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Interrelation between Nitrogen Doses and the Efficiency of *Bt* Genes in the Effective Control of *Spodoptera frugiperda*

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Abstract

The objective of this work was to identify the effect of nitrogen rates on *Bt* transgenic resistance and damage caused by *Spodoptera frugiperda* in corn plants at different developmental stages. The experimental design was a randomized complete block in a 3x6 factorial arrangement, with three potassium rates (0, 80, and 160 kg ha^{-1} kg of N) and six corn hybrids (20A55Hx, 30A77PW, AG1051, MG652PW, NS90VTPro2, and NS92VTPro), with three replicates. The data were analyzed through the analysis of canonical variables. Nitrogen doses interfere directly with the tolerance and resistance conferred by the Cry proteins of the plants in corn hybrids, altering the control of the caterpillar *Spodoptera frugiperda* and possibly favoring the breakdown of resistance in low availability. The corn hybrids showed divergent performance regarding proteins under low nitrogen availability (0 and 80 kg ha^{-1}), and it was impossible to state which would be the most efficient protein. However, pyramiding has greater control under the recommended dose (160 kg ha^{-1} of N), with damage in the opposite direction to the transgenics.

Keywords: *Zea mays.* L, caterpillar, damage, pest, transgenics

1. Introduction

Corn (*Zea mays* L.) is of great importance in the world agricultural scenario, with the United States being the largest producer, followed by China and Brazil, with production around 383, 277, and 129 million tons, totaling 66% of the world production (United States Department of Agriculture [USDA], 2023). Brazil had an average grain yield of 5,675 kg ha^{-1} in the 2022/23 harvest, of which the Midwest region was responsible for 58% of production. Also, the state of Goiás stands out with a high yield, estimated at 6,767 kg ha^{-1} for the 2023/24 harvest, which denotes the importance of the crop (National Supply Company [CONAB], 2023). Thus, using higher quality seeds and more technology inserted provides the producer greater security and financial support, indirectly bringing production stability and lower costs with applications, mainly for pest control.

Among the main corn pests, the ones that cause the greatest direct and indirect damage are the caterpillars, as they can limit the yield and profitability of the crop, which can activate the differential expression of genes when attacked and alter the physiological behavior of the plants (Ye et al. 2022). Among the insects from the order Lepidoptera, the fall armyworm (*Spodoptera frugiperda* JE Smith) is considered the main pest of corn in South America, in addition to causing significant damage throughout the development stages of the plant by the use of leaves, whorl, and ear in its feeding when present in larger populations (Fatoretto et al. 2017).

Among the factors that can affect resistance is plant nutrition. Nitrogen is of great importance in achieving high yields and increasing the ability of plants to tolerate different abiotic and biotic stresses. According to Wang et al. (2022), there is a dose that favors plant defenses, as the dose of 156.6 mg compared to 52.2 mg kg^{-1} of N not only increased the plant biomass but also induced the chemical contents of plant defense compounds, such as volatile organics, against *Spodoptera frugiperda* despite higher consumption. However, increasing nitrogen fertilization causes plants to invest more resources in growth than in defending herbivores, and if extrapolated to other population levels, it could provide a greater understanding of insect-plant interactions and develop optimized management practices, benefiting the capacity and helping develop sustainable agricultural management strategies (Ren et al. 2013).

Given this scenario, the insertion of transgenic events is one of the most important biotechnological alternatives currently, so to overcome the challenges imposed in the control of this pest, corn hybrids that express insecticidal proteins derived from the bacterium *Bacillus thuringiensis* (*Bt*) have been the most adopted technology all around the world. Its use proved a viable and efficient alternative for controlling target insects without harming the population of other important insects, such as predators (Xing et al. 2019). According to Khan et al. (2023), the expression of the "Cry" protein, responsible for the insecticidal effect, can be altered according to the levels of nutrients in the plant and the dose can favor the generation of resistant insects and even delay this condition.

Some of the proteins used to control lepidopteran insects, such as Cry1Ab and Vip3Aa (Niu et al. 2021) or Cry1F (Souza et al. 2018), are already presented as less efficient in control, in some specific regions of the world, in addition, they can transmit this condition to their descendants. Due to the high adaptability of the target insects, linked to the cultivation conditions and the Brazilian climate, there is a risk of resistance breaking in fewer cycles than in other producing countries (Moscardini et al. 2020).

Therefore, research combining *Bts* transgenic events, nitrogen fertilization, and insect-plant interaction is scarce. Thus, this study aimed to identify the effect of nitrogen doses in transgenic corn hybrids with different *Bts* events by analyzing the interrelation of damages and doses.

