SOCIEDADE BRASILEIRA DE ENTOMOLOGIA
FUNDADA EM 1937

REVISTA BRASILEIRA DE
Entomologia
A Journal on Insect Diversity and Evolution

Occurrence of *Heilipus draco* (Coleoptera: Curculionidae) in seeds of *Ocotea puberula* (Lauraceae) and its influence on germination

Marciane Danniela Fleck Pessotto[1*], Ervandil Corrêa Costa[1], Suelen Carpenedo Aimi[2], Maristela Machado Araujo[2], Karine Schoeninger[3], Iyanassô Andrade Haag dos Santos[1], Mateus Alves Saldanha[1], Jardel Boscardin[4]

[1]*Universidade Federal de Santa Maria (UFSM), Departamento de Defesa Fitossanitária, Santa Maria, RS, Brasil.*
[2]*Universidade Federal de Santa Maria (UFSM), Departamento de Ciências Florestais, Santa Maria, RS, Brasil.*
[3]*Instituto Nacional de Pesquisas da Amazônia (INPA), Coordenação de Pesquisas em Entomologia, Manaus, AM, Brasil.*
[4]*Universidade Federal de Uberlândia (UFU), Instituto de Ciências Agrárias, Monte Carmelo, MG, Brasil.*

ARTICLE INFO

Article history:
Received 25 January 2021
Accepted 19 April 2021
Available online 12 May 2021
Associate Editor: Ricardo Siqueira da Silva

Keywords:
Forest entomology
Parasitoid hymenopterans
Granivorous insects
Tri-trophic interactions.

ABSTRACT

This study aims to report the occurrence of *Heilipus draco* in seeds of *Ocotea puberula*, and to describe the main injuries caused to the seeds and the effects on germination, as well as the occurrence of parasitoid hymenopterans. To this end, fruits of seven trees were selected, in the extreme south of the Atlantic Forest, Brazil. Fruit collection was carried out weekly in the middle third of the canopy, forming lots, from the beginning of fruit formation until total dehiscence, for two years. In order to examine the damage caused by the granivorous insects inside the fruits, 100 fruits were sectioned taking into account the batch/year with the aid of a scalpel and analyzed using a binocular stereomicroscope. In the same way, 144 fruits from each lot/year were stored individually in transparent plates in order to verify the occurrence and identification of species of granivorous insects. The proof of the influence of the injury caused to the seeds by the granivorous insects was carried out by the germination test, with four replications of 25 seeds, comparing seeds with and without oviposition holes. The species *H. draco* was found to be associated with the fruits of *O. puberula*. The egg-layings are endophytic, carried out directly on the seed. Larval parasitism of Hymenoptera *Bracon*, *Omeganastatus*, *Scambus* and *Triapsis* was observed. It is concluded that the cycle from egg to adult takes place inside the seed of *O. puberula*, and the injuries caused by the larvae decrease germination.

Introduction

The species *Ocotea puberula* (Rich.) Nees (Lauraceae) popularly known as canela-guaicá is a native tree species that occurs throughout tropical and subtropical America, in almost all forest formations (Farago et al., 2005) being indicated for environmental recovery (Carvalho, 2002) and mixed plantations in degraded areas of permanent preservation (Lorenzi, 1998). In Brazil, the occurrence of this species extends from 14° South latitude in the State of Bahia to 31° in Rio Grande do Sul (Carvalho, 2002).

The tree is 10 to 15 meters tall and has a diameter of 20 to 60 cm at breast height (DBH) (Carvalho, 2003) with a globular crown and dense light green foliage (Marchiori, 1997). The trunk can be cylindrical or irregular, straight or slightly bent, and the shaft can reach up to 12 m in length (Carvalho, 2002).

* Corresponding author.
E-mail: marcianedanniela@gmail.com (M.D.F. Pessotto).

