ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 10, Issue, 09, pp. 40810-40815, September, 2020
https://doi.org/10.37118/ijdr.20053.09.2020

RESEARCH ARTICLE OPEN ACCESS

FIRST RECORD AND DAMAGE RATIO OF *Montella* sp. (COLEOPTERA: CURCULIONIDAE) IN VANILLA PLANTATIONS IN BAHIA, BRAZIL

Jéssica Pereira Jordão*[1]; Ariane Morgana Leal Soares[1]; Eduardo Gross[1]; Matheus Felipe Zazula[2], Miryan Denise Araujo Coracini[3] and Carla Fernanda Fávaro[1]

[1]State University of Santa Cruz, Ilhéus, Bahia, Brazil, [2]Federal University of Paraná, Curitiba, Paraná, Brazil, [3]State University of West Paraná, Cascavel, Paraná, Brazil

ARTICLE INFO

Article History:
Received 17th June 2020
Received in revised form
28th July 2020
Accepted 04th August 2020
Published online 30th September 2020

Key Words:
Vanilla planifolia, Vanila weevil, Field sampling, Economic damage.

Corresponding author:
Jéssica Pereira Jordão

ABSTRACT

The orchid *Vanilla planifolia* (Asparagales: Orchidaceae) is the best known and most economically important species of Orchidaceae in the world, and is widely used in the food, cosmetic and pharmaceutical industries. Commercial crops of this plantsuffer from constant attacks by beetles in southern Bahia, Brazil. This study reports the first occurrence of the weevil *Montella* sp. Bondar, 1948 (Coleoptera: Curculionidae) as a pest and describes and characterizes damage from this insect in commercial plantations. Larval weevils build galleries that interrupt the sap flow in plant stems and leaves. Adult weevils scrape and pierce the petals of the flowers, reducing production of the vanilla beans. The degree of severity was considered high because of the generalized attacks on the plant, with an average of 56% of plants attacked in the stands evaluated. The calculated LED (Level of Economic Damage) was 0.06 insect/plant for productivity interference. There was no difference between the two periods evaluated indicating the non-relationship between the time of sampling with the occurrence of damage. However, plants in the reproductive stage were less damaged by the insect when compared to those in the vegetative stage. These data can be useful for pest monitoring.

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Citation: ***Jéssica Pereira Jordão; Ariane Morgana Leal Soares; Eduardo Gross; Matheus Felipe Zazula, Miryan Denise Araujo Coracini and Carla Fernanda Fávaro. 2020.*** "First record and damage ratio of montellasp. (coleoptera: curculionidae) in vanilla plantations in Bahia, Brazil*", International Journal of Development Research*, 10, (09), 40810-40815.

INTRODUCTION

The genus *Vanilla* belongs to the family Orchidaceae, with about 110 species occurring in the Americas, Africa, Asia, and Oceania (Koch *et al*., 2013). The main producing countries are Indonesia, Madagascar, China, Mexico, and Tonga, which together produce approximately 95% of the world's commercial vanilla (FAO, 2013). The main species of the genus is *Vanilla planifolia* Andrews, which is cultivated commercially due to the high quality and concentration of vanillin in its fruits (Kalimuthu *et al*., 2006). In the state of Bahia, Brazil, there are commercial organic plantations of the *V. planifolia*, and the primary buyer market for this production is the state of São Paulo (Fraife *et al*., 2015). The difficulties in cultivation are due to the biotic and abiotic factors that limit production. In southern Bahia, among biotic factors, the absence of specific pollinator insects hampers the pollination, which is carried out manually by the farmer, but this process can damage reproductive structures and compromise the production (May *et al*., 2006). Surveys on insect pests associated with vanilla culture are scarce. Many species have been described in India and classified according to their severity of incidence, including members of the orders Lepidoptera, Hemiptera, and Orthoptera, and classified as medium to low severity in that order. Insects of the order Coleoptera, *Holotrichia serrata* (Fabricius, 1781) and *H. rufoflava* Brenske, 1894, both of the family Melolonthidae, were classified as high severity and may cause injury to the plant in all stages of the insect development (Vanitha *et al*., 2011). The cochineal *Conchaspis angraeci* Cockerell, 1893, is reported as a critical pest of *V. planifolia* on Réunion Island, causing damage by sucking the plant sap (Richard *et al*., 2003). Another prominent pest is the weevil *Cratopus retusa* Klug, 1832, belonging to the family Curculionidae, which bores into flowers, preventing formation of the pods (Farooqi *et al*., 2005). Beetles of the genus *Montella* (Coleoptera: Curculionidae: Baridinae) are found in orchids, especially during flowering. Studies with *Dichaea pendula* Congniaux, 1903 (Asparagales: Orchidaceae) have demonstrated that this insect is florivorous and a secondary pollinator, due to self-pollination by mating in the flower (Nunes *et al*., 2016). In orchids of *Phragmipedium sargentianum* Rolfe, 1896 (Asparagales: Orchidaceae), this beetle was reported only as a floral visitor (Hansen *et al*., 2012). In *Grobya amherstiae* Lindley, 1835 (Asparagales: Orchidaceae), *Montella* sp. was considered an influencer on reproduction through self-pollination (Mickeliunas *et al*., 2006). In Brazil, there are no reports of pest occurrence in vanilla crops. This study reports the first occurrence, describes the injuries, and characterizes the economic damage caused by insects of the genus *Montella* in commercial plantations of *V. planifolia* in southern Bahia.

