Influence of temperature on the pupal development of the cassava shoot fly (*Neosilba perezi*, Diptera, Lonchaeidae)

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Abstract – The cassava shoot fly (*Neosilba perezi*, Lonchaeidae) (Romero & Ruppel) is a key pest of cassava in Southern Brazil. Females oviposit in shoots and larvae, when feeding, reduces the growth of the plant, after destroying its apical meristem, causing losses in propagative material and root production, depending on the cultivar. Few studies address the biology of this fly. Thus, this study aimed to analyze the influence of different temperatures on the pupal development of the cassava shoot fly. Cassava shoot fly pupae were maintained under laboratory conditions at 10°C, 25°C, and 30°C. Significant differences were found between the temperatures tested. The temperature of 25°C provided the best development and emergence of viable adults while 10°C delayed the development and provided a greater number of deformed adults. At 30°C, the pupal time was shorter, but the number of deformed adults was greater. These results show that the survival of the cassava shoot fly is directly related to temperature. The colder the winter during off-season, the smaller the fly population in the next season.

Index terms: *Manihot esculenta*; integrated pest management; insect rearing; population dynamics; insect biology.

Influência da temperatura no desenvolvimento pupal da mosca-do-broto da parte aérea da mandioca (Neosilba perezi, Diptera, Lonchaeidae)

Resumo – A mosca-do-broto da mandioca, *Neosilba perezi* (Diptera: Lonchaeidae), é uma praga chave da cultura da mandioca no Sul do Brasil. As fêmeas desta espécie ovipositam nas brotações e as larvas, ao se alimentarem, impedem o crescimento da planta, após destruírem seu meristema apical. Isto resulta em perdas de ramas e na produção de raízes dependendo do cultivar. Existem poucos estudos sobre sua biologia, assim, esta pesquisa teve como objetivo analisar a influência da temperatura no desenvolvimento pupal da mosca-do-broto da mandioca. As pupas da mosca-do-broto foram mantidas em laboratório em três diferentes temperaturas constantes: 10°C, 25°C e 30°C. Foram detectadas diferenças significativas entre as temperaturas testadas. A temperatura de 25°C proporcionou o melhor desenvolvimento pupal com maior formação de adultos viáveis, enquanto a baixa temperatura (10°C) retardou o desenvolvimento e resultou em maior número de adultos deformados. A 30°C, o tempo pupal foi menor, porém o número de adultos deformados também foi maior. Esses resultados indicam que a sobrevivência da mosca-do-broto está diretamente relacionada à temperatura. Durante o período de entressafra, ou seja, quanto mais frio for o inverno na região, menor tenderá ser a população de moscas no próximo ciclo de cultivo.

Termos para indexação: *Manihot esculenta*; manejo de pragas; criação de insetos; dinâmica populacional; biologia de insetos.

Introduction

Cassava (*Manihot esculenta* Crantz) is essential for food supply in poor and developing countries due to its high energy content and great capacity to adapt to different climatic and edaphic conditions (ELIAS & NEUBERT, 2018). Despite being tolerant to pest attack, according to some authors (BELLOTTI et al., 1999; OLIVEIRA & PAULA-MORAES, 2011), the cassava shoot fly (*Neosilba perezi*, Diptera, Lonchaeidae) (Romero & Ruppel) has been a key pest of the plant for some decades in Southern Brazil (FARIAS, 1991). Females oviposit in cassava shoots, from where their larvae hatch from two to four days and start to feed and develop for about 15 to 23 days. After this period, the larvae leaves the plant to pupate in the soil, completing their life cycle in 40 or 50 days, depending on the temperature (BELLOTTI, 2002; DE LORENZI & NORA, 2016; GISLOTI & PRADO, 2013).

Feeding larvae in the meristematic region of the plant can delay its growth and consequently cause losses in propagating material and roots, depending on the variety and infestation

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level (DE LORENZI, 2018).

The biology of this fly is poorly known and the existing studies focus on simulating the damage caused by the fly in different cassava genotypes (LOURENÇÃO et al., 1996); on its morphology, biology, and population dynamics (GISLOTI & PRADO, 2011; 2013); on parasitoid records (AROUCA & PENTEADO-DIAS, 2011; GISLOTI & PRADO, 2012); and on the occurrence of the species in other crops and parts of cassava (RAGA et al., 2015; STRIKIS et al., 2012). Few studies address the survival of this species in relation to abiotic factors.

