ANDREISA FABRI LIMA

MAIZE DEFENSE AGAINST *Spodoptera frugiperda* DUE TO LANDRACE GENOTYPES AND SEED TREATMENT

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Thesis presented to Universidade Federal de Lavras as part of the demands of the Post-graduation Program in Entomology, concentration area Entomology, in order to obtain the Ph.D. title.

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ANDREISA FABRI LIMA

MAIZE DEFENSE AGAINST *Spodoptera frugiperda* DUE TO LANDRACE GENOTYPES AND SEED TREATMENT

DEFESA DE MILHO CONTRA *Spodoptera frugiperda* DEVIDO A GENÓTIPOS CRIOULOS E TRATAMENTO DE SEMENTES

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GENERAL ABSTRACT

Maize (*Zea mays* L.) is a commodity of global importance, but its productivity is constantly affected by biotic and abiotic stresses. Aiming to reduce the impacts on productivity caused by insect pests in this crop, the main control methods used are chemical (via spraying or seed treatment) and resistant transgenic cultivars (*Bt* events), which are mainly used to control fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). However, due to recurrent and incorrect use, such measures have not been efficient to control this caterpillar. In addition, seed treatment, as a preventive measure, especially for sucking insects, can alter and compromise plant defenses to other arthropods. Thus, it is important to study the possible effects of seed treatment, as well as other control methods for *S. frugiperda* in maize. The general objectives of this study were: to evaluate the tolerance levels of six maize genotypes (Amarelão, Aztequinha, Branco Antigo, Palha Roxa, São Pedro and BM 207) under natural infestation of insects, as a promising source for integrated management and for breeding programs (see manuscript 1) and to evaluate the effect of neonicotinoid seed treatment on the constitutive and induced defense of maize plants (see manuscript 2). In the manuscript 1, five maize landrace genotype (Amarelão, Aztequinha, Branco Antigo, Palha Roxa and São Pedro) and a conventional hybrid (BM 207) were used. Under natural infestation of *S. frugiperda*, plant height, stem diameter and chlorophyll content at two growth stages were evaluated. Tolerance levels to *S. frugiperda* was calculated, and comparisons between the conventional hybrid and the different landrace genotypes were carried out. Tolerance levels varied depending on the genotype, with Palha Roxa, BM 207, São Pedro and Aztequinha being considered tolerant. Referring to manuscript 2, two maize genotypes (B73 and MC 4050) were used, which seeds were treated with neonicotinoid insecticide (thiamethoxam). In the V4 and V6 growth stages, the effect of seed treatment on the preference and weight gain of *S. frugiperda*, the levels of phytohormones [salicylic acid (SA) and jasmonic acid (JA)] and constitutive and induced plant volatiles by *S. frugiperda* caterpillars were evaluated. Plant response to neonicotinoid seed treatment varied as a function of genotype and growth stage. The B73 genotype was the most affected by the neonicotinoid, being observed suppression of SA and volatiles, and a direct effect on the weight gain of *S. frugiperda*. In conclusion, the landrace genotypes studied have a high tolerance potential, and for this reason should be used in breeding programs aimed at the management of *S. frugiperda*. Furthermore, thiamethoxam seed treatment is a strategy that should be studied more in depth, as it may influences the defenses of maize plants.

Keywords: *Zea mays*. Plants defenses. Fall armyworm. Thiamethoxam. IPM

RESUMO GERAL

O milho (*Zea mays* L.) é uma *commodity* de importância mundial, porém a sua produtividade é constantemente afetada por estresses bióticos e abióticos. Visando reduzir os impactos na produtividade causados por insetos pragas nessa cultura, os principais métodos de controle utilizados são o químico (via pulverização ou tratamento de sementes) e cultivares transgênicas resistentes (eventos *Bt*), as quais são empregadas, principalmente, para controle da lagarta-do-cartucho do milho, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). Porém, devido ao uso recorrente e incorreto, tais medidas não têm sido eficientes para o controle dessa lagarta. Além disso, o tratamento de sementes, como medida preventiva, especialmente para insetos sugadores, pode alterar e comprometer as defesas de plantas a outros artrópodes. Desta forma, é importante estudar os possíveis efeitos do tratamento de sementes, bem como outros métodos de controle para *S. frugiperda* em milho. Os objetivos gerais desse estudo foram: avaliar os níveis de tolerância de seis genótipos de milho (Amarelão, Aztequinha, Branco Antigo, Palha Roxa, São Pedro e BM 207) sob infestação natural de insetos, como um recurso promissor para o manejo integrado e para programas de melhoramento (ver artigo 1) e avaliar o efeito do tratamento de sementes com neonicotinoide na defesa constitutiva e induzida de plantas de milho (ver artigo 2). No artigo 1 foram utilizados cinco genótipos de milho crioulo (Amarelão, Aztequinha, Branco Antigo, Palha Roxa e São Pedro) e um híbrido convencional (BM 207). Sob infestação natural de *S. frugiperda*, foi avaliada a altura da planta, diâmetro do colmo e conteúdo de clorofila em dois estádios de crescimento. Foram calculados os níveis de tolerância à *S. frugiperda*, e realizadas comparações entre o híbrido convencional e os diferentes genótipos crioulos. Os níveis de tolerância variaram em função do genótipo, sendo Palha Roxa, BM 207, São Pedro e Aztequinha considerados tolerantes. Referente ao artigo 2, foram utilizados dois genótipos de milho (B73 e MC 4050), cujas sementes foram tratadas com inseticida neonicotinoide (tiametoxam). Nos estádios de crescimento V4 e V6, foi avaliado o efeito do tratamento de sementes na preferência e ganho de peso de *S. frugiperda*, nos níveis de fitohormônios [ácido salicílico (AS) e ácido jasmônico (AJ)] e voláteis de plantas constitutivos e induzidos por lagartas de *S. frugiperda*. A resposta das plantas ao tratamento de sementes com neonicotinoide variou em função do genótipo e estádio de desenvolvimento. O genótipo B73 foi o mais afetado pelo neonicotinoide, sendo observado supressão de AS e voláteis, e efeito direto no ganho de peso de *S. frugiperda*. Para concluir, os genótipos crioulos estudados apresentam alto potencial de tolerância e por isto podem ser utilizados em programas de melhoramento visando ao manejo de *S. frugiperda*. Além disso, tiametoxam em tratamento de sementes é uma estratégia que deve ser estudada de forma aprofundada, pois pode influenciar nas defesas de plantas de milho.

Palavras-chave: *Zea mays*. Defesa de plantas. Lagarta-do-cartucho. Tiametoxam. MIP

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GENERAL INTRODUCTION

Maize, *Zea mays* L. (Poaceae), is a crop with a wide geographic distribution experiencing an expansion of planted areas in the growing regions (ERENSTEIN; CHAMBERLIN; SONDER, 2021; LEFF; RAMANKUTTY; FOLEY, 2004). Maize products are used as a nutritional and food source for humans and animals (SHIFERAW et al., 2011). In addition, the crop has gained greater visibility and increased demand for use in the production of fuel (VELJKOVIĆ et al., 2018). However, damage (direct or indirect) by insect pests is the main cause of reduction in maize productivity (KARJAGI et al., 2017; PIMENTEL, 2009).

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is one of the most important agricultural pests in the Americas (ANDREWS, 1980; EARLY et al., 2018). *Spodoptera frugiperda* is a major pest of maize, due the direct damage caused by feeding, which decreases crop productivity (SARMENTO et al., 2002). This pest is native to tropical and subtropical regions (EARLY et al., 2018; SPARKS, 1979), and is currently considered invasive in Africa (GOERGEN et al., 2016; OTIM et al., 2018), India (CHORMULE et al., 2019; SHARANABASAPPA et al., 2018), and China (JING et al., 2020). Fall armyworm has more than 350 host plants in 76 botanical families, including important economic species, such as rice, sorghum, soybean, and cotton (MONTEZANO et al., 2018).

The management of *S. frugiperda* occurs mainly with insecticide applications and with the use of genetically modified cultivars containing toxin of the bacterium *Bacillus thuringiensis* (*Bt*). Nonetheless, inappropriate, and excessive spraying results in insecticide-resistant *S. frugiperda* populations. Insects have become resistant to several synthetic insecticide groups, including pyrethroids and organophosphates (CARVALHO et al., 2013; MORILLO; NOTZ, 2001), benzoylurea (NASCIMENTO et al., 2016), spinosyn (LIRA et al., 2020; OKUMA et al., 2018), and diamides (BOLZAN et al., 2019). Moreover, there are reports of field-evolved resistance to *Bt* proteins such as Cry1F and Cry1Ab expressed in maize transgenic lines (BOAVENTURA et al., 2020; FARIAS et al., 2014; FLAGEL et al., 2018; OMOTO et al., 2016).

In addition, neonicotinoids, another group of insecticides, through seed treatment is a routine practice to control insect pests, especially *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae) (RIBEIRO; CANALE, 2021) and is used in almost all planted maize seeds (TOOKER; DOUGLAS; KRUPKE, 2017). However, although neonicotinoid treatment aids in the reduction of target pest species, there is increasing concern around non-target effects

such as altering plant defenses and increasing susceptibility to non-target arthropods (SZCZEPANIEC et al., 2011, 2013; WULFF et al., 2019).

Given this context, it is important to explore integrated pest management (IPM) strategies for efficient and sustainable pest control (DARA, 2019). Maize landraces have high genetic diversity, which have been reported as potential sources of host plant resistance to insect pests (BEDOYA et al., 2017; BRILINGER et al., 2020; COSTA et al., 2018; COSTA; SILVA; OGLIARI, 2017; NOGUEIRA et al., 2019). Host plant resistance (HPR) is categorized into antibiosis (plants traits that affect insect biology), antixenosis (plant traits that affect insect behavior), and tolerance (traits that allow plants to withstand damages without compromising or with a low impact on yield) (PAINTER, 1951; SMITH, 2005; SMITH; CLEMENT, 2012). Host plant resistance is an effective component of IPM because it reduces productivity loss, is commonly cheaper, safe and sustainable (DARA, 2019; KARJAGI et al., 2017). In addition to the need to integrate effective methods for pest control in maize, it is also important to evaluate the direct and indirect effects of one of the most used strategies, neonicotinoid seed treatment (DOUGLAS; TOOKER, 2015; TOOKER; DOUGLAS; KRUPKE, 2017)

Therefore, the main objectives of this study are: I) to evaluate the tolerance levels of five landraces and a conventional hybrid under natural infestation of *S. frugiperda*, and II) to evaluate the effect of neonicotinoid seed treatment on the constitutive and induced defenses of maize.

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1 MAIZE CROP PRODUCTION: IMPORTANCE AND PEST INSECTS

Brazil, one of the largest global food producers, is responsible for producing agricultural products to supply domestic and foreign markets. Among the main commodities, maize (*Zea mays* L., Poaceae) stands out as one of the cereals with the greatest economic importance (ARTUZO et al., 2019; HURLEY; MITCHELL, 2020), and it is cultivated worldwide (LEFF; RAMANKUTTY; FOLEY, 2004; SHIFERAW et al., 2011). In Brazil, maize production is present in all federative units with a strong tendency toward increasing the production area (CONAB - COMPANHIA NACIONAL DE ABASTECIMENTO, 2021).

Maize is especially important due to the use of its products and by-products for human and animal food, production of fuel, and other industrial raw materials (SHIFERAW et al., 2011; VELJKOVIĆ et al., 2018). Further, the demand for maize has been increasing with population growth, especially in developing countries (ANDORF et al., 2019; TANUMIHARDJO et al., 2020). However, poor soil fertility, and the presence of weeds, diseases, droughts and pests are factors leading to loses in maize yield (ANDORF et al., 2019). Besides that, intensive production can favor the occurrence of insect pests (BERNAL; MEDINA, 2018), and may result in decreased productivity.

Maize is a host for a wide range of herbivores, which can occur during the entire crop cycle and attack various plant structures, such as seeds, stems, stalk, leaves and ears (VALICENTE, 2015). The main pest species are of the order Lepidoptera [e.g., *Spodoptera frugiperda* (J. E. Smith) (Noctuidae), *Helicoverpa zea* (Boddie) (Noctuidae), *Helicoverpa armigera* (Hübner) (Noctuidae), *Diatraea saccharalis* Fabricius (Crambidae), *Elasmopalpus lignosellus* Zeller (Pyralidae), *Agrotis ipsilon* (Hufnagel) (Noctuidae), and *Pseudaletia sequax* (Franclemont) (Noctuidae)]; Coleoptera [e.g., *Diabrotica speciosa* (Germar) (Chrysomelidae)]; and Hemiptera [e.g., *Rhopalosiphum maidis* (Fitch.) (Aphididae), *Dalbulus maidis* (DeLong & Wolc.) (Cicadellidae), *Dichelops furcatus* (Fabricius) (Pentatomidae), *Dichelops melacanthus* (Dallas) (Pentatomidae), *Euchistus heros* (Fabricius) (Pentatomidae), and *Leptoglossus zonatus* (Dallas) (Coreidae)] (WORDELL FILHO et al., 2016). The damage caused by herbivory is one of the major factors responsible for a decrease in maize production.

Historically, synthetic insecticides have been used in agricultural fields to protect crops against losses caused by insect herbivory (BEGUM; ALAM; JALAL UDDIN, 2017; GHOSAL; HATI, 2019). There are records of several synthetic insecticides being used on maize via

spraying or seed treatment aiming to reduce pest damage (e.g., chemical group organophosphates, methylcarbamates, pyrethroids, benzoylureas, spinosyn, diamides, and neonicotinoids) (AGROFIT, 2021). Nonetheless, inappropriate and excessive spraying results in the selection of insecticide-resistant insect populations, mainly *S. frugiperda* (BOLZAN et al., 2019; CARVALHO et al., 2013; LIRA et al., 2020; MORILLO; NOTZ, 2001; NASCIMENTO et al., 2016; OKUMA et al., 2018).

In addition, genetically engineered crops that express *Bacillus thuringiensis* (*Bt*) proteins are extensively used in maize to control *S. frugiperda* (BERNARDI et al., 2015; BOTHA et al., 2019; HURLEY; MITCHELL, 2020), which contributed to decreased insect pest damage and application of synthetic insecticides (BROOKES; BARFOOT, 2017; VALICENTE, 2015). However, since the introduction, *Bt* technology has been adopted at a large scale, and as a result, it has contributed to the rapid evolution of insect resistance, which was first reported in 2014 (FARIAS et al., 2014).

Overall, the main forms of pest control currently used in maize production have contributed to the selection of arthropod resistance and inefficient control, mainly *S. frugiperda* populations, which requires more attention to this insect.

2 *Spodoptera frugiperda*

Fall armyworm, *S. frugiperda* is the most important pest in the Americas (EARLY et al., 2018; SPARKS, 1979), and became an invasive pest in Africa in 2016 (GOERGEN et al., 2016). Since then, *S. frugiperda* has spread into various geographic regions (OTIM et al., 2018; SHARANABASAPPA et al., 2018; SUN et al., 2021), raising concern about food security. In addition to maize, *S. frugiperda* is a key pest in others crops of economic and food importance such as rice (*Oryza sativa* L.), sugarcane (*Saccharum officinarum* L.), sorghum (*Sorghum bicolor* L.), cotton (*Gossypium hirsutum* L.), and soybean (*Glycine max* L.) (DAY et al., 2017; MONTEZANO et al., 2018). The polyphagous habit of the species, and consequently the constant supply of hosts, is a factor that makes it difficult to control this pest (CUI et al., 2020; GOUIN et al., 2017; MONTEZANO et al., 2018).

Fall armyworm, is an insect with holometabolous development [complete metamorphosis including egg, larva (caterpillar), pupa and adult stage]. Females can lay between 50-250 eggs, forming a mass distributed in layers, and without a preferred oviposition location on the plant. After approximately three days, the caterpillars hatch and go through six stages of growth. Young caterpillars typically scrape the leaves while beginning to feed, which

is the typical damage symptom of *S. frugiperda.* While, as caterpillars develop, holes and leaf damage increase (CRUZ, 1995). All instars prefer leaves within the whorl and eventually can destroy the plant's growing point, known as deadheart. However, caterpillars my also feed on all maize structures (CRUZ, 1995; TOEPFER et al., 2021). In the last larval instar, the caterpillars migrate into the soil to pupate and later emerge as adults (CRUZ, 1995; SPARKS, 1979).

Fall armyworm adults have strong flight abilities (EARLY et al., 2018; JING et al., 2020; VILARINHO et al., 2011), as well as high fecundity, long adult life span and high spawning rate (CRUZ, 1995; SPARKS, 1979), which contributes to their establishment and maintenance in new regions.

As soon as *S. frugiperda* colonizes a new area, control methods need to be integrated for the effective management of this pest (TAMBO et al., 2020). The main control methods (chemical insecticides and *Bt* cultivars) have shown loss of efficiency due to the selection of resistant populations of *S. frugiperda* in maize (BANERJEE et al., 2017; BOAVENTURA et al., 2020; BOLZAN et al., 2019; CARVALHO et al., 2013; FARIAS et al., 2014; FLAGEL et al., 2018; LIRA et al., 2020; MORILLO; NOTZ, 2001; NASCIMENTO et al., 2016; OKUMA et al., 2018). Overall, considering the need for new control alternatives for *S. frugiperda*, especially due to the high voracity of the pest and ineffective control methods, it is important to study the plants' natural defense capacity, as well as the factors that can change the relationship between insect and plant.

3 MAIZE LANDRACE

The maize germplasm contains distinct crop genotypes, known as landraces. Landraces are dynamic populations, which are often highly genetic diverse, locally adapted, and associated with a set of farmers' practices of seed selection and field management, as well as with traditional knowledge (VILLA et al., 2005). Due to the high genetic variability and adaptability to different environmental conditions, landraces have a wide diversity of morphological and physiological traits (ARTEAGA et al., 2016; BEDOYA et al., 2017; SANTOS et al., 2020; STRIGENS et al., 2013), resulting in different levels of resistance to biotic and abiotic stresses (BRILINGER et al., 2020; COSTA et al., 2018, 2020; DWIVEDI et al., 2016; SANTOS et al., 2020). In this way, landraces offer a great source of genetic material for breeding programs (DWIVEDI et al., 2016; PRASANNA, 2012; STRIGENS et al., 2013).

The allocation of resources to productivity in modern hybrids may result in less investment in defense against insects (CHINCHILLA-RAMÍREZ et al., 2017; FONTES-PUEBLA et al., 2021; FONTES-PUEBLA; BERNAL, 2020; ROSENTHAL; DIRZO, 1997). On the other hand, previous studies have found many maize landraces with potential defenses to arthropod pests, such as *S. frugiperda* (COSTA et al., 2020; NOGUEIRA et al., 2019; SINGH et al., 2021), *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (BRILINGER et al., 2020; NASCIMENTO et al., 2014a, 2014b), and *D. speciosa* (COSTA et al., 2018, 2021). Thus, there is growing evidence of the potential of maize landraces that needs to be explored for insect resistance and tolerance traits.

4 INTEGRATED PEST MANAGEMENT AND HOST PLANT RESISTANCE

Integrated pest management (IPM) combines different tactics and strategies to identify, manage, and reduce the risk from pests' injury in a way that minimizes overall economic, health, and environmental risks to the agroecosystem. In other words, IPM is an approach to manage insect pests in an economically viable, socially acceptable, and environmentally safe manner (DARA, 2019). The goal of IPM is to keep the pest population density below the economic injury level (GREEN; STENBERG; LANKINEN, 2020). For this, IPM considers the knowledge of the environmental conditions and population dynamics of the pest (KOGAN, 1998). In this context, host plant resistance (HPR) is an important and profitable component of IPM (SHARMA; ORTIZ, 2002; STOUT, 2014), either through conventional breeding or genetic engineering (ANDERSON et al., 2019). One of the main advantages of host plant resistance is the compatibility with other control methods, such as chemical, cultural, and biological control (FATHIPOUR et al., 2019; HANSON; KOCH, 2018).

Among the advantages attributed to HPR are the reduction of synthetic insecticide applications, reduction of residues in food and environment, and limited impact on natural enemy populations (BALDIN; VENDRAMIM; LOURENÇÃO, 2019). Also, HPR has been raised as an efficient alternative control method where most farmers have low financial resources destined to sustainable and effective pest management, such as the control of *S. frugiperda* in Africa (MATOVA et al., 2020).

HPR is defined as *"the sum of the genetically inherited qualities that results in a plant of one cultivar or species being less damaged by a pest arthropod than a susceptible plant lacking these qualities"*(SMITH; CLEMENT, 2012). According to these authors, HPR is segmented into three categories: antibiosis (adverse effects on insect biology), antixenosis

(adverse effects on insect behavior), and tolerance (plant's ability to support herbivore injury) (PAINTER, 1951; SMITH, 2005; SMITH; CLEMENT, 2012).