The flowers are small, with colors ranging from white to beige, grouped in dense axillary, multifloral and dioecious panicles, and the flower buds are greenish (Carvalho, 2002). The anthesis is diurnal, completing the event in the morning, and occasionally in the afternoon (Souza and Moscheta, 1999). The reproduction of the species is by allogamy or cross-fertilization (Carvalho, 2002).

The fruits of the species *O. puberula* are drupes that originate from perigynous flowers (Souza, 2006). At the beginning of the development, the floral parts still remain involving the young fruit, but they soon become senescent and fall. At this stage, only the reduced hypanthium remains, which together with the floral receptacle forms the cupule, a structure typical of the fruits of the Lauraceae family. The ripe fruits of the species are spherical, with a single seed, black glabrous and the cupule predominantly red (Souza and Moscheta, 2000).

The fruit is dispersed by zoochory, mainly by birds, which are attracted by the color of the fruits (Carvalho, 2002, 2003). The birds, when feeding

https://doi.org/10.1590/1806-9665-RBENT-2021-0008

on the fruits, can disperse the seeds over long distances such as the Rufous-bellied thrush, *Turdus rufiventris* Vieillot, 1818 (Pereira et al., 1995) and the Rusty-margined guan, *Penelope superciliares* Temminck, 1815 (Mikich, 2002). In addition to the birds, the plant provides resources for the entomofauna, as the flowers are visited by several small insects, belonging to the orders Lepidoptera, Diptera, Thysanoptera, Hemiptera and Hymenoptera, with the bee *Apis mellifera* Linnaeus, 1758 being the one that visits more frequently (Souza and Moscheta, 1999).

However, this interaction can be harmful to the plant, concerning germination, since species of the Lauraceae family commonly have their seeds damaged by Coleoptera of the family Curculionidae. These insects that feed on seeds are called granivorous insects. The granivory, represents a particular type of herbivory in which a mobile predator consumes a sessile prey (the seed) (Menalled and Liebman, 2008). Forest seeds, because they have a high nutrient content, favor the development of larvae of many species of granivorous insects. These insects can consume the whole embryo and the seed reserve, with only the integument remaining in some cases, and this can directly influence germination (Costa et al., 2018).

In Lauraceae seeds, there are records of damage caused by granivorous insects on pau-rosa *Aniba rosaeodora* Ducke in Brazil (Rosa and Ohashi, 1999; Vanin and Gaiger, 2005), canela-fogo *Cryptocarya aschersoniana* Mez (Almeida, 2001; Muxfeldt et al., 2012), canela-do-mato *Nectandra nitidula* Nees & Mart., canela-amarela *Nectandra lanceolata* Nees & Mart. ex. Nees, canela-fedida *Nectandra grandiflora* Nees & Mart. ex. Nees (Carvalho et al., 2009), Brazilian canela sassafras *Ocotea odorifera* (Vell.) Rohwer (Milanesi, 2008), maúba *Licaria mahuba* (A.Samp.) Kosterm., avocado *Persea americana* Mill. (Lima, 1956) and imbuia *Ocotea porosa* (Mez) L. Barroso (Cuaranhua, 2010).

For *O. puberula* the only record of a granivorous insect is *Heilipus* sp. (Coleoptera: Curculionidae) (Hirano, 2004). The genus *Heilipus*, originally described by Germar in 1824, comprises several species distributed in the Neotropical region (O'Brien and Wibner, 1982; Wibner and O'Brien, 1986). In Brazil, 28 species are currently known (Vanin et al., 2019).

Some studies show that the injury caused by insects, in seeds of Lauraceae, significantly reduces the germination. However, in most cases, injuries caused to the internal part of the seed cannot be seen externally, which makes it difficult to select healthy seeds for sowing (Carvalho et al., 2009). In this sense, studies directed at native Brazilian forest trees are important, mainly, in relation to granivorous insects, in order to verify which species of insects cause the injuries, the internal and external symptoms of these attacks in the seeds and, whether or not this damage harms their germination.

Thus, this work aims to report the occurrence of *Heilipus draco* in *Ocotea puberula* seeds, to describe the main injuries caused to the seeds and the effects on germination, as well as the parasitoid hymenopterans associated with it.