Temperature is one of the most crucial factors in insect development, directly affecting the reproductive success and the duration of the stages of larval development (TAYLOR, 1981). The emergence, distribution range, and fluctuation of each insect population are directly regulated by the temperature of the environment (KANG et al., 2009). Thus, understanding the thermal requirements of insect pests is important to develop integrated pest management (IPM) programs.

Regarding the influence of temperature on the population dynamics of the cassava shoot fly, Gisloti & Prado (2011), in field conditions, found that temperatures higher than 23°C negatively influence the population growth of *N. perezi* larvae.

In Southern Santa Catarina, Brazil, cassava shoot flies cause serious problems for farmers (DE LORENZI, 2018). In this region, cassava is usually planted in September and harvested in June or July (SÔNEGO & MASSIGNAN, 2018). Shoot flies usually appear in the crops when the temperature begins to rise (monthly average temperature of about 25°C). Their critical period of occurrence is from November to February, with a peak in January in the beginning of the vegetative development of the crop (DE LORENZI et al., 2021).

In order to contribute to the implementation of IPM programs for cassava crops in this region, this study aimed to analyze the influence of different temperatures on the pupal development of the cassava shoot fly.

Material and methods

Pupae were extracted from 300 5–10cm cassava shoots with symptoms of infestation by *N. perezi*, which were collected from December to January during the 2016/2017 harvest season in commercial crops located in the municipality of Sangão, Santa Catarina (28°36'30.66"S and 49°7'14.30"W). The climate of the municipality is humid subtropical (Cfa, according to the Köppen-Geiger climate classification) and according to the Environmental Resources and Hydrometeorology Information Center of Santa Catarina, its mean temperature is about 25°C in summer and 15°C in winter, reaching 0°C (CIRAM, 2002).

After collection in the field, the shoots were taken to the Laboratory of Entomology, Agricultural Research and Rural Extension (EPAGRI) in the municipality of Urussanga, state of Santa Catarina. In the laboratory, about 30 shoots with few or without leaves were placed in individual plastic boxes with 2cm autoclaved moistened vermiculite, which were covered with voile cloth and maintained at room temperature (25±3°C) until the larvae were transformed into pupae. The humidity of the vermiculite layer was preserved by spraying distilled and autoclaved water every two days.

For 25 days the mean time for the development from larval to pupal stage, according to Gisloti & Prado (2013) the material was screened three times a week. Pupae were transferred to Petri dishes (10 per plate) with autoclaved filter paper and later stored in BOD chambers at 10°C, 25°C, and 30°C (±1°C). The filter paper was moistened with autoclaved water every two days.

Experiments were performed in BOD chambers with relative humidity of 70±10% until adult emergence (adapted from GISLOTI & PRADO, 2013). In total, 200 pupae per temperature were used. Adult emergence was monitored daily. Emerging adults were collected and placed in glass bottles with 70% alcohol. Specimens were identified with the help of a specialist and specific keys for Lonchaeidae.

To evaluate the relationship between temperature and time to adult emergence, generalized linear models (GLM) and the Poisson distribution were used, according to the log link function (NELDER & WEDDERBURN, 1972). All models were built considering simple and second-order polynomial functions and the model that best fit for each treatment was selected.

The effect of temperatures on the emergence of viable (completely developed) and deformed adults was evaluated by the Chi-square test of independence (AGRESTI, 2007). Test data were plotted in mosaic charts with details of the residual values for each category.

All analysis were performed using the R software (R DEVELOPMENT CORE TEAM, 2021) with the packages "vegan" (OKSANEN et al., 2020) and "vcd" (MEYER et al., 2007).

Results and discussion

All male flies that emerged during the experiment were *N. perezi*. The temperatures of 10°C and 30°C had the longest and shortest incubation time and an average pupal period of 19.15 and 9.19 days, respectively. At 10°C, cassava shoot flies started emerging from the 14th day and at 30°C, from the third day. At 25°C, they started emerging from the seventh day and completely emerged in an average of 13.55 days (Figure 1).