MATERIALS AND METHODS

Occurrence of the insect pest: *Montella* sp. adults were collected in the field from August to October 2018, and from January to March 2019. The plantations were located on 11 properties in southern Bahia, Brazil, as shown in Figure 1. Each property was sampled twice, in two different months. The insects adults were collected manually, stored in plastic cages, taken to the laboratory at the State University of Santa Cruz (S 14°47’53.6” and W 39°10’19.1”), and reared under 28 ± 2ºC, photoperiod of 14L: 10D, and 70-80% relative humidity. The rearing chamber consisted of plastic boxes containing a 6cm layer of vermiculite to simulate soil and were fed with stems of vanilla plants, that were kept inside of a 500 mL beaker containing 100 mL water and 5g of Potato Dextrose Agar (PDA) medium. Specimens were sent for identification to Dr. Germano H. Rosado Neto, at the Federal University of Paraná, and to Dr. Sérgio A. Vanin, at University of São Paulo. All *V. planifolia* plants in each plantation were inspected to evaluate the injuries caused by the insect.

Description of injuries: The injuries caused by the larval stages of *Montella* sp. were estimated visually as a percentage, described, and photographed in loco for characterization. The farms where the insects were collected were grouped by stages of plant development: initial vegetative stage (3 farms) and reproductive stage flowering (8 farms). The plants were counted and those that showed signs of insect attacks were marked to determine the percentage of damage. The following criteria were evaluated: the presence of galleries in stems, leaves, and broad beans made by the larvae. Two evaluators independently estimated the severity of attacks for each area, using a scale (Table 1). The evaluations of the injuries observed contributed to describe the insect feeding characteristics on the host plants. Vegetative parts with the presence of galleries and larvae were investigated in the field, through their opening, and laboratory. Each area was inspected once a month to monitor the evolution of the lesions.

