ADRIANO CIRINO TOMAZ

GENETIC DIVERGENCE AND RESISTANCE OF SUGARCANE GENOTYPES TO *Diatraea saccharalis*

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

VIÇOSA
MINAS GERAIS - BRASIL
2014

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T

T655g
2014

Tomaz, Adriano Cirino, 1986-
Genetic divergence and resistance of sugarcane genotypes to *Diatraea saccharalis* / Adriano Cirino Tomaz. - Viçosa, MG, 2014.
vii, 46f. : il. ; 29 cm.

Orientador: Márcio Henrique Pereira Barbosa.
Dissertação (mestrado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Cana-de-açúcar - Doenças e pragas. 2. *Diatraea saccharalis*. 3. Melhoramento de cultivos agrícolas . I. Universidade Federal de Viçosa. Departamento de Fitotecnia. Programa de Pós-graduação em Fitotecnia. II. Título.

CDD 22. ed. 633.61

ADRIANO CIRINO TOMAZ

GENETIC DIVERGENCE AND RESISTANCE OF SUGARCANE GENOTYPES TO *Diatraea saccharalis*

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

APROVADA: 21 de julho de 2014.

Júlio Cláudio Martins

Eugênio Eduardo de Oliveira

Eliseu José Guedes Pereira
(Coorientador)

Márcio Henrique Pereira Barbosa
(Orientador)

À meu pai João, minha mãe Cleusa e minha irmã Adriana, com quem aprendi tudo de importante.

Dedico**.**

AKNOWLEDGEMENTS

First I would like to thank the University Federal of Viçosa for giving me this opportunity.

I would like to thank the "Coordenação de aperfefeiçoamento de pessoal de nível superior" – Capes for providing me the scholarship. I also would like to thank the "Rede interuniversitária para o desenvolvimento do setor sucroalcoeiro" – RIDESA and FAPEMIG and CNPq for the financial support.

I would like to give a special thanks to my adviser Dr. Márcio Henrique Pereira Barbosa for the supervision, assistance and friendship. I am also thankful to my co-advisers Dr. Eliseu José Guedes Pereira and Dr. Luis Alexandre Peternelli for the support and Dr. Eugênio Eduardo and Dr. Júlio Cláudio Martins to participate as members of the committee.

A very special thanks for all the team of CECA – Centro de pesquisa e melhoramento genético da cana-de-açúcar for the assistance in the field experiments. I would like to thank also the people of the labs: biotecnologia e melhoramento vegetal, Ecotoxicologia, Manejo Integrado de Pragas, Fisiologia dos insetos and Interação inseto-planta for the good acquaintanceship.

I would like to thank all the team of sugarcane research for the friendship and knowledge exchange. A very special thanks to the intern Alan for the help, loyalty and friendship.

Finally but no less important, I would like to thank my family: My mother Cleusa, my father João and my sister Adriana for the support, to believe in me and to teach everything I know in my life.

CONTENTS

RESUMO

TOMAZ Adriano Cirino, M.Sc. Universidade Federal de Viçosa, julho de 2014. **Divergência genética e resistência de genótipos de cana-de-açúcar à Diatraea saccharalis.** Orientador: Márcio Henrique Pereira Barbosa. Coorientadores: Luiz Alexandre Peternelli e Eliseu José Guedes Pereira.

A broca-da-cana Diatraea saccharalis Fab. (Lepidoptera: Crambidae) é a principal praga da cana-de-açúcar no Brasil. No entanto, apesar da importância dessa praga, não há métodos precisos para comparação da resistência de genótipos e seleção de genótipos resistentes a serem aplicado por programas de melhoramento genético de cana-de-açúcar. Esse estudo foi conduzido para determinar um método para comparar injúria causada por broca-da-cana no colmo de genótipos de cana-de-açúcar em casa-de-vegetação, investigar características de resistência presentes na folha, superfície e interior do colmo de seis genótipos de cana-de-açúcar e a relação entre esses fatores de resistência. Adicionalmente, foi feito um estudo sobre divergência genética de genótipos de cana-de-açúcar para resistência à broca-da-cana através do uso de análises de agrupamento. A infestação de plantas individuais (não em touceiras) com sete meses de idade com 20 lagartas (3º ou 4º instar) por planta para avaliação do comprimento de galerias foi adequada para comparar a injúria entre genótipos. Os genótipos SP803280, RB928064 e RB835486 apresentaram as menores injúrias no colmo enquanto o genótipo SP891115 apresentou a maior injúria. As lagartas gastaram mais tempo para penetrar nos colmos do genótipo SP803280 em relação ao genótipo SP891115, indicando que esse genótipo apresenta alguma característica na superfície do colmo que atrasa a entrada da lagarta. As lagartas nos últimos instares apresentaram o menor ganho de massa ao se alimentarem no interior do colmo do genótipo SP813250, indicando que esse genótipo apresenta algum fator de resistência afetando o desenvolvimento da larva dentro do colmo. As análises de correlação entre os fatores de resistência indicam que o tempo gasto pelas lagartas para entrar no colmo das plantas está mais diretamente relacionado com a injúria no colmo do que a alimentação das lagartas no interior do colmo. Os genótipos RB867515 e SP891115 causaram a mais alta mortalidade de lagartas novas durante o período em que se alimentam na folha, indicando que a presença de fatores de antibiose na folha desses genótipos. As análises de agrupamento dividiram os genótipos em três grupos indicando divergência genética entre os genótipos.

ABSTRACT

TOMAZ Adriano Cirino, M.Sc. Universidade Federal de Viçosa, July, 2014. **Genetic divergence and resistance of sugarcane genotypes to Diatraea saccharalis.** Adviser: Márcio Henrique Pereira Barbosa. Co-advisers: Luiz Alexandre Peternelli and Eliseu José Guedes Pereira.

The sugarcane stalk borer Diatraea saccharalis Fab. (Lepidoptera: Crambidae) is the most important pest of sugarcane in Brazil. However, despite the importance of this pest, there is a lack of efficient methods to compare the resistance of genotypes and select resistant sugarcane genotypes to be employed by sugarcane breeding programs. This study was conducted to determine a greenhouse method to compare stalk damage among sugarcane genotypes, investigate resistance factors present in the leaves, stalk surface and within the stalks of six Brazilian sugarcane cultivars and the relationship among these resistance factors. In addition, we assessed the genetic divergence of sugarcane genotypes to borer resistance by using cluster analysis. The infestation of 7-month-old single plants with 20 larvae (3^{rd} - 4^{th} instar) per plant for assessment of tunnel length was proved adequate to compare stalk damage among sugarcane genotypes. The genotypes SP803280, RB928064 and RB835486 had the lowest stalk damage while SP891115 had the highest damage. The larvae spent more time to enter the stalk of the genotype SP803280 compared to SP891115, indicating that this genotype has some resistance trait in stalk surface delaying the larvae entrance in the stalk. Late-stages larvae feeding in the stalk of SP813250 had the lowest weight gain indicating that this genotype present some resistance factor affecting larval development within the stalks. Correlation analysis among resistance factors indicated that the time spent by larvae to enter the stalk is more related to stalk damage than the larval feeding within the stalks. The genotypes RB867515 and SP891115 caused the highest mortality of early-stage larvae feeding on the leaves, which indicate the presence of antibiotic factors in the leaves of these genotypes. Cluster analysis divided the genotypes in three groups, indicating the genetic dissimilarity between genotypes. The results from this study may contribute in selection of resistant clones and choice of genitors for crossings in sugarcane breeding programs.

GENERAL INTRODUCTION

Brazil is the world's largest sugarcane producer with nearly 8.5 million cultivated hectares and a production of 600 million tons in 2012/13 crop season. Approximately half of the sugarcane produced in 2012/2013 crop season was used to produce 25 billion liters of ethanol (CONAB, 2012). To supply a significant part of the world's demand of renewable fuel, it is predicted that by 2020, the country will be planting around 14 million hectares, producing more than 1 billion tons of cane and 65 billion liters of ethanol (Matsuoka et al, 2009).

Insect pests constitute an important biotic stress among the various limiting factors that affect sugarcane production (Dinardo-Miranda, 2008). In general, insect pests have been projected to account for more than 10% of yield loss in sugarcane worldwide (Ricaud & Ryan, 1989).

The sugarcane stalk borer Diatraea saccharalis Fabr. (Lepidoptera: Crambidae) is the major pest of sugarcane in Brazil. This lepidopteran is widespread throughout the Americas and is considered a key pest in most countries where sugarcane is cultivated (Botelho, 1992). In Brazil, this pest is present in all states where sugarcane is cultivated, causing severe losses (Guevara & Wiendl, 1980).

D. saccharalis presents a complete biological cycle that includes egg, larvae, pupae and adult stages. The duration of the biological cycle is quite variable ranging from 53 to 60 days (Gallo et al., 2002) and it depends on several factors such as climate and host plant (Dossi et al., 2004). This insect normally presents four generations per year and under favorable climate conditions, it may reach up to five generations per year (Dinardo-Miranda, 2008). The sugarcane borer occurs during all sugarcane development cycle. However, its incidence is lower when the sugarcane is young and do not present developed internodes and increases as the plant develops (Dinardo-Miranda, 2008).

The newly hatched larvae feeds on the foliar parenchymal of sugarcane and then converges to the leaf-sheaths. Nearly 15 days after hatch, the larvae penetrate into the stalk, forming galleries from down upward. Then, when the larvae is about to pupate, it opens a hole in the stalk rind for the moth exit and closes it partially with silk and food waste (Gallo et al., 2002).

Damage is caused by larvae boring the stalks, causing the death of a large number of shoots when the sugarcane plantation is young, and a sharp reduction in

productivity in more developed sugarcane crops. The infested stalks lose weight, become smaller and thinner, and many wither and die, while others are broken by the wind action. Under favorable conditions, secondary pests such as Metamasius hemipterus (L.) (Coleoptera: Curculionidae), can also infest sugarcane stalks through the holes made by the borer, increasing losses in the field. The sugarcane borer favors the entrance of many microorganisms in the stalk, especially fungi that cause the "red rot" disease, which reduces sucrose content in stalks, due to its conversion into glucose and fructose. The microorganisms present in stalks contaminate the broth, hampering industrial processes, hindering the attainment of high-quality sugar and inhibiting fermentation (Botelho & Macedo, 2002; Dinardo-Miranda, 2008; Dinardo-Miranda et al., 2012). Studies conducted by Copersucar, SP, Brazil, in the late 1990's (Arrigoni, 2002), revealed that 1% of bored internodes caused losses of 1.50% in stalk productivity, 0.49% in sugar productivity and 0.28% in alcohol productivity (Dinardo-Miranda et al., 2012).

The development of resistant sugarcane cultivars to D. saccharalis is very important to the sugar and alcohol sectors owing the reduction of production costs and development of the crop (Demetrio et al, 2008). Although development of insect-resistant crop cultivars using classical breeding methods is a time-consuming and expensive process, the benefits may be enormous in terms of monetary return and reduced burdening of the environment with insecticides. The economic advantage of using pest-resistant cultivars is estimated to be a 120-fold greater return on investment and, no less importantly, some developed cultivars of cotton, rice, and vegetables contain insect resistance sufficient to reduce significantly the use of insecticides (Schoonhoven, 2005).

According to Smith (2005), plant resistance to arthropods is the sum of the constitutive, genetically inherited qualities that result in a plant of one cultivar or species being less damaged than a susceptible plant lacking these qualities. Plant resistance to arthropods must always be measured on a relative scale, with the degree of resistance based on comparison to susceptible control plants that are more severely damaged or killed under similar experimental conditions, as well as resistant control plants with a known, predetermined level of resistance. Relative measurements are necessary, since resistance is influenced by environmental fluctuations occurring over both time and space (Smith, 2005).

Three mechanisms of plant resistance are recognized, emphasizing those aspects of insect–plant relations that are relevant to insect resistance: nonpreference or antixenosis, antibiosis, and tolerance. Antixenosis is related to plant traits that affect the choice of that plant by insect for oviposition, food or shelter. Antibiosis is related to some factor in host plants that reduces fecundity, size, or longevity, or cause mortality of the attacking insect. Tolerance is a form of resistance in which the plant shows an ability to reduce insect damage without affecting the insect biology (Schoonhoven, 2005).

Although there is no much information about sugarcane resistance to D. saccharalis some studies in several sugarcane genotypes confirm that the main sugarcane resistance mechanisms to borers are antibiosis and antixenosis (Meagher et al., 1996) and these mechanisms can be divided in resistance in either leaves or stalks and involving either physical or chemical barriers.

Host trichomes affect oviposition behavior in different species of Lepidoptera, stimulating or deterring females to lay their eggs. Sosa Jr (1988, 1990) observed that in glabrous sugarcane cultivars, the density of D. saccharalis eggs clusters per plant was higher than in pubescent cultivars. On the other hand, Dinardo-Miranda et al. (2012) found no relationship between the presence of trichomes and the amount of eggs, suggesting that others factors probably had a more important role in oviposition preference, such as attractants and arrestant substances. Sosa Jr (1988) observed that trichomes on sugarcane leaves may also delay first instar larval movement, which should increase larval mortality by increasing exposure to adverse environmental and biological factors (Dinardo-Miranda et al., 2012).

