amino acid residues that make cycloartenol synthase produce alternative products instead of cycloartenol. Therefore, we hypothesize that the cycloartenol synthase enzyme of the sugarcane genome contains mutations that allow the biosynthesis of friedelin.

Furthermore, Alves et al. (2018) found statistically significant less friedelin biosynthesis with the accumulation of precursors of the ergosterol pathways such as lanosterol. Lanosterol accumulation should be avoided in order to improve friedelin production because the compound is also a product of the 2,3-oxidosqualene

cyclization, competing and consuming the friedelin synthase substrate. Nevertheless, the gene encoding lanosterol synthase cannot be completely deleted without ergosterol addition because it is an essential compound. The ergosterol addition raises the production cost of the reaction by decreasing the substrate competition of 2,3-oxidosqualene by lanosterol and friedelin synthases (Alves et al., 2018). Besides the competition between friedelin and lanosterol accumulation, we observed that lanosterol appears to be associated with sugarcane borer susceptibility in the conditions before infestation and combined analysis (Tables 4 and 6). Therefore, genotypes with lower constitutive lanosterol content are desired for sugarcane borer resistance, contributing to reduce the substrate competition with the friedelin synthase and allowing higher friedelin content in sugarcane plant tissues.

3.2. Logistic regression and relative importance of components in resistance classification

After observing the specific peak pattern variation of friedelin and its level of reproducibility, data from the remaining 17 components were chosen for further statistical analysis. The relation between resistance classes and chemical sugarcane wax composition variables was explored through logistic regression. Friedelin peak data was not included in the analysis because the algorithm of the logistic regression did not converge, as the entire peak area data of the susceptible class were null for this component. Data used for further statistical analyzes was in the format of integrated peak areas obtained from each gas chromatogram, which reflected the fractionation of surface wax volatile components.

Not all of the 17 components exhibited significant effects in the logistic regression model fitted to the resistance classification applied in the BI condition. The smaller AIC value for the model adjustment was obtained with the elimination of these components, as they did not contribute on resistance classification. The complete model presented AIC=38.71, while the reduced model presented AIC=28.92, meaning that when variable selection was performed, the reduced model improved its predictive fitness in relation to the full model. Therefore, only four evaluated components have some influence on the class distinction (Table 4). The four components with significant regressions coefficients were the peak numbers 8, 9, 10 and 18, relating respectively to the octacosanal, triacontane, lanosterol, and tetratriacontane components.

Table 4 – Estimates of the regression coefficients ($\hat{\beta}_j$), standard error of the sample mean (SE), p-value, significance (Signif.) and odds ratio (OR) of the explanatory variables (components) used in the logistic regression model fitted to the genotype classification (Susceptible=0 and Resistant=1) before the infestation with sugarcane borer.

Peak number	Component	RT[†]	$\hat{\beta}_j$	SE	p-value[‡]	Signif.	OR
8	Octacosanal	39.906	0.230	0.114	0.045	*	1.259
9	Triacontane	41.479	2.897	1.587	0.068	.	18.117
10	Lanosterol	42.105	-0.829	0.407	0.042	*	0.436
18	Tetratriacontane	53.848	-1.333	0.718	0.064	.	0.264
	Intercept		2.477	1.583	0.118		

[†]Retention time in minutes; [‡]p-value: probability values associated with the z statistic of Wald; * Significant at 5% of probability by the Wald test; · Significant at 10% of probability by the Wald test.

The parameter estimates of the coefficients of predictor variables were generated from the evaluated samples and interpretation is similar to multiple linear regression. A significant coefficient implies that the predictor variable significantly influences the decision to either classify a plant as resistant or susceptible. Odds ratio statistics has more practical interpretation than the regression coefficients. Values of the odds ratios (exp ($\hat{\beta}_j$)) reflect the effect of each component on the probability of resistance classification by the logistic regression. Therefore, in this order, triacontane, octacosanal, lanosterol and tetratriacontane were the most important constitutive components for class separation in the BI condition. According to the odds ratio, a plant sample with higher constitutive amounts of triacontane has a 18.12 times greater chance of being classified as resistant than a plant with lower triacontante content. OR>1.0 is associated with components that contribute to the sugarcane borer resistance. Meantime, components with OR<1.0 contribute to better class separation of the susceptible genotypes. Therefore, a plant sample with higher constitutive contents of lanosterol and tetratriacontane appears to be associated with borer susceptibility. Moreover, this indicates that when the epicuticular wax of a sugarcane genotype is high in constituent compounds triacontane and octacosanal, that genotype should be categorized as well performing or resistant, while a genotype that is high in compounds lanosterol and tetratriacontane should be classified as susceptible.

