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Feeding preference of *Cladomorphusphyllinus*Gray, 1835 (Phasmida: Phasmatidae) by four species of Myrtaceae under laboratory conditions

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ABSTRACT

The aim of this study was to evaluate and define the feeding preference of *Cladomorphusphyllinus* (stick insect) by four different species of myrtaceae under laboratory conditions, in order to obtain feedingalternatives for maintaining Phasmidaerearing for academic initiation of students in forest entomology. The experiment was carried out at the Forest Protection Laboratory of the Federal University of Paraná - UFPR, where both tests were set with and without the choice for plant species. The following types of myrtaceaswere used in the test: *Psidiumguajava* (Guava), *Psidiumcattleianum* (Araça), *Eucalyptus dunni* (Eucalyptus) and *Eugenia uniflora*(Brazilian cherry). For the test on which the plant species could not be chosen, the number of insects used per replicate was five and they were transferred to cages containing a water bottle and the branches of a single species within the bottle. For the test in which plant species could be chosen, the number of insects used was 15 per replicate, and they were placed into cages containing four bottles with a type of each plant species. Just after harvesting the branches, they were weighed and placed inside the cage and this procedure was repeated every three days during the test to evaluate the amount, in grams, of plants consumed. The insect consumed all Myrtaceae species, preferring *P. guajava* in tests with and without chance of choice. Among the tested species the most indicated for the rearing maintenance was *P. guajava*. The other species were also consumed and can be used as an alternative for rearing maintenance, in the following order of preference: Araça; Cherry; Eucalyptus.

INTRODUCTION

The insects known as stickinsects belong to Phasmidaorderand they use mimicry to protect themselves from predators, resembling sticks or dry twigs (COSTA LIMA, 1938). Females are larger than males and famales are 220 mm in length: this difference is related to laying eggs which are relatively large; males have wings while the female is wingless (Costa Lima, 1938; Sottorriva*et al*., 2008). Besides, laboratory insect rearing is a strategic activity for the knowledge of bioecology of pest-insects, because under these conditions it is possible to study in detail biological and ecological aspects of the pests when they would not be occurring on their natural environment, and by this knowledge the control tools can be planned in a more efficient way and with less environmental risks. Therefore, the study and development of natural or artificial diets are fundamental in order to maintain laboratory rearing.

With this in mind, the Forest Protection Laboratory of the Forest Engineering Graduation Course of the Federal University of Paraná (Brazil) has maintained, for many years, insect rearing for research purposes as well as for academic initiation of forest entomology students. Scientific rearing involve pest insects known as

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the *Atta* and *Acromyrmex* ants, which are used in tests with control alternative that are later replied in field. Didactic rearing have the aim of arousing the interest for forest entomology by studying insects which are not difficult to manipulate, since they consolidate biological and ecological concepts related to insects, for further replicating these on scientific rearing.

Rearing the stick insect (Phasmida) for academic initiation purposes shows the Best results because these insects are great in size, have a long life cycle and call the attention by its mimicry and sexual dimorphism, with distinct and well defined phases, and this fact arouses the students' interest for scientific activity in forest entomology.

According toDorval*et al*. (2003), in the Order Phasmatodea all species present phytophagous feeding habits. Insects are considered as generalist herbivores, though empirical studies on the diet of these populations are scarce (Whitingh*et al*,. 2003; Willig*et al*., 1993; Sandlin and Willig, 1993).

In Brazil, among the various species of stick insect, *Cladomorphusphyllinus* Gray, 1835, is one of the largest,they present monphagous habits and can feed on species such as *Psidiumguajava, Mangiferaindica* and *Ficus* sp. (Costa Lima, 1938;Sottorriva*et al*., 2008; VARGAS *et al*., 2008).

The Forest Protection Laboratory rearing insects are fed with leaves of Guava (*Psidiumguajava*), but these in the winter season are scarce and difficult to maintain the insects feeding. Facing this difficulty arouse the need to find alternative food sources for the rearing maintenance. This challenge together with the aim of arousing interest of graduation students for forest entomology led to the experiments of this study, which aimed to evaluate the feedingbehavior of *Cladomorphusphyllinus* (stick insect) submitted to feeding with four species of myrtaceas, in tests with and without choice under laboratory conditions.

MATERIAL AND METHODS

This experiment was assembled on the Forest Protection Laboratory of Federal University of Parana in the city of Curitiba, state of Paraná. Inside the laboratory, *Cladomorphusphyllinus*(Stick-insects) are reared to be used on experiments, as the adult insects of the species that were used on tests.