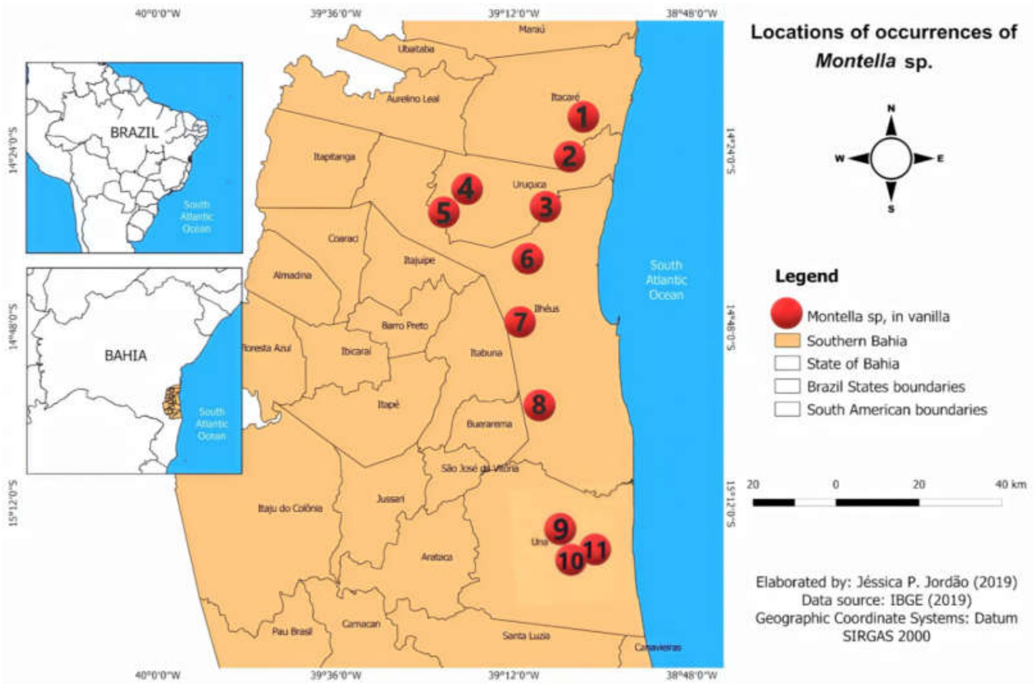

Figure 1. Distribution of the collection sites of *Montella* sp. Bondar, 1948(Coleoptera: Curculionidae) in areas of *Vanilla planifolia* cultivation, in southern Bahia state, Brazil

Table 1. Scale used to estimate the severity of damage, based on the presence of galleries, from larvae of *Montella* sp. Bondar, 1948 (Coleoptera: Curculionidae) in *Vanilla planifolia* on farms in southern Bahia

Scale	Damage level
1	no evidence of larval damage
2	up to 15% of leaves with galleries
3	up to 15% of leaves + stems with galleries
4	up to 15% of leaves + stems + beans with galleries
5	16 to 50% of leaves with galleries
6	16 to 50% of leaves + stems with galleries
7	16 to 50% of leaves + stems + beans with galleries
8	more than 50% of leaves with galleries
9	more than 50% of leaves + stems with galleries
10	more than 50% of leaves + stems + beans with galleries

Evaluation of economic damage: The Control Cost (CC), Productivity Ratio (PR), and Production Value (PV) of the *V. planifolia* plantings with larval damage were estimated in Brazilian currency, from the equations defined by Stone and Pedigo (1972). The CC was based on cultural control with manual collection of insects and removal of the infested parts of the plants. The field service data were collected, as well as the number of days required for capturing insects, on one farm during the year 2018 for one harvest. The PR was defined by the average data collected in this production. The PV was calculated using productivity data in Brazilian Reais (R$) per hectare, using equation (1):

- $PV = Pd \times Pu$

where: Pd = productivity (kg/ha) and Pu = unit price (R$/kg).

The average expected yield was determined for 1 hectare, with the Pd = 300 kg/ha and Pu = 3,300.00 R$/kg. The value of Pu was the average of prices quoted for the first months of 2019. The productivity losses were estimated in percentages for the Levels of Economic Damage (LED), according to Stone and Pedigo (1972), using equation (2):

- $LED = CC \times 100 / PV \times K$

where: CC = Control Cost (R$/ha), PV = Production Value (R$/ha), and K = coefficient of control efficiency (%).

For K, a value of 0.5, corresponding to 50% efficiency, was used because even with cultural control the pest resurgence occurs within a short period of time.

Statistical analysis: The results obtained from field samplings were organized in the form of relative frequency to calculate the similarity of the areas. The data grouping was analyzed using the Similarity Index, based on the Bray-Curtis distance coefficient, and applied in the ANOSIM statistical test, using the programs R version 4.0.0 (R Core Team, 2020), RStudio version 1.2.5033 (RStudio Team, 2019), and the Vegan package version 2.5.6 (Oksanen *et al*., 2019). The aim of the test was to analyze the distance matrix through the Non-Metric Multidimensional Scaling (NMDS), which performs the permutation of this matrix with the rank of the data, generating values of the categorical variables at random. The R statistic is applied to do test relation, where values closer to 0 lead to the interpretation of randomness of the clusters, and values closer to 1 lead to the interpretation of nonrandomity of the clusters (Warton *et al*., 2012). The average of the frequencies between the two times was used for the clustering of the dissimilarities of the observations (Zhang *et al*., 2017).

RESULTS

The adult of *Montella* sp. is uniformly black and measures approximately 3 to 5 mm long (Figure 2). The head projects past the front of the body due to the elongated face. The larva has a chitinized head and a milky-white curved body. Larvae and adults of the insect were found on all 11 farms evaluated. Although the larvae are endophytic, their damage to the plants was apparent. The presence of the larvae in the field was revealed by characteristic damage, such as: galleries on the stem followed by drying of the lesions, yellowing near the predation site, and absence of the apical bud.