The trichotomic framework of HPR was proposed by Painter in 1951. 62 years later, Stout in 2013 proposed a dichotomous classification, since, in some cases, the separation between antibiosis and antixenosis may overlap or is difficult to disentangle. For example, *S. frugiperda* feeding on plant extracts of different species of Solanaceae showed lower larval weight after seven days of feeding. This weight reduction was primarily attributed to adverse effects of the compounds on insect biology (antibiosis). However, this result can also be due to compounds that inhibit feeding (antixenosis) (LIMA et al., 2021). Therefore, due to this complexity, the dichotomous classification divides HPR into resistance and tolerance, which is also compatible with the literature on insect-plant interactions, and these concepts may favor the use by farmers in practice (STOUT, 2013).

In the dichotomic scheme, resistance is used to comprehend plant traits that reduce the extent of injury done to a plant by an herbivore, being subdivided into two categories: constitutive × inducible, and direct × indirect (MITCHELL et al., 2016; STOUT, 2013). While the term tolerance is the plant's ability to withstand damage without compromising plant fitness and productivity, keeping the same definition proposed by Painter (1951). Although Stout (2013) raised concerns about Painters' classification, the trichotomous classification has been widely used in papers and accepted by many researchers (BALDIN; VENDRAMIM; LOURENÇÃO, 2019; COSTA et al., 2018; NOGUEIRA et al., 2019; SMITH, 2005; SMITH; CLEMENT, 2012).

4.1 Antibiosis

Antibiosis is a type of resistance in which the insect uses the plant as a host, however, due to chemical and morphological factors, the plant causes adverse effects on the pest's biology, such as survival, development and fecundity (PAINTER, 1951; SMITH, 2005; SMITH; CLEMENT, 2012). The antibiosis category has been reported for several plant species against arthropods pest (BALDIN et al., 2018; CORREA et al., 2021; COSTA et al., 2018; LIMAJE et al., 2018; NOGUEIRA et al., 2019; PALIAL et al., 2021; QIU et al., 2011). Overall, it is correlated with high mortality of insects in the immature stage (except in cases of phagodeterrence), high percentages of deformation, and reduced fecundity and fertility (VENDRAMIM; GUZZO, 2009). For example, the landrace Pérola caused an increase in the larval period, higher mortality rate, and lower nutritional indices of *S. frugiperda* caterpillars (NOGUEIRA et al., 2019). When infested with *D. speciosa* larvae, Pérola and cultivar SCS

154-Fortuna increased the complete period from larva to adult, caused higher mortality, and a lower fertility rate (COSTA et al., 2018).

Regarding evaluation of antibiosis, depending on the pest species under study, it can be a time-consuming process due to the time needed to assess the development of the young stage, also it can require specific measuring equipment, and controlled conditions (STENBERG; MUOLA, 2017). However, characterizing the effects on the biology of pest insects is important when considering the possibility of incorporating resistant plants into IPM, as the longer duration of the immature stage in the field may increase the insects' exposure and risk of attack by natural enemies like parasitoids, predators, and infection by entomopathogens. In addition, emerged adults could be out of synchrony with the normal population and, therefore, copulation would tend to be difficult, with a consequent decrease in the pest population (VENDRAMIM; GUZZO; RIBEIRO, 2019).

4.2 Antixenosis

Antixenosis or non-preference involves plant traits that negatively affect arthropod behavior, leading to rejection of the plant as a host for food, oviposition and/or shelter (PAINTER, 1951; SMITH, 2005; SMITH; CLEMENT, 2012). As well as antibiosis, the occurrence of antixenosis has been reported for several plant species under attack by pest herbivores (BRILINGER et al., 2020; FARIA et al., 2021; NOGUEIRA et al., 2019; OKI et al., 2017; SMITH; CLEMENT, 2012; SULISTYO; INAYATI, 2016).

In general, antixenosis can be inferred by measuring the injury to the plants (e.g., leaf area consumed by herbivory) and counting the number of insects on plants (e.g., number of eggs, larvae, or adults) (STENBERG; MUOLA, 2017). For example, counting the number of *S. zeamais* adults on maize genotypes, in a free-choice experiment, the landrace Presidente Amarelo received ~4 to 5 times fewer insects than the genotypes Colorado and Cateto, respectively, being considered a potential source of resistance (BRILINGER et al., 2020). In another example under field conditions, by assigning injury scores caused by *S. frugiperda* in different maize genotypes (Davis scale) the landrace Pérola had the lowest scores, and consequently the least injury (COSTA et al., 2020) due to possible repellent and/or deterrent characteristics expressed in that genotype. Typically, assays to assess antixenosis are set up in Petri dishes, common gardens or other simple settings, which is cheap and fast to conduct. However, it may be less robust if insect injury is analyzed by eye (STENBERG; MUOLA, 2017). It is noteworthy to know the generation of the pest and the longevity of the crop to more

accurately infer the resistance potential for antibiosis and/or antixenosis (STENBERG; MUOLA, 2017).

4.3 Tolerance

Tolerance is defined as the plant's ability to withstand damage without compromising plant fitness and yield (MITCHELL et al., 2016; SMITH; CLEMENT, 2012; STOUT, 2013). In other words, tolerance is the plant response to injury caused by pest arthropods, and it is also known as compensation (AGRAWAL, 2000; STOWE et al., 2000). Tolerance responses in plants can be classified into undercompensation (no tolerance), compensation (injured plants do not differ from uninjured plants) and overcompensation (better fitness or performance in injured plants) (AGRAWAL, 2000; STOWE et al., 2000). These tolerance levels are sustainable, especially by the mechanisms of increased photosynthetic rate, growth rates, branching or tillering, resource allocation, detoxification mechanisms, and better use of stored reserves (STRAUSS; AGRAWAL, 1999; TIFFIN, 2000). However, the compensation levels can be compromised by several factors, including intensity of injury, as demonstrated for potato (*Solanum tuberosum*) under infestation by specialized tuber feeders, Guatemalan tuber moth *Tecia solanivora* Povolny (Lepidoptera: Gelechiidae) and potato weevils *Premnotrypes* spp. (Coleoptera: Curculionidae), where 10% tuber injury increases the yield up two fold, but yields decrease as injury levels increase more than 10% (POVEDA; DÍAZ; RAMIREZ, 2018).

Tolerance is a potential source to improve crop productivity (POVEDA; DÍAZ; RAMIREZ, 2018), and has been reported in economically important plant species such as maize (COSTA et al., 2021; GARCIA; EUBANKS, 2019; QU et al., 2016; ROBERT et al., 2014, 2015). Another advantage associated with the use of tolerant plants is the reduction in the application of synthetic insecticides, as the economic injury level in tolerant plants would be greater than in susceptible plants. Also, plant tolerance is a more stable management alternative, since it does not act directly on the pest herbivore, and consequently reduces the selection pressure of resistant populations (KOCH et al., 2016; PETERSON; VARELLA; HIGLEY, 2017). Despite being a promising approach in IPM, plant tolerance has received the least attention from researchers relative to antixenosis and antibiosis (PETERSON; VARELLA; HIGLEY, 2017), consisting of a research field with wide possibility of studies.

5 NEONICOTINOID SEED TREATMENT

Neonicotinoids make up a class of systemic insecticides marketed since the early 1990s, with imidacloprid being the first available compound (JESCHKE et al., 2011; TOMIZAWA; CASIDA, 2011). Thiamethoxam was the next insecticide commercialized in 1998 under the trademarks Actara® for foliar and soil treatment, and Cruiser® for seed treatment (MAIENFISCH et al., 2001). Since introducing neonicotinoids on the market, they have become a widely used class of insecticide for crop protection (JESCHKE; NAUEN, 2008; SMITH; BAUTE; SCHAAFSMA, 2020; TOOKER; DOUGLAS; KRUPKE, 2017). Neonicotinoids can be applied by multiple methods: as foliar spray, as root irrigation, or as seed treatment (JESCHKE; NAUEN, 2008). However, seed treatment is the most frequently used application method (DOUGLAS; TOOKER, 2015; TOOKER; DOUGLAS; KRUPKE, 2017).

Neonicotinoids are highly water soluble and can be translocated (via xylem) throughout the plant tissues to provide pest protection, especially at the beginning of the crop cycle (ALFORD; KRUPKE, 2017; WHALEN et al., 2021). It is applied for the control of sucking insect pests, such as aphids, whiteflies, leafhoppers, thrips and some lepidopteran and coleopteran pests (DING et al., 2018; ELBERT et al., 2008; MAGALHÃES; HUNT; SIEGFRIED, 2009)**.** In the insect, neonicotinoids act as acetylcholine agonists. The insecticidal molecules bind to nicotinic acetylcholine receptors on the postsynaptic neuron, and induce a neural hyper-excitation, which leads to the insect's death (MATSUDA et al., 2001). The rapid control of insect pests, even at relatively low doses, the low toxicity to mammals, and the systemic ability of translocation popularized the use of neonicotinoids via seed treatment (ELBERT et al., 2008; GOULSON, 2013).

Seed treatment with insecticide is a chemical control strategy used prophylactically against persistent pests (DARA, 2019). However, natural enemies and pollinators are also exposed to the toxic compounds (SANCHEZ-BAYO, 2014; SANCHEZ-BAYO; GOKA, 2014). For example, the predator *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) experienced an increased pre-oviposition period after being exposed to thiamethoxam seed-treated soybean plants, and a reduction in survival, oviposition period, and fecundity of females (GONTIJO et al., 2018). Further, cotton plants grown from thiamethoxam-treated seeds caused sublethal and transgenerational effects on *Chrysoperla externa* (Neuroptera: Chrysopidae) and *Harmonia axyridis* (Coleoptera: Coccinellidae) (SÂMIA et al., 2019). Also, the lethal and sublethal effects of neonicotinoids on bees have been extensively reported (MAIN et al., 2021; RUNDLÖF et al., 2015; WOODCOCK et al., 2017). Besides the impact caused by neonicotinoids on beneficial fauna, neonicotinoids have also been linked to outbreaks of

arthropod pests, such as mites (RUCKERT; ALLEN; RAMIREZ, 2018; SMITH et al., 2013; SZCZEPANIEC et al., 2011; SZCZEPANIEC; RAUPP, 2013).

Maize seed treated with clothianidin resulted in higher population growth rates of spider mites *Tetranychus urticae* (Acari: Tetranychidae) by over 100%, due to the direct effect of the neonicotinoid on host-plant defense (SZCZEPANIEC et al., 2013). The authors observed that the insecticide inhibited the expression of genes related to the phytohormones salicylic acid (SA) and jasmonic acid (JA), such as phenylalanine ammonia lyase, coenzyme A ligase, chitinase, and trypsin protease inhibitor in the presence of the spider mites. Also, the neonicotinoids thiamethoxam, imidacloprid, and clothianidin suppress the constitutive levels of 12-oxo-phytodienoic acid (OPDA) in cotton, tomato, and maize (SZCZEPANIEC et al., 2013). The suppressive effect of neonicotinoids was also observed in soybean from thiamethoxam seed treatment, where plants exposed to neonicotinoids downregulated genes involved in plant-pathogen interactions, including the SA, JA and phenylpropanoid pathways, and cell wall biosynthesis. Consequently, the plants became more susceptible to spider mite *Tetranychus cinnabarinus* (Acari: Tetranychidae) herbivory (WULFF et al., 2019). This alteration of plant defense gene expression related to higher susceptibility to herbivory also occurs through different growth stages and under water stress after thiamethoxam treatment (STAMM et al., 2014).

The phytohormones JA and SA play an important role in plant defense against pathogens and herbivores (PIETERSE et al., 2012). Imidacloprid and clothianidin induced a SA response in *Arabidopsis thaliana* plants, which increased plant resistance to the fungus, powdery mildew, *Golovinomyces orontii* (FORD et al., 2010). Taken together all the information on the effect of neonicotinoids on plant defense, further research is needed to understand the complex consequences of interactions between neonicotinoids and plants, and how seed treatment can affect other pest management approaches (DOUGLAS; TOOKER, 2015). An emphasis should be taken to study the effects of neonicotinoids on maize, as it is the crop that is most seed treated with neonicotinoids (TOOKER; DOUGLAS; KRUPKE, 2017).

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ARTICLE 1

Comparative tolerance levels of maize landraces and a hybrid to natural infestation of insects

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Comparative tolerance levels of maize landraces and a hybrid to natural infestation of insects

Abstract

Spodoptera frugiperda is a pest native to the tropical and subtropical Americas, and is invasive in Africa and Asia, where it causes significant losses to a variety of crops, mainly maize (*Zea mays mays*). Control of this pest is difficult, but the use of insect resistant cultivars, including tolerant cultivars, is a promising alternative, and landraces are a potential source of insect resistance. This study investigated tolerance to *S. frugiperda* in six maize genotypes, including five Brazilian landraces, Amarelão, Aztequinha, Branco Antigo, Palha Roxa, and São Pedro, and one conventional (non-*Bt*) hybrid, BM 207, under field conditions. We estimated tolerance ratios for three plant variables, plant height, stem diameter, and leaf chlorophyll content at two plant stages, as indices of tolerance; these were estimated as the ratio of insecticide-free to insecticide-protected plants for each variable. Tolerance ratios varied across the maize genotypes, but inconsistently across plant variables, and cluster analysis revealed three groups based on tolerance ratios. A first group contained genotypes similarly tolerant to *S. frugiperda* (tolerance ratio ≥ 1.0), Palha Roxa, BM 207, São Pedro, and Aztequinha, while the second and third groups each contained single genotypes considered not tolerant (tolerance ratio ≈ 0.9), Amarelão, and Branco Antigo. We concluded that the landraces Palha Roxa, São Pedro, and Aztequinha compared favorably to the commercial hybrid BM 207 in terms of tolerance to *S. frugiperda*, and therefore may be valuable for management of this pest, and as germplasm sources to improve tolerance in other cultivars.

Keywords: Host plant resistance, fall armyworm, compensation, overcompensation, plant defense

1. Introduction

Maize (*Zea mays mays* L.) crops are constantly affected by abiotic and biotic stresses, including pest insect herbivory, which is the main biotic stress impacting crop yield (Karjagi et al., 2017; Pimentel, 2009). The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is one of the most important insect pests affecting maize crops in the Americas (Early et al., 2018; Montezano et al., 2018; Sparks, 1979), mainly due to the polyphagous habit of the species (Cui et al., 2020; Gouin et al., 2017; Montezano et al., 2018). In maize, FAW caterpillars preferentially feed on young leaves, compromising plant growth (Toepfer et al., 2021).

FAW is native to the tropical and subtropical Americas. However, due to its long-distance flight capacity, and broad environmental adaptation (Jing et al., 2020), FAW has become an invasive pest in Africa (Goergen et al., 2016; Otim et al., 2018), and more recently in India (Chormule et al., 2019; Sharanabasappa et al., 2018) and China (Jing et al., 2020). Upon its occurrence in new areas, control methods need to be integrated for the effective management of this pest (Tambo et al., 2020). Control of FAW is usually carried out with insecticide applications and genetically modified cultivars expressing toxic proteins of the bacterium *Bacillus thuringiensis* (*Bt*), in crops and countries where they are available. However, FAW has shown resistance to maize *Bt* cultivars (Banerjee et al., 2017; Boaventura et al., 2020b; Farias et al., 2014; Flagel et al., 2018), as well as to insecticides (Boaventura et al., 2020a; Carvalho et al., 2013; Nascimento et al., 2016; Okuma et al., 2018). Overall, resistance of FAW populations to the main control methods are a challenge for the effective management of this pest, requiring new strategies to ensure the productivity of affected crops, such as maize.

Host plant resistance (HPR) is a fundamental component of integrated pest management (IPM) strategies, and comprises antibiosis, through plant traits that affect pest survival, development, and reproduction; antixenosis, through traits that affect pest colonization; and tolerance, through traits that allow plants to withstand pest injury without substantially compromising productivity (Painter, 1951; Smith, 2005). All three forms of HPR can be incorporated in crop cultivars through traditional breeding or genetic engineering (Dara, 2019). Tolerance may play important roles in crop protection, especially in cases where insect pests do not transmit crop pathogens (Mitchell et al., 2016), or where resistance to pests is low (Bustos-Segura et al., 2014). Tolerance does not directly affect pest insects, thus it is presumed to not contribute to the selection of resistant biotypes (Gagic et al., 2016; Peterson et al., 2017).

Furthermore, tolerant cultivars can sustain greater pest injury before requiring insecticide applications (Peterson et al., 2017).

Tolerance is associated with greater efficiency in plant photosynthetic activity, better use of stored reserves, and appropriate phenological changes (Mitchell et al., 2016; Tiffin, 2000). These mechanisms may generate different levels of tolerance, whether compensation or overcompensation for lost tissues, or non-tolerance, i.e., undercompensation (Strauss and Agrawal, 1999). Overcompensation can occur for vegetative and/or reproductive plant tissues, and may vary according to the plant genotype (Agrawal, 2000; Garcia and Eubanks, 2019), and it can be exploited in agriculture due to the direct impact on crop productivity (Ramula et al., 2019). For instance, potato (*Solanum tuberosum*) plants increase their productivity when injured by *Tecia solanivora* (Povolny) (Lepidoptera: Gelechiidae) caterpillars, and yield can increase by up to 100% when 10% of tubers are damaged by specialist herbivores (Poveda et al., 2018, 2010). In maize, tolerance traits are diverse, and include traits contributing to greater root system growth and biomass in the case of *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) larvae (Qu et al., 2016; Robert et al., 2015), or compensatory shoot growth due to the allocation of photoassimilates (Robert et al., 2014). Tolerance to *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae) in the Brazilian maize landrace Azteca is related to the greater number of photosynthetic pigments (Costa et al., 2021).

Maize landraces are open-pollinated varieties with broad genetic bases that were selected by the environment and farmers over many generations, and that maintain moderate stress resistance and yield characteristics (Dávila-Flores et al., 2013). They contain high genetic diversity, so are valuable genetic resources that can be used in breeding programs, particularly breeding directed at improving agronomic parameters and food security (Prasanna, 2012; Strigens et al., 2013). Several studies reported maize landraces with resistance to arthropod pests (Brilinger et al., 2020; Costa et al., 2020, 2018; Nascimento et al., 2014; Santos et al., 2020). However, tolerance traits are poorly studied and widespread, despite their potential use in IPM and genetic breeding programs, so they merit additional research (Costa et al., 2021; Peterson et al., 2017).

Given the growing demand for effective management strategies for FAW, we evaluated five landraces and one commercial hybrid for their tolerance to this pest. Specifically, we conducted field experiments in which we evaluated five Brazilian maize landraces (Amarelão, Aztequinha, Branco Antigo, Palha Roxa, and São Pedro) for their tolerance to FAW, in comparison to a Brazilian commercial hybrid (BM 207). We inferred tolerance based on plant

growth characteristics in insect-protected relative to unprotected plants, and in the landraces relative to the commercial hybrid.

2. Material and methods

2.1. Experimental conditions and maize genotypes

Field experiments were carried out at the Center for Scientific and Technological Development of the Lavras Federal University (UFLA), Fazenda Muquém, located in the municipality of Lavras, Minas Gerais state, Brazil (21º 14' 45" S, 44º 59' 59" W and 918 m asl). The experiment was replicated in the 2017/2018 (season 1) and 2018/2019 (season 2) summer crop seasons. Sowing was carried out manually on December 21, 2017, and November 15, 2018. Field temperatures had low fluctuations in the two growing seasons, with the average maximum temperature ranging from 27.6 to 30.4 ºC for season 1, and 26.9 to 30.9 ºC for season 2 (INMET - Instituto Nacional de Meteorologia, 2021). On the other hand, rainfall showed high variation between the seasons, being higher in season 2, with monthly accumulation from 143.6 mm (January 2019) to 323.2 mm (December 2018), while in season 1 it varied from 3.2 mm (April 2018) to 240.2 mm (January 2018) (INMET - Instituto Nacional de Meteorologia, 2021).

This study is registered in the National System of Genetic Resource Management and Associated Traditional Knowledge (SisGen) under the code AAFDB1D. We evaluated six maize genotypes: Five landraces, Amarelão, Aztequinha, Branco Antigo, Palha Roxa, and São Pedro, and the conventional (non-Bt), double hybrid, BM 207 (Sementes Biomatrix® Patos de Minas, Minas Gerais, Brazil). The seeds of the landraces were provided by the non-governmental organization AS-PTA Farming Family and Agroecology, located in the municipality of Palmeira, Paraná State, Brazil, from the 2016 harvest. All seeds were stored in a cold chamber at 11 ºC until used.

2.2. Management practices and experimental model

The initial preparation of the experimental site consisted of eliminating weeds by spraying the herbicide atrazine (Nortox® 500 SC) at a commercial dose of 4 L ha^{-1}. This was followed by fertilization with nitrogen, phosphorus, and potassium (NPK 08-28-16). The herbicide was sprayed again 30 days after sowing to ensure cleanliness of the site and between rows of maize, and manual weeding was carried out when necessary. Topdressing fertilization with urea (200 kg ha^{-1}) was performed 40 days after sowing to maintain fertilization.