Kyle & Hensley (1970) conducted studies comparing the establishment and damage of the sugarcane borer on two sugarcane cultivars. Their studies suggested that the resistance of the cultivar NCo 310 (compared to the susceptibility of the cultivar CP 44-101) to sugarcane borer was due primarily to higher mortality of larvae, especially of young larvae prior to tunneling into the stalk. Coburn & Hensley (1972) reported difference in the larval survival at 2, 4 and 10 days after infesting a susceptible and resistant sugarcane genotypes with D. saccharalis neonates. Their studies also suggested that leaf-sheath tightness around the stalk in NCo 310 is partially responsible for the resistance of this variety to D. saccharalis because when young larvae are attempting to establish on plants, the tighter fitting leaf sheaths of NCo 310 inhibit larval establishment more than the relatively loose fitting leaf-sheaths of CP 44-101.

White (1993a) recorded difference in larval survival at 10 and 30 days after infesting three sugarcane genotypes with D. saccharalis neonates, indicating antibiosis as one resistance component. This researcher also reported that, 10 days after infestation, 48% of larvae recovered on the susceptible cultivar had bored into the stalk, while only 19% of the larvae recovered on the intermediate and resistant genotypes had bored into the stalk. This suggests that another component of resistance to sugarcane borer is the prevention of establishment of young larvae within the stalk. Dinardo-Miranda et al (2012) found difference in percentage bored internode indicating that some sugarcane genotypes probably have components, such as high fiber content and rind hardness (White et al., 2006) which inhibits the penetration of larvae in the stalk.

Dinardo-Miranda et al (2012) performed studies to assess resistance mechanisms of 10 sugarcane genotypes to sugarcane borer. In their studies, they found difference in number of larvae recovered in the stalks at 29 days after infesting the plants with D. saccharalis eggs. They also found significant differences in relation to the weight and length of recovered larvae indicating that the genotypes may affect borer performance in addition to cause mortality. White et al. (2011) also suggested antibiosis as a mechanism of resistance to the sugarcane borer in two genotypes from Louisiana breeding program, since they observed low adult emergence and low larval weights in insects from those cultivars.

White & Hensley (1987) reported that plant tolerance may also be a mechanism of resistance because some sugarcane cultivars are able to minimize sugarcane weight and sugar losses following infestation by sugarcane borer.

According to White et al. (2006), the absence of techniques to screen and categorize sugarcane populations for sugarcane borer resistance has hampered the development of resistant genotypes. Sugarcane borer damage is complex because economic damage results from an accumulation of damage over a relatively long growing period (120 days). In addition, cultivars express resistance via complex contributions and interactions of several components. Thus, all of the recognized mechanisms of resistance (antibiosis, antixenosis, and tolerance) may be expressed in cane's resistance to the cane borer (Milligan et al., 2003).

Borer resistance is measured in sugarcane clones in numerous ways in field trials. Bessin et al. (1990) recorded the number of bored internodes per stalk and the number of the internodes with exit holes per stalk, indicating a successful pupation. Using stalk number per area and the number of moth exit holes, these researchers

estimated the number of adult moths produced per area/year/variety. White (1993b) incorporated a damage rating in addition to using percentage of bored internodes in their assessment of damage. Milligan et al. (2003) calculated selection indices using five different measures (percent bored internodes, percent exited internodes, pupation success, moth production, and a damage rating) and found that the most effective single trait to indicate yield loss is percent bored internodes.

A bored internode indicates the successful entry of a larva into the stalk. Clones with a lower percentage of bored internodes indicate the presence of traits that inhibit successful penetration such as high fiber and a hard internode rind. The presence of a pupal gate is an indication of successful larval development and subsequent emergence of a moth. Cultivars with high numbers of successful moth production are assumed to contribute to higher area-wide populations of the sugarcane borer (Bessin et al., 1990). Low numbers of emergence holes is an indirect measure of the existence of a possible resistance factor within the stalk (White et al., 2011).

Field populations of pest insects are normally used by researchers to evaluate plant materials in early stages of plant resistance programs. However, field evaluations of plant material have some inherent problems that may affect the assessment of resistance. Unmanaged insect populations may be either too low or too high or unevenly distributed in space or time to inflict a consistent level of damage (Smith, 2004).

According to Vercambre et al (2001), it is often necessary to combine several methods to be able to characterize the behavior of a variety with regard to its response to attack by one or more borer species. Although differences between varieties may occur in the field, the resistance needs to be clarified under uniforms infestation conditions for further investigation of resistance mechanisms and possible resistance sources against sugarcane borer. This is just possible by growing the varieties within glasshouses where they are submitted to uniforms and controlled infestations (Terán et al., 1986).

Dinardo-Miranda et al (2012) compared the resistance of 10 sugarcane genotypes in a glasshouse under artificial infestation condition and concluded that IACSP94-2094 was the most unfavorable for larvae entrance and development. However, in later study, Dinardo-Miranda et al (2013) compared the resistance of the same cultivars in the field and found that IACSP94-2094 resistance level was intermediate while IAC87-3396 was the most resistant. These contradictory results indicate the need of refining the assessment of sugarcane resistance to stalk borer.

Plant reaction to insect attack may depend on the number of insects per plant, plant vigor, plant age and environmental factors. When insect populations are too high, cultivars with low and moderate resistance may appear susceptible, whereas too few insects may prevent the separation of resistant and susceptible cultivars (Smith, 2004).

According to Smith (2004), the expression of insect resistance in different plant tissues varies tremendously during the life of a plant. In some crops, plants are less resistant to insects in the early stages of development. Resistance to the southwestern corn borer Diatraea grandiosella (Dyar) and European corn borer Ostrinia nubilalis (Hubner) (Lepidoptera: Pyralidae) in resistant corn hybrids is greater in whorl (vegetative) than in tassel (reproductive) stages (Klun & Robinson, 1969, Videla et al., 1992). Conversely, resistance to the rice leaffolder Cnaphalocrosis medinalis (Guené)(Lepidoptera: Pyralidae), is more pronounced in older foliage of resistant varieties than in young foliage (Ramachandan & Khan, 1991).

The age of the test insect is directly proportional to the amount of plant biomass it will consume during the bioassay. For this reason, it is important to determinate an insect age that shows the greatest difference between resistant and susceptible varieties (Smith, 2004).

Therefore, it is necessary to study the ideal plant and insect age, damage measure and infestation levels in order to develop a reliable protocol for assessing sugarcane resistance to D. saccharalis under controlled conditions in addition to investigate the plants traits related to resistance to D. saccharalis.

In addition to the knowledge about resistance traits in sugarcane genotypes against D. saccharalis, another important factor in sugarcane breeding programs in order to develop resistant cultivars is the study of genetic divergence for borer response (Souza et al., 2013). A group of genotypes can be assessed in order to identify divergent genotypes that can be used as progenitors for hybridization in crop breeding programs (Cruz, 2004). Studies regarding genetic divergence for pest response has been successful performed for crop pests such as Tibraca limbativentris Stål (Hemiptera: Pentatomidae) in rice (Souza et al., 2008) and Tuta absoluta Meyrick (Lepidoptera: Geleichidae) in tomato (Suinaga et al, 2000) indicating the importance of studying genetic divergence for pest response. In sugarcane, Souza et al. (2014) studied the genetic divergence of sugarcane genotypes for borer response and reported genetic dissimilarity between genotypes and the possibility of grouping them by using cluster analysis.

The dissimilarity among individuals within a population can be estimated by using multivariate techniques such as principal components analysis, canonic variables and cluster analysis (Cruz, 2004). In cluster analysis, the purpose is to form groups that present homogeneity within the group and heterogeneity between groups.

Several researchers (Oliveira et al., 1999; Machado et al., 2002) recommend the use of genitors with maximum genetic divergence possible, in order to maximize the heterosis in the hybrids, increasing the genetic gain in the segregating population. Thus, individuals belonging to different groups should be used as progenitors in crop breeding programs (Carpenteri-Pípolo et al., 2000). In addition to genetic divergence, the choice of progenitors for hybridization must consider the use of groups presenting more favorable characteristics (Abreu et al.,1999).

This thesis was written in two chapters:

The chapter one aimed to study some parameters that affect the assessment of resistance of sugarcane genotypes to D. saccharalis. The parameters studied were: plant age, plant conduction, stalk damage measure and infestation level for assessing sugarcane borer resistance by assessing stalk damage in greenhouse trials.

The chapter two aimed to study the effect of six sugarcane varieties in survival of early-stages of larvae, survival and performance of late-stages larvae and effect of genotypes in the larvae establishment within the stalk. We also assessed the genetic divergence of sugarcane varieties for borer response.

REFERENCES

Abreu, A.F.B.; Ramalho, M.A.P.; Ferreira (1999). Selection potential for seed yield from intra and inter-racial populations in common bean. Euphytica, v.108, n.2, p.121-127, 1999.

Arrigoni, E.B. (2002) Broca da cana-de-açúcar – importância econômica e situação atual. In: Arrigoni, E.B.; Dinardo-Miranda, L.L.; Rosseto, R. Pragas da cana-de-açúcar – importância econômica e enfoques atuais. Piracicaba: STAB, 2002. p.1-4.

Bessin, R.T; Reagan, T.E.; Martin, F.A. (1990). A moth production index for evaluating sugarcane cultivars for resistance to the sugarcane borer (Lepidoptera:Pyralidae). J. Econ. Entomol. 83:221-225.

Boiça jr, A. L.; Lara, F. M.; Bellodi, M. P. (1997). Influência de variedades de cana-de-açúcar, incorporadas em dieta artificial, no desenvolvimento de Diatraea saccharalis (Fabr.) e no seu parasitismo por Cotesia flavipes (Cam.). Anais da Sociedade Entomológica do Brasil, 26(3): 537-542.

Botelho, P.S.M. (1992) Quinze anos de controle biológico da Diatraea saccharalis utilizando parasitóides. Pesquisa Agropecuária Brasileira, Brasília, v. 27, n. 2, p. 255-262, 1992.

Botelho, P.S.M.; Macedo, N. (2002) Cotesia flavipes para o controle de Diatraea saccharalis. In: Parra, J.R.P.; Botelho, P.S.M.;Correa-Ferreira, B.S.; Bento, J.M.S. Controle biológico no Brasil: parasitóides e predadores. São Paulo: Manole, 2002 .p.409-425.

Carpentieri-Pipolo, V.; Destro, D.; Prete, C.E.C. (2000). Seleção de genótipos parentais de acerola com base na divergência genética multivariada. Pesquisa Agropecuária Brasileira, Brasília, v.35, n.8, p.1613-1619, 2000.

Coburn, G.E.; S.D. Hensley. (1972). Differential survival of Diatraea saccharalis (F.) larvae on two varieties of sugarcane. Proc. Int. Soc. Sugar Cane Technol. 14:440-444.

CONAB, Acompanhamento da safra brasileira: Cana-de-açúcar, primeiro levantamento abril/2012. Companhia Nacional de Abastecimento. CONAB, Brasília, 2012.

Cruz, C.D.; Regazzi, A.J.; Carneiro, P.C.S. Modelos biométricos aplicados ao melhoramento genético. Viçosa: UFV, 2004, 480p.

Demetrio, P.A.; Zonetti, P.P.; Munhoz, R.E.F. (2008) Avaliação de clones de cana-de-acucar promissores RBs quanto à resistência à broca-da-cana (Diatraea saccharalis) na região noroeste do Paraná. Iniciação Científica Cesumar, v.10, p.13-16, 2008.

Dinardo-Miranda, L.L. Pragas. In: Dinardo-Miranda, L.L.; Vasconselos, A.C.M.; Landell, M.G.A. (Eds). Cana-de-açúcar. IAC, São Paulo, p: 349 – 404, 2008.

Dinardo-Miranda, L.L; Anjos, I.A; Da Costa, V.P; Fracasso, J.V. (2012). Resistance of sugarcane cultivars to Diatraea saccharalis. Pesquisa agropecuária brasileira 47(1):1-7.

Dinardo-Miranda, L.L.; Fracasso, J.V.; Anjos, I.A.; Garcia, J.; Costa, V.P. (2013). Influência da infestação de Diatraea saccharalis (Fabr.) sobre parâmetros tecnológicos da cana-de-açúcar. Bragantia, Campinas, 2013.

Dinardo-Miranda, L.L.; Fracasso, J.V.; Costa, V.P.; Anjos, I.A.; Peixoto; D.O.L. (2013), Reação de cultivares de cana-de-açúcar à broca do colmo. Bragantia, Campinas, v. 72, n. 1, p.29-34, 2013.