Materials and Methods

The fruits were collected in the municipality of Taquaruçu do Sul (27°23'48"S and 53°29'55"W), located in the Alto Uruguai region in the state of Rio Grande do Sul, Brazil. The climate in the region is classified as humid subtropical with hot summer (Cfa) because of its well-distributed rainfall throughout the year (Alvares et al., 2013).

Seven visually healthy trees, with a well-formed crown, were selected in forest fragments, which varied from 0.5 to 5 ha in area. *O. puberula* fruits were collected weekly from September to November (fruiting period), in two consecutive years (2014 and 2015). Twelve collections were carried out in 2014, beginning on September 13 and ending on November 30, and eleven collections were carried out in 2015, beginning on September 14 and ending on November 23. This allowed the collection of fruits at various stages of development, each collection being considered a batch of fruits.

In 2014, *O. puberula* fruits were collected directly from seven trees. After the fourth collection, four trees no longer bore fruit, while the others continued to bear fruit. Collections were completed when all the trees had no more fruit. In 2015, only three trees bore fruit throughout the evaluation period.

The fruits were collected with the aid of pole pruner in the middle third of the tree, and randomly in the canopy, with around 100 fruits being collected from each tree for laboratory tests. After collection, the fruits of all the trees were mixed with each other, forming the batch, regardless of their degree of ripeness.

From the batch 144 fruits were randomized per collection/year. They were placed in six transparent plates with dimensions of 8.0 x 12 x 1.5 cm, containing 24 cells each with 16 mm in diameter. These 24 cells contained filter paper, previously cut with a hollow punch and sterilized in a Pasteur oven for one hour at 150°C, to prevent the further proliferation of fungi. In total, 1,728 fruits were packaged in 2014 and 1,584 in 2015.

During the evaluation period, the containers remained in the laboratory, in ambient conditions (average temperature = 17.4 ºC and relative humidity = 80.5%). To avoid excessive drying of the fruits, 0.0015 mL of distilled water were applied weekly to the filter paper with a micropipette, according to the methodology adapted from Dorneles (2014). The emergence of adult insects was followed up weekly, for about three months. The emerged adult insects were removed from the plates and kept in Eppendorf microtubes containing 70% alcohol, for later quantification and assembly and to be sent for identification by specialists.

The insects of the order Coleoptera, family Curculionidae, subfamily Molytinae, were sent to Professor Germano Henrique Rosado Neto from the Federal University of Paraná (UFPR) for identification and deposited in the collection of the Department of Zoology (DZUP), at the Biological Sciences Sector of the Federal University of Paraná. Specimens of the order Hymenoptera were identified by the fifth author of the National Institute for Research in the Amazon (INPA).

Additionally, 100 fruits of each batch/year were sectioned for the evaluation of the damage caused by insects. The fruits were sectioned longitudinally with the aid of a scalpel. Subsequently, they were observed in a binocular stereo microscope, with a 4.2x magnification, for a better visualization of the injuries in the seed and, mainly, indications of the presence of insects, such as the occurrence of egg-laying. In the study 1,200 fruits were sectioned in 2014 and 1,100 fruits in 2015.

For the germination test, ripe fruits, with red cupule and black colored pericarp, were collected from four parent trees on November 20, 2016. To overcome physical dormancy, the fruits were manually processed with a sieve and running water and, with the aid of a scalpel to remove the integuments, when necessary (Vicente, 2014). After removal, *O. puberula* seeds were sterilized according to MAPA (2013), with five drops of neutral detergent in 100 mL of distilled water for a period of five minutes, and then rinsed with distilled water, until complete removal of the detergent. Subsequently, the seeds were left on paper towels for about 20 minutes to remove excess water.

The seeds were separated in two treatments, in order to verify if the damage caused by *H. draco* would influence the germination percentages: T1 - the seeds showing no signs of insects, being intact; T2 - the seeds showed indications of the presence of insects, with orifices caused by the oviposition.

