# Resistance of maize landraces from Brazil to fall armyworm (Lepidoptera: Noctuidae) in the winter and summer seasons

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ABSTRACT: The fall armyworm (Spodoptera frugiperda) is a major maize (Zea mays L.) pest in Brazil, whose larvae cause losses from plant emergence to harvesting stage. This pest has been controlled almost exclusively with chemical insecticides and Bt plants (transgenics); however, resistance evolution has been detected to these two control tactics. Therefore, alternative control tactics are needed for management and control of the fall armyworm. Thus, this study aimed to evaluate the resistance of maize landraces from Brazil to larvae of this herbivore in the winter and summer seasons, studying also the occurrence of the predator Doru luteipes (Dermaptera: Forficulidae). The variety Pérola had the highest resistance levels to fall armyworm in the summer season, with lower values of leaf injury, number of infested plants, and number of larvae. Conversely, this variety did not express resistance to the pest in winter. Doru luteipes was found abundantly in the summer season, showing no differences between varieties. This is the first study to evaluate the resistance of maize landraces from Brazil to fall armyworm in the winter and summer seasons. The next step is the molecular characterization of the variety Pérola, as well as evaluating its effects on the most common natural enemies of the pest in Brazil, such as the predator D. luteipes and the egg parasitoid Trichogramma pretiosum (Hymenoptera: Trichogrammatidae). These results may assist future maize breeding programs aimed at developing cultivars and hybrids resistant to the pest, and hence reducing agriculture's dependence on chemical insecticides and transgenic plants.

**Key words:** Zea mays L., integrated pest management, plant resistance to insects, Spodoptera frugiperda.

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# INTRODUCTION

Maize (*Zea mays* L.) (Cyperales: Poaceae) is one of the world's most important crop plants (Beyene et al. 2016). This plant species has a broad genetic diversity, and therefore it is highly adaptable and responsive to selective pressure. As a result, maize has been grown from the tropics to Southern Canada, a wide biogeographical range that displays high diversity in soil composition, climate, day length, and elevation (Neuffer 1982).

This important crop has had its productivity threatened by different insect pests. The fall armyworm [*Spodoptera frugiperda* (J. E. Smith)] (Lepidoptera: Noctuidae) is a major pest of maize as well as other crops in the Americas (Oliveira

et al. 2014; Nagoshi et al. 2015). The larval feeding on maize plants causes yield reductions of up to 60% depending on severity of the infestations, crop stage development, sowing period, region, genotype, and agricultural practices. This pest attack may occur from seedling emergence to reproductive phase (Cruz 2008; Cruz et al. 2013).

Control of fall armyworm larvae has been historically performed with sprays of insecticides; however, several cases of resistance evolution have limited their effectiveness (Carvalho et al. 2013). In contrast, transgenic maize hybrids that express insecticidal proteins of *Bacillus thuringiensis* (*Bt* maize) have been a widely adopted alternative to insecticides for controlling this insect in Brazil. Nevertheless, most *Bt* hybrids have lost efficacy to control the pest within three years after commercial release (Fatoretto et al. 2017).

In this context, host plant resistance (HPR) is a relevant tool to consider for implementation in the integrated pest management (IPM) programs. Host plant resistance is an efficient and environmentally friendly mode of controlling insects (Dogimont et al. 2010), and it is commonly segmented into three categories: antibiosis, antixenosis, and tolerance (Smith 2005; Smith and Clement 2012). Antibiosis is a resistance category that possess at least one plant characteristic that adversely affects pest growth. The insect may show a lower weight, a delayed developmental period, a lower survival, and reduced rates of fecundity and fertility. Antixenosis exhibits at least one plant characteristic that negatively affects pest behavior. The insect rejects the resistant variety for feeding, oviposition, and/or shelter. Tolerance is a polygenic trait enabling a plant to withstand or recover from pest damage without negatively affecting insect's development or survival. The tolerant variety exhibits a similar yield (or biomass) when compared infested versus uninfested plants (Smith 2005; Smith and Clement 2012).