Greater triacontane content was also evidenced in the total wax of water-stressed cotton leaves, bracts and bolls in comparison to cotton plants under well-watered conditions, suggesting that triacontane may have a defensive role in protecting the plants from adverse environmental conditions such as water-stress (Bondada et al.,

1996). The authors observed that triacontane has a higher contact angle and is less wettable, with greater hydrophobicity of the organs, contributing to reduced penetration of chemicals into the plant organs.

In the AI condition, the full model with the 17 components exhibited an AIC of 33.22. According to the obtained results in the condition of plant interaction with sugarcane borer, we also verified the absence of significant effects of fourteen components in the complete logistic regression model. In this case, we likewise decided to eliminate these components from the analysis for having no influence on the resistance classification. After removing the components with no significant effects, the reduced model with better adjustment was composed by three fatty alcohols with significant regression coefficients (Table 5). The components selected by the adjusted logistic regression model that contributed for the resistance discrimination in decreasing order of importance were the peak numbers 6, 15 and 3, which are respectively associated with the C28, C32 and C26 fatty alcohols.

Table 5 - Estimates of the regression coefficients ($\hat{\beta}_j$), standard error of the sample mean (SE), p-value, significance (Signif.) and odds ratio (OR) of the explanatory variables (components) used in the logistic regression model fitted to the genotype classification (Susceptible=0 and Resistant=1) after the infestation with sugarcane borer.

Peak number	Component	RT[†]	$\hat{\beta}_j$	SE	p-value[‡]	Signif.	OR
3	Hexacosanol	30.811	-0.318	0.138	0.021	*	0.728
6	Octacosanol	36.943	0.048	0.022	0.029	*	1.049
15	Dotriacontanol	48.485	-0.016	0.006	0.010	**	0.984
	Intercept		4.022	1.525	0.008	**	

[†]Retention time in minutes; [‡]p-value: probability values associated with the z statistic of Wald; ** Significant at 1% of probability by the Wald test; * Significant at 5% of probability by the Wald test.

Analyzing the OR statistics, the presence of higher quantities of octacosanol (C28) component is more associated with the resistant class of genotypes. The expression of the C28 alcohol appears to be increased after the interaction between the sugarcane borer and the sugarcane plant, characterizing the inducible resistance. However, a plant with higher hexacosanol (C26) content has greater chances of classification as borer susceptible genotype. Therefore, induced resistance mechanism appears to be associated with longer carbon chain length of fatty alcohols. Moreover, Rutherford and Van Staden (1996) concluded that the fatty alcohol fraction decreases as a portion of the total with susceptibility and the C28 fatty alcohol was associated with

resistance to stalk borer. However, these authors concluded that the C26 fatty alcohol was associated with resistance in disagreement with our result in the AI condition. On the other hand, our results are in conformity with Purcell et al. (2005), which found that C26 fatty alcohol was associated with susceptibility to a particular resistant trait classified by conventional field-based rating systems.

Plant-host resistance occurs by incompatible interactions involving resistant plants and avirulent pest arthropods by producing allelochemical and biophysical plant traits that adversely affect arthropod behavior and survival or that allow plants to tolerate arthropod damage. Both constitutively produced proteins by resistance gene products and proteins via jasmonate and other signaling pathways induced by arthropod herbivory or oviposition mediate these incompatible interactions (Smith & Clement, 2012). The duration of induction of arthropod resistance varies greatly and defense response gene upregulation is still poorly known. According to Smith and Clement (2012), there is sparse evidence that some of the defense genes are downregulated in the initial hours after the beginning of arthropod attack and subsequently upregulated during following days.