Two tests were assembled, with and without choice of plant species by the insect, using many kinds of plant species of Myrtaceae family (Table 1).

Table 1: Myrtaceas species used on the tests with and without choice

Treatment	Scientific Name	Common Name
1	*Psidiumguajava*, Linn	Guava
2	*Psidiumcattleianum*, Sabine	Strawberry guava
3	*Eucaliptusdunni*, Maiden	Eucalyptus
4	*Eugenia uniflora*, O. Berg	Surinam cherry

For the no-choice experiment, there were used five insects per replicate, from which three were females and two were males, of proportional size on both sexes. They were transferred to cages of 0.90 x 0.50 x 0.50 m in size, containing a water reservoir and branches of the plant species, set individually so that the insects could not choose nother species. For the free-choice experiment, there were used 15 insects per replicate, from which eight were females and seven were males, and they were placed in cages of 1.70 x 0.80 x 0.80 m in size, containing four bottles with water and a plant branch, and each pot had a different type of plant species, allowing the insect to choose its preferred species. The position in which the plant pots were placed inside the cages was modified on every new reading, avoiding certain species to be always at the same position inside the cage.

Firstly, the free choice test was assembled with four readings lasting 3 days each, and after their ending, the no-choicetestwasassembled, also with four readings, for each test, every three days, and so the experiment lasted 24 days.

The branches were collecteddirectlyfromtreesthere are locatedinsidethe campus Jardim Botanico, of UFPR, with the aid of a trimmer. These branches with their leaveswere weighed before they were placed into cages,and in each pot 100 grams of branches with the leaves were put to all the species, in all four readings, and every three days they were removed and weighed again, and the difference between the initial and final weight of the branches with leaves was determined as being the quantity of leaves consumed by the insect during the three days when the branches with leaves were exposed to insects in every reading, similar to the methodology postulated by Vargas (2009), who fed nymphs of *C. phyllinus*and swapped the branches every five days. Bythedifference in theweightofthebranches, it wasmeasuredthequantityofleavesofeachspeciesconsumedbyinsectsduringthe gaps ofreadingthatwereestablished.

The no-choice experiment was assembled with a completely randomized design with four treatments and four replicates using the completely at random design. Therefore, for the free choice experiment, a randomized block design was used, with four treatments and four blocks, using the blocks at random design, due to the greater quantity of insects inside each cage and to the size of the cages used.In ordertoestablishwhethertherewas a significantdifference in theconsumption rates ofleavesbytheinsects in bothstages, the data weresubjectedtoanalysisofvariance (ANOVA) andmeanswerecomparedusingtheTukeytestat 5% probability. The amountofleavesthateachinsectconsumed in a threedays gap and per daywasalsoestablished. It wasalsodeterminedhowwastheconsumptionofleavesbyinsectswithandwithoutchoice over the gaps ofreading.

RESULTS AND DISCUSSION

a) For the test in which the plant species could be chosen:

According to the resultsshownbytheexperiments in whichtheplantspeciescouldbechosenbytheinsects, thespecies*C. phyllinus*foodpreferencewas for Guavaleaves, comparedtootherspecies, buttheinsectwasalsoabletofeedregularlybyeatingStrawberryguava, EucalyptusandSurinam cherry, however, in smalleramounts. There was no statistical difference among the blocks,in all blocks the preferences and quantity of leaves consumed by the insects were similar, nevertheless, there was a significant difference between theconsumption rates of leaves in different species, On Table 2, there is the quantity of leaves of every species consumed by the insects from the initial 100 grams put in every pot, along the four readings, with a three days interval.Even changing the position of species inside the cage, insects preferred Guava leaves, no matter its position inside the cage.

Table 2: Consumption quantity in gramsofMyrtaceaes leaves by *C. phyllinus*, with chance of choice, in four gaps of reading, at a three days interval.