The experimental design was a randomized block with four replications (blocks). Each experimental plot consisted of three rows spaced 0.6 m apart and six plants per row spaced 0.25 m (18 plants/plot) for season 1, and eight plants per row for season 2 (24 plants/plot). A spacing of 0.5 m between plots and 1.0 m between blocks was used to facilitate the evaluations.

A control plot was used for calculating tolerance ratios. This plot was located ~7 m distant from the other plots, had the same dimensions established for each season described above, and was treated in the same fashion, except that the insecticide lambda-cyhalothrin (Karate-Zeon® 50 CS) was sprayed biweekly at the recommended dose of 150 mL ha^{-1} for control of FAW (AGROFIT, 2021).

2.3. Data collection

The experiments were carried out under natural infestation of herbivorous insects. We recorded the numbers of *D. maidis* and FAW injury on maize plants because these were frequently found in the plots, and these indices were used as covariables in statistical analyses (see below); additionally, *D. maidis* is a relevant pest with economic importance in Brazil and other Latin America countries (Meneses et al., 2016; Oliveira et al., 2007). Thus, we recorded the numbers of *D. maidis* adults per plant, and the injury caused by FAW to the youngest leaf per plant at developmental stages V4, V6, V8, and V12 and at the beginning of the reproductive stage (Table 1). Evaluations were carried out on three plants randomly selected in each row of the plots (9 plants/plot), and both variables were used as covariates in statistical analyses (see below).

We measured three plant vegetative parameters as indices of tolerance of maize genotypes. Leaf chlorophyll content was measured at V6 growth stage and at reproductive stage (Table 1); growth stage V6 is the beginning of the phase of greatest growth and water consumption (Rozas et al., 1999). Chlorophyll content was measured non-destructively using the SPAD-502 meter (Konica Minolta Sensing, Tecnal, Piracicaba, São Paulo State, Brazil). The readings were carried out on the youngest expanded leaf of two plants per row (6 plants/plot), with two evaluations per plant, recording the average of the readings. Finally, plant growth was measured as plant height and stem diameter at the end of the crop cycle, from three plants per row (9 plants/plot). Height was measured as the length from the soil surface to the insertion of the last expanded leaf (cm), while stem diameter (mm) was measured below the insertion of the first ear, with the aid of a digital caliper (MTX ®). All variables were measured for the blocks without insecticide application and the control block (with insecticide application).

2.4. Data analyses

Each of the three plant variables was converted to ratios, according to the following formula:

$$Tolerance\ ratio = \frac{Cultivar\ without\ insecticide}{Average\ of\ cultivar\ with\ insecticide}.$$

The data analyses consisted of analysis of variance (ANOVA) for the tolerance ratios, and included the independent variables *plant genotype* (Amarelão, Aztequinha, Branco Antigo, Palha Roxa, São Pedro, and BM 207), *season* (1 and 2), and the interaction term *plant genotype* × *season*; additionally, FAW injury score and corn leafhopper number per plant were included as independent covariables. The ratios were normalized by converting them to their log values prior to the ANOVA. Dunnett's post-hoc test ($\alpha = 0.05$) was used to compare averages between each landrace and the hybrid BM 207 within the main effect of plant genotype, while *a priori* contrasts were used to compare averages between each landrace and BM 207 within the interaction effect of plant genotype × season; the critical *P* for each *a priori* contrast was set at 0.010 per Sidak's correction (Abdi, 2007). All statistical analyses were performed using the JMP® Pro 14.0.0 software (SAS Institute, 2018).

To examine whether maize genotypes exposed to FAW compensated for tissue loss, we performed one-sample *t*-tests, with the log-transformed ratio, using the statistical software "R", version 4.0.3 (R CoreTeam, 2020). The one-sample *t*-test tested the null hypothesis that tolerance ratios did not differ from 1 (i.e., $H_0 = 1$, exposure to FAW did not affect the ratio tolerance). For interpretation of results, tolerance ratios < 1 were considered indicative of undercompensation, i.e., no tolerance, and values = 1 or > 1 as indicative of compensation and overcompensation, respectively, i.e., tolerance in both cases (Fontes-Puebla and Bernal, 2020). The critical *P* for each *t*-test was set at 0.014, per the Bonferroni correction (Abdi, 2007).

Finally, hierarchical clustering analysis was performed using the Ward method to group genotypes by tolerance ratios across the plant variables (SAS Institute, 2018). This analysis was conducted on per-maize genotype, average tolerance ratios for each of the plant variables. All results showing tolerance ratios are presented as back-transformed averages of the transformed values used for statistical analyses.

3. Results

ANOVA revealed significant effects of maize genotype, season, and the genotype × season interaction on the tolerance ratios for the parameters plant height, diameter, and chlorophyll index during reproductive stage. However, the chlorophyll content at V6 stage was not significantly affected by season (Table 2). Similarly, FAW injury and numbers of corn leafhoppers did not significantly affect the tolerance ratios (Table 2).

Hybrid BM 207 and the landraces Palha Roxa and São Pedro showed overcompensation for plant height (ratio > 1.0, $P \leq 0.0001$), while Amarelão, Aztequinha, and Branco Antigo displayed undercompensation (Fig. 1A, ratio < 1, $P < 0.0001$). The plant height ratio of genotypes was lower than that of BM 207 ($P < 0.0001$), except for Palha Roxa, which did not differ from the conventional hybrid (Fig. 1A, $P = 0.126$). In season 1, BM 207 showed the highest tolerance height ratio (ratio = 1.45, $P < 0.0001$), which overcompensated for FAW feeding, as well as Palha Roxa and São Pedro landraces (Fig. 1B, $P < 0.0001$). In season 2, Palha Roxa was the only genotype that showed overcompensation (ratio = 1.12, $P < 0.0001$) and had a higher ratio than hybrid BM 207 (Fig. 1C, $P < 0.0001$, $F = 16.844$).

The genotypes Aztequinha, Branco Antigo, and Palha Roxa exhibited tolerance (ratio ≥ 1) according to the plant stem diameter (Fig. 2A). Amarelão, São Pedro, and BM 207 did not display tolerance (Fig. 2A, $P < 0.0001$), and no landrace differed from hybrid BM 207 (Fig. 2A, $P \geq 0.099$). Throughout season 1, Branco Antigo was the only tolerant genotype [overcompensation (Fig. 2B, ratio = 1.02, $t = 7.173$, $P < 0.0001$)]; however, no genotypes differed from BM 207 (Fig. 2B, $P \geq 0.061$). In addition to Branco Antigo in season 2, Aztequinha, Palha Roxa, and São Pedro showed overcompensation for stem diameter ($P < 0.0001$), and the ratio in Aztequinha was greater than in BM 207 (Fig. 2C, $F = 1.745$, $P = 0.007$).

Genotypes Amarelão, Aztequinha, and São Pedro exhibited tolerance per their V6 stage chlorophyll ratios (ratio = 1, $P \geq 0.016$)], but not genotypes Branco Antigo and Palha Roxa [undercompensation (Fig. 3A, ratio < 1, $P < 0.0001$)]. BM 207 showed overcompensation ($P < 0.0001$) and significantly differed from Branco Antigo (Fig. 3A, $P = 0.004$). Season had no significant effect on the relative chlorophyll content in V6 plants (Table 2, $P = 0.193$). There was no significant genotype × season interaction between BM 207 and the genotypes in season 1 (Fig. 3B, $P \geq 0.044$). Conversely, Branco Antigo had the lowest ratio in season 2 (Fig. 3C, ratio = 0.89, $F = 9.522$, $P = 0.002$). Hybrid BM 207 showed overcompensation in both seasons ($P \leq 0.0001$), and São Pedro showed overcompensation in season 1 (Fig. 3B, $P < 0.0001$), and Amarelão and Aztequinha in season 2 (Fig. 3C, $P < 0.0001$).

The landrace Palha Roxa overcompensated for the chlorophyll index during the reproductive stage (Fig. 4A, ratio = 1.25, $F = 79.743$, $P < 0.0001$) and showed a higher tolerance

ratio than hybrid BM 207 (Fig. 4A, $P < 0.0001$). The genotypes Aztequinha and São Pedro were also tolerant by compensation ($P = 0.032$) and overcompensation ($P < 0.0001$), respectively (Fig. 4A). In the genotype × season interaction, Palha Roxa was the only genotype to show overcompensation in both seasons (Fig. 4B, C, $P < 0.0001$). Amarelão, Aztequinha, and Branco Antigo were lower than BM 207 at season 1 (Fig. 4B, $P = 0.0001$), while Aztequinha and Palha Roxa were superior to BM 207 mainly in season 2 (Fig. 4C, $P \leq 0.002$).

Hierarchical cluster analysis revealed three groups based on similarity across tolerance indices (Fig. 5). The first group included Palha Roxa, BM 207, São Pedro, and Aztequinha; all were considered tolerant to FAW because the geometric averages across the four tolerance ratios were ≥ 1.0 for each of these genotypes (Fig. 5). The second group included only Amarelão, and the third group only Branco Antigo; both groups were considered not tolerant to FAW because the geometric average across the four tolerance ratios were ≈ 0.9 for each of these genotypes (Fig. 5).

4. Discussion

This study investigated tolerance of maize genotypes to FAW injury under field conditions in terms of several relevant plant parameters (plant height, stem diameter, and chlorophyll index at two growth stages), considering the natural factors of insect infestation, climate, and soil conditions. According to Pearse et al. (2017), research of this nature adds relevant information to the literature, since results from realistic studies are scarce. We used tolerance indices that were calculated as the average ratio per genotype in the plot without insecticide application and the corresponding average in treated plot with lambda-cyhalothrin (control) for control of FAW. The tolerance responses were classified as undercompensation, compensation, or overcompensation when the calculated tolerance ratios were below, equal to, or above 1.0, respectively (Fontes-Puebla and Bernal, 2020; Strauss and Agrawal, 1999). The maize genotypes compensated for injury caused by FAW in the evaluated parameters (Fig. 5). All genotypes showed tolerance (compensation or overcompensation) in at least one of the plant parameters.

The capacity to compensate for injury caused by herbivory is related to alterations in physiological and/or metabolic processes in the plant, such as increased photosynthetic activity, increased antioxidant metabolism, use of stored reserves, compensatory growth, and increased branching (García-Caparrós et al., 2020; Prins and Verkaar, 1992; Tiffin, 2000). For example, stem herbivory affects the architecture of woody plants by stimulating branch growth (Stephens

and Westoby, 2015). In some cases, mainly in herbivory by chewing insects, these tolerance mechanisms can result in overcompensation for both vegetative and reproductive parameters (Garcia and Eubanks, 2019; Koch et al., 2016; Tito et al., 2016).

Stem diameter was a useful index for tolerance of maize genotypes to FAW. Thus, landrace Branco Antigo showed consistently the highest tolerance index (overcompensation; Fig. 2) though it was less tolerant per the other indices (Fig. 5). Stem diameter is an important agronomic maize plant trait, as it is directly related to greater length of the ear, number of grains per row on the ear (Brambilla et al., 2009; Dourado Neto et al., 2003), as well as to the capacity to withstand environmental adversities (Slewinski, 2012). Increases in stem diameter may be correlated with the ability to allocate photoassimilates from the damaged part to storage structures. The reallocation of resources, mainly carbon, is a key tolerance mechanism to leaf injury, whether natural or mechanical (Hochwender et al., 2000; Holland et al., 1996; Machado et al., 2017; Schwachtje et al., 2006). Biochemically, this plant response can be regulated by mitogen-activated protein kinases (Schwachtje et al., 2006) or by the induction of jasmonate derivatives, which may vary according to the plant species (Machado et al., 2017, 2015).

Maize tolerance to *D. virgifera virgifera* increases with increasing availability of resources for plant growth and reproduction, which may result in changes of metabolite and phytohormone levels (Fontes-Puebla et al., 2021; Fontes-Puebla and Bernal, 2020), and improvement of stem growth (circumference and mass) because of greater carbon allocation (Robert et al., 2014). However, tolerance ratios appear to be mediated by crop domestication, spread, and breeding; for example, the stem diameter compensated for belowground larval injury in Mexican and US maize landraces post *D. virgifera virgifera* infestation, while Balsas teosintes and US inbred maize lines undercompensated (Fontes-Puebla and Bernal, 2020). The stem is a tank of photoassimilates (Scofield et al., 2009), and the gain in stored reserves results in energy for growth or regrowth (Zhou et al., 2015). The reserve of photoassimilates, such as carbon and proteins, stored in the stem of tomatoes (*Solanum lycopersicum*) was used for leaf regrowth after complete defoliation in plants treated with *Manduca sexta* (Linnaeus) (Lepidoptera: Sphingidae) (Korpita et al., 2014).

Plant height has also been evaluated as a response of plant tolerance to herbivory (Boalt et al., 2010; Kasoma et al., 2020; Tayo, 1982). Here, we observed that maize genotype influenced the expression of tolerance (Table 2), and the genotypes Palha Roxa, São Pedro, and BM 207 showed overcompensation, while the other genotypes displayed undercompensation (Fig. 1). Wild cotton plants under artificial defoliation of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) also showed low compensation for plant height in attack levels equal

to or greater than 25% (Quijano-Medina et al., 2019). On the other hand, *Schizotetranychus oryzae* Rossi de Simons (Acari: Tetranychidae) mite infestation did not affect the height of rice plants (*Oryza sativa*) (Buffon et al., 2021). The height overcompensation for the Palha Roxa, São Pedro, and BM 207 genotypes may reflect an important agronomic characteristic, since plant height correlates with increased forage crop yield (Pedersen et al., 2021). As leaf herbivory by FAW can negatively affect plant growth parameters, such as height, further work is needed to assess the relationship between plant height and grain yield in genotypes infested and not infested by FAW (Kasoma et al., 2020); this knowledge would benefit the development of a practical protocol for evaluation of tolerance levels in maize genotypes under field conditions, which usually requires an estimate of maize grains yield upon harvest at the end of crop cycle that is time- and labor-consuming.

Herbivory can affect plant primary growth due changes in primary metabolism (Zhou et al., 2015; Züst and Agrawal, 2017). Primary metabolism is responsible for energy generation (Wen et al., 2015), and changes in the allocation of primary compounds can alter plant defense, growth, and reproduction mechanisms (Züst and Agrawal, 2017). However, plant growth is a complex process, which is affected by many physiological and metabolic pathways, and involves the balance between phytohormones. High levels of jasmonic acid, either endogenous or exogenously applied, are known to reduce plant height in rice, tobacco (*Nicotiana attenuata*), *Arabidopsis thaliana*, and maize (Feng et al., 2012; Heinrich et al., 2013; Qi et al., 2016; Zhang and Turner, 2008). One of the reasons is the inhibition of gibberellin production, a phytohormone used to regulate plant growth and development that plays an important role in stem elongation (Heinrich et al., 2013).

Feeding injury caused by FAW caterpillars can affect chlorophyll content in leaves of V6- and reproductive stage-maize (Fig. 3, 4), and these effects may vary according to maize genotype (Table 2). Chlorophyll content was used to infer possible effects on the photosynthetic rate of the maize genotypes, as chlorophyll is the main photosynthetic pigment and positively correlates with the ability to perform photosynthesis (Chen et al., 2009; Curran et al., 1990; Richardson et al., 2002). Increased photosynthetic activity is one of the main tolerance mechanisms of plants (Strauss and Agrawal, 1999; Tiffin, 2000). Generally, leaf area reduction caused by defoliation increases the photosynthetic activities in the remaining tissues (Eyles et al., 2013; Zhou et al., 2015), which can be explained by the “source-sink hypothesis”.

According to this hypothesis, the photosynthetic rate increases with the reduction of the source supply (Retuerto et al., 2004). The source comprises the tissues responsible for the acquisition and export of resources (e.g., carbon in leaves), while the sink involves the tissues

responsible for its assimilation and importation (e.g., nitrogen in the leaves) (White et al., 2016). The literature reports several examples that support this hypothesis (Moustaka et al., 2021; Retuerto et al., 2004; Thomson et al., 2003). However, this is not a rule that applies to all herbivory situations (Costa et al., 2021; Nabity et al., 2009; Sperdouli et al., 2021). The genotypes Palha Roxa and São Pedro presented higher chlorophyll ratios during the reproductive stage (Fig. 4A), which may be related to increased photosynthetic capacity. Plants of *Cucumis sativus* under herbivory by *Helix aspersa* Muller (Gastropoda, Stylommatophora) showed increased photosynthetic capacity with consequent compensatory plant growth (Thomson et al., 2003). The tolerance ratio of chlorophyll content of the maize genotypes in our study varied according to the growth stage (Fig. 3, 4), which was expected, as plant age can affect several compensation parameters (Capó et al., 2021; Hódar et al., 2008; Lima et al., 2018; Tito et al., 2016).

Our results suggested that the evaluated maize genotypes are capable of compensating for FAW injury under field conditions. However, plants are subject to several biotic and abiotic factors that can affect their compensation for multiple stress. Important sources of variation include soil nutritional levels (Hochwender et al., 2000; Weintraub et al., 2018), light availability (Hódar et al., 2008), abundance of herbivores (Gagic et al., 2016), natural enemies (Cuny et al., 2018), and microorganisms (Allsup and Paige, 2016; Vannette and Hunter, 2009). Furthermore, the capacity of plants to compensate for injury is influenced by plant genotype, as shown in this study (Capó et al., 2021; Hochwender et al., 2000; Scholes et al., 2017; Weintraub et al., 2018), and by other environmental conditions (Villegas et al., 2021), such as rainfall, which was important in our study, as evidenced by the significant effect of growing season of maize.

The domestication and breeding processes of maize are other variables that influence plant tolerance to herbivory, as modern cultivars tend to allocate more resources to productivity (growth and reproduction) than to defense against herbivorous insects (Chinchilla-Ramírez et al., 2017; Fontes-Puebla et al., 2021; Fontes-Puebla and Bernal, 2020). Tolerance of the various landraces relative to the commercial hybrid varied across the measured tolerance indices (Fig. 5). The tolerance of three landraces, Palha Roxa, São Pedro, and Aztequinha were comparable to that of BM 207, as shown by the cluster analysis (Fig. 5). This is broadly consistent with expectations of comparative tolerance levels in landraces and modern maize cultivars (Fontes-Puebla et al., 2021; Fontes-Puebla and Bernal, 2020).

Tolerance through compensation for insect herbivory without a yield tradeoff is a promising plant trait for incorporating to crop cultivars (Zheng et al., 2021). Our work showed

that three maize landraces displayed promising levels of tolerance to herbivory by FAW, compared to a commercial hybrid. Future studies are needed to determine how each of the tolerance indices that we measured affects plant yield under different levels of pest infestation, their heritability, and the mechanisms by which they contribute to enhanced tolerance.

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Table 1. Evaluations of maize genotypes in an experimental site of the Lavras Federal University in the municipality of Lavras, Minas Gerais State, Brazil, and the respective dates.

Parameters evaluated	Developmental stage	Season/Evaluation date	
		2017/2018	2018/2019
Leaf injury caused by *Spodoptera frugiperda* and number of adults of *Dalbulus maidis*	V4	Jan 5	Nov 11
	V6	Jan 19	Dec 26
	V8	Feb 06	Jan 11
	V12	Feb 20	Jan 23
	Reproductive	Mar 21	Feb 12
Chlorophyll content	V6	Jan 19	Dec 26
	Reproductive	Mar 20	Feb 12
Plant growth	Post-reproductive	Apr 28	Apr 1

Table 2. Analysis of covariance (ANOVA) statistics for the independent variables genotype (Amarelão, Aztequinha, Branco Antigo, Palha Roxa, São Pedro, and BM207), season (1 and 2) and genotype × season interaction. The FAW injury and corn leafhopper (CLH) number was added to the model as covariates.