Temporal progression of induced responses can be studied in different time scales. Pre-formed induced responses are observed promptly after injury and are restricted to injured tissues. Rapidly induced responses occur in one or two hours or days of plant injury and may be localized or systemic and remain in effect for days. Delayed-induced responses occur in the next season plant organs and may remain effective for years (Baldwin, 1989). Our study focused on the two first types of induced responses, being concerned with rapid plant responses that operate on relatively short timescales as delayed induced responses might not be effective to deter initial herbivory. The induction costs and effectiveness are established by the parameters time-lag between injury and onset of defense and between injury cessation and defense mitigation. The first time-lag is likely to be determined by physiological constraints of increasing the expression of producing new components. Time-lags regarding chemical defenses tend to be shorter and independent of new tissue development (Karban, 2010). Furthermore, one simple justification of induced resistance mechanism could be genetic redundancy as some of the biosynthetic genes of the identified fatty alcohols may have family members that are not critical for epicuticular wax deposition during plant development but are responsive to sugarcane borer attack.

The combined analysis (BI+AI) was performed in order to generate information regarding the resistance classification in a condition where there is no full assurance of borer infestation in all plants. As is performed with multiple linear regressions, variable selection is used to eliminate non-significant input variables from the model. According to the obtained AIC values, a better fit was observed in the reduced model (AIC = 71.75 for the full model vs. AIC = 45.57 for the reduced model). The smaller values of AIC reflect a better overall fit (Akaike, 1974). The logistic regression analysis with the reduced model generated the regression coefficients, the Wald statistics and the odds ratios that were all highly significant for all genotypes (Table 6). The high level of significance indicates model robustness and prediction reliability. According to Agresti (2007), model prediction is expected to be unreliable if the studied statistics are not significant. Moreover, this condition of high-level significance is an indicative of adequacy of the database for this type of analysis.

Table 6 - Estimates of the regression coefficients ($\hat{\beta}_j$), standard error of the sample mean (SE), p-value, significance (Signif.) and odds ratio (OR) of the explanatory variables (components) used in the logistic regression model fitted to the genotype classification (Susceptible=0 and Resistant=1) in the combined condition: BI and AI with sugarcane borer.

Peak number	Component	RT†	$\hat{\beta}_j$	SE	p-value	Signif.	OR
3	Hexacosanol	30.811	-0.243	0.111	0.029	*	0.784
4	Hexacosanal	33.704	0.155	0.073	0.034	*	1.167
5	Octacosane	35.464	0.420	0.144	0.004	**	1.522
10	Lanosterol	42.105	-0.175	0.080	0.030	*	0.839
11	Triacontanol	42.822	0.037	0.015	0.019	*	1.038
15	Dotriacontanol	48.485	-0.032	0.009	0.000	***	0.968
	Intercept		5.544	1.862	0.003	**	

†Retention time in minutes; ‡p-value: probability values associated with the z statistic of Wald; *** Significant at 0.1% of probability by the Wald test; ** Significant at 1% of probability by the Wald test; * Significant at 5% of probability by the Wald test.

In the reduced model of the combined analysis, the most influential components for the resistance classification probability were in the following order octacosane (C28), hexacosanal (C26), triacontanol (C30), dotriacontanol (C32), lanosterol (C30) and hexacosanol (C26). According to the OR values, plants with higher contents of C28 alkane, C26 aldehyde and C30 fatty alcohol appears to be associated with the mechanism of resistance to the sugarcane borer. On the other hand, plants with increased content of lanosterol or C26 fatty alcohol have increased chances of classification as susceptible to the initial attack of sugarcane borer on the new leaves of

the sugarcane whorl. As a result of the analysis, the final selection and further evaluation of the sugarcane population can focus on identifying genotypes holding the desired combination of the significant components of the sugarcane epicuticular wax.

The C30 alcohol, triacontanol, was also associated to a particular resistance trait in a chemometrics investigation of sugarcane properties based on the molecular composition of epicuticular wax (Purcell et al., 2005). Triacontanol is a plant secondary metabolism compound that exhibits regulatory growth activity, increasing yield, photosynthesis, nitrogen fixation, enzymatic activity, reducing sugars and protein solubilization. Moreover, triacontanol is a growth regulator that increases the cell physiological efficiency and allows exploring the plant genetic potential to a great extent (Naeem et al., 2012). In addition, triacontanol is known to influence insect behavior in other plant species. For instance, the waxes of the Arabidopsis Cer3 mutant exhibited a remarkable increase in the triacontanol content. The mutation caused greater mobility and reduced the acceptance of cabbage aphid (*Brevicoryne brassicae*) on the host plant (Rashotte et al., 1997).