The germination test was conducted with four replications of 25 seeds, in a plastic box (*gerbox*), previously disinfected with 1% sodium hypochlorite solution and 70% alcohol. In the plastic boxes, the previously sterilized vermiculite was stored for four hours in a Pasteur oven at 170 °C. The test was installed one day after fruit collection.

The "between vermiculite" procedure was used, where 27 grams of vermiculite were placed at the bottom of the plastic box (17 grams) and on top of the seeds (10 grams), which were moistened with 43 mL of distilled and autoclaved water. The methodology proposed by Brasil (MAPA, 2013) was adopted to calculate the amount of water to be added to the substrate. During the evaluations, the vermiculite was moistened whenever necessary. The samples were taken to a BOD Incubator Greenhouse at 25 °C and a 12-hour photoperiod of white light.

The count of germinated seeds was carried out weekly, and normal seedlings that showed all essential structures for their development (main root, epicotyl, cotyledons, primary leaves and bud) were considered to be germinated (MAPA, 2009, 2013). The first count was performed at 73 days and the germination test lasted for 163 days.

The germination data (G%) were subjected to the Kolmogorov-Smirnov normality test and to the Bartllet's homogeneity test, when these assumptions were not met, the data were transformed into arcosene $\sqrt{\frac{x}{100}}$ with the aid of the Action statistical supplement of the Microsoft Office Excel 2007 (Ferreira, 2008). After, the data were submitted to analysis of variance and the averages compared to each other by the Tukey test at 5% probability of error, with the aid of the Sisvar statistical software.

Results

Insect of the species *Heilipus draco* (Fabricius, 1801) (Curculionidae, Molytinae, Hylobiini) emerged from *O. puberula* fruits. In 2014, 120 adult insects emerged and in 2015, 34 adult insects emerged. In addition to 101 adult insects that did not emerge, remaining trapped inside the seed.

The occurrence of ovipositions of *H. draco* was verified in ripe and unripe fruits, during the whole process of fruit ripening. In both years of evaluation the oviposition peak occurred in October. During oviposition the female drills a hole in the seed coat, through the action of the rostrum. The egg-laying is performed at the entrance of this orifice and with the aid of the rostrum, the female pushes the egg into the seed (Fig. 1). Only a single oviposition was found in the fruits in the 2,300 fruits sectioned during the study, except for the year 2014, when 29 fruits with two egg-layings and four fruits with three eggs were counted.

When the larva hatches from the egg, it starts to consume the embryo and the reserve the seed, forming galleries inside it. During its development, the larva totally consumes the internal content of the seed, making germination impossible. The larvae remain inside the fruit until the pupae stage. In the two consecutive years of study, there was a progressive increase in the number of fruits with larvae, according to their maturation.

As much as four *H. draco* larvae were counted per seed, and the occurrence of different stages of development such as eggs and larvae in the same fruit were also observed. However, this occurred in only 134 of the fruits sectioned (5.8%). In the stored fruits, the occurrence of fine residues was observed. These residues were removed from the interior of the seed by the larvae, probably consisting of their excrement, representing a visual indication of their presence. The percentage of fruits with the presence of insects was 27% of the 3,312 fruits stored.

Exarate pupae occur inside the seed and only five were seen during sectioning in the laboratory, in fruits collected in the month of November. The adult makes a hole with its jaws to emerge from the inside of the seed. During the sectioning of the fruits, no adult insects were found. The emergence of adults occurs when the fruits are already dispersed from the tree, in the soil.

In addition to the emergence of the *H. draco* species, 36 parasitoid insects were also found. They were identified as *Bracon* sp.1, *Bracon* sp.2 (Ichneumonoidea, Braconidae, Braconinae), *Omeganastatus* sp.1 (Chalcidoidea, Eupelmidae, Eupelminae), *Scambus* sp.1 (Ichneumonoidea, Ichneumonidae, Pimplinae) and *Triapsis* sp.1 (Ichneumonoidea, Braconidae, Brachistinae).