Source	Height ratio		Diameter ratio		Chlorophyll V6 ratio		Chlorophyll reproductive ratio	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Genotype	55.823	**<0.0001**	4.027	**0.001**	3.626	**0.003**	16.383	**<0.0001**
Season	38.573	**<0.0001**	14.186	**0.0002**	1.707	0.193	7.622	**0.006**
Genotype ×Season	20.699	**<0.0001**	2.655	**0.023**	4.604	**0.0005**	7.079	**<0.0001**
FAW Injury	0.015	0.902	1.403	0.237	1.568	0.212	0.418	0.519
CLH	3.508	0.062	0.003	0.960	0.458	0.499	0.479	0.489

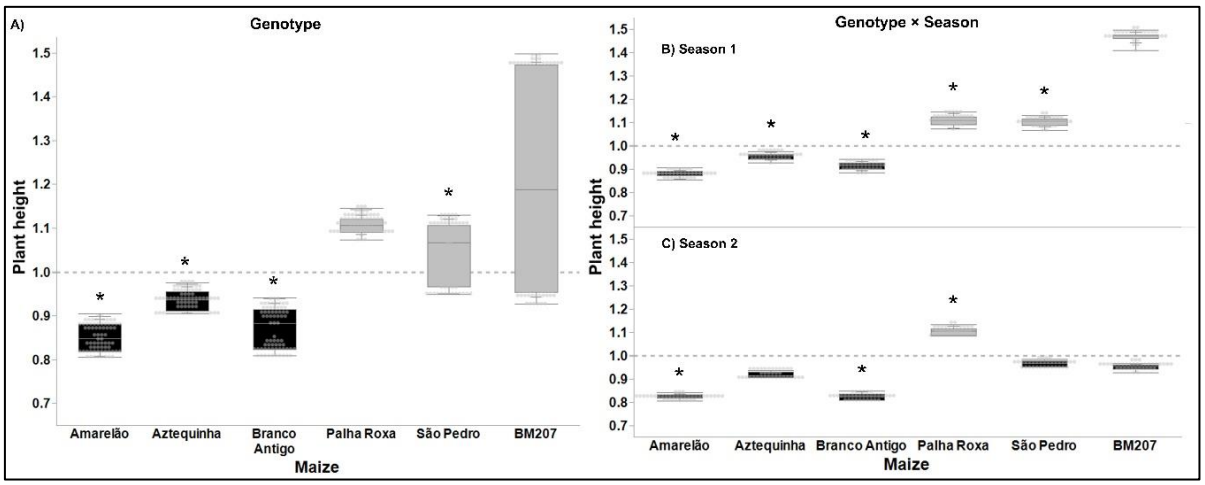

Fig. 1. Tolerance ratio (plant without insecticide application/average of plants with insecticide application) for the parameter plant height in **A)** six maize genotypes across two seasons, **B)** six genotypes in season 1, and **C)** six genotypes in season 2 to FAW. In each plot, asterisks indicate statistical difference relative to BM 207, per Dunnett's test in **A**, and per a *priori* contrasts in **B** and **C** (critical $P \leq 0.010$ per Sidak's correction). In each plot, black filling of boxes indicates undercompensation (ratio < 1), and gray filling indicates overcompensation (ratio > 1) (critical $P = 0.014$ per Bonferroni correction).

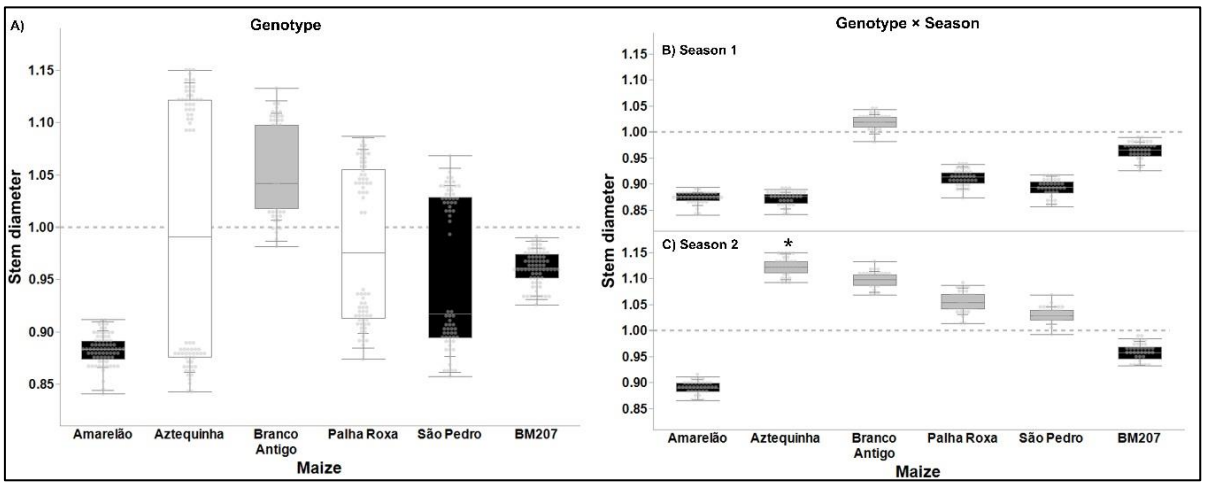

Fig. 2. Tolerance ratio (plant without insecticide application/average of plants with insecticide application) for the parameter stem diameter in **A)** six maize genotypes across two seasons, **B)** six genotypes in season 1, and **C)** six genotypes in season 2 to FAW. In each plot, asterisks indicate statistical difference relative to BM 207, per Dunnett's test in **A**, and per a *priori* contrasts in **B** and **C** (critical $P \leq 0.010$ per Sidak's correction). In each plot, black filling of boxes indicates undercompensation (ratio < 1), gray filling indicates overcompensation (ratio > 1), and white filling indicates compensation (ratio not significantly different from 1.0) (critical $P = 0.014$ per Bonferroni correction).

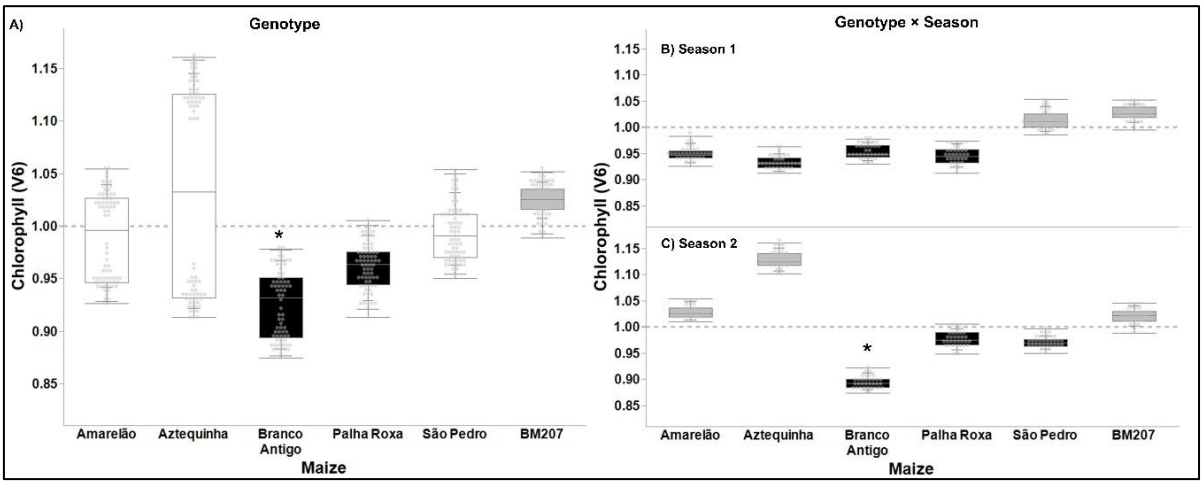

Fig. 3. Tolerance ratio (plant without insecticide application/average of plants with insecticide application) for the parameter chlorophyll content at V6 in **A)** six maize genotypes across two seasons, **B)** six genotypes in season 1, and **C)** six genotypes in season 2 to FAW. In each plot, asterisks indicate statistical difference relative to BM 207, per Dunnett's test in **A**, and per a *priori* contrasts in **B** and **C** (critical $P \leq 0.010$ per Sidak's correction). In each plot, black filling of boxes indicates undercompensation (ratio < 1), gray filling indicates overcompensation (ratio > 1), and white filling indicates compensation (ratio not significantly different from 1.0) (critical $P = 0.014$ per Bonferroni correction).

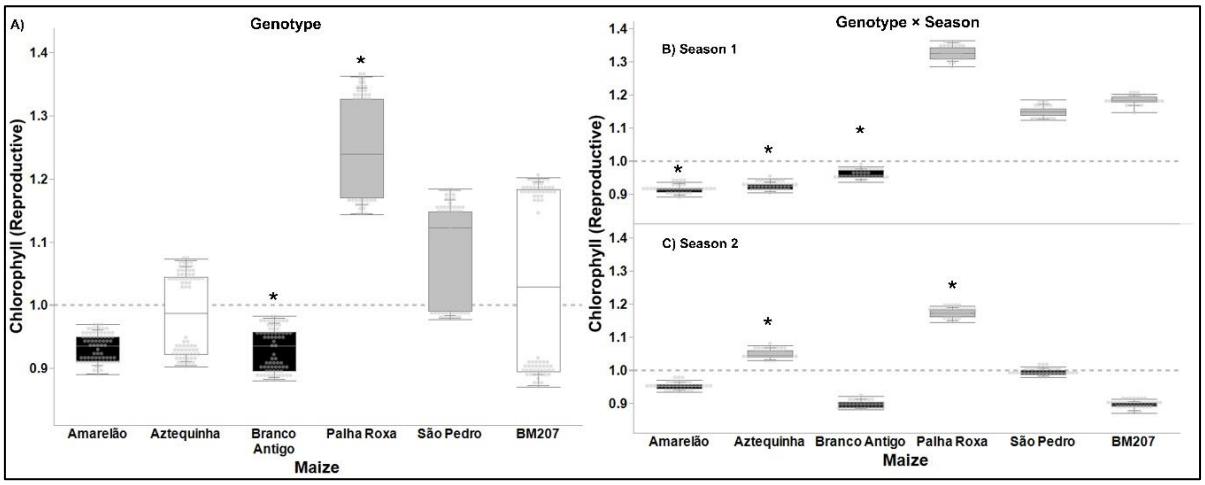

Fig. 4. Tolerance ratio (plant without insecticide application/average of plants with insecticide application) for the parameter chlorophyll content at reproductive in **A)** six maize genotypes across two seasons, **B)** six genotypes in season 1, and **C)** six genotypes in season 2 to FAW. In each plot, asterisks indicate statistical difference relative to BM 207, per Dunnett's test in **A**, and per a *priori* contrasts in **B** and **C** (critical $P \leq 0.010$ per Sidak's correction). In each plot, black filling of boxes indicates undercompensation (ratio < 1), gray filling indicates overcompensation (ratio > 1), and white filling indicates compensation (ratio not significantly different from 1.0) (critical $P = 0.014$ per Bonferroni correction).

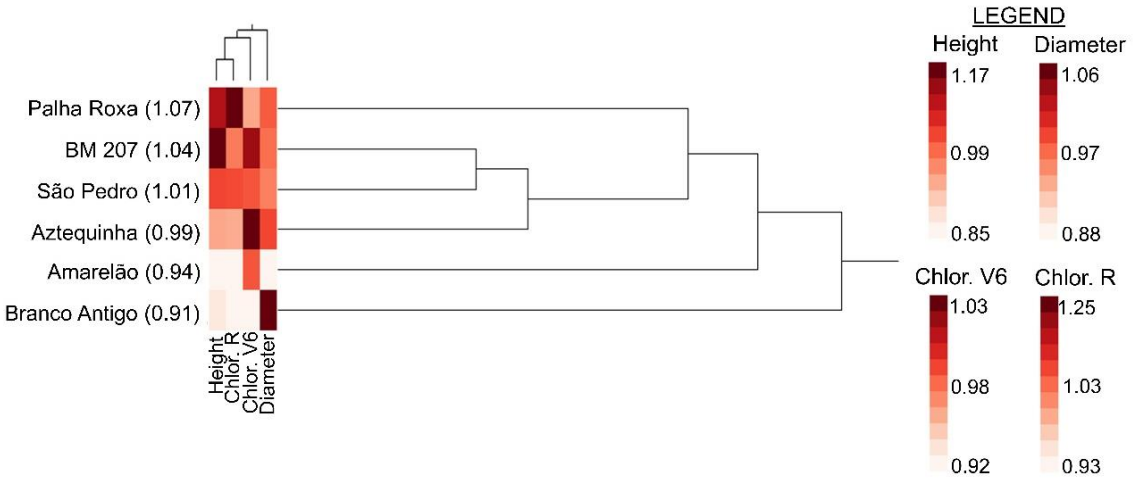

Fig. 5. Hierarchical clustering and heat map for three FAW tolerance indices (tolerance ratios, see text): Plant height (Height), chlorophyll content at V6 stage (Chlor. V6) and at reproductive stage (Chlor. R), and stem diameter (Diameter). The heat map shows changes (within columns) in tolerance ratio across plant genotype (see Legend: intense color = highest tolerance, light color = lowest tolerance). Numbers following the genotypes are geometric averages of the four tolerance ratios.

ARTICLE 2

Effects of neonicotinoid seed treatment on anti-herbivore defenses of two maize genotypes

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Abstract

Neonicotinoid seed treatment (NST) is a routine practice used worldwide to control insect pests in a variety of crops, including maize (*Zea mays* L.). However, previous work indicates that systemic insecticides can compromise plant defenses, counteracting efforts to control insect pests. Our goal was to evaluate the effect of thiamethoxam neonicotinoid seed treatment on the resistance of two maize genotypes (B73 and MC 4050) against a major non-target pest fall armyworm (*Spodoptera frugiperda* Lepidoptera: Noctuidae). In preference and performance assays, we evaluated the effect of NST on fall armyworm behavior and biology. We also determined the influence of NST on induced plant defenses, quantifying phytohormone levels and plant volatile emissions, in treatments with and without fall armyworm herbivory. NST did not affect caterpillar host preference, however it reduced caterpillar performance on the genotype B73 across both maize growth stages (V4 and V6). NST also reduced the release of diurnal volatiles in V4 stage and on the amount of salicylic acid inV6 stage B73 plants. In contrast, MC 4050 appears to be less impacted by the insecticide, regardless of the growth stage. In conclusion, we found that NST can affect maize defenses. However, the effects were dependent on the plant genotype and growth stage, suggesting growers may need to tailor their selection of plant genotypes to NST to avoid negative impacts on plant resistance and ultimately pest control.

Keywords: Thiamethoxam, *Spodoptera frugiperda*, fall armyworm, plant defense, volatiles, phytohormones

1. Introduction

Neonicotinoid insecticides are the most used pesticide worldwide for protection of crops against insect pests and are mainly applied through seed treatment (Jeschke et al. 2011; Douglas and Tooker 2015; Tooker et al. 2017). Neonicotinoid seed treatment (NST - e.g., active ingredients clothianidin, imidacloprid, and thiamethoxam) is a routine and prophylactic practice (Alford and Krupke 2017; Tooker et al. 2017), which aims to reduce pest damage in crops such as maize (*Zea mays mays* L.), soybean (*Glycine max* (Merr) L.) and cotton (*Gossypium* spp.) (Douglas and Tooker 2015), mainly against early season pests during crop establishment (Alford and Krupke 2017). Because they are soluble in water, neonicotinoid insecticides have the ability to translocate and spread throughout the plant tissues (Jeschke and Nauen 2008; Bonmatin et al. 2015). Once ingested by insects, neonicotinoids acts as a nicotinic acetylcholine receptor (nAChR) competitive modulator, causing hyperactivity by collapse of the nervous system (Tomizawa and Casida 2005). They are usually used to suppress populations of sucking arthropods, such as aphids and leafhoppers (Oliveira et al. 2008; Magalhães et al. 2009; Krupke et al. 2017; Ding et al. 2018). Some characteristics that popularized the use of neonicotinoids are their systemic nature, efficiency at low doses and relatively low toxicity to mammals (Elbert et al. 2008; Goulson 2013).

However, despite their advantages, neonicotinoid insecticides can negatively affect populations of beneficial insects and contribute to pest outbreaks. For example, neonicotinoids applied through seed treatment can cause lethal and sub-lethal effects on beneficial organisms that feed on plant resources such as pollen, floral and extrafloral nectar, tissue and sap (Moscardini et al. 2014, 2015; Gontijo et al. 2014, 2018; Rundlöf et al. 2015; Oliveira et al. 2018; Sâmia et al. 2019; Wu et al. 2021). Further, beneficial insect populations, such as parasitoids and predators, can be second-handedly exposed to the toxic effects of neonicotinoids by contacting untreated adjacent plants (Botías et al. 2016; Bredeson and Lundgren 2019), feeding on neonicotinoid-contaminated prey (Wanumen et al. 2016; Korenko et al. 2019) and on honeydew excreted by neonicotinoid-contaminated insects (Calvo-Agudo et al. 2019, 2021). NST can also cause complex and variable effects on primary and secondary plant metabolism. For instance, neonicotinoids can alter leaf photosynthetic pigments (Preetha and Stanley 2012; Macedo et al. 2013; Todorenko et al. 2021), increase root development, and improve yield (Macedo and Castro 2011; Macedo et al. 2013) even under water stress (Endres et al. 2016). On the other hand, neonicotinoids have also been associated with outbreaks of arthropod pests under diverse environmental conditions (Szczepaniec et al. 2011; Smith et al. 2013;

Szczepaniec and Raupp 2013; Ruckert et al. 2018). A few studies have shown that neonicotinoid treatment can alter the plant's ability to defend itself against biotic factors due to changes in defense signaling pathways that modulate the synthesis of defensive metabolites against pathogens and insects (Ford et al. 2010; Szczepaniec et al. 2013; Zhou et al. 2019).

Jasmonic acid (JA), salicylic acid (SA) and ethylene are the main phytohormones involved in modulation of plant defense signaling pathways (Pieterse et al. 2012). The phytohormone JA is generally responsible for modulating induced plant defenses against herbivores, while SA is involved in modulating defenses against biotrophic pathogens and some arthropod herbivores (Thaler et al. 2010; Pieterse et al. 2012; Lazebnik et al. 2014). The SA signaling pathway often interacts antagonistically with the JA signaling pathway, leading to greater plant susceptibility to herbivores following activation of the SA pathway (Kawazu et al. 2012; Schweiger et al. 2014). It is noteworthy that neonicotinoids can interfere with SA and JA signaling, which seems to be responsible for the reduced resistance of neonicotinoid-treated plants to arthropod pests (Szczepaniec et al. 2013; Wulff et al. 2019). At the same time, the activation of the SA signaling pathway makes neonicotinoid-treated plants more resistant to pathogens (Ford et al. 2010). These effects of NST on defense signaling pathways and differential expression of genes associated with defenses seem to depend on the plant species and neonicotinoid molecule (Szczepaniec et al. 2013; Wulff et al. 2019), and further studies are needed to gain understanding on the influence of NST on plant resistance to pests.

Given these findings, our hypothesis is that neonicotinoid seed treatment can decrease plant resistance against insect. We chose maize as our study system due to its world economic and social importance (Shiferaw et al. 2011) and because NST is widely used as a chemical control in maize. In the US, most of the maize seeds planted are treated with neonicotinoid insecticides to control populations of early season pests, such as aphids and leafhoppers (Douglas and Tooker 2015; Tooker et al. 2017). Studying the effects of NST on a non-target maize pest allows us to detangle the direct insecticidal effect of NST on herbivores from the effect of NST on plant defense and resistance. The fall armyworm [FAW; *Spodoptera frugiperda* (J. E. Smith)] is a major pest of maize in the Americas (Sparks 1979; Early et al. 2018) and has also recently spread into different geographic regions (Goergen et al. 2016; Otim et al. 2018; Sharanabasappa et al. 2018; Chormule et al. 2019; Jing et al. 2020). NST is not recommended for FAW control thus, our objective was to evaluate whether NST affects the resistance and induced defenses of two maize genotypes and ultimately compromises crop resistance to FAW attack.

2. Material and methods

2.1. Plants, insects, and neonicotinoid treatment

We evaluated the effect of NST on two maize genotypes: the hybrid Masters Choice® 4050 (MC 4050) and the inbred B73. MC 4050 is a field corn genotype commercially available without NST that is planted in the United States. The B73 is a genotype widely used in studies, mainly due to the knowledge about its genome (Schnable et al. 2009). First, the seeds were sterilized in a 10% bleach solution for 10 min and rinsed in distilled water. Once the seeds were completely dried, they were treated with the neonicotinoid insecticide thiamethoxam (Cruiser® 5FS, Syngenta) at 0.47 mg AI/kernel, following the maximum recommended concentration for the management of *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae) in the maize crop in Brazil (AGROFIT 2021), where it is a major pest (Ribeiro and Canale 2021).

The seeds were individually planted in pots (10 x 10 x 9 cm) filled with commercial potting soil (BACCTO Premium Potting soil 85–15–10, Michigan Peat, TX, USA), without additional fertilizer and were kept in a climate-controlled room at 25 ± 3°C, 40 ± 10% RH and 12:12 photoperiod (Light: Dark) with broad-spectrum LED lighting (Fluence, TX, USA). The seedlings were watered whenever necessary with a minimum volume of water to prevent leaching of the insecticide. All experiments were conducted at the growing stage V4 (25 days after planting) and V6 (36 days after planting) of seed-treated plants (treatment named Neo) and seed-untreated plants (treatment named control) of MC 4050 and B73 under the same controlled conditions described above.

FAW caterpillars used in assays were obtained from eggs purchased from a commercial supplier (Benzon Research, PA, USA). In experiments with 4th instar FAW larvae, caterpillars were fed an artificial diet and then transferred to feed on maize leaves for 24 hours prior to the experiment.

2.2. Plant shoot growth

To assess whether neonicotinoid treatment affects plant shoot growth, we measured height (cm) and stem diameter (mm). Plant height was measured from the soil line to the insertion of the last expanded leaf. Two perpendicular stem diameters were measured using a digital micrometer (Pittsburgh®, Harbor Freight Tools, Camarillo, CA, United States) and an average diameter was reported. All measurements were carried out at the V4 (n = 28) and V6 ($n \geq 24$) stages.