Maize landraces are classified as open-pollinated varieties (OPVs), and hence they underwent long-term natural and artificial selection in the past centuries. A great number of these materials have arisen over time, selected by farmers according to the adaptation to the environmental conditions (Cömertpay et al. 2012). Landraces might possess novel favorable alleles for agronomic traits such as yield, tolerance to abiotic stress, and resistance to arthropod pests (Harlan 1975; Zeven 1998). Knowing the extent and nature of the available diversity of maize landraces is essential for their potential application in plant breeding and conservation programs (Sharma et al. 2010).

In this study, was tested the hypothesis that the variety Pérola, adopted as a resistance standard for showing resistance in the laboratory and in the greenhouse to fall armyworm (Nogueira et al. 2018) and *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae) (Costa et al. 2018), would also be resistant to the mentioned lepidopteran species in field conditions. Thus, the objective of this study was to evaluate the resistance to fall armyworm in maize landraces from Brazil in the winter and summer seasons, assessing the occurrence of the predator *Doru luteipes* (Scudder) (Dermaptera: Forficulidae).

## MATERIAL AND METHODS

## **Experimental site**

The experiments were performed from June 2018 to January 2019, corresponding to the maize sowings of the winter (sowing on 15 May 2018) and summer (sowing on 10 December 2018) seasons, which were considered separate trials. These experiments were conducted in an experimental area (22° 19' South latitude, 54° 937' West longitude, 454 m altitude) of Federal University of Grande Dourados (Universidade Federal da Grande Dourados - UFGD), Dourados, MS, Brazil.

The climate in the experimental site is classified as Cwa (humid subtropical climate with hot summer and dry winter), because the temperature of the coldest period (June and July) is inferior to 18 °C and of the hottest month is superior to 29 °C (Fietz and Fisch 2017). The soil of the experimental site is categorized as dystroferric red latosol (Bergamin et al. 2010).

## Cultural practices

In both sowing periods, the soil was prepared by plowing and harrowing operations. Spacing of 0.45 m between each row was adopted in the experiments and six seeds were sown per linear meter. Thinning was carried out 20 days after sowing,

keeping three plants of maize per linear meter. Each plot had four rows (10 m long), totaling 18 m<sup>2</sup> of total area and 9 m<sup>2</sup> of useful area. The experiment total area was 288 m<sup>2</sup>.

Four hundred and fifty kg·ha<sup>-1</sup> of the formula 00-20-20 (N-P-K) was used for crop fertilization, with an additional 90 kg·ha<sup>-1</sup> of N in urea form. No chemical insecticides and fungicides were used for pest control and the plots were irrigated whenever necessary. Manual weeding was done at the V3 (three fully expanded leaves) maize vegetative stage according to the Ritchie scale (Ritchie et al. 1993).

A randomized block experimental design was used, constituted by four treatments (maize landraces) and three sampling dates. Four blocks were used as replicates in each growing season.

#### Maize landraces

Four maize landrace varieties were assessed in the experiments performed during the winter and summer seasons: Pérola (resistance standard), Asteca Palha Roxa (resistant), Asteca Amarelo (susceptible), and Milho Roxo (susceptible), selected according with the results obtained in previous laboratory and greenhouse experiments and seeds availability. The variety Pérola was adopted as resistance standard according to earlier laboratory and greenhouse results with fall armyworm (Nogueira et al. 2018) (compared to other seven genotypes) and *D. speciosa* (Costa et al. 2018) (compared to other 18 genotypes).

## Assessment methodology

In the winter season, the evaluations were performed at the V4, V6, and V8 vegetative stages. In the summer season, the evaluations were done at the V6, V7, and V8 vegetative stages. The maize plants were assessed from V4 to V8 vegetative stages because this is the period in which maize plants are more susceptible to fall armyworm larval feeding (Cruz 1995). In general, this period is extremely critical for the formation of maize ears and consequently in the final yield of the crop (Cruz and Turpin 1982). In summer, evaluations started at V6 stage because the pest was not found at V4 or V5 stages.

Five maize plants of the two central rows of every plot were sampled on each date, totaling 10 sampled plants per plot. The evaluated plants were randomly chosen before arriving in the field (determined in the laboratory). The maize whorl was removed from the plant, and then opened to evaluate the presence of fall armyworm larvae and *D. luteipes*. Data were expressed as total number of larvae, leaf injury rating (Davis et al. 1992), number of infested plants (injured whorl), and number of individuals of the predator *D. luteipes*. The 1-9 Davis scale (Davis et al. 1992) was used to evaluate leaf injury rating, whereby a score of 1 indicates no damage up to three very small injuries on maize whorl and a score of 9 indicates whorl and expanded leaves almost or totally destroyed. Each of the five plants of the two central rows in each plot were rated for injury and then an average was calculated.