Gallo, D.; Nakamo, O.; Silveira, S.; Carvalho, R.P.L.; Batista, G.C.; Berti Filho, E.; Parra, J. R.P.; Zuchi, R.A.; Alves, S. B.; Vendramim, J. D.; Marchini, L. C.; Lopes, J. R. S.; Omoto, C. Entomologia Agrícola. Piracicaba: FEALQ, 2002, 920p.

Guevara, L.A.C.; Wiendl, F.M. (1980). Aspectos da biologia, em condições de campo, da broca da cana-de-açúcar (Diatraea sacccharalis (Fabr, 1794)). Agropecuária técnica, Areia, v. 1, n.1, p. 32-39, 1980.

Kyle, M.L., Hensley, S.D.(1970). Sugarcane borer host plant resistance studies. Proc. La. Acad. Sci. (In press).

Klun, J.A.; Robinson, J.F. (1969), Concentration of two 1,4-benzoxazinones in dente corn at various stages of development of the plant and its relations to resistance of the host plant to the European corn borer. Journal of economics entomology, 62, 214, 1969.

Machado, C.F.; Nunes, G.H.S.; Ferreira, D.F. (2002). Divergência genética entre genótipos de feijoeiro a partir de técnicas multivariadas. Ciência Rural, Santa Maria, v.32, n.2,p.251-258, 2002.

Matsuoka, S.; Garcia, A.; Arizono, H. Melhoramento da cana-de-açúcar. In: Borém, A. (Ed). Melhoramento de espécies cultivadas. Editora UFV, Viçosa, MG, p: 225 – 274, 2005.

Meagher, R. L., Jr., J. E. Irvine, R. G. Breene, R. S. Pfannenstiel Gallo, M. (1996). Resistance mechanisms of sugarcane to Mexican rice borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 89: 749-760.

Milligan, S. B., M. Balzarini, and W. H. White, (2003): Broad-sense heritabilities, genetic correlations, and selection indices for sugar-cane borer resistance and their relation to yield loss. Crop Sci.43,1729—1735.

Oliveira, V.R.; Casali, V.W.D.; Cruz, C.D. (1999). Avaliação da diversidade genética em pimentão através de análise multivariada. Horticultura Brasileira, Brasília, v.17, n.1, p.19-
24, 1999.

Ramanchadran, R.; Khan, Z.R. (1991); feeding site selection of first ínstar larvae of Cnaphalocrocis medinalis on susceptible and resistant rice plants. Entomology exp. Appl., 60, 43, 1991.

Schoonhoven, L.M., Van Loon, J.J.A., Dicke M. (2005) Insect-Plant Biology, Ed 2. Oxford University Press, Oxford.

Smith, C.M. 2005. Plant resistance to arthropods: molecular and conventional approaches. The Netherlands: Springer, 2005. 423p.

Sosa Jr, O. (1990). Oviposition preference by the sugarcane borer(Lepidoptera: Pyralidae). Journal of Economic Entomology,v.83, n.3, p.866–868, 1990.

Sosa Jr, O. (1988). Pubescence in sugarcane as a plant resistance character affecting oviposition and mobility by the sugarcane borer (Lepidoptera; Pyralidae). Journal of Economic Entomology,v.81, n.2, p.663-667, 1988.

Souza, J.R.; Boiça Jr, A.R.; Perecin, D.; Cargnelutti Filho, D.; Costa, J.T. (2013). Divergência genética de cultivares de cana-de-açúcar quanto à resistência a Diatraea saccharalis. Semina: Ciências Agrárias, Londrina, v. 34, n. 6, suplemento 1, p. 3367-3376, 2013

Souza, J.R.; Ferreira, E.; Cargnelutti Filho, A; Boiça Jr, A.L.; Chagas, E.F.; Mondego, J.M. (2009). Divergência genética de cultivares de arroz quanto à resistência a Tibraca limbativentris Stål (Hemiptera: Pentatomidae). Neotropical Entomology, Londrina, v. 38, n. 5, p. 671-676, 2009.

Suinaga, F.A.; Casali, V.W.D.; Silva, D.J.H.; Picanço, M.C. Dissimilaridade genética de fontes de resistência de Lycopersicon spp a Tuta absoluta (Meyrick, 1917)(Lepidoptera: Gelechiidae) (2000). Revista Brasileira de Agrociência, Pelotas, v. 9, n. 4, p.371-376, 2003.

Terán, F.O.; Sánchez, A.G.; Precetti, A.A.C.M. (1986). Estudos sobre resistência da cana à broca em telado – III. Boletim Técnico Copersucar, v.34, p.53-60, 1986.

Vercambre, B.; Goebel, R., A, D.C.;Rochat, J. (2001). Available methods for assessing varietal resistance to sugarcane stalk borers. Proc S Afr Sug Technol Ass (2001) 75.

Videla, G.W., Davis,F.M.; Willians, W.P.; Ng ,S.S. (1992). Fall armyworm (Lepidoptero: noctuidae) larval growth and survivorship on susceptible and resistance corn at different vegetative growth stages, Journal of economic entomology, 85, 2486, 1992.

Ricaud, C.; Ryan, C. Leaf scald. In: Ricaud, C.; Ryan, C.; Egan, B.T. Gillaspie, E.G.; Rughes, C.G. (Eds). Diseases of sugarcane. Amsterdan. P: 36 – 53, 1989.

White, W.H., (1993a). Movement and establishment of sugarcane borer (Lepidoptera: Pyralidae) larvae on resistant and susceptible sugarcane. Fl. Entomol. 76, 465–473.

White, W. H. (1993b). Cluster analysis for assessing sugarcane borer resistance in sugarcane line trials. Field Crops Res. 33: 159-168.

White, W.H; Tew, T.L; Richard, E.P. (2006). Association of sugarcane pith, rind hardness, and fiber with resistance to the sugarcane borer. Journal American Society Sugar Cane Technologists, 26:87-100.

White, W.H.; Hale A.L.; Veremis, J.C.; Tew, T.L.; Richard Jr, E.P. (2011). Registration of two sugarcane germoplasm clones with antibiosis to the sugarcane borer (Lepidoptera: Crambidae). Journal of Plant Registration, v.5, n.2, p.248-253, 2011.

White, W.H., Hensley. S.D. (1987). Techniques to quantify the effect of Diatraea saccharalis (Lepidoptera: Pyralidae) on sugarcane quality. Field Crops Res. 15:341-348.

CHAPTER 1

A METHODOLOGY FOR SCREENING SUGARCANE RESISTANCE TO *Diatraea saccharalis* BY ASSESSING STALK DAMAGE

ABSTRACT

The sugarcane borer is one of the most important pests in sugarcane in Brazil. Deployment of resistant sugarcane varieties is an important tool to reduce the yield loss caused by this pest. However, the absence of fast and efficient techniques to screen large amount of sugarcane genotypes for borer resistance in greenhouse trials has hampered the development of resistant varieties. Therefore, this work aimed to study some parameters that may affect the screening of sugarcane resistance to D. saccharalis by assessing the stalk damage. We assessed optimum plant age, damage measure and infestation level in greenhouse trials. Tunnel length was damage measure that proved more adequate to compare stalk damage among the sugarcane genotypes. The high correlation between seven and 10-month-old plants indicates that plant traits affecting stalk damage are maintained during the stalk growth. Therefore, the assessment of resistance may be performed in 7-month-old plants as it enables saving time and resources. The lack of correlation between single plants and stools indicates that the difference in stalk number per pot affect comparison of resistance. Therefore, the tillers should be removed in order to keep only a single plant or the same number of plants per pots when assessing stalk damage. The optimum infestation level to compare stalk damage among genotypes is 20 larvae per plant, as it enables clear distinction between resistant and susceptible genotypes. Our method may be employed by sugarcane breeding programs to select resistant clones within large sugarcane populations.

Keywords: sugarcane borer, plant-insect interactions, Saccharum officinarum

1. Introduction

The sugarcane stalk borer Diatraea saccharalis Fab. (Lepidoptera: Crambidae) is one of the major pests in sugarcane in Brazil and is widely distributed in sugarcane belts across the country (Dinardo-Miranda et al., 2008). Damage is caused by larvae tunneling into the stalks, causing the death of a large number of shoots when the sugarcane crop is young and yield loss in more developed crops. In addition, the borer favors the entrance of fungi that causes the disease known as sugarcane red rot (Botelho & Macedo, 2002; Dinardo-Miranda, 2008; Dinardo-Miranda et al., 2012).

The development of resistant sugarcane cultivars to D. saccharalis is very important to the sugarcane sector owing the reduction of production costs and development of the crop (Demetrio et al, 2008). According to Rutherford (2014), there is a consensus that growing genetically resistant varieties is the most cost effective and appropriate means for managing pests and diseases. However, according to White et al. (2006), the absence of techniques to screen and categorize sugarcane populations for sugarcane borer resistance has hampered the development of resistant genotypes. Sugarcane borer damage is complex because economic damage results from an accumulation of damage over a relatively long growing period (120 days). In addition, cultivars express resistance via complex contributions and interactions of several components. Thus, all of the recognized mechanisms of resistance (antibiosis, antizenosis, and tolerance) may be expressed in cane's resistance to the cane borer (Painter, 1951; Milligan et al., 2003).

Borer resistance is measured in sugarcane clones in numerous ways in field trials. Bessin et al. (1990) recorded the number of bored internodes per stalk and the number of the internodes with exit holes per stalk, indicating a successful pupation. Using stalk number per area and the number of moth exit holes, these researchers estimated the number of adult moths produced per area/year/variety. White et al. (1993a) incorporated a damage rating in addition to using percentage of bored internodes in their assessment of damage. Milligan et al. (2003) calculated selection indices using five different measures (percent bored internodes, percent exited internodes, pupation success, moth production, and a damage rating) and found that the most effective single trait to indicate yield loss is percent bored internodes.

Field populations of pest insects are normally used to evaluate plant materials in early stages of plant resistance program. However, field evaluations of plant material

have some inherent problems that may affect the assessment of resistance. Unmanaged insect populations may be either too low or too high or unevenly distributed in space or time to inflict a consistent level of damage. Year-to-year variation in population levels of the target pest insect may also make interpreting the results of field evaluations difficult. Finally, unmanaged field populations may be contaminated with non-target pest insects that cause feeding damage similar to the target insects (Smith, 2005).

Although differences between varieties may occur in the field, the resistance needs to be clarified under uniforms infestation conditions for further investigation of resistance mechanisms and possible resistance sources against sugarcane borer. This is just possible by growing the varieties within glasshouses where they are submitted to uniforms and controlled infestations (Terán et al., 1986).

Dinardo-Miranda et al (2012) compared the resistance of 10 sugarcane genotypes in a glasshouse under artificial infestation condition and concluded that IACSP94-2094 was the most unfavorable for larvae entrance and development. However, in later study, Dinardo-Miranda et al (2013) compared the resistance of the same cultivars in the field and found that IACSP94-2094 behavior was intermediate while IAC87-3396 was the most resistant. These contradictory results indicate the need of refine the assessment of sugarcane resistance to stalk borer.

Plant reaction to insect attack may depend on the number of insects per plant, plant vigor, plant age and environmental factors. When insect populations are too high, cultivars with low and moderate resistance may appear susceptible, whereas too few insects may prevent the separation of resistant and susceptible cultivars (Smith, 2004).

According to Smith (2004), the expression of insect resistance in different plant tissues varies tremendously during the life of a plant. In some crops, plants are less resistant to insects in the early stages of development. Resistance to the southwestern corn borer Diatraea grandiosella (Dyar) and European corn borer Ostrinia nubilalis (Hubner) (Lepidoptera: Pyralidae) in resistant corn hybrids is greater in whorl (vegetative) than in tassel (reproductive) stages (Klun & Robinson, 1969, Videla et al., 1992). Conversely, resistance to the rice leaffolder Cnaphalocrosis medinalis (Guené)(Lepidoptera: Pyralidae) is more pronounced in older foliage of resistant varieties than in young foliage (Ramachandan & Khan, 1991).

Therefore, this work aimed to determinate optimum plant age and growth conduction, damage measure and infestation level for assessing sugarcane borer resistance by assessing stalk damage in greenhouse trials.

2. Material and Methods

The experiments were carried out in the Sugarcane Research and Breeding Center (CECA) of the Federal University of Viçosa, located in Oratórios (20º 25´ 5´´ S, 42º 47´ 28´´ W; h = 492 m), Minas Gerais state, Brazil, and in the "Diogo Alves Mello" experimental field of Federal University of Viçosa located in Viçosa (20º45´ S, 42º52´ W; H = 650 m), Minas Gerais state, Brazil from July/2012 to March/2014.

The insects used in all experiments were obtained from a mass rearing in an artificial diet according to the methodology proposed by Hensley and Hammond (1968), adapted by Araújo et al. (1985).