2.3. FAW preference assay

We evaluated whether NST influences preference (host-choice and food consumed) of neonate FAW caterpillars (< 24h) in dual-choice assays. The youngest completely developed leaf (either the fourth or sixth leaf) of control and Neo plants of the same genotype were placed inside Petri dishes (diameter 140 mm x 15 mm height) and closed without damaging the plant. Ten larvae were introduced in the middle of the dish, and the Petri dishes were sealed using Parafilm®. After 24 h, the number of larvae on each leaf was counted to assess host preference and the leaf fragments were excised and scanned to assess the food preference by the area consumed (mm^2) using the software ImageJ (O'Neal et al. 2002). The assay was performed in a completely randomized design, with eight replicates for each genotype and growth stage.

2.4. FAW performance assay

To evaluate FAW performance, we measured caterpillar mortality and the fresh weight gain (mg) of the surviving caterpillars after seven days of feeding on Neo or control plants in a no-choice assay. Three neonate FAW were placed in a Petri dish (diameter 100 mm x 15 mm height) with a leaf section of one of the treatments (Neo or control) and closed and sealed as described above. We kept the leaf segments attached to the plant throughout the experiment and, whenever necessary, the dish was moved to a new leaf section to provide enough food supply for the caterpillars. We conducted 7-14 replicates with the two maize genotypes at the V4 and V6 stages, as described in the previous section.

2.5. Collection and analysis of plant volatiles

We characterized the constitutive and herbivore-induced plant volatile emissions from Neo and control maize plants using a dynamic headspace sampling technique. For the FAW herbivore-damage treatments (control + FAW or Neo + FAW), plants received a single fourth-instar caterpillar, which was starved for approximately 3 hours. The youngest (either the fourth or sixth leaf) and the whorl leaf of each plant were enclosed inside individual nylon collection bags (Reynolds Consumer Products Inc., IL, USA), either with or without FAW. We sampled 8-12 Neo and control plants at V4 and V6 stages for each maize genotype.

During the collections, filtered air was delivered into each collection bag at 0.7 L min^{-1} and pulled out of the bag through an adsorbent filter containing 60 mg of HaySep® Q (Hayes Separations, Inc., TX, USA) at 0.5 L min^{-1}. We collected volatiles for 8 h during photophase (10:00–18:00) and on a separate set of filters for 8 h during scotophase (23:00–07:00). Volatiles were also collected from empty bags containing only air to control for background volatiles.

After collections, volatile compounds were eluted from filters exposed to odors using 150 µL dichloromethane solvent. As an internal standard, 5 µL of a standard solution containing nonyl acetate (80 ng/µL) was added to each sample. The leaves were harvested, dried, and the dry mass was recorded.

We analyzed the volatiles using an Agilent 7890B gas chromatograph and 5977B mass spectrometer with a splitless injector held at 250°C and helium as the carrier gas. After sample injection (1 µL), the column (HP-5MS 30 m × 0.250 mm-ID, 0.25 µm film thickness, Agilent Technologies) was held at 40°C for 5 min before the temperature was increased at 20°C/min to 250°C. Compounds were ionized by electron impact ionization at 70 eV and mass spectra were acquired by scanning from 40 to 300 m/z at 5.30 scans/s. The compound identities were tentatively determined by comparison with mass spectral libraries (NIST17, Adams2 [Allured Publishing Corporation]) and confirmed using authentic standards when possible. Compounds were quantified relative to standard concentrations and calculated as ng g^{-1} dried leaf mass. We included a compound in the analysis only if it was detected in at least 50% of the samples.

2.6. Phytohormones

Immediately after the volatile collections, we collected leaf tissue from the whorl of each plant (~ 100 mg tissue) to measure the levels of *cis*-JA (JA) and SA as indicators of plant defense. The tissue was flash frozen in liquid nitrogen and stored at -80°C until analyzed. To quantify JA and SA, endogenous plant hormones were extracted and derivatized to methyl esters, which were isolated using vapor-phase extraction (Schmelz et al. 2004). These compounds were then analyzed by coupled GC/CI-MS using isobutane and selected ion monitoring (SIM). We quantified relative amounts of JA and SA by adding 100 ng dihydro-JA and labelled 2-hydroxy-benzoic acid, added as internal standards to each sample. Finally, we compared the retention times and spectra of our samples with standards of the pure compounds.

2.7. Data analyses

We carried out all data analyses using the software R version 4.0.3 (R CoreTeam 2020). All data were tested for normality and homogeneity of variances according to Shapiro-Wilk and Bartlett tests ($p < 0.05$), respectively. Whenever necessary, data were transformed with the Box-Cox method (Box and Cox 1964), using the function of the package MASS, or by square-root transformation. The plant parameters (height and diameter) and caterpillar mass gain were compared using Student's *t*-tests, while the consumed leaf area was analyzed using a paired *t*-test. The number of insects on each maize leaf (preference assay) and the FAW mortality

(performance assay) were inferred using generalized linear models (GLM) (Nelder and Wedderburn 2000) with quasipoisson and quasibinomial distribution, respectively. The goodness of fit was evaluated using half-normal plots with a simulated envelope of hnp package (Moral et al. 2017).Volatile blend data (herbivory and NST treatments) were compared using permutational multivariate analysis of variance (PERMANOVA) calculated using the VEGAN package. Random forest analysis was used to identify compounds with the greatest contribution to variation among treatments. We also compared total volatile production (sum of all detected volatiles) and individual compounds and phytohormones using one-way ANOVA. For this, the GLM family with the best quality of fit was used and multiple comparisons tests were performed (Tukey's *post hoc* test, $p < 0.05$) in cases of significative differences.

3. Results

3.1. Plant shoot growth

Shoot height of Neo and control maize plants of both genotypes was similar at the V4 stage (Table 1; MC 4050: t = -1.253, df = 53.633, $p = 0.216$; B73: t = 0.244, df = 51.227, $p = 0.809$), and V6 stage (Table 1; MC 4050: t = 0.669, df = 45.223, $p = 0.507$; B73: t = -0.656, df = 43.042, $p = 0.516$). Furthermore, the neonicotinoid did not affect the stem diameter at either growth stage of MC 4050 (Table 1; V4: t = - 0.173, df = 53.985, $p = 0.864$; V6: t = 0.377, df = 45.976, $p = 0.708$) and B73 plants (Table 1; V4: t = 0.329, df = 53,730, $p = 0.744$; V6: t = 0.094, df = 47.995, $p = 0.926$).

3.2. Preference assay

At the V4 stage, no significant differences were found in Neo and control plants for leaf area consumed by FAW neonate larvae (Fig. 1A; B73: t = -0.402, df = 7, $p = 0.699$; MC 4050: t = 1.012, df = 7, $p = 0.345$) or FAW host preference (Fig. 1B; B73: $F_{1, 14} = 0.389$, $p = 0.543$ and MC 4050: $F_{1, 14} = 2.572$, $p = 0.131$). On the other hand, at the V6 stage, although the leaf area consumed was similar in treated and untreated leaves of B73 (Fig. 1C; t = 1.843, df = 7, $p = 0.108$), more FAW caterpillars were found on control B73 than on Neo B73 plants at the end of the assay (Fig. 1D; $F_{1, 14} = 5.289$, $p = 0.037$). Neonicotinoid treatment did not affect leaf area consumed (Fig. 1C; t = -0.403, df = 7, $p = 0.699$) or host preference (Fig. 1D; $F_{1, 14} = 2.095$, $p = 0.169$) of FAW on MC 4050 plants at V6.

3.3. Performance assay

There was no lethal effect of Neo compared to control plants after seven days of FAW feeding. These results were consistent for both maize genotypes at V4 (Fig. 2A; B73: $F_{1, 21}$ = 4.036, p = 0.058; MC 4050: $F_{1, 24}$ = 0.253, p = 0.619) and V6 stages (Fig. 2C; B73: $F_{1, 19}$ = 1.647, p = 0.215; MC 4050: $F_{1, 24}$ = 1.084, p = 0.308). The surviving FAW caterpillars gained more weight when fed on control B73 than those fed on Neo B73 plants at both stages of growth (Fig. 2B; V4: t = 2.198, df = 17.464, p = 0.042; Fig. 2D; V6: t = 2.627, df = 8.738, p = 0.028). NST did not influence the weight gain of caterpillars fed on MC 4050 plants at either stage (Fig. 2B; V4: t = 1.299, df = 21.976, p = 0.208; Fig. 2D; V6: t = 0.208, df = 22.771, p = 0.837).

3.4. Plant volatiles

Overall, we observed mixed effects of noenicitinoid treatment and FAW herbivory on volatile emissions from the two maize genotypes across the V4 and V6 growth stages. Neonicotinoid treatment induced a distinct diurnal constitutive volatile blend in B73 plants at the V4 stage (Fig. 3A; PERMANOVA $F_{1, 43}$ = 3.694, R_2 = 0.079, p = 0.011), but there was only a marginal effect of NST on the composition of herbivore-damaged volatile emissions in V4-stage B73 (Fig. 3A; PERMANOVA $F_{1, 43}$ = 2.319, R_2 = 0.049, p = 0.065). In contrast, for B73 plants at the V6 stage, multivariate analysis revealed significant differences only due herbivore damage (Fig. 3C; PERMANOVA $F_{1, 42}$ = 4.055, R_2 = 0.091, p = 0.004), but not by NST (Fig. 3C; PERMANOVA $F_{1, 42}$ = 0.474, R_2 = 0.011, p = 0.836). Similarly, the volatile blend of MC 4050 was influenced solely by herbivore damage (Fig. 3B; V4: PERMANOVA $F_{1, 47}$ = 2.558, R_2 = 0.054, p = 0.030; Fig. 3D; V6: PERMANOVA $F_{1, 46}$ = 2.438, R_2 = 0.051, p = 0.032), but not neonicotinoid treatment (Fig. 3B; V4: PERMANOVA $F_{1, 47}$ = 0.407, R_2 = 0.009, p = 0.913; Fig. 3D; V6: PERMANOVA $F_{1, 46}$ = 1.382, R_2 = 0.0291, p = 0.218).

Random forest analysis revealed that the compounds that contributed most to the variation across treatments varied according to genotype and stage (Fig. S1). These compounds were highlighted (in bold) in the Table 2 and 3. In B73, random forest identified eight compounds (Table 2). Undamaged Neo plants emitted smaller amounts of nonanal and the aromatic benzyl acetate relative to undamaged control plants of B73 at V4 stage (Table 2; nonanal: $F_{3, 40}$ = 5.356, p = 0.001; benzyl acetate: $F_{3, 40}$ = 10.916, p < 0.0001). Six compounds were released in lower amounts by herbivore-damaged Neo plants compared to the emission from herbivore-damaged control plants of the B73 genotype at V4 [Table 2: Nonanal ($F_{3, 40}$ = 5.356, p = 0.001); α-pinene ($F_{3, 40}$ = 2.911, p = 0.046); (*E*)-β-ocimene ($F_{3, 40}$ = 4.574, p = 0.008); (3*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) ($F_{3, 40}$ = 5.345, p = 0.003); (*E*)-α-bergamotene ($F_{3,}$

$_{40}$ = 6.042, p = 0.002) and (*E*)-β-farnesene ($F_{3, 40}$ = 5.838, p = 0.001)]. When considering the major compound groups, herbivore-damaged Neo plants emitted a blend with reduced quantities of fatty acids derivates ($F_{3, 40}$ = 2.973, p = 0.043) and terpenes ($F_{3, 40}$ = 4.396, p = 0.009) relative to that emitted by herbivore-damaged control B73 plants at the V4 stage. These differences did not persist, however, and for B73 plants at the V6 stage, only a marginal difference was observed between treatments for the total of fatty acids derivatives (Table 2; $F_{3, 38}$ = 2.452, p = 0.078), while herbivore-damaged control or Neo emitted greater amounts of terpenes relative to respective undamaged treatments (Table 2; $F_{3, 38}$ = 3.291, p = 0.019). Notably, undamaged Neo plants emitted a blend containing greater amounts of β-caryophyllene ($F_{3, 38}$ = 3.754, p = 0.010) and benzyl acetate ($F_{3, 38}$ = 3.140, p = 0.024) than that of B73 control plants at V6 stage.

For MC 4050 plants at the V4 stage, herbivore-damaged plants emitted a similar blend of compounds in the Neo and control treatments, with greater production in herbivore-damaged than respective undamaged plants for fatty acids derivates ($F_{3, 44}$ = 4.586, p = 0.003), aromatic compounds ($F_{3, 44}$ = 7.398, p < 0.0001), and terpenes in Neo plants ($F_{3, 44}$ = 3.207, p = 0.022). On the other hand, for MC 4050 at the V6 stage, there was no difference in the total compounds released in each group among the treatments [fatty acids derivates ($F_{3, 42}$ = 2.745, p = 0.055), terpenes ($F_{3, 42}$ = 1.024, p = 0.392), and aromatics ($F_{3, 42}$ = 0.287, p = 0.835)]. The individual analysis of selected compounds in the random forest for MC 4050 at V4 stage (Fig. S1) revealed that NST suppressed the emission of nonanal released by herbivore-damaged plants (Table 3; $F_{3, 44}$ = 3.174, p = 0.023) and (*E*)-β-farnesene of undamaged plants of (Table 3; $F_{3, 44}$ = 3.539, p = 0.014). At the same time, NST up-regulated the emission of β-pinene, which was 3.85 times higher in the blend of herbivore-damaged Neo plant than of that emitted by herbivore-damaged control plants of the same genotype and growth stage (Table 3; $F_{3, 44}$ = 2.975, p = 0.030). For MC 4050 plants at the V6 stage, NST suppressed the compound (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) in the blend of herbivore-damaged volatiles (Table 3; $F_{3, 42}$ = 4.084, p = 0.007). In addition, herbivore-damaged plants, irrespective of the neonicotinoid treatment, released greater amounts (*Z*)-3-hexenyl acetate (Table 3; $F_{3, 42}$ = 6.031, p = 0.002) and hexyl acetate (Table 3; $F_{3, 42}$ = 3.441, p = 0.016) relative to undamaged plants.

Concerning nocturnal volatile blends, NST did not alter the composition of constitutive volatile blends in B73 [Fig. S2 PERMANOVA V4 ($F_{1, 41}$ = 0.742, R_2 = 0.017, p = 0.615); V6 ($F_{1, 42}$ = 0.702, R_2 = 0.154, p = 0.625)] or MC 4050 [Fig. S2 PERMANOVA V4 ($F_{1, 44}$ = 0.424, R_2 = 0.008, p = 0.859); V6 ($F_{1, 46}$ = 1.106, R_2 = 0.022, p = 0.335). However, FAW damage modified the composition of nocturnal volatile emissions for both genotypes and growth stages

[Fig. S2; PERMANOVA B73: V4 ($F_{1, 41}$ = 4.584, R_2 = 0.104, p = 0.006); V6 ($F_{1, 42}$ = 5.487, R_2 = 0.121, p = 0.001); MC 4050: V4 ($F_{1, 44}$ = 8.769, R_2 = 0.174, p = 0.001); V6 ($F_{1, 43}$ = 4.968, R_2 = 0.099, p = 0.005)]. For B73 at the V4 stage, herbivore-damaged plants released a larger total amount of fatty acid derivates compared to undamaged plants (Table 4; $F_{3, 38}$ = 3.088, p = 0.038), but not at the V6 (Table 4; $F_{3, 38}$ = 2.409, p = 0.082). Notably, FAW herbivory did not change the total amount of nocturnal volatile terpenes emitted by B73 at either growth stage (Table 4; V4: $F_{3, 38}$ = 0.850, p = 0.475; V6: $F_{3, 38}$ = 1.248, p = 0.306). Among the compounds selected in the random forest (Fig. S3), DMNT was significantly decreased in Neo B73 plants at V4 (Table 4; $F_{3, 38}$ = 6.188, p = 0.0003), but at V6 the differences of DMNT were due FAW herbivory (Table 4; $F_{3, 38}$ = 3.692, p = 0.011).

For MC 4050 plants, there was a greater released of fatty acids derivates and terpenes in herbivore-damaged plants regardless of neonicotinoid at V4 stage (Table 5; fatty acids derivates: $F_{3, 41}$ = 6.734, p = 0.0001; terpenes: $F_{3, 41}$ = 6.423, p = 0.0002). At V6 stage, fatty acids derivates released by MC 4050 herbivore-damaged plants were greater than their respective controls (Table 5; $F_{3, 43}$ = 5.940, p = 0.0005), while NST increased the total terpenes in herbivore-damaged plants relative to Neo plants ($F_{3, 43}$ = 3.148, p = 0.035). For MC 4050 plants at the V6 stage we observed an increase for some compounds from NST (Table 5). Specifically, MC 4050 Neo damaged plants had increased production of the terpenes DMNT ($F_{3, 43}$ = 20.190, p < 0.0001), β-caryophyllene ($F_{3, 43}$ = 14.871, p < 0.0001), and (*E*)-α-bergamotene ($F_{3, 43}$ = 12.660, p < 0.0001) than control damaged plants.

3.5. Phytohormones

SA levels in the two maize genotypes were not affected by neonicotinoid treatment or FAW damage at V4 (Fig. 4A; B73: $F_{3, 38}$ = 1.915, p = 0.144; Fig. 4B; MC 4050: $F_{3, 40}$ = 1.649, p = 0.193). However, at the V6 stage, the levels of SA were increased by herbivore-damage in MC 4050 control and Neo plants (Fig. 4D; $F_{3, 37}$ = 9.584, p < 0.0001). While in B73, we observed that NST suppressed SA levels in undamaged plants (Fig. 4C; $F_{3, 34}$ = 2.929, p = 0.032), but Neo and control plants were not different from each other when damaged (Fig. 4C).

Herbivore-damaged plants of MC 4050 showed the highest amounts of JA at both growth stages, regardless of neonicotinoid treatment (Fig. 4B; $F_{3, 41}$ = 9.563, p < 0.0001; Fig. 4D; $F_{3, 37}$ = 18.816, p < 0.0001). On the other hand, in B73, the JA levels were not affected by neonicotinoid or FAW damage at V4 (Fig. 4A; $F_{3, 37}$ = 1.842, p = 0.157). However, the concentration of JA increased due to herbivore damage in B73 plants at the V6 stage (Fig. 4C; $F_{3, 37}$ = 14.704, p < 0.0001).

4. Discussion

The neonicotinoids translocation and effects of NST on plant physiology vary depending on the plant species (Szczepaniec et al. 2013; Yang et al. 2018; Whalen et al. 2021). In this study, we report that NST influences anti-herbivore plant defenses and plant defense signaling differently within the same plant species, as the maize genotypes B73 and MC 4050 responded differently to thiamethoxam seed treatment. In B73 plants, NST negatively affected the behavior and biology of FAW caterpillars, suppressed the emission of herbivore-induced volatile compounds and constitutive levels of SA. In contrast, NST on the MC 4050 genotype did not affect plant resistance to FAW or induced plant defenses, measured in terms of herbivore-induced plant volatiles and phytohormone levels.

The difference across maize is likely due to a large intraspecific variation (e.g., genotypic, and phenotypic) present in the species (Degen et al. 2004; Stupar and Springer 2006; Chen et al. 2018; Luo et al. 2019). It is noteworthy that we used an inbred (B73) and a hybrid (MC 4050) in this study, and both groups are known to have distinct homozygosis and traits (Gama and Hallauer 1977; Betrán et al. 2003; Yendrek et al. 2017; Hisse et al. 2019). Traits like plant growth rate, growth stage and physiological variations can affect the translocation of insecticides throughout the plants (Cloyd et al. 2011), and consequently their interactions on the plant. Noticeably, MC 4050 plants were about 27% and 37% taller than B73, at the V4 and V6 stage, respectively (Table 1) and this may be one of the reasons that contributed to the lower effect of thiamethoxam on MC 4050. Fast growing plants might have lower concentration of insecticides in leaf tissue, and higher concentration in the soil resulting from a dilution of relatively low soil insecticide and unavailability for plant absorption (Whalen et al. 2021). In maize, the effect of neonicotinoid on the plant seems to depend on the genotype and application technique. For example, when thiamethoxam was applied into the soil by root irrigation, a hybrid genotype showed reduced photosynthetic pigment content, hence being more susceptible to the insecticide than an inbred genotype (Todorenko et al. 2021). However, when applied via seed treatment, the amount of photosynthetic pigments were inversely proportional to the concentration of thiamethoxam in the maize hybrid (Macedo and Castro 2018).