Species	Readings (grams)			
	1st Reading	2nd Reading	3rd Reading	4th Reading
Guava	12,31 a	14,33 a	12,82 a	13,31 a
Strawberryguava	5,72 b	7,53 b	6,92 b	8,77 b
Eucalyptus	3,86 b	3,95 c	4,27 b	4,94 c
Surinam cherry	3,61 b	3,25 c	3,87 b	3,15 c
F treatments	26,19**	56,96**	20,81**	55,93**
F blocks	$0,50^{ns}$	$1,32^{ns}$	$0,18^{ns}$	$1,41^{ns}$
DMS	3,51	2,96	4,0	2,66
CV(%)	24,9	18,47	25,94	15,96

The means that are followed by the same letter in columns do not present any difference between them, at level 5% of probability by the Tukey's test.
Note: **meaningful at 1% probability ($p < .01$).
ns: not meaningful ($p >= .05$).

It is clear that the consumption of Guava leaves was higher than all other species in all readings, and Strawberry guava was the second species most chosen by the insects. The preference presented for these two species (Guava and Strawberry guava) could be explained by the fact their leaves remained preserved for a longer period, becoming more turgid than others. The same observation was highlighted by Sottorriva*et al*. (2008), in a comparison between the consumption of Guava (*Psidiumguajava*), Strawberry guava (*Psidiumfirmum*), Mulberry (*Moruscelsa*) and Crisp lettuce (*Lactuca sativa*) leaves by this insect.

The higher preference for Guava in comparison to Strawberry guava, considering that leaves of both species tend to remain more turgid and both belong to the same genus, could be explained by particular characteristics of their leaves' formation. According to Sottorriva*et al*. (2008), one of the greater differences between the two plant species is the presence of hairs on Strawberry guava trees and absence of them on Guava. This may be the main reason the stick-insects feed on Strawberry guava less than on Guava. Parra*et al*. (1991 and 2001) highlights that the quality of the food depends on physical attributes such as: stiffness, surfacepilosity, shapeandothers, whichinfluencetheabilityoftheinsectto consume anddigestfood.

EucalyptusalongwithSurinam cherry hadthelowest rates ofconsumption, appearingtobetheleastpreferredspecies, andshowingthatmaybethey do notpresentthenecessaryconditionsto complete thedevelopmentoftheinsect. Ferrera*et al*. (2007) reportedthatEucalyptusleaves are notsuitabletosupply*C. phyllinus*, sincethereis a 100% ofmortalityof males whenfedontheseplantspeciesonly. In females, it wasobserved a reduction in longevity as well as in theamountandmaturityoftheeggs.

Analyzingtheconsumptionbehaviorofleavesalongthe gaps ofreading, therecanbenoticedthatwhensuppliedsimultaneouslywithallspecies, insectskept a similar rate ofconsumption for allreadings, showinglittlevariation in consumptionwhenspecieswereanalyzedseparately in thesame test. In otherwords, in allreadings, theinsectsconsumed similar rates ofleaves in allspecies, as shown in Figure 1.

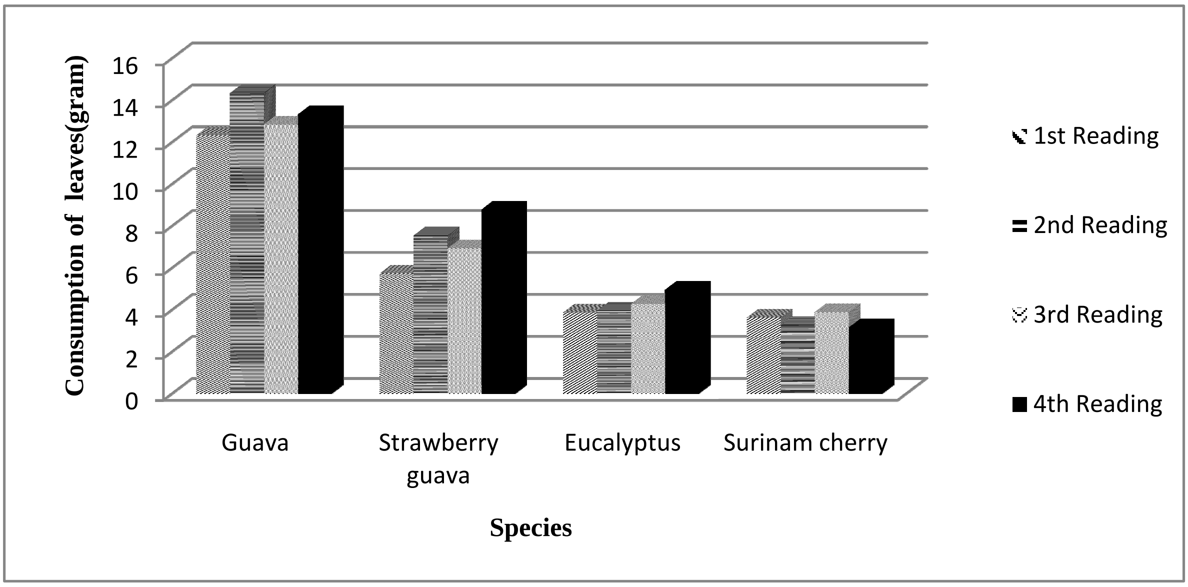

Fig. 1: Consumption in gramsof Myrtaceas leaves by *C. phyllinus*, along four gaps of reading,with chance of choice.