With the objective of evaluating the performance of the varieties in practical terms, a figure was built to check whether the varieties would show  $\geq$  20% plants with an injury rating  $\geq$  3, the control level established by the Brazilian Association of Seeds and Seedlings (Associação Brasileira de Sementes e Mudas) (ABRASEM 2014).

## **Statistical Analysis**

Data on the leaf injury caused by the larval feeding of fall armyworm were analyzed using repeated measures analysis of variance followed by the Tukey's test at 5% probability. The variety and the sampling time were considered the fixed factors, whilst the four replications within each cropping season were considered the random factor. The count data (number of infested plants, number of fall armyworm larvae, and number of *D. luteipes* individuals) were analyzed using the generalized linear model (Poisson regression), and the means separated by the Tukey's test at 5% probability. All analyses were performed using the statistical software R (R Core Team 2019).

## **RESULTS AND DISCUSSION**

#### Winter season

No significant differences were observed for leaf injury considering variety (F = 2.15; df = 3, 9; p = 0.5419) (values ranged from 3.15 to 3.84), sampling time (F = 0.08; df = 3, 9; p = 0.7720) (variation of 2.83 to 4.77 for leaf injury rating), or the interaction between variety versus sampling time (F = 0.72; df = 3, 9; p = 0.8687). Statistical differences were not found considering the number of fall armyworm larvae at V4 stage ( $X^2 = 1.55$ ; df = 3, 9; p = 0.6697) (from 6.25 to 8.25 larvae), at V6 stage ( $X^2 = 3.74$ ; df = 3, 9; p = 0.2913) (from 4.25 to 7.50 larvae), or at V8 stage ( $X^2 = 2.45$ ; df = 3, 9; p = 0.4845) (from 0.75 to 2.00 larvae). However, a marginally significant difference (i.e. statistical result with a P value near the standard criterion of p = 0.05) was recorded for number of infested plants ( $X^2 = 7.03$ ; df = 3, 9; p = 0.0708), which had values between 4.50 (variety Pérola) to 9.00 (variety Milho Roxo) infested plants. Because no significant differences were noted in the winter season, the statistical details are not shown.

In this study, it was evaluated the resistance of the variety Pérola (compared to other three varieties) in the summer and winter seasons, popularly known as first and second crops, respectively. In Brazil, maize is cultivated in these two different cropping seasons, which show differences regarding abiotic factors as temperature, light length, and relative humidity, variables that can affect plant resistance. For example, the duration, quantity, and quality of light have each been shown to affect the expression of plant resistance to arthropods (Smith 2005).

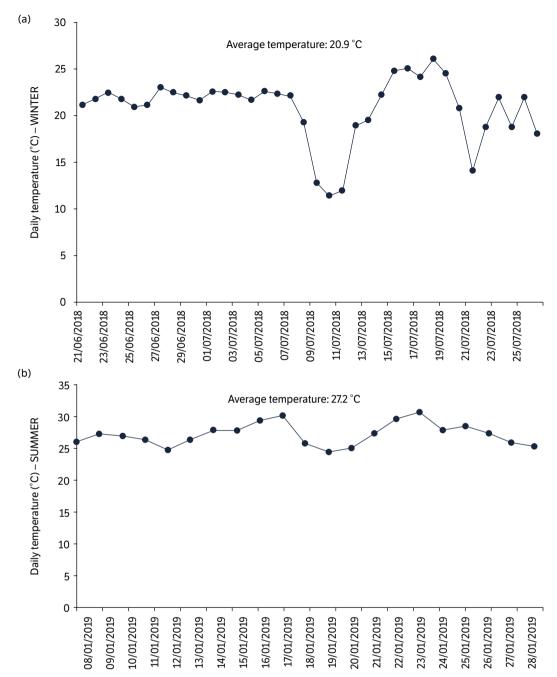
The resistance of the variety Pérola was not expressed in the winter season. It is found in the literature some examples in which the resistance was not expressed when plants were cultivated in short-day periods. For instance, Kennedy et al. (1981) found that *Manduca sexta* (L.) (Lepidoptera: Sphingidae) larvae fed on plants of *Lycopersicon hirsutum* f. *glabratum* grown under long-day condition displayed greater mortality than larvae fed on leaves from plants grown under short-day condition. Similarly, Patterson et al. (1994) revealed that resistance of strawberry (*Fragaria ananassa* Duch) plants to *Tetranychus urticae* Koch (Acari: Tetranychidae) is greater in plants cultivated under long-day condition compared to short-day regime. Smith (2005) mentioned that the higher resistance levels observed under long-day regime occur as a result of a greater plant toxin production. Therefore, it is important to take care when growing resistant varieties in the winter season (non-preferential season), because their resistance expression can be compromised.