2. Method

Two experiments were conducted during the summer harvest of 2014/2015 and 2015/2016 at Universidade Estadual de Goiás in Ipameri - GO. The experimental site was located at 17°43'19" S, 48°09'35" W, and an altitude of 773 m.

The randomized block design with three replications was used. The treatments were arranged in a 6 x 3 factorial scheme (six corn hybrids and three nitrogen doses). Each experimental unit was composed of two rows, 3 m long, spaced 0.5 m apart (3 m^2).

The following corn hybrids and their respective *Bts* events were evaluated: 20A55Hx – "A" (Cry1F), 30A77PW – "B" (Cry1A.105/Cry2Ab2/Cry1F), AG1051 – "C" (conventional – control), MG652PW – "D" (Cry1A.105/Cry2Ab2/Cry1F), NS90VTPro2 – "E" (Cry1A.105/Cry2Ab2/CP4-EPSPS) and NS92VTPro – "F" (Cry1A.105/Cry2Ab2). The following nitrogen doses were evaluated: 0, 80, and 160 kg ha^{-1} of N.

One plowing and two harrowing operations were conducted to prepare the area. The fertilization was performed according with soil analysis (Table 1) and an expected grain yield of 8 tons ha^{-1}. In the sowing fertilization, only the doses of nitrogen (urea) varied, being 0, 80, and 160 kg ha^{-1} of N, plus 120 kg ha^{-1} of P_2O_5 and 90 kg of ha^{-1} of KCl, 20% being applied, at sowing, and 80%, in topdressing, at the V_6 stage, distributed manually, next to the sowing furrow.

Sowing was conducted manually in November, distributing five seeds per meter, with subsequent thinning at the V_3 stage, obtaining a final stand of 55,000 plants per hectare. At the V_4 stage, weed was controlled with benzoyl cyclohexanedione at 240 ml ha^{-1}, applied with a knapsack sprayer, and manual weeding in the subsequent months. The control of the caterpillars was performed only in the conventional corn hybrids with flubendiamide at a dose of 150 ml ha^{-1}, at the V_5 stage, after monitoring, and only once.

Visual estimates were made for each plot, using a score ranging from 0 to 9, to assess the damage of *S. frugiperda* to the corn whorl and leaves. The scale was adapted by Davis et al. (1992) for estimates of damage at different stages, 30 (DAMG30), 70 (DAMG70), and 110 (DAMG110) days after sowing (DAS). In each evaluation, three plants were used randomly and considered as the average of the plot. At 70 days after sowing, photosynthetic rates were evaluated using a chlorophyll meter model CFL 1030 in five plants per plot, after measurements, transformed on average per plot, called leaf chlorophyll index (LCI), in FALKER units.

Harvesting was performed when the ears reached the point of physiological maturation, with humidity around 30%, in which all the ears of the plots were harvested manually. Subsequently, ear diameter (DIAMETER) was evaluated, obtained from the average of five ears taken randomly. With the aid of a graduated caliper, the average was assigned to the plot in millimeters. Ear length (LENGH), with the aid of a graduated ruler, the length of five ears taken at random was measured, with the arithmetic mean attributed to the plot in centimeters.

Also, the percentage of ears without damage (PEWD) was evaluated from the count of ears without injuries caused by caterpillars, based on the total number of ears of the plot, later transformed into a percentage, calculated the presence or absence of damage in the ear. Grain yield (YIELD) was obtained from the grain mass, in kg, resulting from the threshing of the total ears of each plot; the values obtained were corrected for 13% moisture, later transformed to kg ha^{-1} and, then, the 1000-grain weight (1000W), which one thousand grains were separated at random from each plot, weighing was carried out and then corrected to 13% moisture, in grams.

The univariate analysis of variance was performed,and the significance of the F-test was verified, followed by the multivariate analysis of variance, in which the set of evaluated variables was grouped according to their characteristics and in coordinate axes (biplot) through the analysis of canonical discriminant variables (Hair Jr. et al., 2005). The results of these analyzes were represented in graphs, together with the ellipses (95% confidence) for the treatments, which inferred about the equality of the treatments from the superposition of their confidence ellipses, using the R software (R Core Team, 2022) and the candisc package (Friendly & Fox, 2021).

3. Results and Discussion

It was verified that the condition for interpreting the data through the analysis of canonical variables was reached since it was possible in a relatively small number of variables, explaining most of the variability of the original data. It was observed that there was significance in the univariate and multivariate analysis ($p<0.001$) and also that the first two canonical variables explained 60.2 and 27% of the total variance between hybrids in the 2014/15 and 2015/16 harvests, respectively (Figure 1A and 1B). It was also detected that the average variation explained by each canonical variable at the dose of 80 and 160 kg ha^{-1} were 90 and 6.5% and 68.1 and 14.2% of the total variance for the harvests (Figure 2 and 3).