2.1 Plants cultivation

The varieties tested were: SP803280, SP813250, RB928064, RB835486, RB867515 and SP891115 obtained from the Germplasm Unit of the sugarcane breeding program of Federal University of Viçosa in partnership with RIDESA (Rede Interuniversitária para o desenvolvimento do setor sucroenergético)(Barbosa et al., 2012). The variety SP891115 was used as susceptible standard (Dinardo-Miranda et al., 2012).

Single-node sugarcane stems cuttings containing one lateral bud were germinated in trays filled with agricultural substrate (Tropstrato®). Then, 60 days after planting, the seedlings were transplanted to 12 L plastic pots filled with soil and maintained in a greenhouse (26 ± 10ºC temperature; 75 ± 20% relative humidity and 12 ± 2 hours photophase) to avoid the infestation by field borer population. Spacing between rows was one meter, while pots within rows were 0.8 meters distant from each other. The soil correction and fertilization were performed based in previous soil analysis and following the indication to the crop as described by Korndorfer et al (1999). Plants were irrigated using drippers at 0.5 to 2.0 l/pot/day.

2.2 Assessment of stalk damage in 10-month-old single plants

For this experiment, we removed the tillers periodically in order to keep just one plant per pot. When the plants reached 10-month-old they were infested with 15-days-old larvae of D. saccharalis. We used 15-days-old larvae for infesting the plants

because this is the nearly age that the larvae enter the stalk (Gallo et al. 2008). The larvae were transferred to 50 ml plastic cups and then, the cups were placed in the middle portion of the stalk with the opening toward the stalk.

The experiment was carried out in a randomized block design with 3 replicates in factorial scheme 6 x 6. Each pot was considered as a replicate. Plants of each variety previously cited were submitted to seven infestation levels: 0, 5, 10, 15, 20, 30 and 40 larvae per plant. The level zero was used to ensure there was no natural infestation by D. saccharalis and was not used for statistical analysis. Then, at 15 days after infestation, the plants were cut off at the soil level, split and the percentage bored internodes (infestation index) and total tunnel length (damage extension) were recorded.

2.3 Assessment of stalk damage in 7-month-old single plants

For this experiment, we removed the tillers periodically in order to keep just one plant per pot. When the plants reached 7-month-old they were infested with 15-day-old larvae of D. saccharalis. The larvae were transferred to 50 ml plastic cups and then the cups were placed in the middle portion of the stalk with the opening toward the stalk.

The experiment was carried out in a randomized block design with three replicates in factorial scheme 6 x 4. Each pot was considered as a replicate. Plants of each variety were submitted to five infestation levels: 0, 5, 10, 20 and 40 larvae per plant. At 15 days after infestation, the plants were cut off at the soil level, split and the percentage bored internodes (infestation index) and total tunnel length (injury extension) were recorded.

2.4 Assessment of stalk damage in sugarcane plants at tillering stage

For this experiment the tillers were not removed, thus the plants were allowed to develop normal stools. When the plants reached 5-month-old, the stools were infested with larvae of D. saccharalis. 15-days-old larvae were transferred to 50 ml plastic cups and then, the cups were placed in the basal portion of a central stalk, in order to allow the larvae to move throughout the plants.

The experiment was carried out in a randomized block design with three replicates in factorial scheme 6 x 4. Each pot was considered as a replicate. Plants of

each variety previously cited were submitted to five infestation levels: 0, 5, 10, 20 and 40 larvae per plant. The level zero was not used for statistical analysis.

The plants were cut off at the soil level at 15 days after infestation. The total number of tillers and tillers with dead heart symptom was recorded for calculating the percentage of tillers with dead heart symptom. The plants with less than one developed internode were considered as tillers. The plants with more than one developed internode were split and the total infestation index and the injury extension were recorded.

2.5 Statistical analysis

The data were submitted to analysis of variance (Two-way ANOVA). The data were transformed into square root (x+1) when necessary in order to meet the Anova assumptions. The means were compared by the Fisher procedure ($P < 0.05$). The statistical program SAS 9.0 was used in all analyses. To determinate optimum infestation level to compare genotypes, regression analysis were performed between infestation levels and injury extension. The Pearson correlation analysis was used to assess the relationship between seven and 10-month-old plants and single plants with stools.

3. Results and discussion

In single plants, we found no difference between genotypes for infestation index for both 7-months-old plants ($F = 1.69$ and $P = 0.1669$) and 10-months-old plants ($F = 1.42$ and $P = 0.2345$). However, we found significant difference among genotypes for injury extension for both seven-months-old plants ($F = 2.69$ and $P = 0.0398$) and 10-months-old plants ($F = 5$ and $P = 0.0009$). When the injury extension was assessed, the variety SP803280 suffered the smallest damage and the variety RB867515 the largest damage in relation to the susceptible standard SP891115 (Figure 1).

In five months-old-plants in stools, we found no difference between genotypes for percentage dead heart tillers ($F = 1.47$ and $P = 0.23$). However, there was difference between genotypes for total injury extension ($F = 5.62$ and $P = 0.0009$) and percentage bored internodes ($F = 4.10$ and $P = 0.0059$)(Figure 1). When the infestation index was assessed, only the variety SP813250 was less damaged than the control SP891115. Nevertheless, when the injury extension was assessed, the varieties RB928064 and RB835486 were also less damaged than the control SP891115.

Although some researchers successfully differentiated the resistance of sugarcane genotypes to sugarcane borer by assessing the infestation index (percentage bored internode) in greenhouses trials (Terán et al., 1986; Dinardo-Miranda et al., 2012), in this present work, the assessment of injury extension allowed better comparison among genotypes. The tunnel length is used as damage measure for assessing sugarcane resistance to other stalk borers such as the African sugarcane stalk borer (Eldana saccharina Walker (Lepidoptera: Pyralidae))(Keeping, 2006a; Keeping & Meyer, 2006b; Kvedaras et al., 2007). According to Keeping & Meyer (2006b), borer damage is best examined in terms of stalk length bored, as this is the most inclusive and precise measure of damage although this measure is more time-consuming than counting bored internodes (Keeping, 2006a).

We found a strong correlation between seven and 10-months-old single plants ($r = 0.822$ and $P = 0.0445$). In the plants at both age, the variety SP803280 suffered the smallest damage and the variety RB867515 the largest damage in relation to the control SP891115.

The strong correlation of stalk damage in seven and 10-month-old plants indicates that the possible mechanisms of resistance present in stalks such as rind hardness and fiber (White et al., 2006) are maintained during the stalk development. The early assessment of plant resistance is desirable in sugarcane breeding programs as it enables saving time and resources.

We found no correlation between the stalk damage in single plants with plants in stools ($r = 0.557$ and $P = 0.251$). When the plants were in stools, the variety SP803280 had the stalk damage similar to the susceptible control SP891115, and variety SP813250 had the smallest damage. The lack of correlation between the assessment of stalk damage in single plants and plants in stools suggests that different resistance mechanisms are presents in different genotypes. The variety SP813250 has more tillers (in average 4.86) than the others varieties (In average 2.8). This enables the larvae to spread out more throughout the plants, concentrating less damage in the main stalks. According to keeping (2006a), the extent of borer damage per pot, is known to be significantly positively correlated with the number and sum of the length of stalks per pot. This has the undesirable result that strongly tillering cultivars support a larger borer population and accrued more damage by the end of a trial than weakly tillering cultivars, thereby confounding the assessment of resistance with an unrelated trait (Keeping, 2006a). Some studies suggest that the sugarcane tolerate damage by stalk

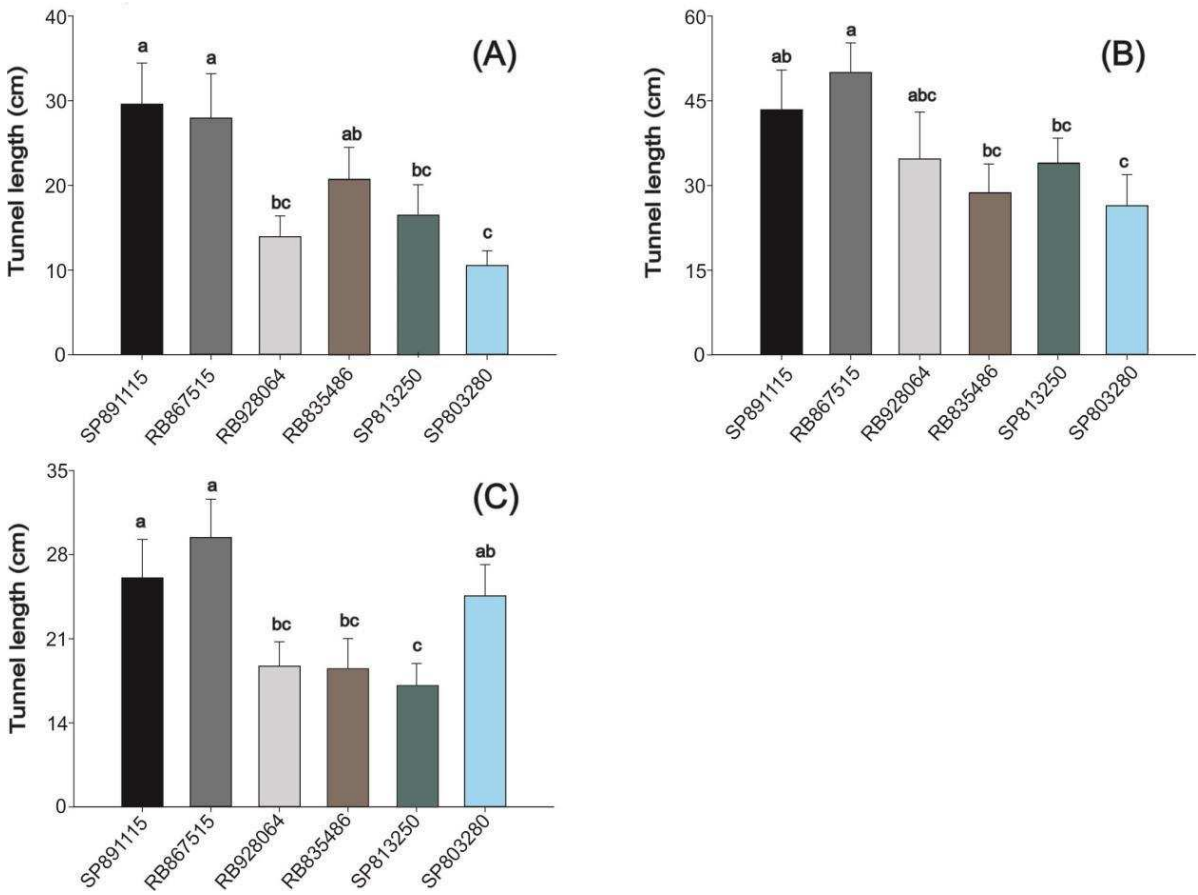

Figure 1. Tunnel length caused by Diatraea saccharalis in stalks of six sugarcane genotypes infested with 15-day-old larvae in greenhouse. (A) Ten-month-old single plants; (B) Seven-month old single plants; (C) Five-month-old plants in stools. Means ± standard error followed by the same letter are not different according to Fisher´s LSD procedure, protected by ANOVA $P < 0.05$).

borers such as Chilo infuscatellus Snellen (Lepidoptera: Pyralidae), Scirpophaga exerptalis Wlk (Lepidoptera: Pyralidae) and Sesamia sp. by producing many tillers (Abbasipour & Askarianzadeh, 2010). Therefore, this present study suggests that the assessment of sugarcane resistance to D. saccharalis should consider the evaluation of damage in single plants and plants forming stools to elucidate different resistance mechanisms present in the genotypes.

The regression model that better explained the relationship between infestation level and injury extension was the sigmoid (table 1). According to the graphs, the most damaged genotypes (SP891115 e RB867515) reached the maximum damage (nearly 40 cm) when they were infested with 20 larvae per plant. On the other hand, the less damaged variety (SP803280) is poorly damaged in the level of 20 larvae per plant (nearly 10 cm borer tunnels)(figure 2).

According to Davis (1985), the appropriate insect infestation level is the minimum number of insects required to place the susceptible check cultivar consistently in the susceptible category. The most damaged genotypes, when infested with more than 20 larvae per plant, do not have increase in the stalk damage. This probably occurs due the competition among larvae. Therefore, these results suggest that the optimum infestation level to assess the stalk damage by borer is 20 larvae per plant.

Although this work suggests a protocol for comparing sugarcane resistance to D. saccharalis by assessing stalk damage, further studies must be performed in order to assess other mechanisms of resistance such as mortality of early-stages larvae (Kyle and Hensley, 1972) and larval performance (Dinardo-Miranda et al., 2012). Complementary studies may help to clarify the different sugarcane mechanisms of resistance against sugarcane borer and the resistance sources in order to use these mechanisms in sugarcane breeding programs.

Table 1. Regression analysis between infestation levels (number of larvae per plant) and tunnel length (cm) in stalks of 10-month-old plants of six sugarcane varieties grown in greenhouse.