The R^2 test showed an explanation

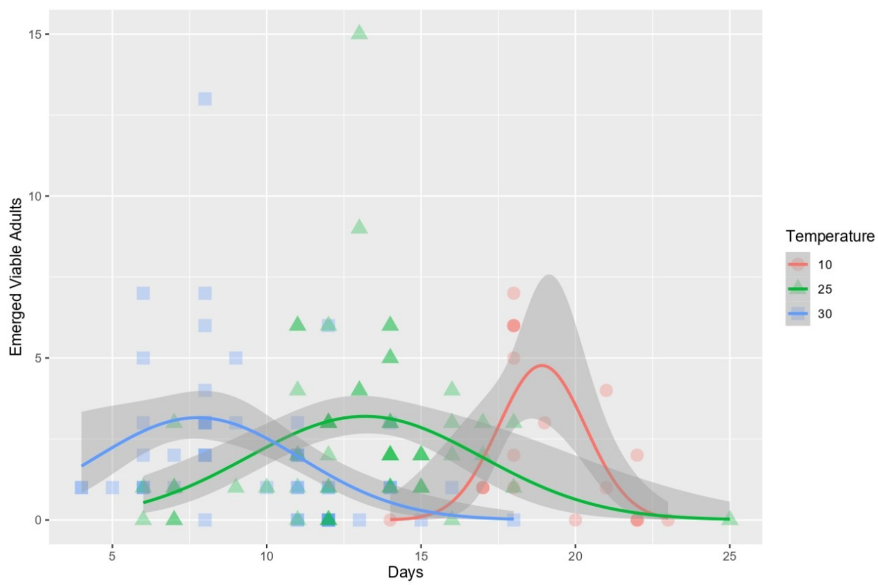

Figure 1. Development time of viable adult cassava shoot flies at different temperatures, according to the GLM test
Figura 1. Tempo de desenvolvimento de adultos viáveis da mosca-do-broto da mandioca em diferentes temperaturas, de acordo com o teste GLM

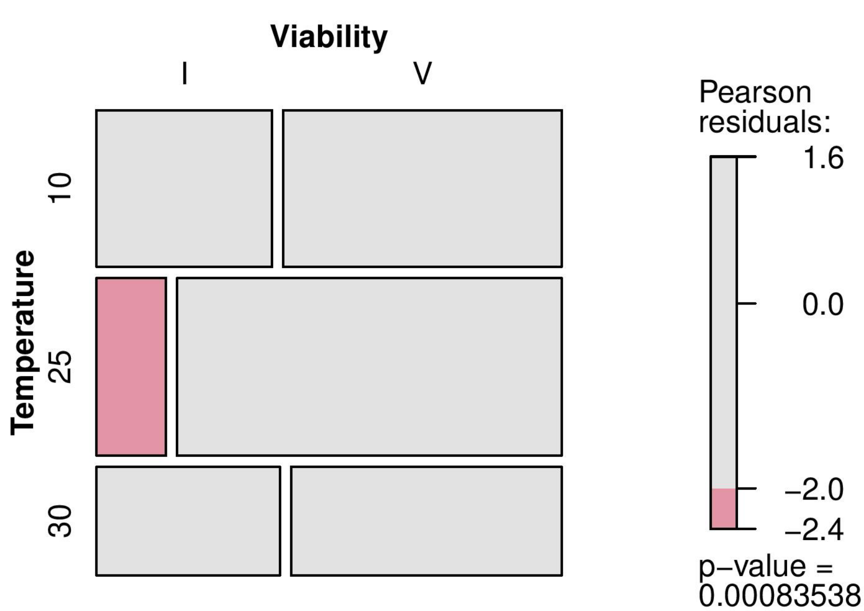

Figure 2. Emergence of viable (V) and deformed (I) adults exposed to 10°C, 25°C, and 30°C, with emphasis on the Pearson residue
Figura 2. Emergência de adultos viáveis (V) e deformados (I) expostos às temperaturas de 10, 25 e 30°C, com destaque para o resíduo de Pearson

potential of 49.6% for 10°C, followed by 18.4% for 30°C and 17.8% for 25°C. Therefore, cassava shoot flies take longer to develop at lower temperatures (Figure 1).