FAW neonates consumed similar amounts of maize irrespective of NST treatment or the genotype or growth stage (Fig. 1). This assay was performed for a short time interval (24 hours), so it is possible that the food area consumed could change if more time was given for larvae to settle on the treatments. However, we observed almost two-fold more FAW neonates on control plants than Neo B73 plants at the V6 stage (Fig. 1D). As FAW neonates are capable

for selecting better hosts (Rojas et al., 2018), this preference for control plants might be explained by our performance assay, in which FAW performed better (measured in terms of gain weight) on control than Neo B73 plants. Thiamethoxam treatment reduced the weight of FAW caterpillars by 53.55% (V4) and 84.29% (V6). Larvae of monarch butterflies *Danaus plexippus* (Linnaeus, 1758) (Lepidoptera: Nymphalidae) also had lower weight, smaller body length and longer duration of the first larval instar when fed leaf segments treated with clothianidin (Pecenka and Lundgren 2015), which is a thiamethoxam metabolite (Nauen et al. 2003).

It was not expected that NST would negatively affect the biology of FAW since there is a rapid decrease in the concentration of neonicotinoid in the plant with the development and growth of maize (Myresiotis et al. 2015; Alford and Krupke 2017; Whalen et al. 2021) and it is not recommended for controlling the FAW (AGROFIT 2021). The lower weight of caterpillars feeding on Neo plants of B73 may have at least two possible explanations. First, a feeding inhibition activity of the caterpillars by thiamethoxam, which is one of the sublethal effects caused by neonicotinoids (Barrania 2013; Sanchez-Bayo 2014; Uhl et al. 2015; Gontijo et al. 2018; Basley and Goulson 2018). In particular for FAW, it has been shown that soybean seed treated with thiamethoxam reduced the leaf area consumed by caterpillars (Gontijo et al. 2018). A second explanation is an increase in energy demand for detoxification and coping with insecticide stress, as demonstrated for wood crickets *Nemobius sylvestris* (Bosc, 1792) (Orthoptera: Gryllidae) after feeding on strawberry leaves treated with low concentrations of imidacloprid (Uhl et al. 2015) and for Madagascar cockroach *Gromphadorhina portentosa* (Olivier, 1789) (Blattodea, Blaberidae), which change the sugar distribution and midgut glucose absorption to increase energy needs induced by insecticides (Sawczyn et al. 2012).

However, the effects of neonicotinoids on biological and biochemical parameters of lepidopteran are diverse, complex and may vary depending on the species of insect. For example, fourth instar larvae of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) when fed fresh leaves of castor treated with thiamethoxam showed a decrease in emergence, fecundity and fertility, and higher mortality in concentrations greater than or equal to 40 µg mL^{-1}, these biological changes were related to changes in DNA and oxidative stress caused by the interaction of the insecticide with the insect (Jameel et al. 2020). In lepidopterans, during the final instar the neonicotinoid imidacloprid acts on the nervous system and disrupts the pupae change for adults (Krishnan et al. 2021). However, sublethal effects of neonicotinoid on lepidopteran pest is not a rule. *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae, for example, rapidly eliminated thiamethoxam without toxicity to the pest (Fan and Shi 2017).

In general, previous studies have shown that neonicotinoids suppress the JA signaling pathway, resulting in greater plant susceptibility to arthropods (Szczepaniec et al. 2013; Wulff et al. 2019). Contrary to these findings, we found that thiamethoxam did not alter the levels of JA in undamaged or herbivore-damaged plants of either genotype at the V4 or V6 stage. However, thiamethoxam decreased the total of constitutive SA in our experiment by about three-fold in B73 at V6, although this effect was no longer detected in the plant upon FAW damage. The suppressive effect of thiamethoxam on the SA pathway was also found in soybean treated plants (Wulff et al. 2019). In contrast, studies have shown that neonicotinoid treatment activates the SA signaling pathway, as in *A. thaliana* and tomato (Ford et al. 2010; Szczepaniec et al. 2013). The suppression of SA is a relevant information that may impact maize defense responses, in particular those against biotrophic pathogens (Yuan et al. 2019). Furthermore, SA influences several important plants parameters such as vegetative growth, photosynthesis, respiration and response to abiotic stress (Vos et al. 2013), which can be compromised by the reduction of SA due thiamethoxam treatment.

Interestingly, at the V4 stage, we did not detect that NST influenced SA levels, similarly to what was observed after seed treatment with clothianidin in 4-week-old maize plants (Szczepaniec et al. 2013). In addition, the same study reported that spider mite *T. urticae* induces gene expression modulated by SA (phenylalanine ammonia lyase, co-enzyme A ligase and chitinase) and JA (trypsin protease), but the neonicotinoid hinders this induction, and decreased 12-oxo-phytodienoic acid (OPDA), a precursor of JA, resulting in an increase in the spider mite population. We observed that damaged treatments (Neo or control) induced higher concentrations of JA, and the constitutive levels were not affected by NST. Overall, the phytohormones, as a proxy of plant induced defenses, do not explain the results of the performance assay in our study, once the low weight of caterpillars in B73 Neo were consistent across the V4 and V6 stages.

In our study, neonicotinoid seed treatment played an important role in changing plant volatile emissions, especially in B73 plants. Both B73 and MC 4050 plants released different volatiles blends across the treatments evaluated, which was expected due the wide natural variability in maize volatile composition (Hoballah et al. 2002; Degen et al. 2004; Block et al. 2018; Yactayo-Chang et al. 2021). Thiamethoxam had a suppressive effect on diurnal volatile emissions of the B73 maize genotype at V4 stage, including compounds of varying groups, such as fatty acid derivates, terpenes, and aromatics. The suppression effect of volatile compounds caused by neonicotinoid was previously observed in tea plants (*Camellia sinensis*) sprayed with imidacloprid, which emitted lower amounts of the green leaf volatiles (*Z*)-3-

hexenal, *n*-hexenal, (*Z*)-3-hexene-1-ol and (*Z*)-3-hexenyl acetate (Zhou et al. 2019). Among the compounds suppressed, we notice that the fatty acid derivative nonanal was consistently suppressed at stage V4 in both maize genotypes in herbivore-damaged Neo plants. Nonanal is a potential repellent compound to larvae and gravid female of Asian corn borer, *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae) (Huang et al. 2009), which is an important maize pest in China and India (Nafus and Schreiner 1991).

The suppression of constitutive and herbivore-induced plant volatiles caused by NST can have implications to the interactions among maize, second and third-trophic levels. Terpenes are important defensive compounds that can directly and indirectly impact the growth and reproduction of maize pests (Block et al. 2019). We observed that some terpenes linked with insect attraction or repellence were affect by NST. For example, in B73 at V4, (*E*)-α-bergamotene was suppressed in the blend emitted by herbivore-damaged Neo plants, this suppression may compromise the attraction of the FAW parasitoid *Cotesia marginiventris* Cameron (Hymenoptera: Braconidae) (Schnee et al. 2006). Besides that, (*E*)-α-bergamotene is an attractive compound for FAW oviposition (Yactayo-Chang et al. 2021). On the other hand, Neo B73 at V6 increased the amount of β-caryophyllene, which is correlated with increase attraction of *C. marginiventris* (Köllner et al. 2008). But, because it is difficult to infer which changes in volatile blend would influence the recruitment of natural enemies, future studies should investigate whether the changes in maize volatile emission induced by NST are ecologically relevant for the third trophic level.

In summary, we found that the effects of neonicotinoid on plant resistance against FAW and defense signaling are highly dependent on the plant genotype and growth stage. Both parameters have already been reported that influence the expression of maize genes that modulate defenses against FAW herbivory (Chuang et al. 2014). Another critical point that may have influenced the difference between the growth stages is the possible reduction in the insecticide concentration in the plant, which tends to decrease over time (Myresiotis et al. 2015; Alford and Krupke 2017; Whalen et al. 2021). Additional tests under field conditions are necessary to substantiate whether the changes in phytohormones and volatiles after thiamethoxam seed treatment can affect the maize defenses under realistic conditions of herbivore and pathogen infestation.

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Table 1 Height and diameter (means ± SE) of B73 and MC 4050 at V4 and V6 stage. The plants from neonicotinoid treatment (Neo) did not differ from control (untreated plants) at V4 and V6 according to *t*-test.

		B73		MC 4050	
Treatment	**Stage**	**Height (cm)**	**Diameter (cm)**	**Height (cm)**	**Diameter (cm)**
Control	V4	14.625 ± 0.306	5.048 ± 0.297	17.946 ± 0.589	5.265 ± 0.331
Neo		14.500 ± 0.387	4.969 ± 0.297	19.036 ± 0.639	5.347 ± 0.336
Control	V6	20.900 ± 0.719	5.846 ± 0.307	29.956 ± 1.518	6.424 ± 0.383
Neo		21.720 ± 1.024	5.805 ± 0.309	28.417 ± 1.732	6.217 ± 0.392

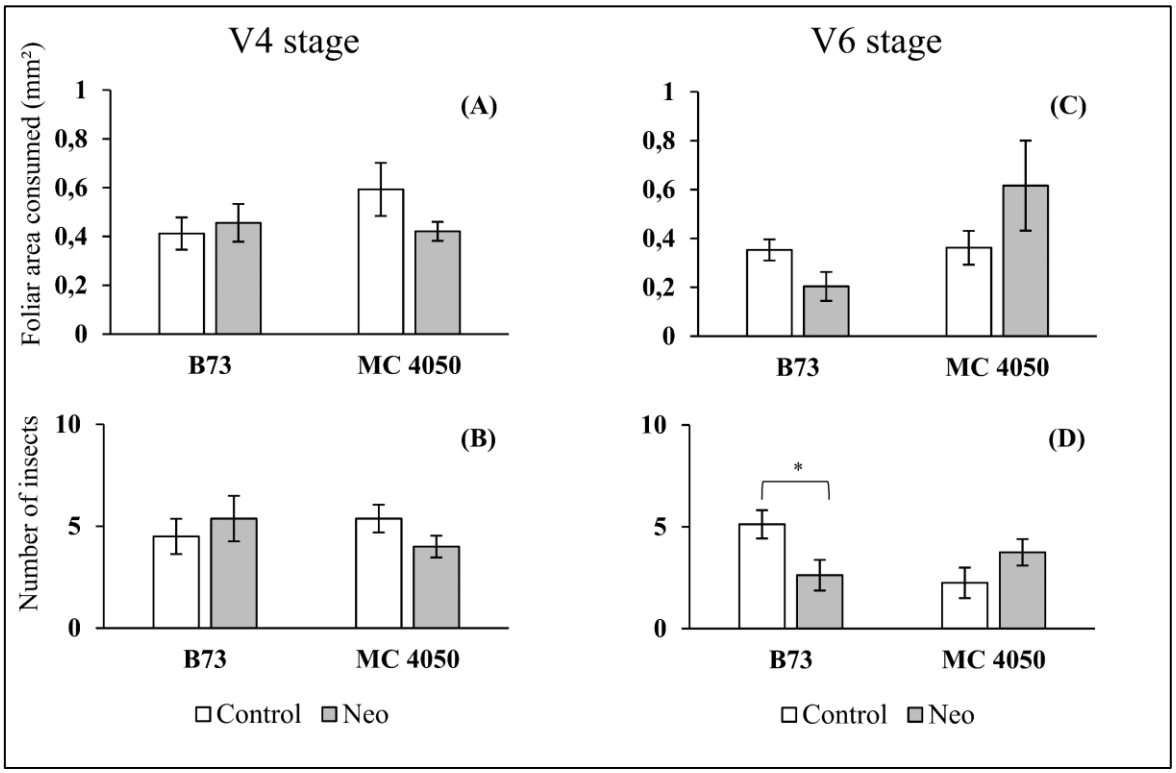

Fig. 1 Preference assay of *Spodoptera frugiperda* caterpillars feed on leaves of B73 and MC 4050 from control (untreated) and Neo (neonicotinoid) treatments at V4 and V6 stage after 24 h of caging. Food preference was measured by foliar area consumed (mean ± SE) (A, C) and host preference as number of insects in each leaf segments (mean ± SE) (B, D). * Means statistical differences by GLM and the absence means no statical differences by paired *t*-test (foliar area consumed) or GLM (number of insects).

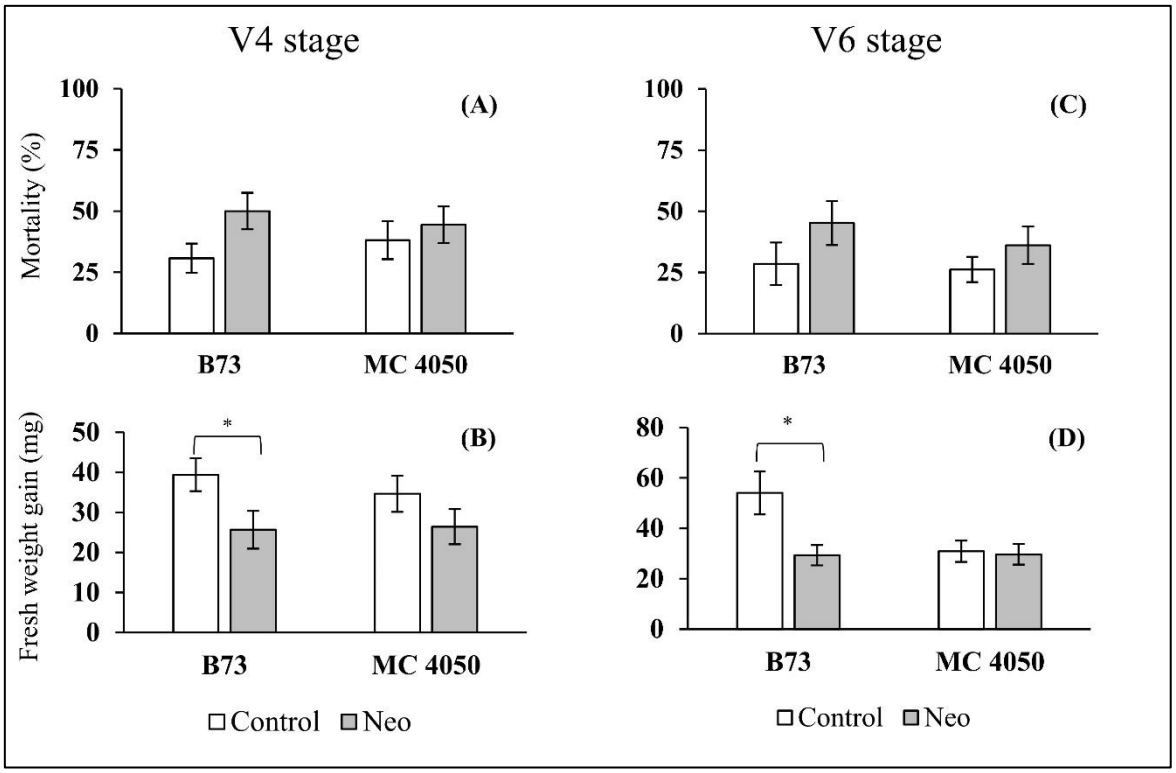

Fig. 2 *Spodoptera frugiperda* performance based on mortality (mean ± SE) (A, C) and fresh weight gain (mean ± SE) (B, D) found after seven days of feeding on maize plants (B73 and MC 4050) from control (untreated) and Neo (neonicotinoid) treatment. There was no lethal effect of neonicotinoid treatment (A, C) by GLM. * Means significant differences in caterpillars fresh gain weight feeding on B73 plants (B, D), however no difference was observed on MC 4050 plants by *t*-test.

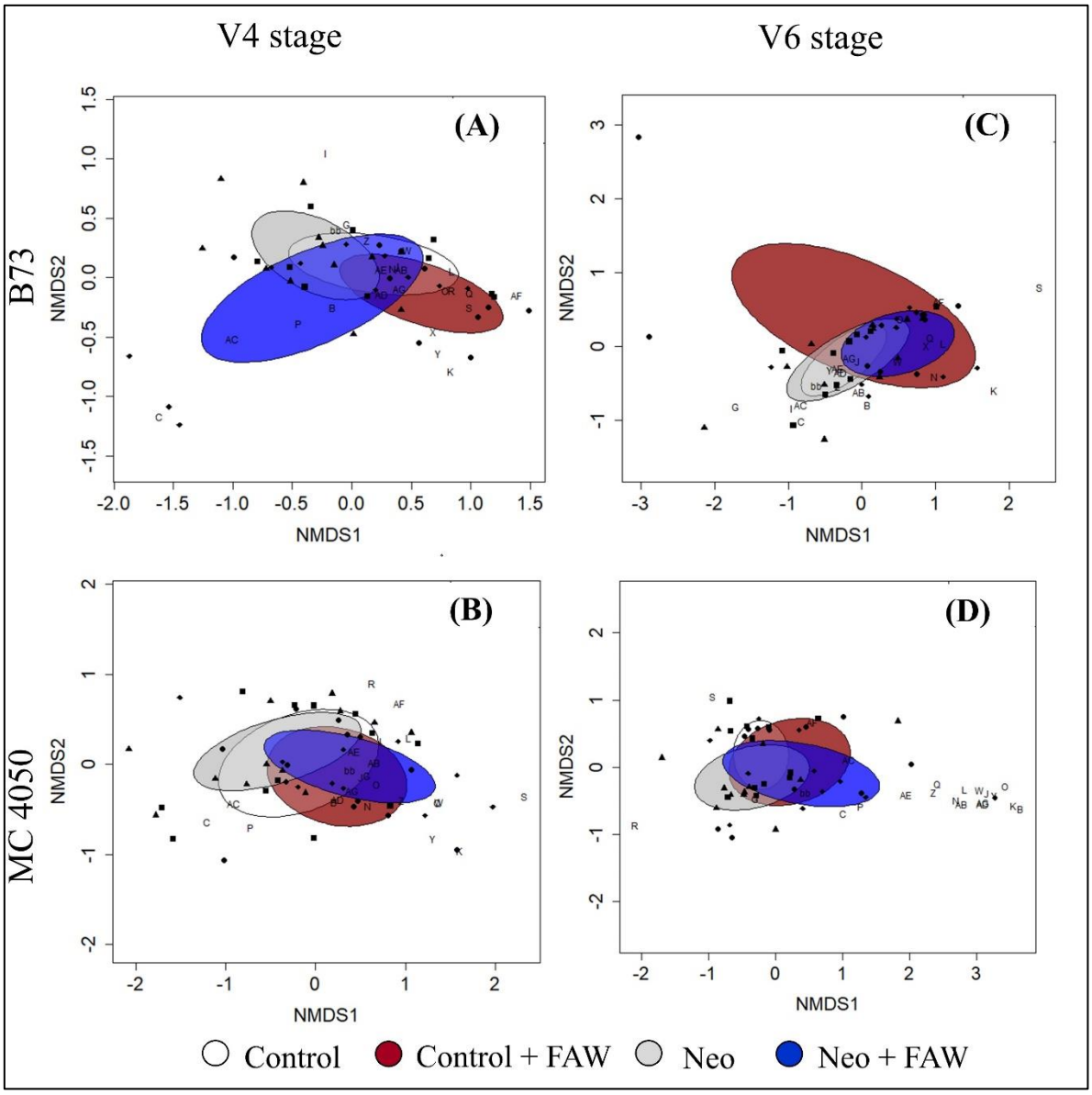

Fig. 3 Diurnal blends emitted by B73 [V4 (A) and V6 (C)] and MC 4050 [V4 (B) and V6 (D)] after eight hours of volatile collections. Treatments: Control (untreated); Control + FAW (untreated + fall armyworm; Neo (neonicotinoid); and Neo + FAW (neonicotinoid + fall armyworm).

Table 2 B73 diurnal individual compound and total of volatile (means ng g^{-1} ± SE) released by control, control + FAW, Neo and Neo + FAW treatments at V4 and V6 stage. Bold value indicates compounds observed in random forest that contributed most to the variation in each treatment. Different letters in the line indicate significant differences across treatment for individual compound and total by group according to GLM (by Tukey's *post hoc* test; $p < 0.05$) and the absence of letters indicates equality between treatments.