Variety	Model	Estimated parameters (± SE)			F	P	R^2
		a	b	x0			
RB867515	Y = a/(1+exp(-(x-x0)/b))	39.06 ± 3.71	4.73 ± 1.67	11.00 ± 1.95	26.55	0.005	0.93
SP891115	Y = a/(1+exp(-(x-x0)/b))	42.01 ± 2.90	4.36 ± 1.15	11.29 ±1.36	52.15	0.001	0.96
RB928064	Y = a/(1+exp(-(x-x0)/b))	20.53 ± 3.12	6.41 ± 3.06	12.23 ± 3.49	13.84	0.016	0.88
RB835486	Y = a/(1+exp(-(x-x0)/b))	29.87 ± 2.72	3.49 ± 1.35	11.77 ± 1.62	29.31	0.004	0.94
SP813250	Y = a/(1+exp(-(x-x0)/b))	32.76 ± 8.02	5.05 ± 3.90	19.43 ± 5.02	8.09	0.039	0.8
SP803280	Y = a/(1+exp(-(x-x0)/b))	16.06 ± 4.50	8.91 ± 6.37	13.05 ± 7.39	6.60	0.054	0.77

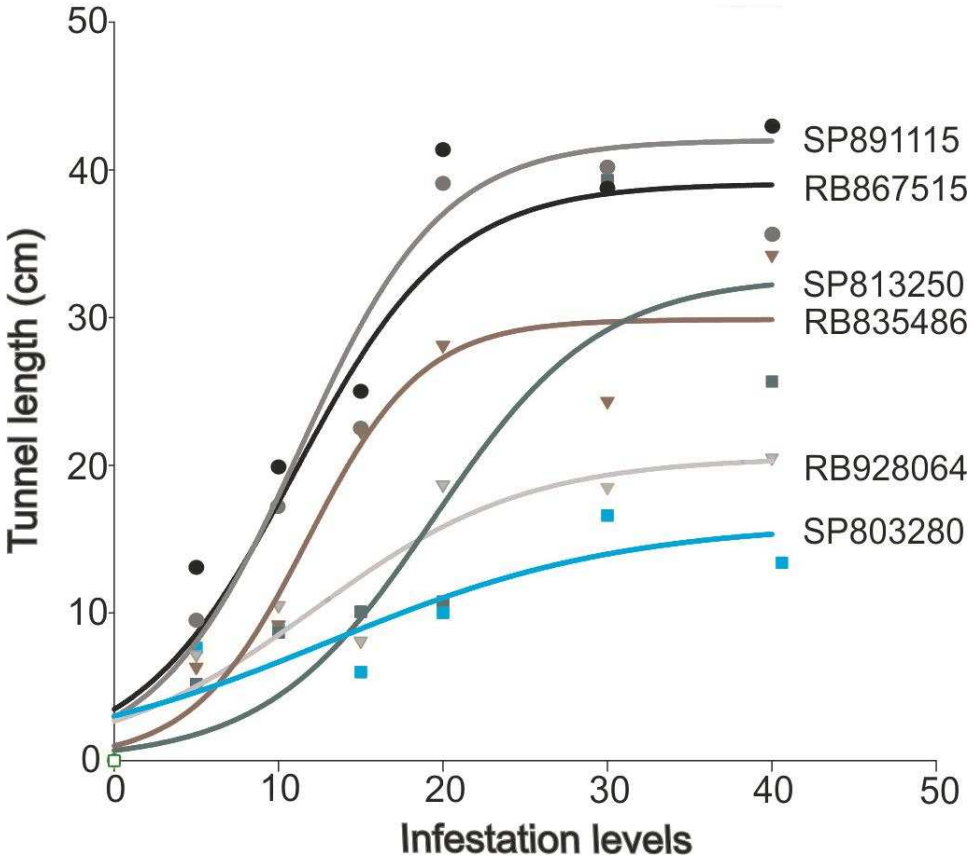

Figure 2. Relationship between infestation levels (number of larvae) and stalk damage in 10-month-old single plants of six sugarcane varieties infested with 15-day-old larvae of Diatraea saccharalis in greenhouses.

4. Conclusion

The injury extension (tunnel length) is a more precise measure of stalk damage than infestation index (percentage bored internodes) in greenhouse trials for assessing sugarcane resistance by assessing stalk damage. The stalk damage may be assessed in 7-months-old plants instead of 10-month-old plants as it enables saving time and resources. It is necessary to keep a single plant or the same number of plant per pot as stalk number affect resistance assessment. The infestation with 20 larvae per plant enable clear distinction between susceptible and resistant genotypes. It is necessary to study the mechanisms of resistance affecting the larvae during the time that they feed on the leaves and leaf sheaths in addition to mechanisms of resistance present in the stalk. The method we developed enable a rapid screening of resistance of large sugarcane population and may be employed by breeding programs in selection of resistant clones.

5. Acknowledgements

Technical assistance provided by RIDESA - UFV is greatly appreciated. The financial support provided by the National Council of Scientific and Technological Development (CNPq), CAPES Foundation, FAPEMIG Foundation and the RIDESA Sugarcane breeding program was greatly appreciated and acknowledged here.

6. References

Abbasipour, H., Askarianzadeh, A. (2010) Crop loss and identification of Physical and biochemical agents related to resistance in different sugarcane cultivars to stalk borers Sesamia spp. (Lepdoptera.: Noctuidae). In. Advances in environmental biology, 4(2), 234 – 240, 2010.

Araújo, J.R.; Botelho, P.S.M.; Araújo, S.M.S.S.; Almeida, L.C.; Degaspari, N. 1985. Nova dieta artificial para criação da Diatraea saccharalis (Fabr.). Saccharum, São Paulo, v. 36, n. 2, p. 45-48, 1985.

Barbosa, M.H.P., Resende, M.D.V., Dias, L.A.S., Barbosa, G.V.S., Oliveira; R.A., Peternelli, L.A., Daros, E. (2012) Genetic improvement of sugar cane for bioenergy: the Brazilian experience in network research with RIDESA. Crop Breeding and Applied Biotechnology S2, 87 - 98.

Botelho, P.S.M., Macedo, N. (2002) Cotesia flavipes para o controle de Diatraea saccharalis. In: Parra, R.P., Botelho, P.S.M., Correa-Ferreira, B.S, Bento, J.M.S. Controle biológico no Brasil: parasitóides e predadores. São Paulo: Manole, 2002. 409 - 425.

Butrón, A., Romay, M.C., Peña-Asín, J.,Alvarez, A., Malvar, R.A. (2012) Genetic Relationship Between Maize Resistance to Corn Borer Attack and Yield. in Crop Sci. 52, 1176 - 1180.

Demetrio, P.A., Zonetti, P.P., Munhoz, R.E.F. (2008) Avaliação de clones de cana-de-açúcar promissores RBs quanto à resistência à broca da cana (Diatraea saccharalis) na região noroeste do Paraná. Iniciação Científica Cesumar, v.10, 13 -16.

Dinardo-Miranda, L.L., Anjos, I.A., Costa, V.P., Fracasso, J.V. (2012) Resistance of Sugarcane cultivars to sugarcane borer. Pesquisa Agropecuária Brasileira, v.47, 1 - 7.

Dinardo-Miranda, L.L. Pragas. In: Dinardo-Miranda, L.L., Vasconselos, A.C.M. de, Landell, M.G. e A. (2008) (Ed.). Cana-de-açucar. Campinas: Instituto Agronômico, 349 - 404.

Dinardo-Miranda, L.L., Fracasso, J.V., Costa, V.P., Anjos, I.A., Lopes, D.O.P. (2013) Reação de cultivares de cana-de-açúcar à broca do colmo. Bragantia, Campinas, v. 72, n. 1, 29-34.

Hensley, S.D.; Hammond, A.H. 1968. Laboratory techniques for rearing the sugar cane borer on an artificial diet. Journal of Economic Entomology.Lanham, v. 61, n. 6, p. 1742-1743, 1968.

Keeping M.G.; Meyer, J.H., (2006b) Silicon-mediated resistance of sugarcane to Eldana saccharina Walker (Lepidoptera: Pyralidae): effects of silicon source and cultivar. J. Appl. Entomol. 130(8), 410 - 420.

Keeping M.G., (2006a) Screening of South African sugarcane cultivars for resistance to the stalk borer Eldana saccharina Walker (Lepidoptera: Pyralidae). Afr. Entomol. 14,(in press).

Klun, J.A. & Robinson, J.F. 1969, Concentration of two 1,4-benzoxazinones in dente corn at various stages of development of the plant and its relations to resistance of the host plant to the European corn borer. Journal of economics entomology, 62, 214, 1969.

Korndorfer, G.H.; Ribeiro, A.C.; Andrade, L.A.B Cana de açúcar. In: Ribeiro, A.C.; Guimarães, P. T.G.; Alvarez V., (Eds.). Recomendação para o uso de corretivos e fertilizantes em Minas Gerais: 5. Aproximação. Viçosa: Comissão de Fertilidade do Solo do Estado de Minas Gerais, 1999. p.25-32.

Kumar H. (1997) Resistance in maize to Chile partdhzs (Swinhoe) (Lepidoptera: Pyralidae): Role of stalk damage parameters and biological control. crop Prorecrron Vol. 16, No. 4, 375-381.

Kvedaras, O.L., Keeping, M.G., Goebel, F.R., Byrne, M.J (2007) Larval performance of the pyralid borer Eldana saccharina Walker and stalk damage in sugarcane: Influence of plant silicon, cultivar and feeding site. International Journal of Pest Management, July – September; 53(3): 183 - 194.

Malvar, R.A., Butrón, A., Alvarez, A., Ordás, B., Soengas, P., Revilla, P., Ordás, A. (2004) Evaluation of the European Union Maize Landrace Core Collection for Resistance to Sesamia nonagrioides (Lepidoptera: Noctuidae) and Ostrinia nubilalis (Lepidoptera: Crambidae). Journal of Economic Entomology, 97(2), 628-634.

Milligan, S. B., M. Balzarini, and W. H. White, (2003) Broad-sense heritabilities, genetic correlations, and selection indices for sugar-cane borer resistance and their relation to yield loss. Crop Sci. 43, 1729-1735.

Ordas, B., Alvarez, A., Revilla, P., Butron, A., Malvar, R.A. (2013) Relationship between Time to Flowering and Stalk and Ear Damage by Second Generation Corn Borers. Journal of Economic Entomology, 106(3), 1234 - 1139.

Ramanchadran, R. & Khan, Z.R. 1991; feeding site selection of first ínstar larvae of Cnaphalocrocis medinalis on susceptible and resistant rice plants. Entomology exp. Appl., 60, 43, 1991.

Schoonhoven, L.M., Van Loon, J.J.A., Dicke, M. (2005) Insect-Plant Biology, Ed 2. Oxford University Press, Oxford.

Smith, D.M., Khan, Z.R., Pathak, M.D. (1994) Book: Techniques for evaluating Insect Resistance in Crop Plants. CRC press. Inc. 320, 1994. USA.

Terán, F.O., Sánchez, A.G., Precetti, A.A.C.M. (1986) Estudos sobre resistência da cana à broca em telado – III. Boletim Técnico Copersucar, v.34, 53 – 60.

Vercambre, B., Goebel, R., A, D.C., Rochat, J. (2001) Available methods for assessing varietal resistance to sugarcane stalk borers. Proc S Afr Sug Technol Ass 75.

Videla, G.W., Davis,F.M.; Willians, W.P.; Ng ,S.S. Fall armyworm (Lepidoptero: noctuidae) larval growth and survivorship on susceptible and resistance corn at different vegetative growth stages, Journal of economic entomology, 85, 2486, 1992.

White, W.H., Dunckelman, J.W. (1989) The response of sugarcane selections to sugarcane borer in greenhouses and field. American Society of Sugar Cane Technologists. Vol. 9, 56 - 61.

White, W.H., (1993b) Movement and establishment of sugarcane borer (Lepidoptera: Pyralidae) larvae on resistant and susceptible sugarcane. Fl. Entomol. 76, 465 - 473.

White, W. H. (1993a) Cluster analysis for assessing sugarcane borer resistance in sugarcane line trials. Field Crops Res. 33: 159 - 168.

White, W.H, Tew, T.L, Richard, E.P. (2006) Association of sugarcane pith, rind hardness, and fiber with resistance to the sugarcane borer. Journal American Society Sugar Cane Technologists, 26, 87-100.