b) For the test in which the plant species could not be chosen:

At this stage, the species were offered separately to the insects, being the only available food for their consumption.It is noticed that the consumption of Guava leaves was higher than other species, in all lectures, except for the first reading, in which Strawberry guava also had a similar consumption.

When *C. phyllinus*had no chance offoodchoice, alsoshowedfeedingpreference for Guavaleaves in relationtotheotherplantspeciesthatwereused, however, theinsectwasabletofeedregularlyonStrawberryguava, EucalyptusandSurinam cherry, but in smallerquantities, as occured in thestepwhentheplantspeciescouldbechosen. There was significant difference the different consumption rates of leaves of different species, there was a significant difference On Table 3, there is the quantity of leaves of every species consumed by the insects from the initial 100 grams put in each pot, along the four readings, with a three days interval each.

Table 3: Consumption quantity in gramsof Myrtaceaes leaves by *C. phyllinus*, when plant species could not be chosen, in four gaps of reading, at a three days interval.

Species	Readings (g)			
	1st Reading	2nd Reading	3rd Reading	4th Reading
Guava	16,61 a	21,99 a	33,82 a	14,56 a
Strawberry guava	15,73 a	14,76 b	9,18 b	8,75 b
Eucalyptus	5,12 b	8,85 bc	10.94 b	6,5 b
Surinam cherry	5,58 b	5,29 c	4,71 b	5,25 b
Ftreatments	10,77**	21,49**	38,79**	13,14**
DMS	8,00	6.62	8,79	4,79
CV(%)	35,41	24,78	28,54	25,95

The means that are followed by the same letter in columns do not present any difference between them, at level 5% of probability by the Tukey's test.
Note: **meaningful at 1% probability ($p < .01$).

The insectsfedregularlyonStrawberryguava, EucalyptusandSurinam cherry duringtheexperiment, but in smallerproportionsthanonGuava. For thesethreespecies, therewere no differences in consumption, except in thesecondreadingwhenSurinam cherry got a lower rate thantheothers.

Regardingtheanalysisoftheconsumptionbehaviorofleaves over the gaps ofreading, it wasclearthatwhenthespecieswereprovidedseparately, theinsectsshowedlargevariations in theconsumption rates ofleaves in four readings, except for Surinam cherry, whichshowed a more uniformconsumptionthanothersatdifferentreadings as shown in Figure 2.