Group	Compound	V4				V6			
		control	control + FAW	Neo	Neo + FAW	control	control + FAW	Neo	Neo + FAW
Fatty acids derivates	Hexenal	58.919 ± 11.791	96.764 ± 39.905	79.876 ± 27.644	36.448 ± 8.153	**23.962 ± 5.918**	**40.513 ± 18.959**	**40.098 ± 16.521**	**96.798 ± 44.101**
	2-hexanol	59.929 ± 21.587	93.342 ± 39.172	139.831 ± 71.236	118.107 ± 42.167	95.253 ± 39.069	69.826 ± 35.798	65.826 ± 27.376	116.96 ± 64.825
	2-ethyl hexanal	28.402 ± 8.450	45.052 ± 22.319	18.722 ± 7.334	20.723 ± 10.234	6.451 ± 2.617	4.705 ± 3.017	8.452 ± 4.967	5.733 ± 1.850
	(*Z*)-3-hexenyl acetate	**23.764 ± 8.087 b**	**325.229 ± 213.146 a**	**10.456 ± 2.557 b**	**146.649 ± 54.089 a**	**14.764 ± 6.581 b**	**282.761 ± 220.556 a**	**11.693 ± 5.111 b**	**484.856 ± 280.402 a**
	Hexyl acetate	26.001 ± 10.086	21.822 ± 5.777	5.255 ± 1.608	8.455 ± 2.731	1.945 ± 0.703	7.022 ± 1.999	2.175 ± 0.933	8.665 ± 3.257
	Ethylhexyl acetate	223.281 ± 97.05	231.026 ± 139.48	19.271 ± 6.516	33.710 ± 14.422	-	-	-	-
	Nonanal	**93.078 ± 30.516 ab**	**186.001 ± 106.511 a**	**39.506 ± 10.613 c**	**52.043 ± 13.899 bc**	-	-	-	-
	Total	513.373 ± 105.129 b	999.236 ± 383.927 a	312.917 ± 107.314 b	416.135 ± 60.990 b	142.375 ± 47.046	404.827 ± 272.108	128.244 ± 49.687	713.012 ± 387.554
Terpenes	α-pinene	**70.413 ± 15.767 ab**	**116.041 ± 21.784 a**	**73.096 ± 21.549 ab**	**46.219 ± 12.452 b**	**37.846 ± 5.792**	**57.197 ± 17.351**	**53.626 ± 16.328**	**89.714 ± 29.053**
	β-pinene	83.519 ± 27.140	115.319 ± 50.001	70.071 ± 28.850	46.564 ± 15.252	21.443 ± 6.254	28.101 ± 16.780	31.266 ± 16.693	56.707 ± 33.539
	β-myrcene	103.019 ± 19.421	180.167 ± 38.531	64.898 ± 18.060	65.670 ± 16.981	56.188 ± 12.128	137.035 ± 31.517	84.110 ± 30.715	109.374 ± 26.710
	(*E*)-β-ocimene	**338.052 ± 90.640 ab**	**503.007 ± 130.475 a**	**203.822 ± 58.269 bc**	**141.750 ± 33.726 c**	**60.846 ± 17.387 b**	**179.184 ± 37.607 ab**	**85.939 ± 29.024 b**	**328.701 ± 153.342 a**
	Linalool	1070.903 ± 364.039	1597.095 ± 382.059	471.849 ± 117.630	625.946 ± 208.220	574.931 ± 210.825	1468.370 ± 405.885	779.861 ± 220.403	1315.068 ± 369.813

Terpenes	DMNT	**971.916 ± 340.076 b**	**2228.501 ± 602.479 a**	**387.130 ± 90.589 b**	**663.184 ± 234.957 b**	**655.211 ± 228.931 c**	**1872.698 ± 446.24 ab**	**794.098 ± 198.788 bc**	**2288.874 ± 687.739 a**
	TMTT	553.696 ± 282.135	740.776 ± 320.045	106.020 ± 29.453	239.794 ± 106.144	507.435 ± 309.508	1098.712 ± 560.695	369.531 ± 97.661	949.530 ± 378.533
	β-caryophyllene	5.693 ± 1.458	9.690 ± 2.410	3.538 ± 0.827	3.706 ± 1.121	**0.592 ± 0.338 b**	**4.810 ± 0.941 a**	**3.716 ± 1.556 a**	**3.832 ± 1.404 a**
	(*E*)-α-bergamotene	**18.166 ± 5.407 b**	**56.620 ± 16.952 a**	**9.230 ± 2.875 b**	**15.892 ± 4.436 b**	23.874 ± 9.658	49.931 ± 17.985	30.066 ± 13.721	65.794 ± 20.435
	(*E*)-β-farnesene	**18.159 ± 5.209 b**	**68.124 ± 21.673 a**	**7.288 ± 2.742 b**	**14.973 ± 4.999 b**	27.585 ± 17.704	18.950 ± 8.320	38.179 ± 19.789	29.777 ± 13.820
	α-caryophyllene	3.390 ± 0.689	4.674 ± 0.689	2.112 ± 0.647	1.719 ± 0.425	1.482 ± 0.881	2.996 ± 0.99	2.038 ± 1.219	1.657 ± 0.841
	β-cubebene	36.100 ± 8.715	65.799 ± 19.523	25.137 ± 6.987	22.220 ± 5.614	6.733 ± 2.326	14.699 ± 6.165	22.796 ± 15.413	15.704 ± 9.250
	α-selinene	37.413 ± 5.703	60.937 ± 24.629	60.310 ± 23.250	57.248 ± 9.590	33.743 ± 10.117	37.463 ± 16.997	26.905 ± 9.521	54.730 ± 21.171
	δ-cadinene	40.706 ± 9.093	76.307 ± 16.924	26.757 ± 8.609	24.203 ± 6.375	28.904 ± 11.248	76.755 ± 22.122	42.224 ± 18.760	42.956 ± 12.125
	α-cubebene	32.177 ± 6.296	53.382 ± 9.824	21.712 ± 4.864	19.052 ± 4.445	17.433 ± 4.641	36.308 ± 9.672	26.266 ± 11.130	22.649 ± 6.285
	Unk sesq	34.267 ± 11.267	41.215 ± 9.035	16.724 ± 5.030	16.661 ± 4.930	**9.770 ± 2.422**	**29.082 ± 5.762**	**14.052 ± 4.648**	**16.132 ± 3.569**
	Total	3417.589 ± 1068.623 ab	5917.654 ± 1380.979 a	1549.694 ± 331.154 b	2004.801 ± 627.334 b	2064.016 ± 784.331 c	5112.291 ± 1374.641 ab	2404.673 ± 550.501 bc	5391.199 ± 1372.862 a
Aromatic	Benzyl acetate	**11.220 ± 6.261 b**	**59.966 ± 18.309 a**	**0.060 ± 0.060 c**	**39.174 ± 23.449 ab**	**6.083 ± 4.798 b**	**162.146 ± 105.362 a**	**62.657 ± 50.994 a**	**412.809 ± 313.371 a**
	Total	11.220 ± 6.261 b	59.966 ± 18.309 a	0.060 ± 0.060 c	39.174 ± 23.449 ab	6.083 ± 4.798 b	162.146 ± 105.362 a	62.657 ± 50.994 a	412.809 ± 313.371 a

Table 3 MC 4050 diurnal individual compound and total of volatile (means ng g^{-1} ± SE) released by control, control + FAW, Neo and Neo + FAW treatments at V4 and V6 stage. Bold value indicates compounds observed in random forest that contributed most to the variation in each treatment. Different letters in the line indicate significant differences across treatment for individual compound and total by group according to GLM (by Tukey's *post hoc* test; $p < 0.05$) and the absence of letters indicates equality between treatments.

Group	Compound	V4				V6			
		control	control + FAW	Neo	Neo + FAW	control	control + FAW	Neo	Neo + FAW
Fatty acids derivates	Hexenal	**54.997 ± 19.612**	**79.316 ± 28.849**	**31.065 ± 6.108**	**79.639 ± 38.373**	35.031 ± 10.191	69.828 ± 38.863	40.368 ± 12.351	98.495 ± 30.001
	2-hexanol	103.174 ± 36.798	131.385 ± 54.211	84.184 ± 27.649	105.243 ± 32.974	**94.224 ± 29.767**	**108.021 ± 45.873**	**135.648 ± 30.780**	**165.768 ± 44.067**
	2-ethyl hexanal	**14.838 ± 8.268**	**29.655 ± 11.029**	**10.676 ± 6.832**	**28.952 ± 18.697**	**5.690 ± 1.849**	**10.501 ± 6.692**	**7.375 ± 4.392**	**11.526 ± 4.205**
	(*Z*)-3-hexenyl acetate	**17.610 ± 4.846 b**	**656.541 ± 387.752 a**	**12.789 ± 3.412 b**	**404.741 ± 203.819 a**	**18.347 ± 5.106 b**	**319.193 ± 179.388 a**	**21.861 ± 7.039 b**	**534.884 ± 238.517 a**
	Hexyl acetate	12.624 ± 6.548	14.761 ± 4.766	12.024 ± 6.562	25.138 ± 11.909	**3.119 ± 0.823 bc**	**8.895 ± 2.949 a**	**2.277 ± 0.877 c**	**8.288 ± 2.583 ab**
	Ethylhexyl acetate	95.378 ± 45.998	46.993 ± 21.705	118.969 ± 74.268	85.867 ± 41.605	16.208 ± 7.779	106.961 ± 101.878	95.844 ± 89.018	15.776 ± 7.836
	Nonanal	**90.626 ± 51.208 ab**	**191.430 ± 62.053 a**	**38.474 ± 17.081 b**	**65.064 ± 10.977 b**	73.988 ± 28.884	143.840 ± 69.220	43.300 ± 19.114	47.844 ± 13.190
	Total	389.247 ± 109.741 bc	1150.081 ± 506.797 a	308.181 ± 80.64 c	794.644 ± 300.195 ab	246.607 ± 70.971	767.239 ± 364.583	346.673 ± 117.674	882.581 ± 299.609
Terpenes	α-pinene	60.756 ± 22.031	49.586 ± 11.508	35.369 ± 10.710	107.524 ± 61.478	38.601 ± 11.435	102.438 ± 41.099	42.433 ± 7.433	112.799 ± 40.699
	β-pinene	**51.735 ± 15.532 ab**	**35.359 ± 6.835 b**	**41.676 ± 21.849 b**	**135.471 ± 86.534 a**	33.652 ± 15.549	98.149 ± 50.246	29.177 ± 7.209	112.407 ± 53.157
	β-myrcene	**31.347 ± 9.186**	**49.905 ± 17.232**	**22.823 ± 6.265**	**65.619 ± 19.671**	34.148 ± 9.458	30.905 ± 8.169	37.460 ± 19.552	34.867 ± 9.930
	(*E*)-β-ocimene	53.361 ± 23.778	90.448 ± 43.339	29.142 ± 7.934	72.314 ± 22.955	**26.697 ± 8.092**	**29.418 ± 13.581**	**44.467 ± 16.560**	**76.032 ± 35.034**
	Linalool	348.152 ± 128.459	468.674 ± 178.899	217.082 ± 62.770	644.163 ± 220.876	227.388 ± 50.508	228.852 ± 54.953	469.507 ± 359.037	226.493 ± 62.406

	DMNT	543.029 ± 222.752	1718.534 ± 894.617	285.178 ± 108.472	2055.971 ± 880.554	**304.243 ± 81.784**	**858.204 ± 388.838**	**635.393 ± 448.743**	**1211.076 ± 515.831**
	TMTT	423.364 ± 216.092	209.404 ± 64.288	391.898 ± 184.270	698.878 ± 326.341	**191.639 ± 62.723 ab**	**558.856 ± 287.961 a**	**522.160 ± 481.949 a**	**86.439 ± 21.945 b**
	β-caryophyllene	**20.151 ± 7.958 b**	**109.801 ± 45.439 a**	**18.239 ± 7.439 b**	**208.378 ± 83.024 a**	19.865 ± 4.676	41.079 ± 21.205	37.749 ± 24.171	45.086 ± 17.765
	(*E*)-β-farnesene	**44.296 ± 41.439 a**	**74.909 ± 41.799 a**	**2.013 ± 0.959 b**	**88.120 ± 39.962 a**	9.438 ± 3.853	62.855 ± 36.256	5.872 ± 2.745	27.666 ± 11.603
	α-caryophyllene	**2.439 ± 0.737 b**	**9.430 ± 3.458 a**	**2.133 ± 0.762 b**	**13.851 ± 4.991 a**	3.179 ± 0.711	3.172 ± 1.471	3.792 ± 1.483	4.122 ± 1.619
	β-cubebene	5.620 ± 1.893	8.019 ± 2.770	4.807 ± 2.402	13.309 ± 4.025	2.587 ± 0.876	2.299 ± 1.489	2.222 ± 0.758	4.132 ± 1.741
Terpenes	α-selinene	58.610 ± 22.166	55.855 ± 15.115	34.542 ± 4.999	65.123 ± 14.685	**28.956 ± 7.739**	**59.016 ± 19.928**	**75.752 ± 26.058**	**69.202 ± 26.649**
	δ-cadinene	9.781 ± 3.743	21.197 ± 7.896	7.475 ± 1.914	24.279 ± 8.022	28.994 ± 12.562	21.361 ± 8.959	19.882 ± 3.935	38.395 ± 14.394
	α-cubebene	11.426 ± 2.737	16.895 ± 4.031	10.628 ± 3.068	28.399 ± 8.789	18.940 ± 5.124	16.712 ± 4.859	15.633 ± 2.500	21.475 ± 5.920
	Unk sesq	11.208 ± 3.988	19.708 ± 6.831	9.462 ± 2.357	30.637 ± 10.001	24.126 ± 8.362	18.544 ± 6.630	18.200 ± 3.239	37.064 ± 16.628
	Total	1675.275 ± 568.135 bc	2937.724 ± 1234.921 ab	1112.468 ± 377.586 c	4252.036 ± 1702.317 a	992.453 ± 193.857	2131.860 ± 705.645	1959.699 ± 1346.868	2107.255 ± 682.190
Aromatic	Benzyl acetate	14.298 ± 6.453	193.059 ± 115.509	24.596 ± 21.166	940.464 ± 560.569	108.812 ± 59.335	238.254 ± 150.381	143.917 ± 131.560	104.844 ± 61.964
	Total	14.298 ± 6.453 b	193.059 ± 115.509 a	24.596 ± 21.166 b	940.464 ± 560.569 a	108.812 ± 59.335	238.254 ± 150.381	143.917 ± 131.560	104.844 ± 61.964

Table 4 B73 nocturnal individual compound and total of volatile (means ng g^{-1} ± SE) released by control, control + FAW, Neo and Neo + FAW treatments at V4 and V6 stage. Bold value indicates compounds observed in random forest that contributed most to the variation in each treatment. Different letters in the line indicate significant differences across treatment for individual compound and total by group according to GLM (by Tukey's *post hoc* test; $p < 0.05$) and the absence of letters indicates equality between treatments.

Group	Compound	V4				V6			
		control	control + FAW	Neo	Neo + FAW	control	control + FAW	Neo	Neo + FAW
Fatty acids derivates	(*Z*)-3-hexenal	**1.038 ± 0.621 b**	**133.531 ± 60.095 a**	**1.564 ± 1.115 b**	**66.480 ± 27.075 a**	3.297 ± 2.204	83.136 ± 35.721	10.093 ± 7.002	116.762 ± 57.793
	Hexenal	55.203 ± 18.852	88.388 ± 27.189	67.943 ± 22.632	47.903 ± 13.807	33.093 ± 11.189	43.138 ± 13.527	50.188 ± 10.041	51.121 ± 18.760
	2-hexanol	83.789 ± 38.746	76.578 ± 40.270	105.216 ± 55.553	92.683 ± 38.171	108.467 ± 44.582	80.467 ± 40.363	67.647 ± 28.276	102.461 ± 58.425
	(*E*)-2-hexenal	-	-	-	-	0.934 ± 0.934	28.421 ± 12.178	23.365 ± 22.049	105.391 ± 55.601
	(*Z*)-3-hexen-1-ol	-	-	-	-	0.272 ± 0.272	58.454 ± 27.629	12.873 ± 9.388	174.532 ± 96.610
	2-ethyl hexanal	**35.924 ± 11.441**	**60.429 ± 26.548**	**38.570 ± 12.037**	**47.045 ± 21.428**	11.383 ± 18.866	4.295 ± 1.977	9.470 ± 3.499	6.722 ± 1.964
	(*Z*)-3-hexenyl acetate	**14.018 ± 6.503 b**	**255.859 ± 108.012 a**	**7.151 ± 2.863 b**	**356.691 ± 140.838 a**	**2.948 ± 1.072 b**	**187.097 ± 81.578 a**	**8.407 ± 3.279 b**	**201.356 ± 78.290 a**
	Hexyl acetate	-	3.134 ± 1.576	0.429 ± 0.312	3.692 ± 1.518	-	2.232 ± 0.749	0.704 ± 0.704	2.663 ± 1.226
	Nonanal	7.837 ± 2.897	35.780 ± 21.132	36.049 ± 15.389	31.282 ± 15.666	28.272 ± 13.915	30.391 ± 23.881	91.904 ± 62.528	5.925 ± 3.855
	Total	197.809 ± 51.985 b	653.699 ± 245.023 a	256.922 ± 90.992 b	645.776 ± 180.864 a	188.666 ± 72.702	517.631 ± 193.192	274.651 ± 138.991	766.933 ± 355.136
Terpenes	α-pinene	**58.614 ± 10.319**	**80.861 ± 13.237**	**88.079 ± 26.491**	**68.832 ± 22.304**	**37.186 ± 7.071**	**58.792 ± 9.798**	**69.158 ± 16.153**	**78.275 ± 18.384**
	β-pinene	**88.988 ± 32.403**	**139.329 ± 33.085**	**100.186 ± 26.499**	**123.398 ± 49.143**	**28.403 ± 9.390**	**52.085 ± 17.295**	**60.277 ± 22.804**	**70.704 ± 22.872**
	β-myrcene	3.639 ± 2.148	6.646 ± 2.488	2.029 ± 0.984	7.572 ± 2.918	2.709 ± 2.087	6.059 ± 1.554	6.696 ± 3.652	5.883 ± 1.854

	(*E*)-β-ocimene	-	-	-	-	0.076 ± 0.076	3.758 ± 1.739	0.562 ± 0.413	1.803 ± 0.756
	Linalool	21.065 ± 15.482	20.459 ± 5.500	10.390 ± 4.399	24.753 ± 8.005	12.116 ± 6.562	34.368 ± 11.131	24.344 ± 15.444	32.478 ± 17.658
	DMNT	**11.984 ± 7.262 b**	**39.375 ± 10.245 ab**	**1.828 ± 0.872 c**	**58.887 ± 20.838 a**	**8.009 ± 4.110 b**	**55.469 ± 17.932 a**	**18.066 ± 10.470 ab**	**38.051 ± 15.347 a**
	TMTT	4.290 ± 1.801	19.188 ± 26.108	2.058 ± 0.705	13.831 ± 5.294	8.459 ± 3.796	39.048 ± 21.934	17.884 ± 12.759	45.514 ± 36.348
	(*E*)-α-bergamotene	0.699 ± 0.654	9.136 ± 3.238	0.166 ± 0.166	8.232 ± 2.707	2.925 ± 2.455	6.054 ± 2.702	1.744 ± 1.214	5.146 ± 2.337
	α-selinene	29.446 ± 5.348	38.780 ± 9.468	55.045 ± 20.929	39.369 ± 8.273	**31.192 ± 9.883**	**42.971 ± 22.311**	**25.363 ± 8.403**	**18.786 ± 7.292**
	δ-cadinene	0.828 ± 0.289	1.439 ± 0.536	0.551 ± 0.341	0.852 ± 0.359	-	-	-	-
	α-cubebene	0.944 ± 0.442	1.602 ± 0.442	0.610 ± 0.319	1.554 ± 0.634	-	-	-	-
Terpenes	Unk sesq	2.338 ± 0.924	4.724 ± 1.544	1.265 ± 0.673	2.409 ± 0.981	1.592 ± 0.877	3.263 ± 1.341	7.149 ± 4.411	1.673 ± 0.892
	Total	222.835 ± 53.587	361.539 ± 64.245	262.207 ± 62.331	349.689 ± 101.790	132.667 ± 31.981	301.867 ± 67.949	231.243 ± 68.572	298.313 ± 88.946

Table 5 MC 4050 nocturnal individual compound and total of volatile (means ng g^{-1} ± SE) released by control, control + FAW, Neo and Neo + FAW treatments at V4 and V6 stage. Bold value indicates compounds observed in random forest that contributed most to the variation in each treatment. Different letters in the line indicate significant differences across treatment for individual compound and total by group according to GLM (by Tukey's *post hoc* test; $p < 0.05$) and the absence of letters indicates equality between treatments.