CHAPTER 2

GENETIC DIVERGENCE AND RESISTANCE OF SUGARCANE VARIETIES TO *Diatraea saccharalis*

ABSTRACT

The sugarcane borer is one of the most important pests in sugarcane in Brazil. Therefore, this work aimed to study resistance traits against D. saccharalis and genetic divergence of six sugarcane genotypes. We assessed the survival of early-stage larvae feeding on the leaves; survival and performance of late-stage larvae feeding within the stalk, time spent by larvae to enter into the stalks and stalk damage. We also assessed the correlation between resistance traits and the genetic divergence of sugarcane varieties for borer response. The genotypes SP803280, RB928064 and RB835486 had the lowest stalk damage while SP891115 had the highest damage. The larvae spent more time to enter the stalk of SP803280 indicating that this genotype has some resistance trait in stalk surface delaying larvae entrance in the stalk. Correlation analysis among resistance factors indicated that the time spent by larvae to enter the stalk is the main factor affecting stalk damage. Late-stages larvae feeding in the stalk of SP813250 had the lowest weight gain indicating that this genotype present some resistance factor affecting larval development within the stalks. The genotypes RB867515 and SP891115 caused the highest mortality of early-stage larvae feeding on the leaves indicating the presence of antibiotic factors in the leaves of these genotypes. Cluster analysis divided the genotypes in three groups, indicating the genetic dissimilarity between genotypes. The results from this study may contribute in selection of resistant clones and choice of genitors for crossings in sugarcane breeding programs.

Keywords: sugarcane borer, genetic divergence, Saccharum officinarum

1. Introduction

The sugarcane stalk borer Diatraea saccharalis Fab. (Lepidoptera: Crambidae) is one of the most important pest in sugarcane in Brazil and is widely distributed in sugarcane belts across the country (Dinardo-Miranda et al., 2008). Damage is caused by larvae tunneling into the stalks, causing the death of a large number of shoots when the sugarcane crop is young and yield loss in more developed crops. In addition, the borer favors the entrance of fungi that causes the disease known as sugarcane red rot (Botelho & Macedo, 2002; Dinardo-Miranda et al., 2008; Dinardo-Miranda et al., 2012).

The development of resistant sugarcane cultivars to D. saccharalis is very important to the sugar and alcohol sectors owing the reduction of production costs and development of the crop (Demetrio et al., 2008). According to Rutherford (2014), there is a consensus that growing genetically resistant varieties is the most cost effective and appropriate means for managing pests and diseases. Therefore, introgression of resistance genes into productive varieties is a key component of sugarcane breeding strategies. It is also generally recognized that a better knowledge of both pests and the host plant defense mechanisms will lead to the development of novel and improved approaches to enhance the durability of resistance.

Although there is no much information about sugarcane resistance to D. saccharalis (Boiça Jr. et al., 1997) some studies in several sugarcane genotypes confirm that the main sugarcane resistance mechanisms to borers are antibiosis and antixenosis (Meagher et al., 1996) and these mechanisms can be divided in resistance in either leaves or stalks and involving either physical or chemical barriers.

Studies comparing the establishment and damage of the sugarcane borer on two sugarcane cultivars suggested that the resistance of the cultivar NCo 310 (compared to the susceptibility of the cultivar CP 44-101) to sugarcane borer was due primarily to higher mortality of larvae, especially of young larvae prior to tunneling into the stalk (Kyle & Hensley, 1970). Coburn & Hensley (1972) reported difference in survival of early-instars larvae in susceptible and resistant sugarcane genotypes, indicating the presence of resistance traits in the leaves and leaf sheaths. Their studies also suggested that leaf-sheath tightness around the stalk in NCo 310 is partially responsible for the resistance of this variety to D. saccharalis because when young larvae are attempting to establish on plants, the tighter fitting leaf sheaths of NCo 310 inhibit larval establishment more than the relatively loose fitting leaf-sheaths of CP 44-101.

White (1993a) reported difference in larval survival in 3 sugarcane genotypes, indicating antibiosis as a resistance mechanism. This researcher also reported difference in the time the larvae take to enter the stalks suggesting that another component of resistance to sugarcane borer is the prevention of establishment of young larvae within the stalk. White et al. (2011) also suggested antibiosis as a mechanism of resistance to the sugarcane borer in two genotypes from Louisiana breeding program, since they observed low adult emergence and low larval weights in insects from those cultivars.

In studies performed by Dinardo-Miranda et al (2012) to assess resistance mechanisms of 10 sugarcane genotypes to sugarcane borer, difference was found in number of larvae recovered in the stalks at 29 days after infesting the plants with D. saccharalis eggs. They also found significant differences in relation to the weight and length of recovered larvae indicating that the genotypes may affect borer performance in addition to cause mortality. They also found difference in percentage bored internodes indicating that some sugarcane genotypes probably have components such as high fiber content and rind hardness (White et al., 2006) which inhibits the penetration of larvae in the stalk.

In addition to the knowledge about resistance traits in sugarcane genotypes against D. saccharalis, another important factor in sugarcane breeding programs in order to develop resistant cultivars is the study of genetic divergence for borer response (Souza et al., 2014). A group of genotypes can be assessed in order to identify divergent genotypes that can be used as progenitors for hybridization in crop breeding programs (Cruz et al., 2004). Studies regarding genetic divergence for pest response has been successful performed for other crop pests such as Tibraca limbativentris Stål (Hemiptera: Pentatomidae) in rice (Souza et al, 2008) and Tuta absoluta Meyrick (Lepidoptera: Geleichidae) in tomato (Suinaga et al., 2000) indicating the importance of studying genetic divergence for pest response. In sugarcane, Souza et al. (2014) studied the genetic divergence of sugarcane genotypes for borer response and reported genetic dissimilarity between genotypes and the possibility of grouping them by using multivariate analysis.

The dissimilarity among individuals within a population can be estimated by using multivariate techniques such as principal components analysis, canonic variables and grouping analysis (Cruz et al, 2004). In grouping analysis, the purpose is to form groups that present homogeneity within the group and heterogeneity between groups.

Several researchers (Oliveira et al., 1999; Machado et al., 2002) recommend the use of genitors with maximum genetic divergence possible, in order to maximize the heterosis in the hybrids, increasing the genetic gain in the segregating population. Thus individuals belonging to different groups should be used as progenitors in crop breeding programs (Carpentieri-Pípolo et al., 2000). In addition to genetic divergence, the choice of progenitors for hybridization must consider the use of groups presenting more favorable characteristics (Abreu et al.,1999).

Therefore, this work aimed to assess the effect of genotypes in survival of early-stages larvae; effect of genotypes in the survival and performance of late-stages larvae; effect of genotypes in the larvae establishment within the stalk. We also assessed the genetic divergence of sugarcane varieties for borer response.

2. Material and Methods

The varieties tested SP803280, SP813250, RB928064, RB835486, RB867515 and SP891115 were obtained from the Germplasm Unit of the sugarcane breeding program of Federal University of Viçosa in partnership with RIDESA (Barbosa, 2012). The variety SP891115 was used as susceptible standard (Dinardo-Miranda et al., 2012). The insects were obtained from a mass rearing maintained in artificial diet according to Hensley and Hammond (1968), adapted by Araújo et al. (1985).

The experiments were carried out at the experimental field Diogo Alves Mello and in the Laboratory of Insect-Plant Interaction of Entomology department of Federal University of Viçosa (UFV), municipality of Viçosa (20º45´ S, 42º52´ W; h = 650 m), Minas Gerais state, Brazil, from October/2013 to April/2014.

For obtaining the plants for all experiments, single-node sugarcane stems cuttings containing one lateral bud were germinated in trays filled with agricultural substrate (Tropstrato®). Then, 60 days after planting, the seedlings were transplanted to 7 L plastic pots filled with soil and maintained in a greenhouse (26 ± 10ºC temperature; 75 ± 20% relative humidity and 12 ± 2 hours photophase). The soil correction and fertilization were performed based in previous soil analysis and following the indication to the crop as described by Korndorfer et al (1999). Plants were irrigated using drippers at 0.5 to 2.0 l/pot/day. All the tillers were removed to keep just a single plant per pot. When the plants reached 6 to 7-month-old, the experiments were initiated as following.

2.1 Survival of early-stage larvae

This experiment was carried out in the laboratory (26 ± 2^0 C; 70 ± 10% RH; and 12 h photoperiod). Pots (5 L) containing one 4-month-old single plant were placed in trays containing water to prevent ant predation. Then, 40 first-instar larvae of D. saccharalis were transferred to the youngest leaf of each plant by using a camel hair. The plants were dissected nine days after infestation and the larval survival and foliar damage were assessed. The foliar damage was assessed by using a scale ranging from 1 to 5, where the score 1 was assigned to low feeding damage in the leaves (< 20% foliar damage in young leaves) and score 5 was assigned to high feeding damage (> 80% of foliar damage in young leaves). The experiment was conducted in a completely randomized design with four replications.

2.2 Survival and performance of late-stages larvae

For this bioassay, 5 plants of each tested variety were harvested, the leaves and the leaf sheaths were removed and a 30 cm section of the upper portion of the stalks was taken to the laboratory. Each stalk section was placed in a 2L polyethylene terephthalate (PET) bottle. The bottles had side-openings covered with organza for ventilation. Then, twenty larvae (15-day-old) were weighted and released within each cage. The cages were maintained in a room (26 ± 2^o C temperature; 70 ± 10% relative humidity and 12 h photophase). At 10 days later, the stalks were dissected and the total amount of insects, average weight gain of larvae and the percentage of pupae were recorded. The average weight gain of larvae was calculated by using the formula: (final total weight) / (final number of insects) – (initial total weight) / (initial number of insects). The experiment was carried out in a completely randomized design with five replicates. Each replicate was composed by one pet bottle containing one stalk section.

2.3 Stalk damage

For this experiment, five 7-month-old plants of each genotype were infested with larvae of D. saccharalis. Nine-day-old larvae (3^{rd} instar) were transferred to 50 ml falcon tubes®. Then, one falcon tube® containing 20 larvae was placed in leaf sheaths in the middle portion of each plant. 20 days later, the stalks were cut off at the soil level and the total tunnel length and percentage of stalk length damaged were recorded. The

experiment was carried out in a completely randomized design with five replicates. Each replicate was composed by one plant.

2.4 Time spent by larvae to enter the stalk

For this experiment, 7-month-old plants were taken to the laboratory (26 ± 10ºC temperature; 75 ± 20% relative humidity and 12 ± 2 hours photophase) and the experiment was initiated. The experiment was carried out in completely randomized design with 20 replicates per treatment. Four plants of each genotype were infested with five larvae 9-day-old, totaling 20 larvae per genotype. Each larvae was transferred to an Eppendorf tube (2.5 mL) and then, each tube was adhered in the stalk by using an adhesive tape with the opening toward the stalk surface. The larvae entrance in the stalks was assessed at 24, 48, 72 and 96 h after infestation.

2.5 Statistical analysis and cluster analysis

The data from each variable were first submitted to analysis of variance and the means compared by the LSD Fisher's procedure. Correlation analysis were performed between variables pairs to study the relationship between them. The software SAS 9.0 was used in all analysis.

The data of time spent by larvae to enter the stalks were analyzed by using the non-parametric procedure PROC LIFETEST of SAS. The Kaplan-Meier method was used to estimate the entrance time curves and the curves were compared by the Log-Rank test ($P<0.05$). The larvae entrance in the stalk was considered as the events and the larvae that did not enter the stalk up to 96 h after infestation were considered as censored data. The difference in mean time spent by larvae to enter the stalks of the six genotypes were compared by Pairwise Multiple Comparison Procedure Holm-Sidak method.

For cluster analysis we used the variables: larval mortality in the leaves and foliar damage; stalk damage; survival and weight gain of larvae and percentage of pupation in the stalk to calculate the standardized mean Euclidean distance as genetic dissimilarity measure and proceed Ward's method, Tocher's grouping optimization method and principal component analysis. The software Genes (Cruz, 2013) was used in all analysis.

3. Results and discussion

There was difference among genotypes in stalk damage ($F_{5;24}$ = 4.896; P = 0.003). The variety SP891115 had the largest tunnel length (61.75 cm) while SP803280 had the lowest tunnel length (27.55 cm)(Figure 1). The result of this experiment was consistent with the previous experiments as SP803280 remained as the least damaged and SP891115 as the most injured genotype.

There was difference in the curves of larval entrance time (Log-Rank statistic = 15.605, df = 5 and P = 0.008). The larvae spent less time to enter the stalk of the genotype SP891115 (46.00 ± 7.13 hours) and more time to enter the stalk of the variety SP803280 (80.5 ± 6.46 hours)(P = 0.0347)(Figure 1). For the other genotypes, the time spent by larvae to enter the stalk ranged from 69.6 to 76.9 hours. In the genotype SP891115, only 15% of larvae did not enter the stalks during the assessment time while in the others genotypes, 50% of larvae in average did not enter the stalks during this time, being these larvae considered as censored data.

The larvae spent in average 34.5 less hours to enter the stalk of SP891115 in comparison to SP803280 and the number of larvae that entered the stalks of the SP891115 up to 96 h after infestation was nearly the double of the larvae that entered the stalk of the SP803280. Difference in the time spent by D. saccharalis larvae to enter the stalks has been reported for sugarcane (White, 2003a) and rice (Sidhu et al., 2013) genotypes. Correlation analysis confirmed that difference in stalk length among genotypes was more related to the presence of some trait in the stalk surface delaying larvae entrance in the stalk than traits affecting the development of larvae established within the stalks (Table 1). In field studies to compare resistance of genotypes, the researchers assess bored internode as damage measure as it is an indication of successful entry of a larva into the stalk (Milligan et al, 2003; White et al. 2001, 2006). According to White et al. (2011), clones with lower percentage of bored internodes indicate the presence of traits that inhibit successful larva penetration in the stalk, such as high fiber content and hard internode rind (White et al., 2011).