Overall, when the plants are cultivated in winter they are subjected to adverse environmental conditions, for example lower temperatures, contributing to a reduction in the expression of genes associated with the resistance to insect pests (Smith 2005). In this study, the average temperature observed in winter was 20.9 °C (Fig. 1A), whereas in summer the average temperature was 27.2 °C (Fig. 1B). Thus, the low temperatures observed in winter may have inhibited the expression of resistance of the variety Pérola.

In winter, there was a greater number of fall armyworm-infested plants than in summer. Perhaps this might be explained by the rare occurrence of natural enemies in winter (what did not allow us to record their population), especially *D. luteipes*, an efficient predator of the pest (Reis et al. 1988). Ni et al. (2014) inferred that the greater abundance and diversity of predators in 2010 than 2009 (summer experiments) might have contributed to a lower level of maize leaf injury caused by this pest on all varieties examined in 2010. Based on the current study data, it is suggested the maize cultivation in winter may result in greater leaf damage and consequent economic losses to growers whether efficient control tactics are not available.

#### Summer season

The variety Pérola showed the lowest value of leaf injury rating (F = 13.49; df = 3, 33; p = 0.0037) compared to the varieties Asteca Amarelo and Asteca Palha Roxa (Fig. 2). There were no statistical differences for leaf injury rating when considering the sampling date (F = 2.07; df = 2, 33; p = 0.1500), with a variation from 1.93 to 2.27 of leaf injury, nor for the interaction between variety versus sampling date (F = 1.13; df = 6, 33; p = 0.7697). The leaf injury recorded for the variety Pérola in summer (1.6) was lower than the leaf injury exhibited by the most resistant varieties shown by Ni et al. (2014)



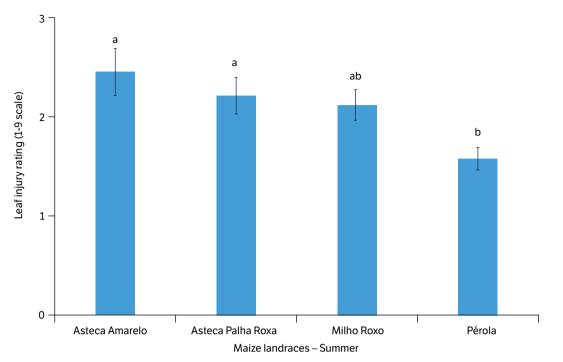
Source: Embrapa Agropecuária Oeste.

Figure 1. Daily temperature recorded during conduction of the experiments in the winter (a) and summer (b) seasons.

(injury rating from 3.0 to 5.0), in a summer season study performed in 2009 at Tifton, GA, USA. This comparison provides an additional parameter to understand the potential of the variety Pérola as an important resistance source to manage fall armyworm infestations.

At V6 stage, the variety Pérola had a lower number of infested plants ( $X^2 = 11.95$ ; df = 3, 9; p = 0.0076) than the varieties Asteca Amarelo and Milho Roxo (Table 1). However, no statistical differences were registered for number of infested plants at V7 ( $X^2 = 3.64$ ; df = 3, 9; p = 0.3031) and V8 stages ( $X^2 = 2.65$ ; df = 3, 9; p = 0.4485) (Table 1).