Group	Compound	V4				V6			
		control	control + FAW	Neo	Neo + FAW	control	control + FAW	Neo	Neo + FAW
Fatty acids derivates	(*Z*)-3-hexenal	**0.353 ± 0.202 b**	**100.779 ± 35.891 a**	**2.136 ± 1.878 b**	**62.733 ± 23.821 a**	-	-	-	-
	Hexenal	37.612 ± 9.670	68.991 ± 25.125	45.417 ± 10.963	57.341 ± 11.714	**35.241 ± 8.968 b**	**77.506 ± 29.140 ab**	**53.152 ± 10.641 b**	**134.487 ± 40.862 a**
	2-hexanol	91.523 ± 37.153	96.881 ± 49.362	57.516 ± 28.092	76.971 ± 30.232	94.309 ± 29.621	138.122 ± 48.413	139.333 ± 30.403	170.404 ± 46.293
	(*E*)-2-hexenal	-	-	-	-	-	81.052 ± 47.834	1.341 ± 1.341	119.221 ± 64.553
	(*Z*)-3-hexen-1-ol	-	-	-	-	1.222 ± 0.763	83.969 ± 48.624	-	175.329 ± 61.475
	2-ethyl hexanal	**18.522 ± 6.039**	**20.724 ± 8.275**	**35.374 ± 16.177**	**27.335 ± 14.673**	4.105 ± 1.697	10.235 ± 3.825	7.773 ± 3.789	12.641 ± 3.772
	(*Z*)-3-hexenyl acetate	**5.505 ± 3.132 b**	**421.372 ± 167.808 a**	**13.264 ± 6.444 b**	**233.856 ± 85.680 a**	**4.319 ± 1.809 b**	**237.202 ± 158.272 a**	**3.365 ± 1.437 b**	**485.471 ± 164.825 a**
	Nonanal	19.488 ± 10.219	14.889 ± 5.626	27.857 ± 16.069	19.191 ± 5.695	26.897 ± 12.508	34.947 ± 17.915	53.143 ± 32.714	40.631 ± 22.566
	Total	173.003 ± 53.227 b	723.636 ± 269.314 a	181.564 ± 47.644 b	477.427 ± 110.501 a	166.093 ± 45.729 c	663.033 ± 303.699 ab	258.107 ± 65.137 bc	1138.184 ± 316.157 a
Terpenes	α-pinene	48.221 ± 10.884	63.350 ± 15.751	70.696 ± 18.262	78.645 ± 19.158	**40.845 ± 7.244**	**73.465 ± 18.249**	**63.876 ± 10.784**	**116.688 ± 35.192**
	β-pinene	75.467 ± 21.896	75.453 ± 15.354	100.127 ± 30.728	101.667 ± 41.063	**36.214 ± 9.397**	**63.106 ± 17.998**	**61.279 ± 13.658**	**121.361 ± 42.975**
	β-myrcene	-	-	-	-	0.271 ± 0.169	1.973 ± 1.017	0.424 ± 0.209	3.959 ± 1.715
	Linalool	**4.446 ± 3.840 b**	**37.504 ± 17.189 a**	**0.475 ± 0.269 b**	**36.177 ± 19.710 a**	3.989 ± 1.642	13.489 ± 6.476	1.641 ± 0.585	16.917 ± 5.147
	DMNT	**1.121 ± 0.625 b**	**155.868 ± 44.518 a**	**2.286 ± 1.102 b**	**154.907 ± 57.411 a**	**1.416 ± 0.660 c**	**59.851 ± 35.117 b**	**1.703 ± 1.102 c**	**255.266 ± 101.168 a**

	TMTT	1.617 ± 0.436	10.114 ± 3.451	7.916 ± 4.538	26.358 ± 10.773	3.113 ± 0.708	3.979 ± 1.638	2.074 ± 0.778	10.717
	β-caryophyllene	**0.213 ± 0.213 b**	**41.633 ± 9.295 a**	**1.469 ± 0.429 b**	**59.262 ± 23.986 a**	**2.789 ± 1.343 c**	**12.582 ± 3.701 b**	**1.447 ± 0.563 c**	**41.189 ± 12.824 a**
	(*E*)-α-bergamotene	-	12.403 ± 3.623	-	12.886 ± 5.185	**0.507 ± 0.503 b**	**1.694 ± 1.049 b**	**-**	**10.818 ± 3.340 a**
	(*E*)-β-farnesene	2.962 ± 7.811	51.306 ± 16.302	1.604 ± 1.102	59.651 ± 24.749	1.276 ± 0.899	6.457 ± 3.808	-	35.238 ± 15.966
	α-caryophyllene	-	3.158 ± 0.842	0.049 ± 0.034	3.804 ± 1.507	-	1.190 ± 0.632	0.078 ± 0.645	2.831 ± 0.995
	α-selinene	46.476 ± 12.564	55.378 ± 15.721	31.334 ± 7.723	34.808 ± 8.578	38.917 ± 12.530	54.848 ± 24.837	39.250 ± 16.194	47.657 ± 10.415
	Unk sesq	0.293 ± 0.119	4.595 ± 2.033	1.066 ± 0.436	1.854 ± 0.651	4.075 ± 2.057	2.032 ± 1.208	3.047 ± 0.944	12.413 ± 4.457
	Total	180.816 ± 37.059 b	510.762 ± 109.173 a	217.022 ± 50.565 b	570.019 ± 169.259 a	133.412 ± 26.452 b	294.666 ± 88.445 ab	174.819 ± 31.577 b	675.054 ± 191.766 a

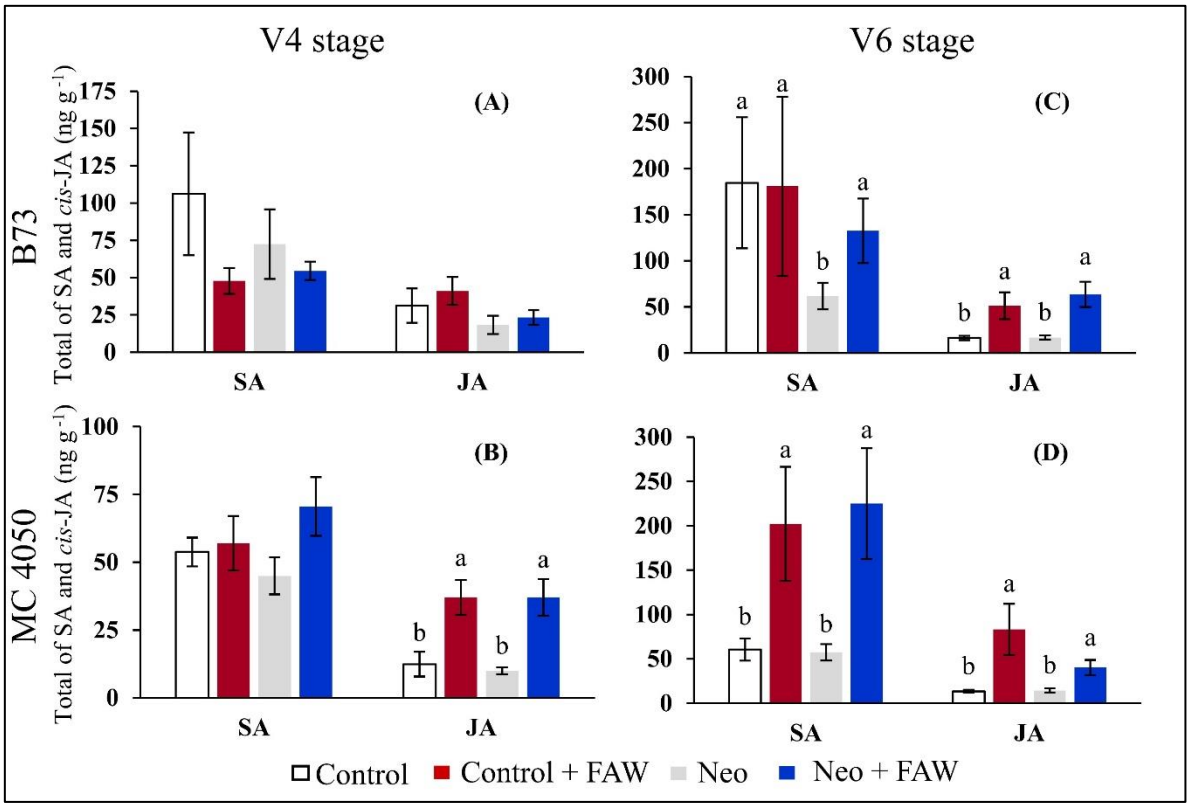

Fig. 4 Total of salicylic acid and cis-jasmonic acid (ng g^{-1}) of the treatments control, control + FAW, Neo and Neo + FAW in B73 and MC 4050 plants at V4 (A, B respectively) and V6 (C, D respectively). Lowercase letters indicate statistical difference between treatments according to GLM.

Supplementary information

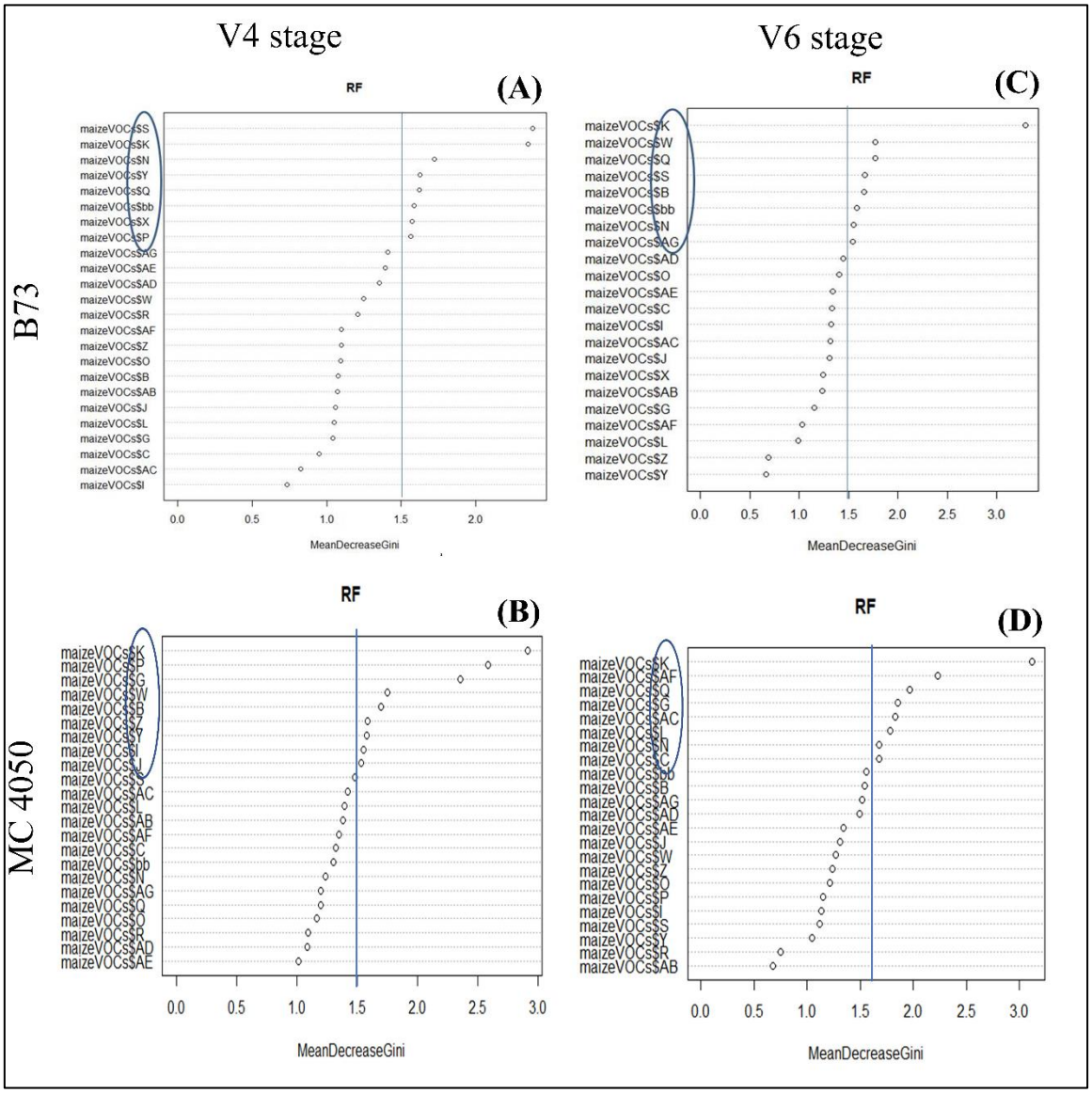

Fig. S1 Random forest analysis of maize (B73 and MC 4050) diurnal volatiles after eight hours of collection. **A)** B73 at V4, **B)** MC 4050 at V4, **C)** B73 at V6, **D)** MC 4050 at V6. Legend: A = (*Z*)-3-hexenal; B = Hexenal; C = 2-hexanol; D = (*E*)-2-hexenal; E = (*Z*)-3-hexen-1-ol; G = 2-ethyl hexanal; I = β-pinene; J = β-myrcene; K = (*Z*)-3-hexenyl acetate; L = Hexyl acetate; M = (*Z*)-β-ocimene; N = (*E*)-β-ocimene; O = Linalool; P = Nonanal; Q = (3*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT); R = Ethylhexyl acetate; S = Benzyl acetate; W = β-caryophyllene; X = (*E*)-α-bergamotene; Y = (*E*)-β-farnesene; Z = α-caryophyllene; AB = β-cubebene; AC = α-selinene; AD = δ-cadinene; AE = α-cubebene; AF = (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT); AG = Unknown sesquiterpenoid; bb = α-pinene.

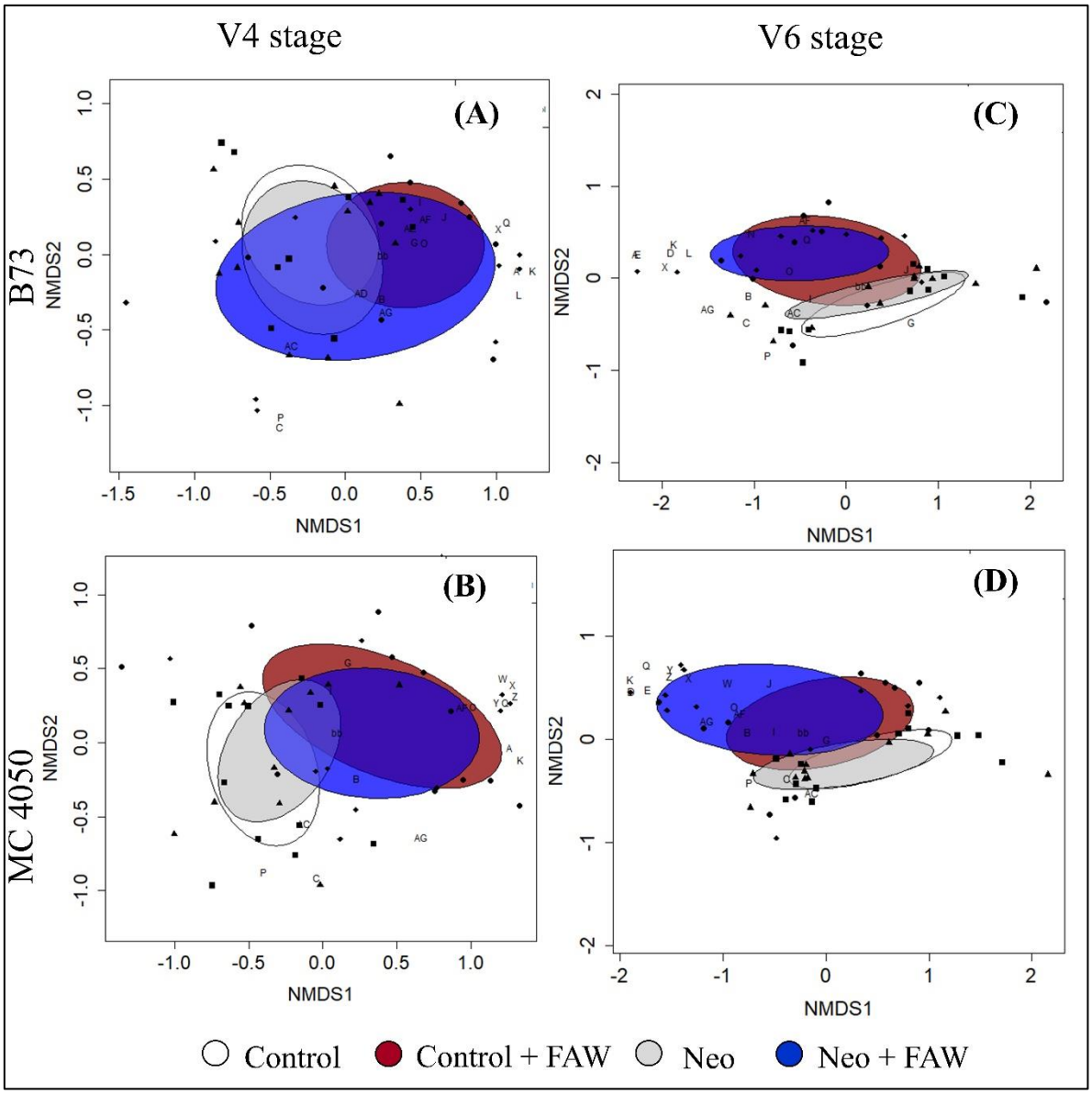

Fig. S2 Nocturnal blends emitted by B73 [V4 (A) and V6 (C)] and MC 4050 [V4 (B) and V6 (D)] after eight hours of volatile collections.

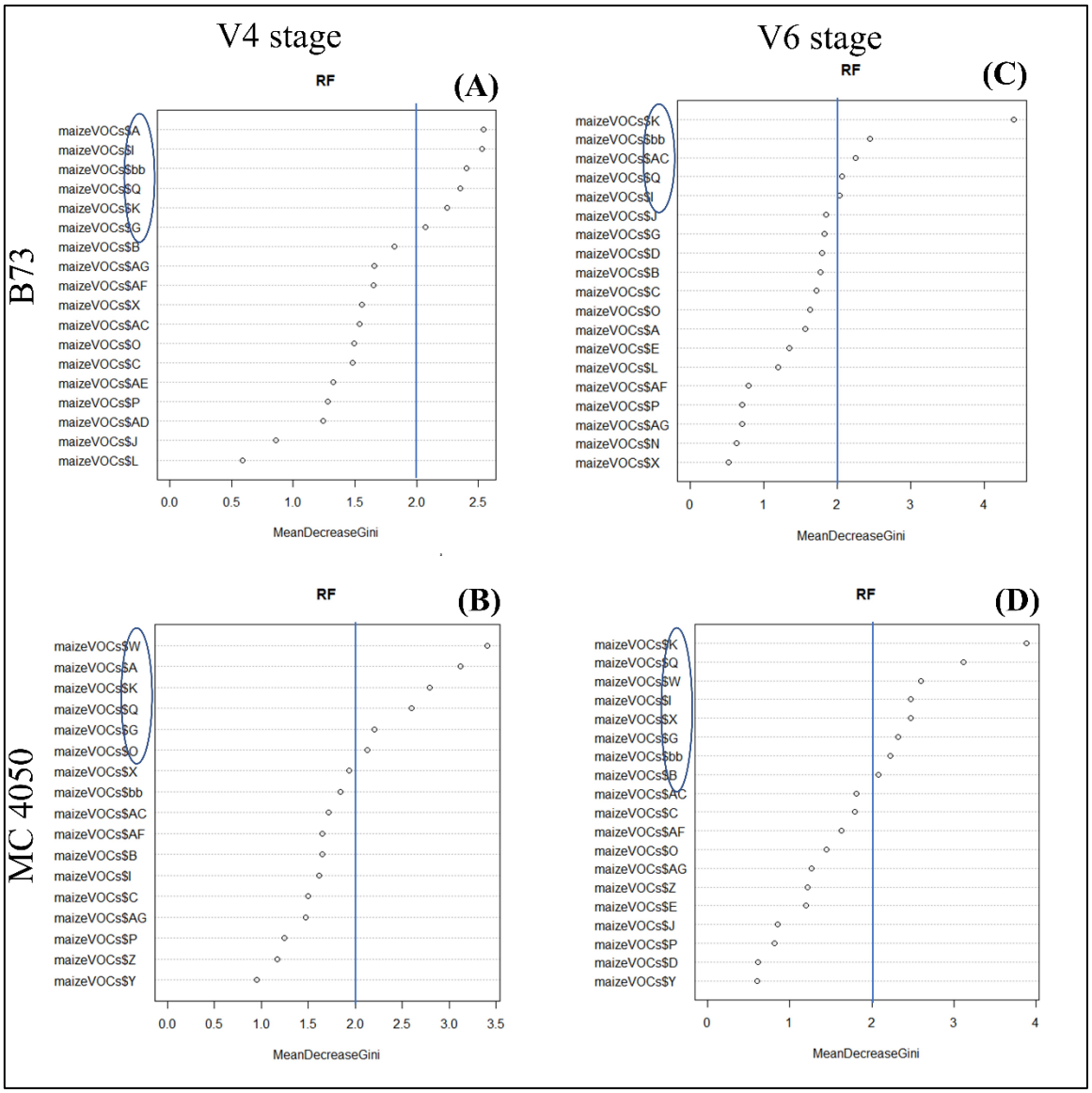

Fig. S3 Random forest analysis of maize (B73 and MC 4050) nocturnal volatiles after eight hours of collection. **A)** B73 at V4, **B)** MC 4050 at V4, **C)** B73 at V6, **D)** MC 4050 at V6. Legend: A = (*Z*)-3-hexenal; B = Hexenal; C = 2-hexanol; D = (*E*)-2-hexenal; E = (*Z*)-3-hexen-1-ol; G = 2-ethyl hexanal; I = β-pinene; J = β-myrcene; K = (*Z*)-3-hexenyl acetate; L = Hexyl acetate; M = (*Z*)-β-ocimene; N = (*E*)-β-ocimene; O = Linalool; P = Nonanal; Q = (3*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT); R = Ethylhexyl acetate; S = Benzyl acetate; W = β-caryophyllene; X = (*E*)-α-bergamotene; Y = (*E*)-β-farnesene; Z = α-caryophyllene; AB = β-cubebene; AC = α-selinene; AD = δ-cadinene; AE = α-cubebene; AF = (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT); AG = Unknown sesquiterpenoid; bb = α-pinene.