Surface wax variation in plants has considerable extent dependence on regulatory genes as well as biosynthetic genes (Jenks & Ashworth, 2010), which could be manipulated. Moreover, contact with surface wax chemicals often suffices to prevent insects from further investigation of the plant and may cause inhibitory effect on test bite or feeding behavior from an intact plant. Behaviorally active phytochemicals include attractants, repellents, and deterrents with a primarily non-toxic mode of action and a safe and long-lasting method of insect management.

Once ingested, the compounds can reduce the efficiency of food utilization or interfere with vital metabolic processes. Antifeedants require very low doses to be effective, in the order of less than 1.0 part per million (ppm) (Schoonhoven et al., 2005). Antifeedants are more effective when acting concomitantly on several behavioral and physiological mechanisms. Possibly sugarcane borer responds to a combination of wax components in the host identification and acceptance phase. The complex mixture of wax components found in sugarcane may provide synergistic effects in defense against arthropod herbivory and hinders borer adaptations. For instance, Hummelbrunner and Isman (2001) found that a combination of two monoterpenoids has increased toxicity on

Spodoptera litura (tobacco cutworm) in almost ten times in comparison to the predicted simple additive effect.

The inconsistencies between our results and those found by Rutherford and Van Staden (1996) and Purcell et al. (2005) do not challenge the validity of their findings. Firstly, we analyzed the epicuticular wax composition of the sugarcane whorl while they analyzed the stalk wax. Secondly, Rutherford and Van Staden (1996) studied the sugarcane stalk borer *Eldana sacharina* whereas we investigated the sugarcane borer *Diatraea saccharalis* and the two species even related may somewhat differ in their reactions to wax components.

3.3. Prediction from calibration set of sugarcane borer resistance classification based on adjusted logistic regression models

The reduced logistic regression models fitted to the conditions of BI, AI, and combined analysis were efficient in predicting the probability ($\hat{p}_i$) of borer resistance classification of the calibration set of sampled individuals. In total, 29 individuals reached the resistance classification threshold ($\hat{p}_i >$ 0.5) in the condition AI with sugarcane borer (Table 7). In the combined analysis, 54 individuals exhibited a resistance probability above 0.5 and this logistic regression model exhibited nearly 95% prediction accuracy.

Table 7 – Confusion matrix with the resistance classifications using the logistic regression models in the conditions before infestation (BI), after infestation (AI) and combined analysis (BI+AI), accompanied by the measures of interest apparent error rate and true positive rate.

Experiment	Real	Predicted		Total	AER†	TPR
		Susceptible	Resistant			
BI	Susceptible	14	2	16	13.63	0.857
	Resistant	4	24	28		
	Total	18	26	44		
AI	Susceptible	13	3	16	11.36	0.929
	Resistant	2	26	28		
	Total	15	29	44		
Combined Analysis	Susceptible	30	2	32	6.81	0.929
	Resistant	4	52	56		
	Total	34	54	88		

† Apparent error rate in percentage of individuals classified incorrectly.

These results demonstrate the efficiency of logistic regression for determining the relative importance of each sugarcane epicuticular wax component in the sugarcane borer resistance classification. Furthermore, the values for AER were low for all tested conditions when the reduced models of logistic regression were utilized. The mean AER values for BI, AI, and combined analysis were respectively of 13.63, 11.36 and 6.81%. Therefore, an AER of 6.81% in the combined analysis signifies that on average only 6.81% of the sampled individuals were misclassified for their reaction to the sugarcane borer.

Regarding the TPR measure of interest, the logistic regression models presented satisfactory prediction performance. The mean TPR values for BI, AI, and combined analysis were respectively of 0.857, 0.929 and 0.929. Thus, a TPR value of 0.929 suggests that the classifier under study was able to classify as resistant 92.9% of the individuals that were also characterized with resistant reaction to the sugarcane borer by the method considered real by the field-based trials in several environments. The results of the comparison between the classifications obtained by the predictions based on the reduced logistic regression models and the real field-based ratings were graphically represented (Figure 2).