During the experiment, a percentage of emerging adults was deformed, that is, they were unable to fully open their wings or free themselves from the pupae after 24 hours. The Chi-square test of independence showed that the non-viability of adults that were exposed to 25°C during larval stage was significantly low. Thus, this temperature promoted the best pupal development. On the other hand, 10°C and 30°C caused high non-viability among adults (Figure 2).

Among the temperatures evaluated in this study, 25°C was the most suitable for pupal development, with a lower rate of deformed adults and 87.5% of viable adults. According to Gisloti & Prado (2013), under laboratory conditions and at 22±1°C and 70±10% UR, pupal viability was 60.5% and the mean pupal period was 23±0.35 days. In Colombia, Peñaranda et al. (1986) found, under laboratory conditions and at 23±1°C and 74.3% UR, an average pupal period of 12.9 days and pupal viability of 40.93% for *Dasiops inedulis* Steyskal. However, in this study, 25°C was more suitable than the temperatures used by these authors (22°C and 23°C).

The temperature of 30°C, despite providing the shortest development time, with an emergence peak on the eighth day (Figure 1) and a percentage of 67,85% of viable adults, provided the largest number of deformed adults, which could have occurred due to low humidity in the BOD chamber, but may show that higher temperatures can negatively influence the development of cassava shoot flies.

In this study, at 10°C, the rate of viable adults was 61.33% and the number of deformed adults was high, which shows that low temperatures also can negatively affect plant development. On the other hand, Gisloti & Prado (2013) found that 10°C promotes a higher emergence of viable adults than 22°C, but the authors used autoclaved and humidified sand as substrate for pupae. In Florida, Waddill (1978) showed that severe winters were responsible for lowering the shoot fly population levels in crops. Calado & Silva (2002) studied the influence of temperature on the development of *Aedes albopictus* under laboratory conditions and showed that flies in immature stages are more susceptible to low temperatures due to the longer time required to complete their cycle, which may help in the development of control strategies. However, lowering the metabolism to enter in diapause or temporary hibernation in low temperature conditions may be a survival strategy of insects (GALLO et al., 2002).

Cassava is cultivated in a wide range of temperatures and the optimum range is 20°C to 27°C (SÔNEGO & MASSIGNAN, 2018). Average temperatures of about 15°C, a typical condition in Southern Brazil, delay germination and can paralyze the vegetative activity, causing dormancy (NUNES, 2018; SÔNEGO & MASSIGNAN, 2018). In Santa Catarina, according to the agroecological zoning, the period from July to October is the most suitable for cassava cultivation.

This implies the need to store branches for about two months in some regions to avoid losses in propagating material due to frost or water deficit soon after planting (SÔNEGO & MASSIGNAN, 2018). Considering that cassava is cultivated from July to October in Santa Catarina, the most important period to monitor and control the pest is the first four months after planting. Gisloti & Prado (2011) reported preferential infestation of younger plants by immature shoot flies and Lourenção et al. (1996) corroborated these results, since pest damage after this period does not result in loss of propagating material or roots.

According to the GLM test, the temperature of 10°C best explains the relationship between temperature and the pupal period, as its average adult emergence time was 19.15 days. Thus, the temperature drop at the end of the productive cycle during off-season delays adult emergence, as flies remain in the pupal stage for a longer time.

On the other hand, according to our results, about 62% of pupae at 10°C emerged viable. During off-season, viable flies are able to survive if the temperature increases in crops left for two productive cycles, in stems stored in the crop area, or even in possible alternative host plants, which increases their population.

Therefore, shoot fly infestation in the field is related to temperature and during off-season, if conditions are favorable for their increase, these flies can cause more damage to cassava crops.

However, besides abiotic factors, we should consider biotic factors that can influence the population dynamics of insects, such as food availability, possibly existing host plants adjacent to cassava crops, and the presence of natural enemies (THOMAZINI, 2002). Therefore, in order to establish IPM programs for cassava crops in the region, future studies should consider these biotic factors, aiming to find alternative hosts and natural enemies.

Conclusions

- The temperature of 25°C was the most suitable for pupal development and provided a higher rate of viable adult cassava shoot flies;
- The temperature of 10°C increased the pupal period and at 30°C, this time was shorter. At both temperatures, the number of deformed adults was high;
- These results show that the survival of the cassava shoot fly is directly related to temperature and, thus, the colder the winter during off-season, the smaller the fly population in the next season.

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