Differences were not found concerning the number of fall armyworm larvae on V6 plants ( $X^2 = 3.37$ ; df = 3, 9; p = 0.3381), although marginally significant differences were observed for V7 plants ( $X^2 = 7.49$ ; df = 3, 9; p = 0.0578), when the variety



**Figure 2.** Leaf injury rating (mean  $\pm$  SE) of maize landrace varieties exposed to fall armyworm larvae in the summer season. Means followed by the same letter above bars did not differ significantly by Tukey's test at 5% probability (repeated measures ANOVA).

| Variety           | V6 plants              |                        |                           |
|-------------------|------------------------|------------------------|---------------------------|
|                   | Infested plants (n)    | FAW larvae (n)         | Doru luteipes (n)         |
| Asteca Amarelo    | $5.00\pm1.96\text{a}$  | $2.25\pm1.44\text{a}$  | $4.25 \pm 1.70$ a         |
| Asteca Palha Roxa | $4.50\pm1.66\text{ab}$ | $3.50\pm2.18~\text{a}$ | $4.75\pm0.85a$            |
| Milho Roxo        | 5.75 ± 1.25 a          | $2.50\pm1.50~\text{a}$ | $4.50\pm1.50~\text{a}$    |
| Pérola            | $1.50\pm0.29~\text{b}$ | $1.50\pm0.29\text{a}$  | $6.50 \pm 1.71\mathrm{a}$ |
| Variety           | V7 plants              |                        |                           |
|                   | Infested plants (n)    | FAW larvae (n)         | Doru luteipes (n)         |
| Asteca Amarelo    | 5.75 ± 0.75 a          | $4.00\pm0.41\text{a}$  | $10.00 \pm 1.83$ a        |
| Asteca Palha Roxa | 4.75 ± 1.03 a          | $1.75\pm0.48\text{a}$  | $6.25 \pm 0.75$ a         |
| Milho Roxo        | $4.75\pm0.95a$         | $3.00\pm0.71\text{a}$  | $8.00\pm1.58\text{a}$     |
| Pérola            | $3.00\pm0.58~\text{a}$ | $1.25\pm0.48\text{a}$  | $5.25 \pm 1.44$ a         |
| Variety           | V8 plants              |                        |                           |
|                   | Infested plants (n)    | FAW larvae (n)         | Doru luteipes (n)         |
| Asteca Amarelo    | 5.75 ± 1.25 a          | $2.25\pm1.60\text{ab}$ | $8.50\pm1.19\mathrm{a}$   |
| Asteca Palha Roxa | 5.75 ± 1.75 a          | $5.50\pm1.71\text{a}$  | $9.00\pm1.41\text{a}$     |
| Milho Roxo        | $4.25\pm1.03\text{a}$  | $2.75\pm0.25\text{ab}$ | $10.00\pm1.87\text{a}$    |
| Pérola            | 3.75 ± 0.48 a          | $1.50\pm0.29~\text{b}$ | 10.25 ± 2.95 a            |

Table 1. Number of infested plants, fall armyworm (FAW) larvae, and individuals of Doru luteipes (mean ± SE) in the summer cropping season.

Means followed by different letters within columns are significantly different by Tukey's test at 5% probability (Poisson regression). (n = number).

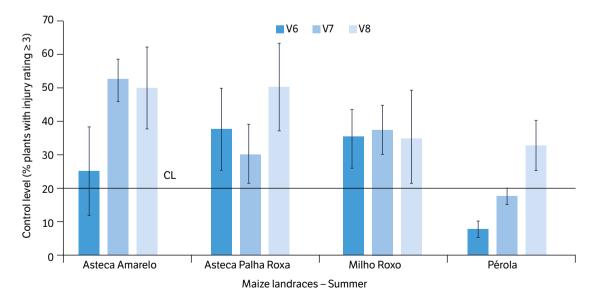
Pérola showed one of the lowest number of larvae. However, this trend was confirmed when evaluating V8 plants ( $X^2 = 11.26$ ; df = 3, 9; p = 0.0104), with the variety Pérola displaying a lower number of fall armyworm larvae compared to the variety Asteca Palha Roxa (Table 1).

Due to the fact that plants of the variety Pérola exhibited lower values of leaf injury rating, number of infested plants, and number of fall armyworm larvae, it is possible to affirm that this variety expressed resistance to the pest in summer, the preferential growing season for maize in Brazil.