Flawlessly, all the green dots should be placed above the classification threshold of 0.5 and all the red dots under the threshold. In the BI condition, only two samples of the susceptible class were misclassified, while four samples of the resistant class were misplaced. If the threshold would be lowered to 0.4, three susceptible samples would be classified as resistant (false positive) and only one sample of the resistant class would be classified as susceptible (false negative), reducing the AER and increasing the prediction accuracy. Meantime in the combined analysis, only four false negatives (green dots below the threshold) and two false positives (red dots above the threshold) were found. Therefore, if genotypes predicted to be susceptible were discarded from a selection program, only 4.5% of resistant genotypes would be lost based on the model classification. Thus, the adjusted logistic regression models presented proper sample discrimination of the dataset.

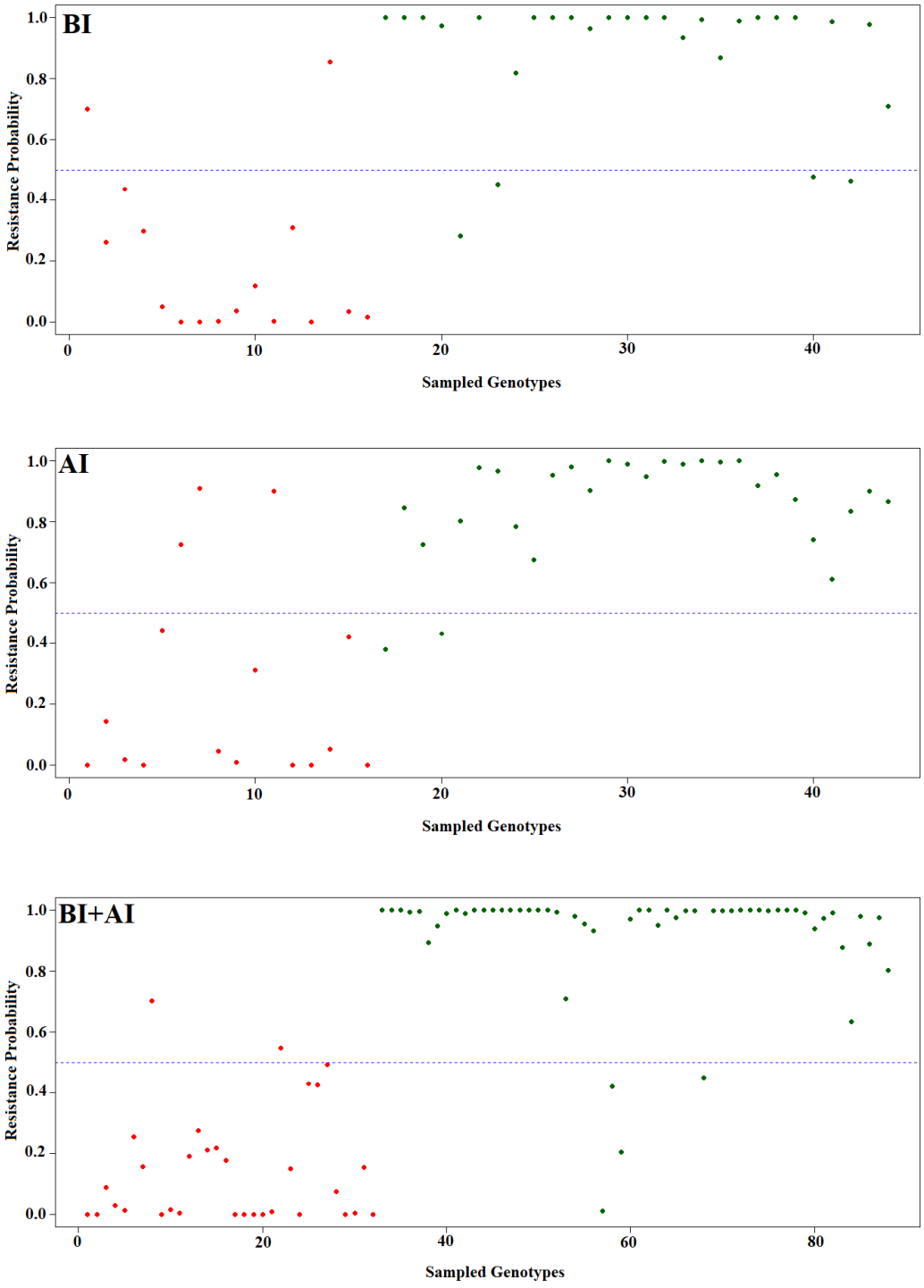

Figure 2 – Predicted classification of the GC-MS data set in the classes of resistance and susceptibility to the sugarcane borer in the conditions before infestation (BI), after infestation (AI) and combined analysis (BI+AI) respectively with 44, 44 and 88 samples. Green dots represent samples classified in conventional field-based rating experiments as resistant and red dots represent samples classified as susceptible to the sugarcane borer. The dashed black line indicates the selected classification threshold of 0.5.

The results obtained in our study can be helpful for plant breeding seeking to develop host-plant resistance to sugarcane borer, deploying resistant cultivars with

broader, more durable resistance that reduces the occurrence of arthropod virulence. Therefore, polygenic resistance is not per se more durable, especially when it involves the concentration of a single chemical compound. Durable and long-lasting resistance combining multiple chemical, physiological, or morphological mechanisms reduces the selection pressure of an arthropod breaking resistance. The structures of plant secondary cell walls have developed to allow physical protection against insect attack by feeding and tissue entrance. The plant cell wall composition is dynamic and structure modifications can be observed as induced defense responses (Zhao & Dixon, 2014), which are associated with expression of a set of insect defense genes. A consistent trend in differences regarding cell wall structure of stalks of genotypes discriminated as resistant or susceptible to the sugarcane borer was not identified (Table 1). Solid differences in fiber content in the stalks is not expected as these genotypes were obtained from crossing between nobilized parental clones and tend to exhibit steady fiber content in the stalks. Nobilization refers to the natural interspecific hybridization events between the wild cane *S. spontaneum* and the noble cane *S. officinarum* and further backcrossing of the progenies with the noble one (Yu et al., 2018). Greater fiber content in the stalks of the IM76-228 is expected because it is germplasm in domestication as an interspecific hybrid of *Saccharum robustum*. However, this genotype was not assessed in these field trials.