Analyzing the seeds with and without *H. draco* oviposition, it was found that germination was higher in seeds without it and that it differed statistically from seeds with it (Tukey test, $p < 0.05$).

Discussion

The occurrence of *Heilipus* sp., causing injuries to the seeds of *O. puberula* had already been verified by Hirano (2004). However, the author did not determine to which species of the genus *Heilipus* it belonged. In Costa Rica, *H. draco* is mentioned as a granivorous insect of *Ocotea veraguensis* (Meisn.) Mez (Janzen, 1987) and in Canoinhas, SC, of the imbuia *O. porosa* (Hirano, 2004). The species *H. draco* occurs from Mexico, passing through Central America and reaching the south of South America (O'Brien and Wibner, 1982; Wibner and O'Brien, 1986).

The genus *Heilipus* has been reported in Brazil in other species of Lauraceae causing damage to seeds, as for example *Heilipus odoratus* Vanin & Gaiger, 2005 in *A. rosaeodora* seeds (Vanin and Gaiger, 2005) *Heilipus hopei* Boheman, 1843 in *L. mahuba* seeds (Lima, 1956) *Heilipus* sp. in seeds of *N. nitidula* (Carvalho, 2006) and *Heilipus montei* C. Lima, 1935 in seeds of *P. americana* (Lima, 1956). Milanesi (2008) verified in the field that the species *O. odorifera* presented holes in the immature fruits, which the author said were possibly caused by the predation of insect larvae. However, the author did not identify the species of insect that was causing the damage. According to Lima (1956), most Curculionidae grow in plants of the same family or species, which is in line with the results obtained in this work.

For *O. porosa* there are records of various insects causing damage to the seeds, in addition to *H. draco*, as for example *Heilipus* sp, *Heilipus tricolor* Perty, 1832, *Pantomorus postfasciatus* Hustache, 1947 (Hirano, 2004), and *Heilipus parvulus* Bohn, 1943 (Vernalha, 1953).

There are species of *Heilipus* that do not cause damage to seeds, as there are records in avocado trees in Brazil of adult insects of *Heilipus catagraphus* Germar, 1824 feeding on young fruits, without reaching the seed. The larvae of this species bores trunks and branches of plants of the families Lauraceae and Annonaceae (Lourenção et al., 1984). Other species, such as *Heilipus rufipes* Perty, 1832 bore the base of avocado trees (Lourenção et al., 2003).

The egg-layings of *H. draco* seen during the process of sectioning the fruits in the laboratory were located inside an orifice made by the female, a behavior similar to that observed by Vanin and Gaiger (2005) in seeds of *A. rosaeodora*. This condition, as emphasized by Grisales et al. (2017) for the species *Heilipus lauri* (Boheman, 1845) represents an advantage to the immature stages of the insect, because besides being a safe source of food for the larva, it also constitutes a shelter for larva and pupa, avoiding direct exposure to pathogens, predators and parasitoids that can affect their development. Thus, the seed represents the food resource for a larva, as well as shelter to complete its development until the adult emerges (Vanin and Gaiger, 2005).

Even though there are fruits of *O. puberula* with more than one egg-laying or larva, or even different stages of development in a single seed, only a single adult insect emerged per fruit. This result was also observed in other species of Lauraceae (Vanin and Gaiger, 2005; Cuaranhua, 2010), as the larvae have cannibalistic behavior (Grisales et al., 2017), that is, they consume competing insects.