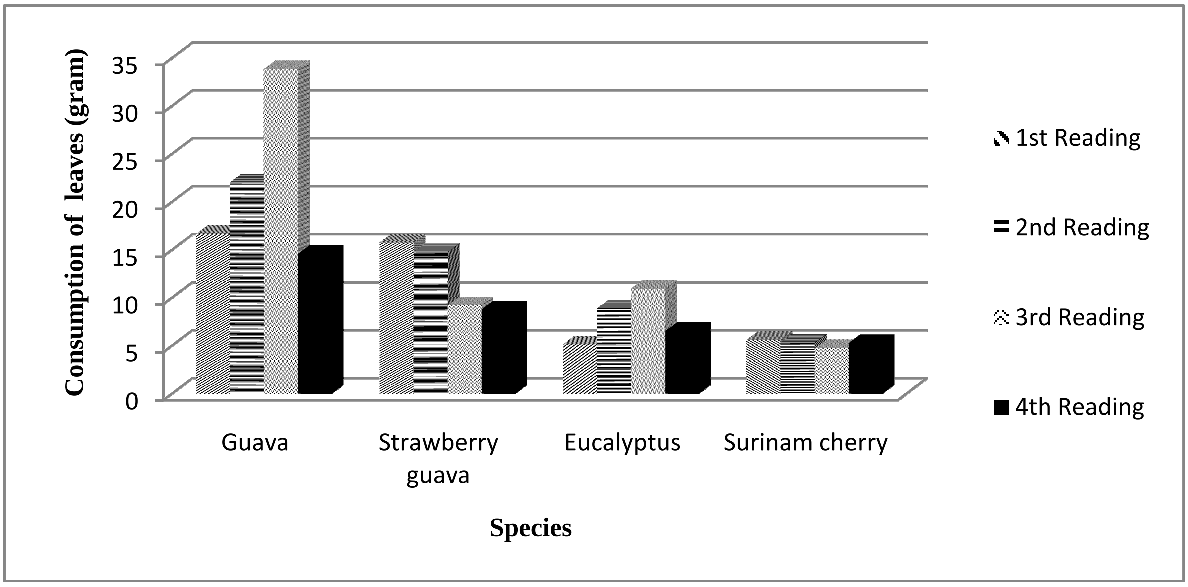

Fig. 2: Consumption of Myrtaceas leaves by *C. phyllinus*, along four gaps of reading, when plant species could not be chosen freely.

c) Comparison Between Both Tests (with and without choice of plant species):

It isclear in bothteststhatall four specieswereconsumedby*C. phyllinus*. Thisfactdiffersfromtheworkpresentedby Vargas,(2009)whoverifiedthatthisinsectdidnot consume Surinam cherry and Mango leaveswhenexposedto it altogetherwithGuavaleaves. Sottorriva*et al*.(2008)alsopointedthatMulberry*(Morus celsa)*andLettuce*(Lactuca sativa)*leaves, werenotattractivetotheseinsectswhenofferedwithGuava.

Nevertheless, otherstudieshaveshowntheconsumptionofotherplantspeciesbytheinsect. Sottorriva*et al*.(2008)reportedthatStrawberryguava*(Psidiumfirmum)* leavescanalsofeed*C. Phyllinus*becausewhentheseleaveswereprovidedalongwithGuava, theconsumptionreached 25% oftheleafarea. CostaLima(1938), alsoreportsthat*Ficus*sp. Leavescanbeofferedtotheseinsects. Butnotonlytheconsumptionlevelsshouldbeusedtoindicatethe ideal food for insectspecies.

Despitethefactallplantspecieswereconsumed, lower rates ofconsumption, in bothtests, ofSurinam cherry andEucalyptuscouldbenoticed, provingthey are lessatractivetotherthestick-insects. AccordingtoWaldbauerandFriedman (1991), thefoodprovidedtoinsects in laboratoryrearingshouldbethemostpreferredbythespecies, andtheysouldalsoprovidethecorrectnutrientsandconsequently a betterdevelopment. The authoralsoreportsthat it isnecessarythat a certainenergythresholdisreached, sothatthe processes ofdevelopmentoftheinsect as survival, fecundity, longevity, movement, andotherscanoccurnormally, andthisthresholdenergyisobtainedthroughfood. The smallintakeoftheseplantspeciesmaynotbeenoughtoprovidethepossibility for theinsectto complete its lifecyclewithquality.

In bothtests it wasevidentthehigherconsumptionofGuavaleavesby*C. phyllinus*in relationtootherspecies, confirmingotherstudies (Sottorriva*et al*., 2008; Vargas, 2009). Besidesbeingthepreferredfood, Guavaleaves proves tobethefoodthatprovidetheinsects a betterdevelopment. Vargas(2009) observedthatwhenthisinsectfedonleavesofGuava, theinsecthad a betterdevelopment, andalso a higher chance ofsurvival, in comparisontothosefedwithleavesof Mango andSurinam cherry tree, reachingupto 93.3% ofsurvivalattheendofthenymphalstage. Ferrera(2007) alsofoundthatthenumber, sizeandweightoftheeggsof*C. phyllinus*werehigherwhenfedonGuavaleaves.

Conclusion:

Based on the analysisit can be concluded that when the food was offered without choice the consumption tends to be greater than when more than one species were provided at the same time. On both tests (with and without choice), the species *Psidiumguajava* (guava) was the most consumed, being the most recommended for *C. phyllinum* laboratory rearing. During the winter, in times of scarcity of *P. guajava* leaves *C. phyllinum*rearing can be maintained with *Psidiumcattleianum* leaves and in their absence with Surinam cherry (*Eugenia uniflora*)leaves, about Eucalyptus (*Eucalyptus dunni*) leaves, these should only be used for rearing when there is no other option, because they were the least consumed by insects.

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