Application of GC-MS metabolic profiling enabled the characterization of epicuticular wax composition in the sugarcane whorl and provided relative quantitative comparisons among sampled genotypes, thus contributing to the understanding of wax composition associated with an important phenotypic trait in a major crop. Furthermore, findings from an initial feasibility study suggest that chemical differences exist in sugarcane epicuticular wax that can be associated with resistance or susceptibility to the sugarcane borer. According to Barbosa et al. (2012b), the sugarcane cultivar development begins with several hybridization among the chosen parental genotypes. Millions of seedlings are evaluated annually in the first phase (T1). Fewer following selected clones are assessed in the second and third phases under representative environmental conditions. Our results can contribute to the development of a screening methodology to assess sugarcane borer reaction in the initial phase of the selection cycle, where greater genetic variability is found. Besides including early selection to borer resistance, reduced time required by plant breeders to rank and select the plants is

expected. Cause and effect relations between GC-MS identified components and resistance or susceptibility to sugarcane borer remain to be shown. Experiments can be design to investigate behavioral or feeding responses to different epicuticular wax components and demonstrate cause and effect.

4. CONCLUSION

The evaluation of chemical composition of epicuticular wax of sugarcane leaves suggested the existence of chemical differences between the tested genotypes and allowed discrimination between susceptibility and resistance reaction to sugarcane borer.

Reduced logistic regression models allowed the evaluation of the relative importance of the epicuticular wax components in the resistance classification, generating more robust and interpretative models and predicting the individuals of the calibration set most likely classified as resistant or susceptible in the conditions before and after infestation and combined analysis in early selection stages.

Triacontane, octacosanal, lanosterol and tetratriacontane were the most important components in the classification of resistant and susceptible genotypes before borer infestation. After infestation, the compounds selected by the adjusted model were octacosanol, dotriacontanol and hexacosanol. In the combined analysis, the most relevant components were octacosane, hexacosanal, triacontanol, dotriacontanol, lanosterol and hexacosanol.

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