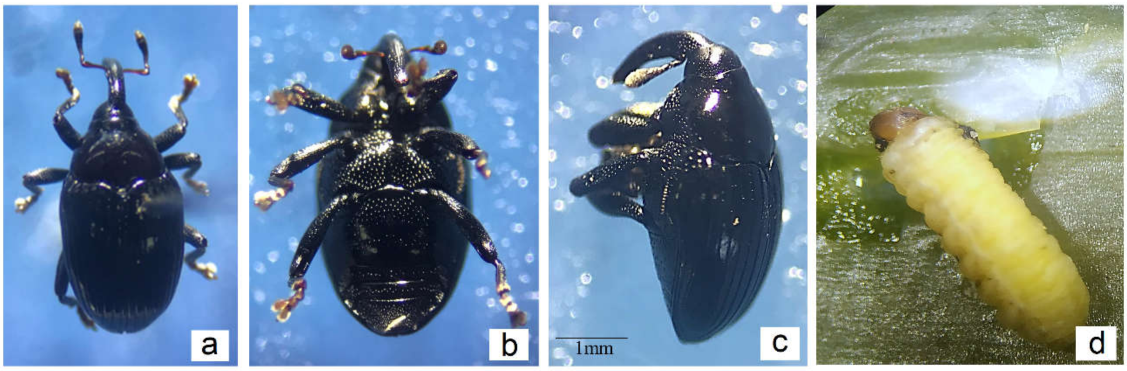

Figure 2. (a) Dorsal, (b) ventral, (c) lateral view of the adult, and (d) larva of *Montella* sp. Bondar, 1948 (Coleoptera: Curculionidae).

Table 2. Degree of severity of attack by *Montella* sp. Bondar, 1948(Coleoptera: Curculionidae)in *Vanilla planifolia*, determined by visual inspection of plants in vegetative (VS) and reproductive (RS) stages on farms in southern Bahia

Farms	Stage	Total plants	Score									
			1	2	3	4	5	6	7	8	9	10
1	RS	500	492	-	-	-	-	-	-	-	8	-
2	RS	300	200	-	58	-	-	-	-	-	30	12
3	VS	155	-	-	-	-	-	-	-	-	155	-
4	VS	200	120	-	-	-	-	-	-	-	80	-
5	RS	400	132	-	197	-	-	-	-	-	67	4
6	VS	380	298	-	-	-	-	-	-	-	82	-
7	RS	676	662	-	-	-	-	-	-	-	14	-
8	RS	1076	715	-	120	-	11	-	-	-	217	13
9	RS	744	596	-	24	-	-	-	-	-	148	2
10	RS	360	287	-	23	-	-	-	-	-	50	-
11	RS	500	297	-	47	-	-	52	-	46	57	1

Source: survey data. Score: 1 = no evidence of beetle attack; 2 = up to 15% of leaves with galleries; 3 = up to 15% of leaves + stems with galleries; 4 = up to 15% of leaves + stems + beans with galleries; 5 = 16 to 50% of leaves with galleries; 6 = 16 to 50% of leaves + stems with galleries; 7 = 16 to 50% of leaves + stems + beans with galleries; 8 = more than 50% of leaves with galleries; 9 = more than 50% of leaves + stems with galleries; and 10 = more than 50% of leaves + stems + beans with galleries.