There are few studies published on resistance of maize landraces from Brazil to insect pests. One study evaluated the oviposition preference and antibiosis to fall armyworm in different maize landrace varieties, in laboratory and greenhouse assays (Nogueira et al. 2018). The authors found that the variety Pérola affected the oviposition preference, and larvae fed on this variety consumed a lower amount of leaves and had longer development time and lower survival compared to seven other landraces. Another study assessed resistance to *Diabrotica speciosa* in 19 maize genotypes (17 landraces and 2 cultivars), and concluded that variety Pérola was one of the most resistant to the pest for delaying the maturation period from larva to adult and decreasing survivorship (Costa et al. 2018). These laboratory and greenhouse studies were very important to classify the variety Pérola as resistant to insect pests. However, there was a gap regarding studies on resistance of the variety Pérola with evaluations of leaf injury rating in field conditions. It is important to evaluate the leaf injury rating because this variable is commonly used to determine whether fall armyworm has reached its control level in maize crops, as defined by programs of integrated pest management.

According to ABRASEM (2014), it is necessary to control fall armyworm larvae when 20% (or more) of the evaluated plants show injury rating  $\geq$  3. In summer, plants of the variety Pérola showed a fall armyworm population level below this control level at V6 and V7 stages (Fig. 3). Conversely, the other varieties had an infestation level above the control level in all evaluations (considering a total of 40 plants evaluated in each vegetative stage). Thus, according to this recommendation, plants expressing resistance of the variety Pérola will be more independent of exogenous intervention (e.g., insecticide application) than the modern commercial cultivars. In a context of integrated pest management, the variety Pérola should be classified as moderately resistant, because the control level for the pest was observed at least in one of the evaluations. Smith (2005) affirmed that plants expressing moderate resistance levels exhibit several advantages, for example, it is much more difficult to occur insect resistance evolution and negative impacts on natural enemies compared to varieties expressing high resistance levels.

It may be inferred that the resistance of the variety Pérola to fall armyworm is strongly associated to antixenosis (lower number of larvae), corroborating a previous greenhouse study (Nogueira et al. 2018), now in a more realistic condition (agricultural field). Due to the fact that the variety Pérola expressed resistance to fall armyworm larval feeding using detached leaf sections (Nogueira et al. 2018), we suggest its resistance is constitutively governed by the presence of pre-formed resistance factors, such as toxic allelochemicals and structures that stiffen the leaf cell wall (lignin, cellulose, silicon etc.). The knowledge that constitutive genes are playing an important role for the resistance of the variety Pérola may be very useful for supporting plant breeding programs.



**Figure 3.** Percentage of plants of different maize landrace varieties displaying a leaf injury rating  $\geq$  3 in the summer season. CL = control level.

With respect to the number of individuals of the predator *D. luteipes*, significant differences were not recorded between varieties when assessing plants at V6 stage ( $X^2 = 2.37$ ; df = 3, 9; p = 0.4982), at V7 stage ( $X^2 = 7.02$ ; df = 3, 9; p = 0.0714), or at V8 stage ( $X^2 = 0.87$ ; df = 3, 9; P = 0.8323) (Table 1). Thus, it seems that the resistance of the variety Pérola did not exert a negative impact on the population of *D. luteipes*, although detailed laboratory studies are required to affirm that this predator is not affected when fed on fall armyworm larvae previously fed on variety Pérola.

## CONCLUSION

The variety Pérola may be promising for protection of maize crops. Further studies are necessary to investigate the resistance of the variety Pérola to fall armyworm larvae in the winter season, perhaps with an earlier sowing time than May. Investigations on which genes are associated to the resistance of the variety Pérola and their effects on natural enemies will contribute to future maize breeding programs, with the aim of developing fall armyworm-resistant cultivars and hybrids and hence reducing agriculture's dependence on synthetic insecticides and transgenic plants.

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## **AUTHOR'S CONTRIBUTION**

Conceptualization, Costa E. N.; Methodology, Costa E. N. and Fernandes M. G.; Investigation, Costa E. N., Medeiros P. H. and Evangelista B. M. D.; Writing – Original Draft, Costa E. N.; Writing – Review and Editing, Costa E. N.; Funding Acquisition, Costa E. N.; Resources, Costa E. N. and Fernandes M. G.; Supervision, Fernandes M. G.

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