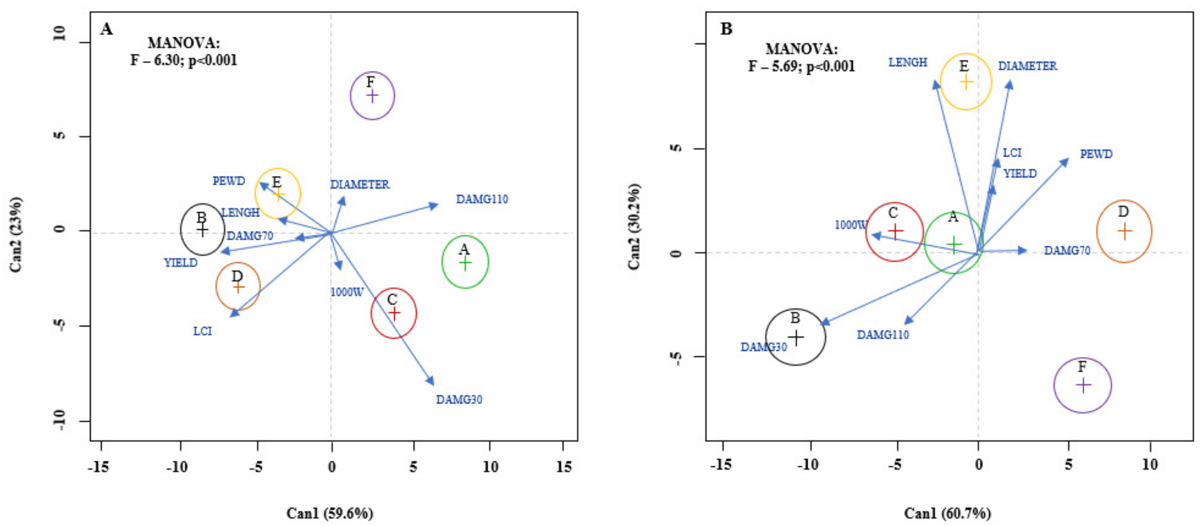

Figure 1. Analysis of canonical variables by the dispersion of corn (*Zea mays*) hybrids, without the application of nitrogen (0 kg ha^{-1} N), in the 2014/2015 (A) and 2015/2016 (B) harvests. The evaluated hybrids and respective proteins between parenthesis were: A - 20A55Hx (Cry1F); B - 30A77PW (Cry1A.105/Cry2Ab2/Cry1F); C - AG1051 (conventional, without Cry proteins, used as the control); D - MG652PW (Cry1A.105/Cry2Ab2/Cry1F); E - NS90VTPro2 (Cry1A.105/Cry2Ab2/CP4-EPSPS); and F - NS92VTPro (Cry1A.105/Cry2Ab2), in the variables DAMG30 - damage at 30 days after sowing; DAMG70 - damage at 70 days after sowing; DAMG110 - damage at 110 days after sowing; LCI - leaf chlorophyll index; DIAMETER - ear diameter; LENGH - Ear length; 1000W - 1,000-grain weight; PEWD - percentage of ears without damage and YIELD - Grain yield.

It was observed that during the 2014/15 harvest, there was a greater discrepancy between the observed values, as an example, the rainfall was higher compared to the 2015/16 harvest, however, poorly distributed between the crop cycle and, as an aggravating factor, water deficit occurred at the beginning of the reproductive stage. According to Nóia Júnior & Sentelhas (2019), the complex interaction of precipitation, solar radiation, air temperature, and other meteorological parameters, in addition to the interaction of the genotype with the environment, make it challenging to determine the best sowing date in soybean succession in different Brazilian producing regions, mainly due to problems caused by the effects of El Niño or La Niña. This influence of precipitation and temperature fluctuation was also verified by Faria et al. (2023), when evaluating effect of potassium rates on *Bt* transgenic resistance and damage caused by *Spodoptera frugiperda* in corn plants at different developmental stages eand detected that it can alter the performance of hybrids and favor herboria by weakening the plant.