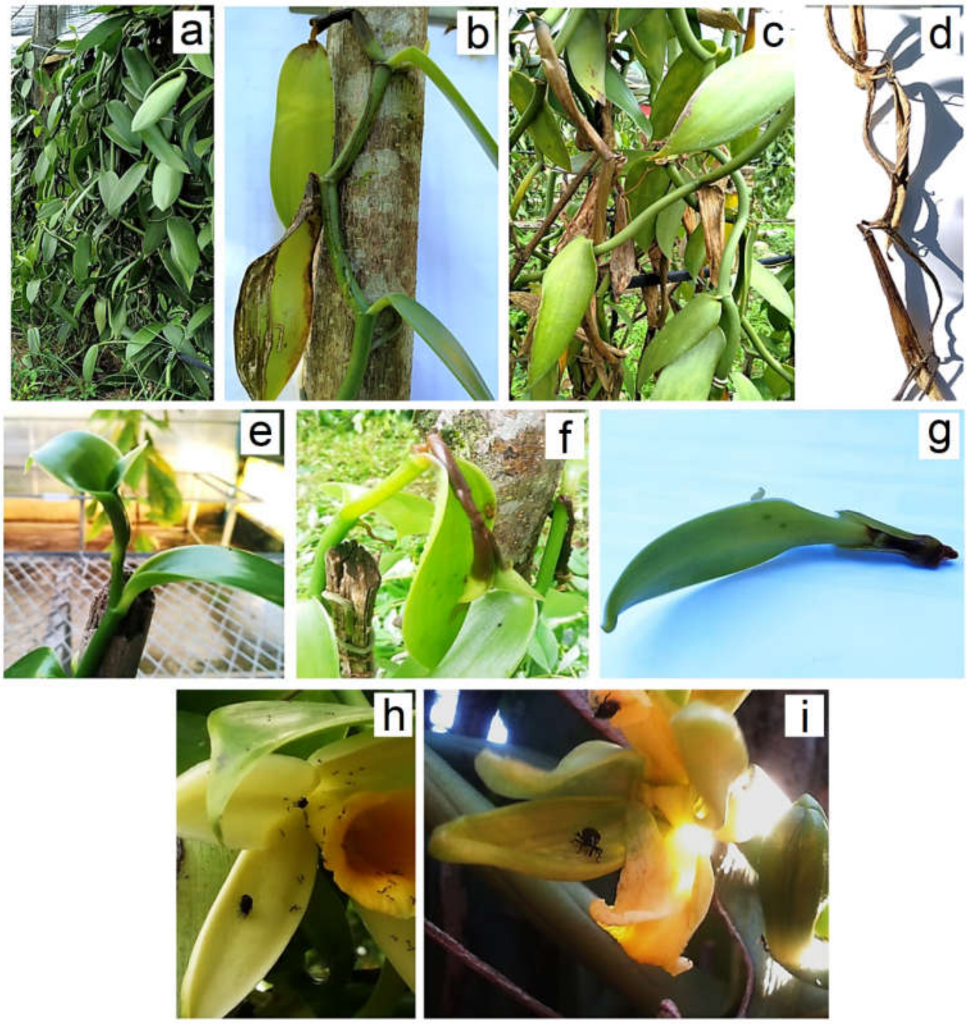

Figure 3. Injuries caused by *Montella* sp. Bondar, 1948(Coleoptera: Curculionidae) in *Vanilla planifolia*. (a), (b), (c), (d) Increasing levels of attack on stems and leaves; (e) Healthy apical tip; (f) Damaged apical shoot tip; (g) Apical bud abortion; (h) Healthy flower; (i) Damaged flower

These characteristics helped to determine the severity of herbivory in plants. The severity of plant damage differed between the reproductive and vegetative stages (Table 2). All plants in the reproductive stage received score 9, with injuries in more than 50% of the leaves and stems with galleries. Of the eight properties with reproductive plants, six were scored as 3, with up to 15% of the plants with damaged leaves and stems with galleries. On average, 43% of plants in the vegetative stage and 13% of plants in the reproductive stage were attacked. Young plants had more injuries, because the plants have a softer structure and tissues in the primary development (vegetative) phase. Farms with well-grown plants also sustained considerable damage. Plants with established structure and development are more able to recover from injuries and can produce new apical buds and heal the galleries, restarting growth. Damage by larvae was characterized by opening the galleries in the plants. When the larvae consumed the stem, the sap flow was interrupted, causing necrosis of the constituent tissues. In the leaves, these galleries reduced the photosynthetic leaf area. In some cases of intense attack, young plants were killed by obstruction of conducting vessels and tissue deterioration. Adult weevils scraped and pierced the apical buds and flowers (Figure 3). Injuries to flowers caused by perforations in the superficial tissues of the petals, causing irregularities in the development of the pods, were observed. In severe cases of attack, the flowers aborted, affecting productivity.