There was no difference among genotypes for number of insect survival and percentage of pupation. However, there was difference among genotypes for weight gain of larvae ($F_{5;24}$ = 5.07; P = 0.003). The larvae feeding in stalks of SP891115 and RB928064 gained more weight (34.61 and 31.39 mg, respectively). On the other hand, the larvae feeding on the stalks of SP813250 had the least weight gain (16.03 mg)(Figure 1).

Larvae feeding on stalks of varieties SP891115 and RB928064 gained nearly 2-fold the weight gained by the larvae feeding on stalks of the variety SP813250. Thus, there appears to be some antibiotic factors in SP813250 stalk as previously observed for other cultivars (White et al., 2011; Dinardo-Miranda et al., 2012). However, it is difficult to distinguish how much of this effect is due to deterrence, low plant quality for the insect, or antibiosis (Dinardo-Miranda et al.; 2012). The lack of correlation among tunnel length and larval development within the stalks indicated that once the larvae are established within the stalks, other resistance factors affect their development. In previous field studies to compare resistance of sugarcane cultivars, the researchers assessed the presence of pupal gate in the stalks as an indicative of successful larval development within the stalk and subsequent moth emergence (Bessin et al., 1990; Milligan et al., 2003). According to White et al (2011), cultivars with low numbers of emergence holes is an indirect measure of the existence of a possible resistance factor within the stalk.

There was no difference among genotypes in foliar damage. However, there was difference among genotypes in larval survival ($F_{5;18} = 4.84$; $P = 0.006$)(Figure 1). The early-stage larvae had lowest survival rate on the leaves of SP891115 and RB867515 (0.63 and 1.88%, respectively). On the other hand, the larvae feeding on the leaves of SP803280 had the highest survival rate (23.75%). It indicates the presence of antibiotic factors present on the leaves of SP891115 and RB867515.

Our findings also indicate that sugarcane genotypes have different effects on survival of early-stage larvae feeding on the leaves, as previous observed in other sugarcane genotypes (Coburn and Hensley, 1972; White, 1993b). Despite we found no significant difference among genotypes in foliar damage, our data showed a high correlation between foliar damage and early-stage larvae survival, which is indicates obviously that antibiotic factors affecting survival of larvae results in less foliar damage. A damage rating based in leaf-sheaths feeding signs, presence of dead tops and lateral shoots has been developed to compare resistance of sugarcane genotypes (see Milligan et al. 2003). These damage rating is likely to be associated to presence of resistance factors on the leaves and green tissues. The general lack of association between damage

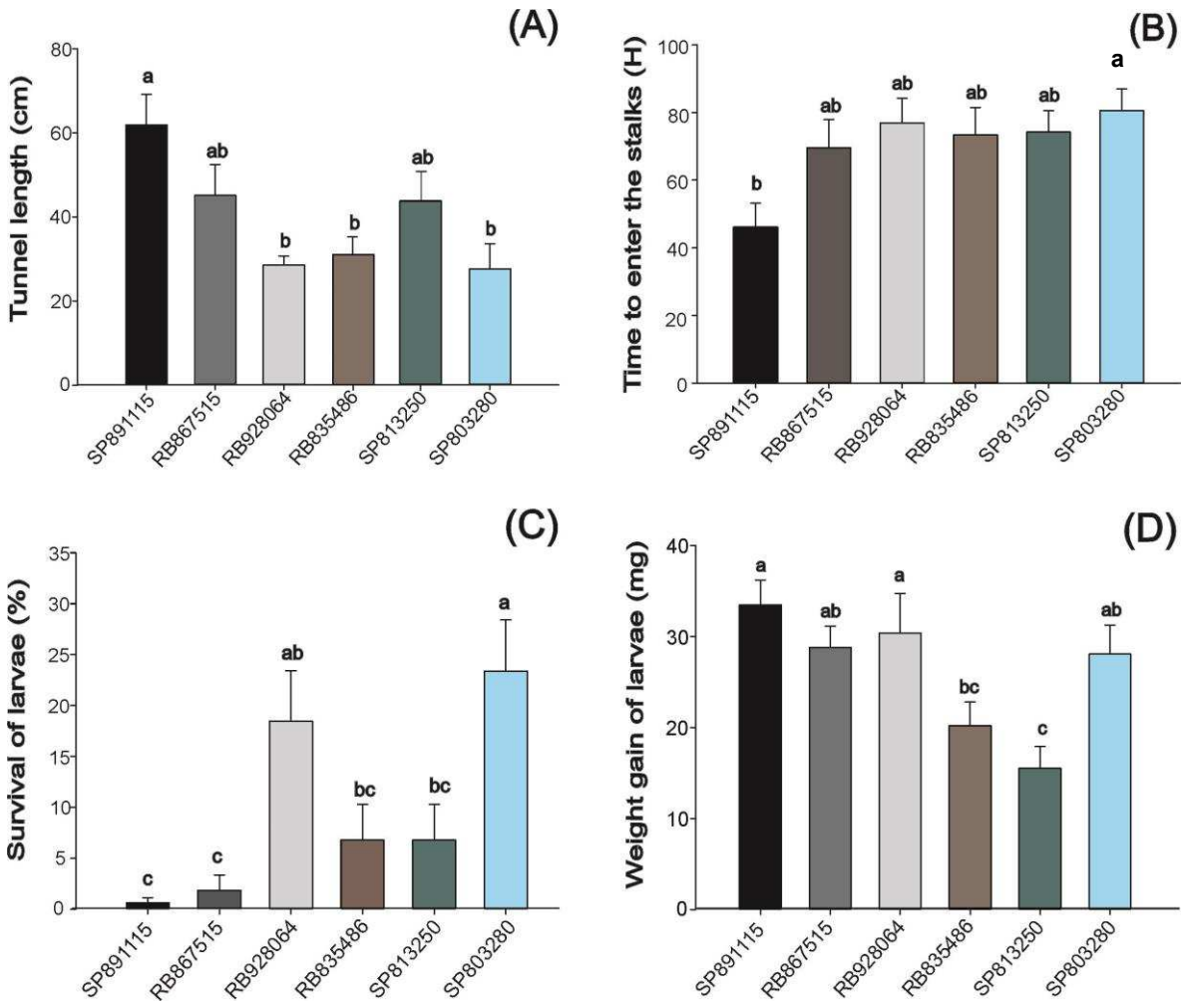

Figure 1. Resistance traits of six sugarcane cultivars against Diatraea saccharalis. (A) Tunnel length in stalks of 7-month-old plants infested with 9-day-old larvae in greenhouse; (B) Mean time spent by 3rd instar larvae to enter stalk of 7-month-old plants; (C) Survival of early-stages larvae feeding on leaves of 4-month-old plants in laboratory (26±2º C; 70±10% RH; and 12 h photoperiod); (D) Weight gain of late-stages larvae feeding in stalks of 7-month-old plants in laboratory (26±2º C; 70±10% RH; and 12 h photoperiod). Means ± standard error topped by the same letter are not different according to Fisher´s LSD procedure, protected by ANOVA $P < 0.05$).

Table 1. Correlation analysis between variables pairs.

Variable	Survival within the stalk (%)	Pupation within the stalk (%)	Tunnel length (cm)	Mean time to enter the stalks (h)	Survival in the leaves (%)	Foliar injury (visual scale)
Weight gain of larvae within the stalk (mg)	r = -0.0994; P = 0.851	r = 0.489; P = 0.325	r = 0.256; P = 0.624	r = -0.458; P = 0.361	r = 0.0779; P= 0.883	r = 0.179; P= 0.735
Survival within the stalk (%)		r = -0.484; P = 0.33	r = 0.681; P = 0.136	r = -0.716; P = 0.109	**r = -0.885; P = 0.0192**	r = -0.594; P = 0.214
Pupation within the stalk (%)			r = -0.15; P = 0.777	r = -0.0746; P = 0.888	r = 0.498; P = 0.315	r = 0.59; P = 0.217
Tunnel length (cm)				**r = -0.916; P = 0.010**	r = -0.807; P = 0.0522	r = -0.542; P = 0.267
Mean time to enter the stalks (h)					r = 0.708; P = 0.115	r = 0.342; P = 0.507
Survival in the leaves (%)						**r = 0.867; P= 0.0253**

Pearson correlation analysis between variables obtained from six sugarcane genotypes. *significant at P<0.05.

ratings and percentage bored internodes supports the notion that different mechanisms of resistance are being expressed among the different varieties (White, 1993a). This present study confirmed this notion as our data indicated a significant negative correlation among survival of early-stages larvae feeding on the leaves and late-stages larvae feeding within the stalk.

Regarding the genetic divergence as revealed by principal components analysis, the first principal component represented 58.63%, and the second principal component represented 83.48% of the total accumulate variance, respectively. Thus, a two-dimensional graph was adequate to represent the genetic divergence among cultivars (Cruz, 2014)(Figure 4). According to the graph, it is possible to distinguish four divergent groups. The group 1 is composed by SP891115, the group 2 is composed by RB867515, the group 3 is composed by SP813250 and RB835486 and the group 4 is composed by SP803280 and RB928064.

In the cluster dendogram, the cophenetic correlation coefficient (r) was 0.93, indicating a high adjustment between the dissimilarity matrix and the cluster dendrogram. Thus the Ward's Method was adequate for providing the cluster dendrogram (Cruz, 2014). In a cluster dendogram a high change of level indicates the union of heterogeneous varieties. Thus, using 55% of dissimilarity among genotypes as a criterion for definition of the groups, three groups were formed (Figure 5). The groups formed by the Tocher's grouping optimization method were in agreement with the groups formed by Ward's method. In both methods, the group 1 was composed by SP891115, the group 2 was composed by RB867515, SP813250 and RB835486 and the group 3 was composed by SP803280 and RB928064 (Table 2).

In our study, cluster analysis was found to be a useful tool for interpreting data from varietal trials and was beneficial as a descriptive tool, complementing standard analysis of variance as it enabled us to split the treatments, into non overlapping homogeneous groups. Cluster analysis has been used in previous field studies to group sugarcane genotypes according to their resistance to sugarcane borer, indicating the genetic dissimilarity between sugarcane genotypes for borer resistance (White, 1993a). In our work, three divergent groups of sugarcane genotypes were formed by Ward's method and Tocher's optimization method and the groups formed were in agreement in both methods. The principal component analysis formed four groups in agreement with the other methods. The only difference was that the variety RB867515 was allocated in a different group. The use of genotypes with high genetic dissimilarity and favorable

means for resistance traits as progenitors is indicated in breeding programs to increase the occurrence of valuable segregants (Pitta et al., 2010).

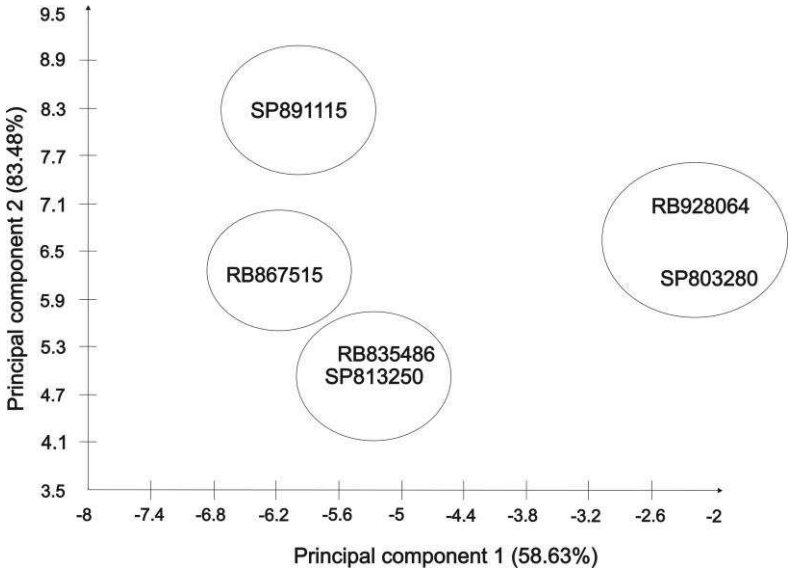

Figure 1. Cluster of six sugarcane varieties by using principal components analysis. The first and the second principal components represent 58.63% and 83.48% of the total accumulate variance respectively.