In the 2014/15 harvest, there were two stresses combined, water deficit and nutritional stress, which had varying impacts on yield (Figure 1A). Damage after 110 days has greater importance on the plants than damage after 30 days, with low influence at 70 days: with length and lower pest index on the ear, with higher photosynthetic rate, the variables most correlated with yield, in 2014/15 harvest. In the later harvest, the diameter had greater importance along with the length of the ear, which the damages influenced in a similar way, as well as the photosynthetic rate.This change in the rainfall regime, such as floods, droughts, and summers, limits grain yield, depending on the stage, also its duration, and interferes with the damage caused by *S. frugiperda* and the photosynthetic rate. In the future, extreme weather and/or its oscillations are already expected and should contribute directly and indirectly to the damage caused by pests, such as the greater use of chemical pesticides, difficulty in adjusting integrated management and mitigation strategies, as a consequence due to pressure and the frequent weakness of the plant under different stresses and with greater oscillations in photosynthesis (Olatinwo & Hoogenboom 2014).

The NS90VTPro2 (Cry1A.105/Cry2Ab2) hybrid showed the highest resistance at different stages and possibly the highest tolerance in both harvests. However, the hybrids with the highest profitability were 30A77PW and MG652PW in the 2014/15 harvest (Figure 1A), which has the expression of three proteins and, for the 2015/16 harvest (Figure 1B), the hybrids 20A55Hx and NS90VTPro2, with one and two events, however, even under conditions of biotic stress combined with abiotic, the hybrids showed effective control against the herbivory of the caterpillar *S. frugiperda*.

It was observed that with the increase in the N dose, there was a greater correlation between damage at 30 days and a greater influence on yield, in addition to damage at 70 and 110 days, more correlated with injuries to the ears in the 2014/15 harvest (Figure 2A). However, in the 2015/16 harvest, the yield was more influenced by damage at 110 days, and at 70 days, more correlated with damage to the ear, in addition to profitability being more linked to the diameter and length of the ears and the 1000-grain weight (Figure 2B). It is noted that the 20A55Hx hybrid showed greater damage when the stresses were combined, and that the situation is reversed when the stress is only due to nitrogen. In addition, it became more visible that more proteins, due to increased nitrogen, generate greater security and less attack by *S. frugiperda*.

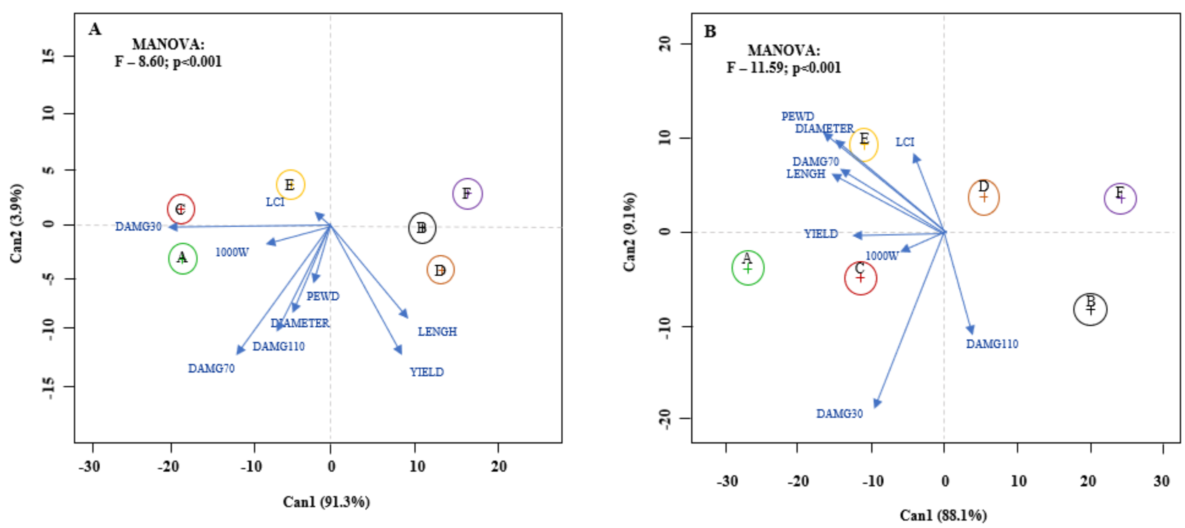

Figure 2. Analysis of canonical variables by the dispersion of corn (*Zea mays*) hybrids, with the application of nitrogen (80 kg ha^{-1} N), in the 2014/2015 (A) and 2015/2016 (B) harvests. The evaluated hybrids and respective proteins between parenthesis were: A - 20A55Hx (Cry1F); B - 30A77PW (Cry1A.105/Cry2Ab2/Cry1F); C - AG1051 (conventional, without Cry proteins, used as the control); D - MG652PW (Cry1A.105/Cry2Ab2/Cry1F); E - NS90VTPro2 (Cry1A.105/Cry2Ab2/CP4-EPSPS); and F - NS92VTPro (Cry1A.105/Cry2Ab2), in the variables DAMG30 - damage at 30 days after sowing; DAMG70 - damage at 70 days after sowing; DAMG110 - damage at 110 days after sowing; LCI - leaf chlorophyll index; DIAMETER - ear diameter; LENGH - Ear length; 1000W - 1,000-grain weight; PEWD - percentage of ears without damage and YIELD - Grain yield.