During the inspections, 156 beetles were collected on one farm. A decrease in the beetle population was observed during the period with high temperatures. The species *Montella* sp. is diurnal. The beetles agglomerate mainly between 06:00 and 08:00 a.m., when the flower buds open. Insects observed in the field were most active between 07:00 and 10:00 a.m. After this time, the beetles were no longer seen probably because of the high temperatures. During the flowering period, adults were found grouped within the flowers, probably for protection and for mating. When assessing the distribution of similarities (Figure 4), it was observed that most of the data show dissimilarities less than 0.4, indicating arbitrariness in the clusters ($R2 = 0.997$ NMDS). Dissimilarity index values lower than 0.4 indicate that the 11 properties are similar, which means that the frequency of damage by *Montella* sp. in all properties is similar. After calculating the dissimilarity index, isolated parameters were selected to be compared between the properties. Analyzing the groupings according to the farms, it is noted that the grouping value is ordered (Figure 5), thus, the occurrence of damage found on the farms, regardless of the period of the year or the life stage of the plants, is significant. ($R = 0.6686$; $p < 0.001$; Per = 9999). Taking into account the life stage of the plants it was possible to observe that a less orderly grouping occurred ($R = 0.3665$; $p = 0.0137$; Per = 9999), showing that the life stage of the plants did not strongly influenced the occurrence of damage. Moreover, it was verified that the plants in the reproductive stage were less damaged by the insect when compared to those in the vegetative stage.

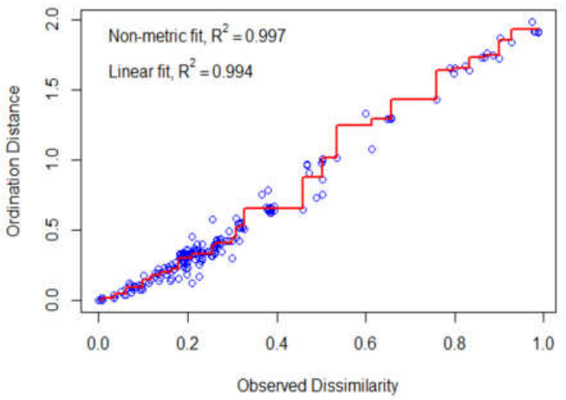

Figure 4. Stressplot of the Distribution of the Dissimilarity Distances of the observations. Values less than 0.4 mean non-randomness of the observations

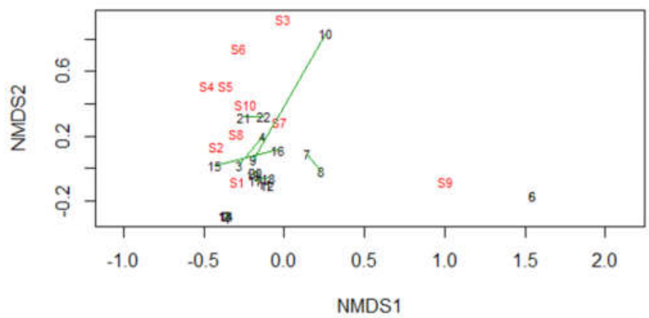

Figure 5. NMDS plot of *Montella* sp. damage in *Vanilla planifolia* in all properties. Values less than 0.4 mean non-randomness of the observations

When evaluating the time, there was no difference between the two periods evaluated (R = 0.006; p = 0.4998; Per = 9999), indicating the non-relationship between the time of sampling with the occurrence of damage. Therefore, only the average values of *Montella* sp. damage of the 11 properties were taken into account for the rank of similarities, through Pearson's correlation (t = 3.3091; df = 53; p <0.001; color = 0.4138) by the method nearest neighbor (Figure 6). The ranking showed higher similarity in damage caused by *Montella* sp. in six properties (11, 8, 7, 3, 9 and 4). The lower similarity was found in properties 10 and 5.

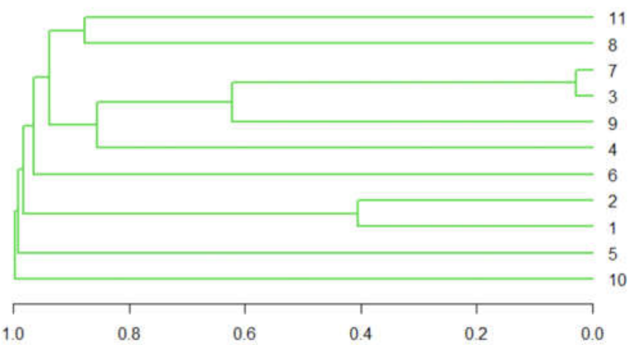

Figure 6. Dendogram of the Property Grouping in relation to the Dissimilarities of occurrence of *Montella* sp. damage in the two sampling periods

The economic damage caused by *Montella* sp. was calculated, and the value obtained for the cost of control (CC) was R$ 308.00/ha. The values were estimated based on the control procedures used on the farms. The productivity value (PV) reached R$ 990,000/ha. The level of economic damage calculated for *Montella* sp. in vanilla by the percentage of losses in productivity (LED) corresponded to 0.06 beetles/plant. To minimize the persistence and permanence of the larvae in the field, the infested plant parts were pruned and burned. Similar damage is commonly found for several species belonging to the family Curculionidae (Toksoz *et al*., 2017).