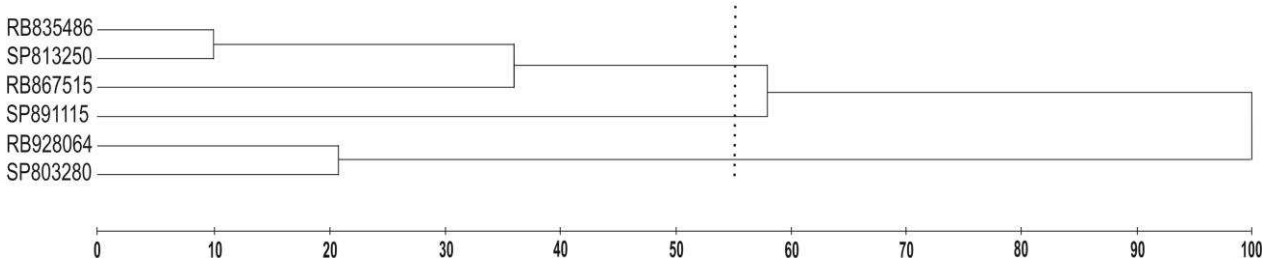

Figure 2. Cluster dendrogram obtained by the Ward method estimated by using the standardized mean Euclidean distance as dissimilarity measure among genotypes pairs. Cophentetic correlation coeficient (r) = 0.93.

Table 2. Cluster of six sugarcane varieties by the Tocher's optimization method based on standardized mean Euclidean distance, estimated by six traits

Group	Varieties	Leaves		Stalks			
		Larval survival (%) †	Damage rating†	Larval survival (%) ‡	Pupation (%)‡	Weight gain of larvae (mg) ‡	Tunnel length (cm) §
1	SP891115	0.63	2.5	86.00	11.03	35.00	61.75
2	RB867515 - RB835486 - SP813250	5.21	2.08	81.67	5.42	22.33	39.87
3	SP803280 - RB928064	21.25	3.38	74.00	12.35	30.00	27.98

Group mean for each variable. † Experiments carried out in laboratory (26±2º C; 70±10% RH; and 12 h photoperiod) with 4-month-old plants, infested with D. saccharalis neonates. ‡ Experiment carried out in out in laboratory (26±2º C; 70±10% RH; and 12 h photoperiod) with stalks of 7-month-old plants infested with 15-day-old larvae of D. saccharalis; § Experiment carried out in greenhouse with 7-month-old plants infested with 9-day-old larvae of D. saccharalis .

4. Conclusions

We can conclude that sugarcane genotypes present different resistance mechanisms. Some sugarcane genotypes present resistance in the leaves and leaf-sheaths affecting survival of early-stages larvae; some genotypes present resistance in stalk surface which delay larvae entrance within the stalk; some genotypes present resistance within the stalk, affecting feeding and development of late-stages larvae. The time spent by larvae to enter the stalk is the resistance trait more related to stalk damage. There is genetic dissimilarity among genotypes for borer resistance and assessment of multiple resistance traits and cluster analysis enable selection of genotypes with genetic divergence and favorable means to be used as progenitors in sugarcane breeding programs regarding resistance to D. saccharalis.

5. Acknowledgements

Technical assistance provided by RIDESA - UFV was greatly appreciated. The financial support provided by the National Council of Scientific and Technological Development (CNPq), CAPES Foundation, FAPEMIG Foundation and the RIDESA Sugarcane breeding program was greatly appreciated and acknowledged here.

5. References

Abreu, A.F.B.; Ramalho, M.A.P.; Ferreira (1999).Selection potential for seed yield from intra end inter-racial populations in common bean. Euphytica, v.108, n.2, p.121-127, 1999.

Araújo, J.R.; Botelho, P.S.M.; Araújo, S.M.S.S.; Almeida, L.C.; Degaspari, N. 1985. Nova dieta artificial para criação da Diatraea saccharalis (Fabr.). Saccharum, São Paulo, v. 36, n. 2, p. 45-48, 1985.

Barbosa, M.H.P., Resende, M.D.V., Dias, L.A.S., Barbosa, G.V.S., Oliveira; R.A., Peternelli, L.A., Daros, E. (2012) Genetic improvement of sugar cane for bioenergy: the Brazilian experience in network research with RIDESA. Crop Breeding and Applied Biotechnology S2, 87 - 98.

Boiça jr, A.L.; Lara, F.M.; Bellodi, M.P. (1997). Influência de variedades de cana-de-açúcar, incorporadas em dieta artificial, no desenvolvimento de Diatraea saccharalis (Fabr.) e no seu parasitismo por Cotesia flavipes (Cam.). Anais da Sociedade Entomológica do Brasil, 26(3): 537-542.

Botelho, P.S.M.; Macedo, N. (2002) Cotesia flavipes para o controle de Diatraea saccharalis. In: Parra, J.R.P.; Botelho, P.S.M.;Correa-Ferreira, B.S.; Bento, J.M.S. Controle biológico no Brasil: parasitóides e predadores. São Paulo: Manole, 2002 .p.409-425.

Carpentieri-Pipolo, V.; Destro, D.; Prete, C.E.C. (2000). Seleção de genótipos parentais de acerola com base na divergência genética multivariada. Pesquisa Agropecuária Brasileira, Brasília, v.35, n.8, p.1613-1619, 2000.

Coburn, G.E.; S.D. Hensley. (1972). Differential survival of Diatraea saccharalis (F.) larvae on two varieties of sugarcane. Proc. Int. Soc. Sugar Cane Technol. 14:440-444.

Cruz, C.D.; Regazzi, A.J.; Carneiro, P.C.S. Modelos biométricos aplicados ao melhoramento genético. Viçosa: UFV, 2004, 480p.

Cruz, C.D. Programa GENES: análise multivariada e simulação. Viçosa: UFV, 2006. 175 p. 2006.

Demetrio, P.A.; Zonetti, P.P.; Munhoz, R.E.F. (2008) Avaliação de clones de cana-de-acucar promissores RBs quanto à resistência à broca-da-cana (Diatraea saccharalis) na região noroeste do Paraná. Iniciação Científica Cesumar, v.10, p.13-16, 2008.

Dinardo-Miranda, L.L. Pragas. In: Dinardo-Miranda, L.L.; Vasconselos, A.C.M.; Landell, M.G.A. (Eds). Cana-de-açúcar. IAC, São Paulo, p: 349 – 404, 2008.

Dinardo-Miranda, L.L; Anjos, I.A; Da Costa, V.P; Fracasso, J.V. (2012). Resistance of sugarcane cultivars to Diatraea saccharalis. Pesquisa agropecuária brasileira 47(1):1-7.

Dinardo-Miranda, L.L.; Fracasso, J.V.; Costa, V.P.; Anjos, I.A.; Peixoto; D.O.L. (2013), Reação de cultivares de cana-de-açúcar à broca do colmo. Bragantia, Campinas, v. 72, n. 1, p.29-34, 2013.

Gallo, D.; Nakamo, O.; Silveira, S.; Carvalho, R.P.L.; Batista, G.C.; Berti Filho, E.; Parra, J. R.P.; Zuchi, R.A.; Alves, S. B.; Vendramim, J. D.; Marchini, L. C.; Lopes, J. R. S.; Omoto, C. Entomologia Agrícola. Piracicaba: FEALQ, 2002, 920p.

Hensley, S.D.; Hammond, A.H. 1968. Laboratory techniques for rearing the sugar cane borer on an artificial diet. Journal of Economic Entomology.Lanham, v. 61, n. 6, p. 1742-1743, 1968.

Kyle, M.L., Hensley, S.D.(1970). Sugarcane borer host plant resistance studies. Proc. La. Acad. Sci. (In press).

Korndorfer, G.H.; Ribeiro, A.C.; Andrade, L.A.B Cana de açúcar. In: Ribeiro, A.C.; Guimarães, P. T.G.; Alvarez V., (Eds.). Recomendação para o uso de corretivos e fertilizantes em Minas Gerais: 5. Aproximação. Viçosa: Comissão de Fertilidade do Solo do Estado de Minas Gerais, 1999. p.25-32.

Machado, C.F.; Nunes, G.H.S.; Ferreira, D.F. (2002). Divergência genética entre genótipos de feijoeiro a partir de técnicas multivariadas. Ciência Rural, Santa Maria, v.32, n.2,p.251-258, 2002.

Meagher, R.L., Jr., J.E. Irvine, R.G. Breene, R.S. Pfannenstiel Gallo, M. (1996). Resistance mechanisms of sugarcane to Mexican rice borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 89: 749-760.

Milligan, S. B., M. Balzarini, and W. H. White, (2003): Broad-sense heritabilities, genetic correlations, and selection indices for sugar-cane borer resistance and their relation to yield loss. Crop Sci.43,1729—1735.

Oliveira, V.R.; Casali, V.W.D.; Cruz, C.D. (1999). Avaliação da diversidade genética em pimentão através de análise multivariada. Horticultura Brasileira, Brasília, v.17, n.1, p.19-
24, 1999.

Schoonhoven, L.M., Van Loon, J.J.A., Dicke M. (2005) Insect-Plant Biology, Ed 2. Oxford University Press, Oxford.

Souza, J.R.; Boiça Jr, A.R.; Perecin, D.; Cargnelutti Filho, D.; Costa, J.T. (2013). Divergência genética de cultivares de cana-de-açúcar quanto à resistência a Diatraea saccharalis. Semina: Ciências Agrárias, Londrina, v. 34, n. 6, suplemento 1, p. 3367-3376, 2013

Souza, J.R.; Ferreira, E.; Cargnelutti Filho, A; Boiça Jr, A.L.; Chagas, E.F.; Mondego, J.M. (2009). Divergência genética de cultivares de arroz quanto à resistência a Tibraca

limbativentris Stål (Hemiptera: Pentatomidae). Neotropical Entomology, Londrina, v. 38, n. 5, p. 671-676, 2009.

Suinaga, F.A.; Casali, V.W.D.; Silva, D.J.H.; Picanço, M.C. Dissimilaridade genética de fontes de resistência de Lycopersicon spp a Tuta absoluta (Meyrick, 1917)(Lepidoptera: Gelechiidae) (2000). Revista Brasileira de Agrociência, Pelotas, v. 9, n. 4, p.371-376, 2003.

Smith, C.M. 2005. Plant resistance to arthropods: molecular and conventional approaches. The Netherlands: Springer, 2005. 423p.

Vercambre, B.; Goebel, R., A, D.C.;Rochat, J. (2001). Available methods for assessing varietal resistance to sugarcane stalk borers. Proc S Afr Sug Technol Ass (2001) 75.

Ricaud, C.; Ryan, C. Leaf scald. In: Ricaud, C.; Ryan, C.; Egan, B.T. Gillaspie, E.G.; Rughes, C.G. (Eds). Diseases of sugarcane. Amsterdan. P: 36 – 53, 1989.

White, W.H., (1993a). Movement and establishment of sugarcane borer (Lepidoptera: Pyralidae) larvae on resistant and susceptible sugarcane. Fl. Entomol. 76, 465–473.

White, W.H. (1993b). Cluster analysis for assessing sugarcane borer resistance in sugarcane line trials. Field Crops Res. 33: 159-168.

White, W.H; Tew, T.L; Richard, E.P. (2006). Association of sugarcane pith, rind hardness, and fiber with resistance to the sugarcane borer. Journal American Society Sugar Cane Technologists, 26:87-100.

White, W.H.; Hale A.L.; Veremis, J.C.; Tew, T.L.; Richard Jr, E.P. (2011). Registration of two sugarcane germoplasm clones with antibiosis to the sugarcane borer (Lepidoptera: Crambidae). Journal of Plant Registration, v.5, n.2, p.248-253, 2011.

White, W.H., Hensley. S.D. (1987). Techniques to quantify the effect of Diatraea saccharalis (Lepidoptera: Pyralidae) on sugarcane quality. Field Crops Res. 15:341-348.

Zalucki, M.P., Clarke, A.R., Malcolm, S.B. (2002) Ecology and behavior of first instar larval lepidoptera. Annu. Rev. Entomol. 47,361 - 93.

GENERAL CONCLUSION

The comparison of stalk damage in sugarcane genotypes caused by Diatraea saccharalis larvae may be assessed by infesting 7-month-old single plants with 20 larvae (3^{rd} – 4^{th} instar) and measuring injury extension (tunnel length) in greenhouse trials.

The varieties SP891115 and RB867515 caused the highest mortality of early-stages larvae. The varieties RB928064, RB835486 and SP803280 have some resistance trait delaying larvae entrance in the stalks once they showed shorter tunnel length in the stalks. The stalks of the varieties SP813250 and RB835486 are the least nutritionally adequate for D. saccharalis larvae. The main factor affecting tunnel length is the time spent by larvae to enter the stalk.

There is genetic divergence between sugarcane genotypes for borer resistance and the genotypes can be divided into 3 groups: the group 1 was composed by the genotypes RB835486, RB867515 and SP813250, the group 2 was composed by the genotypes RB928064 and SP803280 and the group 3 by the genotype SP891115. The genotypes belonging to the group 1 and 2 may be used as progenitors in sugarcane breeding programs regarding borer resistance.