According to Chen et al. (2010), plant-herbivore interactions, along with N availability, can interfere with plant quality from the nutritional perspective of the herbivore, and the nutritional effects can directly extend to natural enemies, as they may not favor consumption. In this way, they can generate a condition similar to defense and increase the number of insects. It was verified that with the increase of the doses, there was a greater attack, in which the vectors were directed towards the hybrid AG1051 (control), with the increase of N, which also indicated greater attractiveness or undesirable nutritional quality for the natural enemies, however, not observed for the hybrids with the presence of proteins (Figure 1, 2, and 3). Jaramillo-Barrios et al. (2019) to establish the population fluctuation of *S. frugiperda* during 2014 – 2016, in the hybrids 30F35R and 30F35HR (Cry1F), reported that when the plant is in the vegetative phase, there is a higher larval population, and, when it is not properly managed, it can cause damages of up to 52% and 72% in hybrids, with and without Cry1F, respectively. The authors reported that the behavior suggests measures should be established, such as refuge areas and strategies such as pest monitoring, as the caterpillar can generate resistance to the Cry1F endotoxin. It should be noted that due to the market time of Hx technology present in the 20A55 corn hybrid, it is possible that there is a break in resistance or that it may be broken in a few years, but there are still no confirmations. In addition, it was clear that the increase of the dose from 80 to 160 kg of N ha^{-1} positively changed the performance, based on the damage and directing it in the same direction and less correlated with grain yield, with the opposite behavior of the hybrids with the presence of proteins in the 2015/16 harvest (Figure 2B and 3B).

It was verified that the combined stress harmed yields and interfered with the damage caused, increasing the intensity and that the presence of more proteins increases yield, which indicated that N is responsible for plant maintenance and increased tolerance in combination with the *Bts* events (Figure 1, 2, and 3). According to Resende et al. (2019), the agronomic efficiency of hybrids concerning N is very divergent, with the stress being total (0 kg ha^{-1}) or partial (80 kg ha^{-1}) , which is correlated with the technological level adopted by the producer, as well as being highly influenced by the rainfall index presented during the cultivation period and also has combinations with other nutrients such as phosphorus and potassium as well.