DISCUSSION

This is the first evidence of *Montella* sp. as a potential pest in orchids of the genus *Vanilla* or other commercial crops. This beetle is described as a floral visitor and colonizer of orchids native to the Atlantic Forest (Hansen *et al*., 2012; Lima, 1940). A research of insects in Brazil by Casey (1922) recorded 13 tribes of Baridinae. Years later, Bondar, after a study in Bahia state, referred to the subfamily Baridinae as a future threat for attacks on palm trees and grasses (Costa Lima, 1956). However, there are currently no records of the occurrence, description, or identification of economic damage caused to commercial cultivars in Brazil. The damage caused to *Vanilla planifolia* by *Montella* sp. is considered severe because the attacks are widespread, affecting the stems, leaves, and flowers. The vast majority of members of the family Curculionidae are pests of important farm crops and are borers, constructing galleries in their host plants. Other commercially important insect pests that cause significant damage by opening galleries are *Metamasius hemipterus*, *Heillipus catagraphus*, *Cosmopolites sordidus*, *Rhinostomus barbirostris*, and *Rhynchophorus palmarum* (Ferreira, 2008). Attacked and weakened plants become targets for colonization by other pests and diseases. In Mexico, the baridinid *Peltophorus adustus* was reported causing injuries in the seeds of *Agave vivipara* L. and *A. cupreata* Trel. & Berger in commercial plantations (Figueroa-Castro *et al*., 2016).

The phenological stages were indicative of weakening of the plant by the beetle attack. Plants in vegetative stages were more susceptible to attack than plants in the reproductive stage. However, the loss of productivity is highest at flowering; floral abortion is the most significant economic damage, since a pod will no longer be produced. Energy loss from healing attacked tissues delays development, with subsequent delays in production (Gayler *et al*., 2004). In the two phenological periods, numerous plants had over 50% of their structures affected due to the formation of galleries, showing the potential of *Montella* sp. as a pest and requiring its control in plantations of *V. planifolia*. There are no control methods or registered insecticides in the Ministério da Agricultura, Pecuária e Abastecimento (MAPA, Brazil) for *Montella* sp. in vanilla, and cultural control is presently the most viable alternative. Determination of the levels of economic damage is essential to accurately describe the effect of attack intensity on productivity, but this is difficult to achieve in field conditions (Hao *et al*., 2002; Higley and Pedigo, 1996). The LED of 0.06 insects/plant demonstrates the need for efficient controls methods. According to the high degree of severity recorded on most properties, constant attacks sharply reduce productivity. The simulation of LED = 1 insect/plant can cause losses up to 30% in the production value. Vanilla crops in southern Bahia require 60 to 70% shade. This partial blocking of luminosity lowers the temperature, providing a favorable environment for *Montella* sp. activity. The biological development of insects, i.e., growth, feeding, oviposition, number of annual generations, and behavior are dependent on abiotic factors (Speight *et al*., 1999), which can be used to predict potential changes in population dynamics of *Montella* sp. This contribution reports the first occurrence, describes the injuries, and characterizes the economic damage caused by insects of the genus *Montella* in commercial plantations of *Vanilla planifolia* in southern Bahia, Brazil. This is the first report of *Montella* sp. as an agricultural pest, and also of the occurrence of insect pests in vanilla plantations in Brazil. This information will enable development of control methods for this weevil.

Acknowledgments

We thank the Coordination for Improvement of Higher Education Personnel (CAPES) and the National Institute of Science and Technology (INCT) Semiochemicals in Agriculture (CNPq Process 465511/2014-7 and FAPESP Process 2014/50871-0) for supporting this research. We also thank Dr. Germano H. Rosado Neto from the Federal University of Paraná and Dr. Sérgio A. Vanin from the University of São Paulo for identifying the genus of the weevils.

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