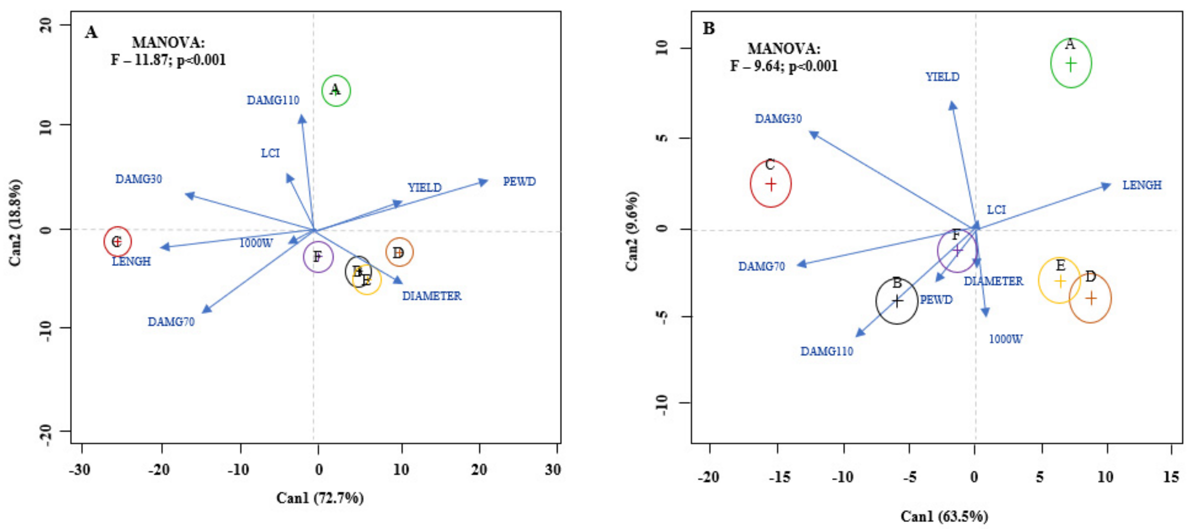

Figure 3. Analysis of canonical variables by the dispersion of corn (*Zea mays*) hybrids, with the application of nitrogen (80 kg ha^{-1} N), in the 2014/2015 (A) and 2015/2016 (B) harvests. The evaluated hybrids and respective proteins between parenthesis were: A - 20A55Hx (Cry1F); B - 30A77PW (Cry1A.105/Cry2Ab2/Cry1F); C - AG1051 (conventional, without Cry proteins, used as the control); D - MG652PW (Cry1A.105/Cry2Ab2/Cry1F); E - NS90VTPro2 (Cry1A.105/Cry2Ab2/CP4-EPSPS); and F - NS92VTPro (Cry1A.105/Cry2Ab2), in the variables DAMG30 - damage at 30 days after sowing; DAMG70 - damage at 70 days after sowing; DAMG110 - damage at 110 days after sowing; LCI - leaf chlorophyll index; DIAMETER - ear diameter; LENGH - Ear length; 1000W - 1,000-grain weight; PEWD - percentage of ears without damage and YIELD - Grain yield.

It was found that, in the 2015/16 harvest, the relative chlorophyll content had little influence on yield and that it possibly demonstrated stabilization of the photosynthetic rate in the different hybrids. In the same condition, the diameter of the ears, in which the difference in grain yield, may be linked to the ear length (Figure 3). According to Wu et al. (2011), the more efficient use of N expresses a high genotypic and phenotypic correlation with grain yield and the number of grains per ear, correlated with the length and diameter of the ear and grain weight, confirming the results obtained. It is noteworthy that the chlorophyll index did not present a strong influence on the variability of the hybrids, but with possible stabilization and increase even for the grain yield; thus, it did not directly influence the primary components.

Waquil et al. (2013) found that the *Bt* corn hybrid that expresses only the Cry1A(b) protein reduces the damage caused by *S. frugiperda* but requires monitoring for decision-making on the use of chemical control and that the DKB 390 hybrid, with a pyramidal structure event that expresses Cry1A.105 and Cry2Ab2 proteins, confers greater protection against *S. frugiperda* and *Diatraea saccharalis* and dispenses chemical control, and those with transgenics also present less damage to the ear. The 30A77PW hybrid has pyramiding with three cry proteins and, however, did not perform better than those with only one or two but had greater control compared to the conventional AG1051 (Figure 3B). Michelotto et al. (2011) also reported that damage at 40 days is not so harmful with little variation, similar to the study, and that hybrids with transgenics only showed a difference in damage after 80 days of germination, however, with the same technology and without pyramiding.

Storer et al. (2012) reported that there are already insects resistant to Cry1F and that tropical countries tend to be more prone to the formation of populations that survive in high numbers and with a greater chance of breaking resistance. However, a population will have more difficulty in breaking the resistance of hybrids that have more than one technology, as individuals need to overcome the resistance of proteins present simultaneously and, consequently, reproduce and increase the population for these alleles, in a specific way. According to the data from the work, nitrogen has a fundamental role in maintaining the technology and reductions in doses must be planned and monitored, thus, the technology will have greater viability and will increase the effectiveness of resistance to caterpillar attacks for longer and will maintain the protein effective control.

Barcelos & Angeline (2018) studied the control of *Spodoptera frugiperda* using *Bt* technologies in corn and concluded that Powercore (Cry 1A.105/Cry 2Ab2/Cry 1F), Viptera3 (IP3Aa20), and VT Pro (Cry 1A105/Cry2Ab2) technologies were the most efficient in controlling *S. frugiperda* and without requiring insecticide applications, demonstrating an effect linked to hybrid tolerance, correlated to other alleles. In this case, the AG1051 hybrid may have greater tolerance because it is a double hybrid and possibly greater capacity to withstand attacks and less variation in its performance, compared to single hybrids, with the presence of proteins and greater uniformity and also vulnerability. According to Faria et al. (2023), at low levels of potassium, the effectiveness of corn hybrids decreases in controlling *Spodoptera frugiperda* and the presence of more Cry proteins is not capable of preventing attacks and is only efficient at the appropriate rate (90 kg ha^{-1} KCl).

In Brazil, multiple *Bt* proteins are registered for the control of fall armyworm, whether in simple or pyramidal combinations, Fatoretto et al (2017) described that the Cry1Ab protein was introduced in 2008 but showed unexpected damage caused by fall armyworm after 3 years. Likewise, Cry1F was introduced in 2009 and with damage in 2012; even the pyramided ones, such as the one containing Cry1A.105 and Cry2Ab2, which were launched in 2010, already showed unexpected damage in the field in 2013. Despite indications of resistance breaks, hybrids with the presence of proteins and with the appropriate dose showed effective control against *S. frugiperda*, even under water deficit, in the 2014/15 harvest (Figure 1A, 2A and 3A). Pyramiding increases efficiency and improves the ability of plants to withstand attacks, but the tolerance offered by the hybrid must also be taken into account, as well as the population level of the pest.

4. Conclusion

Nitrogen doses interfere directly with the tolerance and also resistance conferred by the Cry proteins of the plants in corn hybrids, decreasing the control of the caterpillar Spodoptera frugiperda and possibly favoring the increase in damage at different stages of development.

Corn hybrids showed divergent performance in terms of proteins under low nitrogen availability (0 and 80 kg ha^{-1}), making it impossible to say which protein would be more efficient and pyramiding increases the caterpillar control efficiency at the recommended dose (160 kg ha^{-1} of N), with damage in the opposite direction to transgenics.

References

Barcelos, P. H. S., & Angeline, M. R. (2018). Controle de *Spodoptera frugiperda* (Smith, 1797) em diferentes tecnologias *Bt*s (*Bacillus thuringienses*) na cultura do milho. *Revista de Agricultura Neotropical, 5*(1), 35-40. https://doi.org/10.32404/rean.v5i1.1824

Chen, Y., Olson, D. M., & Ruberson, J. R. (2010). Effects of nitrogen fertilization on tritrophic interactions. *Arthropod-Plant Interactions, 4*(2), 81-94. https://doi.org/10.1007/s11829-010-9092-5

CONAB – National Supply Company. (2023). *Boletim da Safra de Grãos*. Retrieved October 26, 2023, from https://www.conab.gov.br/info-agro/safras/graos

Davis, F. M., Ng, S. S., & Williams, W. P. (1992). *Visual rating scales for screening whorl-stage corn for resistance to fall armyworm*. (Technical Bulletin, 186).

Faria, M. M., Cintra, P. H. N., Amorim, V. A., Campos, T. S., Rocha, E. C. & Rodrigues, F. (2023). Interrelationship between potassium rates and the efficiency of *Bt* genes in the control of *Spodoptera frugiperda*. *Pesquisa Agropecuária Brasileira, 58*(e03241), 1-9. https://doi.org/10.1590/S1678-3921.pab2023.v58.03241.

Fatoretto, J. C., Michel, A. P., Silva Filho, M. C., & Silva, N. (2017). Adaptive potential of fall armyworm (Lepidoptera: Noctuidae) limits *Bt* trait durability in Brazil. *Journal of Integrated Pest Management, 8*(1), 1-10. https://doi.org/10.1093/jipm/pmx011

Friendly, M., & Fox, J. (2021). *Candisc: visualizing generalized canonical discriminant and canonical correlation analysis*. R package version 0.8-6. 2021. Retrieved October 26, 2023, from https://CRAN.Rproject. org/package=heplots

Hair Jr, J. F., Anderson, R. E., Tatham, R. L., & Black, W. C. (2005). *Análise multivariada de dados*. 5.ed. Porto Alegre: Bookman. Retrieved October 26, 2023, from https://books.google.com.br/books?hl=pt-BR&lr=&id=oFQs_zJI2GwC&oi=fnd&pg=PA7&dq=An%C3%A1lise+multivariada+de+dados&ots=KJ6OQd18rp&sig=DWRAQUSnbPOO3-AE9_YSOZqtiYY#v=onepage&q=An%C3%A1lise%20multivariada%20de%20dados&f=false

Jaramillo-Barrios, C. I., Quijano, E. B., & Andrade, B. M. (2019). Populations of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) cause significant damage to genetically modified corn crops. *Revista Facultad Nacional de Agronomía Medellín, 72*(3), 8953-8962. https://doi.org/10.15446/rfnam.v72n3.75730

Khan, S. U., Ali, S., Shah, S. H., Zia, M. A., Shoukat, S., Hussain, Z., & Shahzad, A. (2021). Impact of nitrogen and phosphorus fertilizers on Cry1Ac protein contents in transgenic cotton. *Brazilian Journal of Biology, 83*(e246436). https://doi.org/10.1590/1519-6984.246436

Moscardini, V. F., Marques, L. H., Santos, A. C., Rossetto, J., Silva, O. A., Rampazzo, P. E., & Castro, B. A. (2020). Efficacy of *Bacillus thuringiensis* (*Bt*) maize expressing Cry1F, Cry1A. 105, Cry2Ab2 and Vip3Aa20 proteins to manage the fall armyworm (Lepidoptera: Noctuidae) in Brazil. *Crop protection, 137*, 1-8. https://doi.org/10.1016/j.cropro.2020.105269

Niu, Y, Oyediran, I., Yu, W., Lin, S., Dimase, M., Brown, S., Reay-Jones, F. P. F., Cook, D., Reisig, D., Thrash, B., Ni, X., Paula-Moraes, S. V., Zhang, Y., Chen, J. S., Wen, Z., & Huang, F. (2021). Populations of *Helicoverpa zea* (Boddie) in the southeastern united states are commonly resistant to cry1ab, but still susceptible to vip3aa20 expressed in mir 162 corn. *Toxins, 13*(1), 1–14. https://doi.org/10.3390/toxins13010063

Nóia Júnior, R. D. S. N., & Sentelhas, P. C. (2019). Soybean-maize off-season double crop system in Brazil as affected by El Niño Southern Oscillation phases. *Agricultural Systems, 173*, 254-267. https://doi.org/10.1016/j.agsy.2019.03.012

Olatinwo, R., & Hoogenboom, G. (2014). Weather-based Pest Forecasting for Efficient Crop Protection. *Integrated Pest Management*, 59-78.

R Core Team. (2022). R*: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Ren, L. L., Hardy, G., Liu, Z. D., Wei, W., & Dai, H. G. (2013). Corn defense responses to nitrogen availability and subsequent performance and feeding preferences of beet armyworm (Lepidoptera: Noctuidae). *Journal of economic entomology, 106*(3), 1240-1249.

Resende, C. L., Damaso, L. F., Ávila, M. C., Carvalho, D. D., Melo, P. G., & Rodrigues, F. (2019). Agronomic efficiency of hybrids of corn to nitrogen, phosphorus and potassium targeting fresh corn. *Journal of Agricultural Science, 11*(9), 120-133. https://doi.org/10.5539/jas.v11n9p120

Souza, C. S. F., Silveira, L. C. P., Paula, D. P., Andow, A. D., & Mendes, S. M. (2018). Transfer of Cry1F from *Bt* maize to eggs of resistant *Spodoptera frugiperda*. *Plos One, 13*(9), 1–11. https://doi.org/10.1371/journal.pone.0203791

USDA. United States Department of Agriculture. (2023). *Corn 2023 World Production*. Retrieved October 26, 2023, from https://ipad.fas.usda.gov/cropexplorer/cropview/commodityView.aspx?cropid=0440000

Wang, W., Wang, X., Liao, H., Feng, Y., Guo, Y., Shu, Y., & Wang, J. (2022). Effects of nitrogen supply on induced defense in maize (*Zea mays*) against fall armyworm (*Spodoptera frugiperda*). *International journal of molecular sciences, 23*(18), 1-19. https://doi.org/10.3390/ijms231810457

Waquil, J. M., Dourado, P. M., Carvalho, R. A., Oliveira, W. S., Berger, G. U., Head, G. P., & Martinelli, S. (2013). Manejo de lepidópteros-praga na cultura do milho com evento *Bt* piramidado Cry1A.105 e Cry2Ab2. *Pesquisa Agropecuária Brasileira, 48*(12), 1529-1537. https://doi.org/10.1590/S0100-204X2013001200001

Wu, Y., Liu, W., Li, X., Li, M., Zhang, D., Hao, Z., Weng, J., Xu, Y., Zhang, S., & Xie, C. (2011). Low-nitrogen stress tolerance and nitrogen agronomic efficiency among maize inbreds: comparison of multiple indices and evaluation of genetic variation. *Euphytica, 180*(2), 281-290. https://doi.org/10.1007/s10681-011-0409-y

Xing, Y., Qin, Z., Feng, M., & Li, A., Zhang, L., Wang, Y., Dong, X., Zhang, Y., Tan, S., & Shi, W. (2019). The impact of *Bt* maize expressing the cry1ac protein on non-target arthropods. *Environmental Science and Pollution Research, 26*(6), 5814–5819. https://doi.org/10.1007/s11356-018-4025-4

Ye, W., Bustos ‐ Segura, C., Degen, T., Erb, M., & Turlings, T. C. (2022). Belowground and aboveground herbivory differentially affect the transcriptome in roots and shoots of maize. *Plant direct, 6*(7), 1-17. https://doi.org/10.1002/pld3.426

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Data sharing statement

No additional data are available.