The pupal stage occurred inside the seed, which according to Castañeda-Vildózola et al. (2009) occurs in all species of *Heilipus*

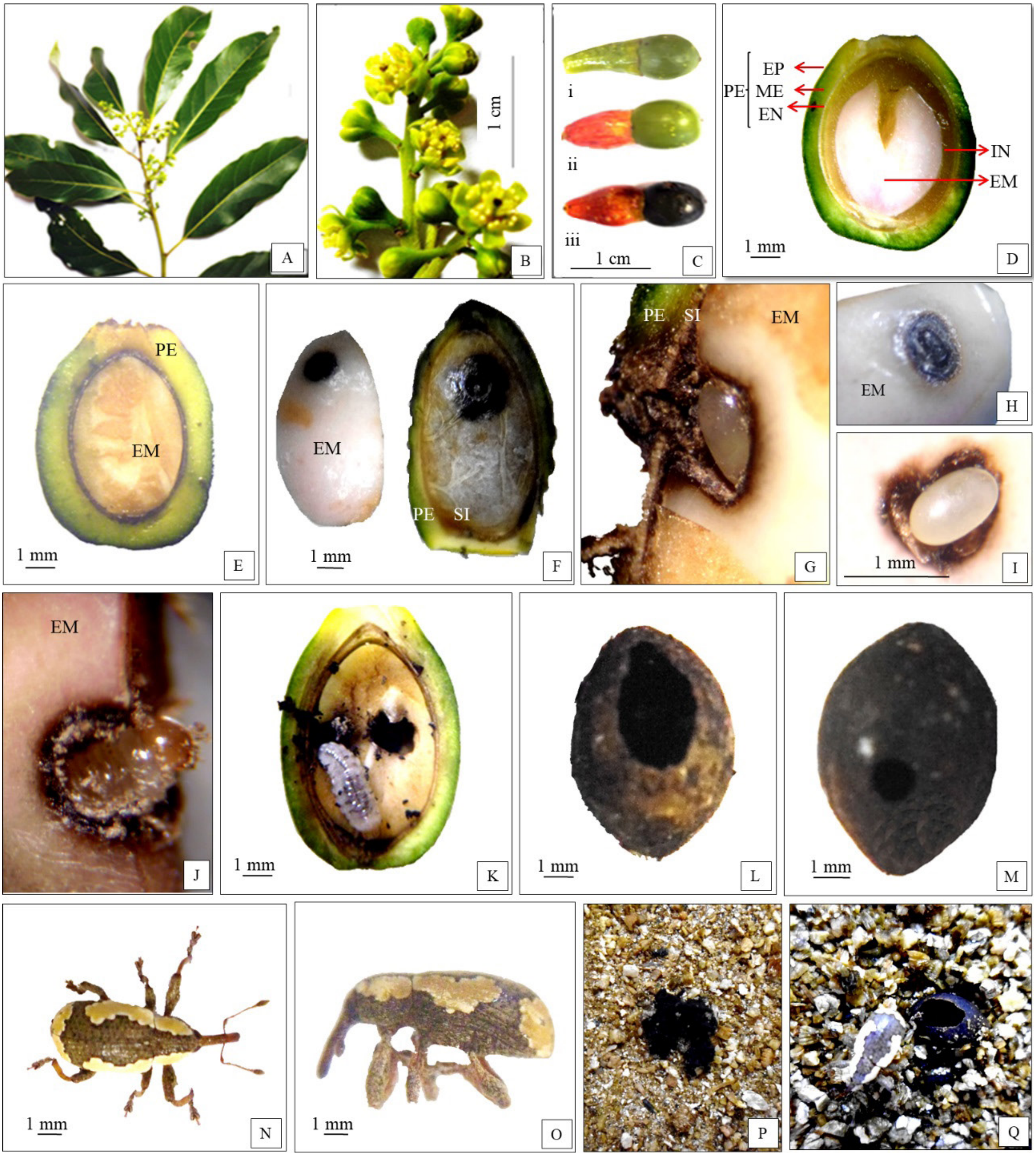

Figure 1 A – *Ocotea puberula* branch with inflorescence; B - Flower buds and open flowers; C - Different colors of fruits during ripening, (i) green colored fruit and green cupule, (ii) green colored fruit and red cupule and (iii) ripe black colored fruit and red cupule; D - Fruit in development; E - Healthy fruit with the developed embryo; F - Endophytic egg layings in ripe fruit, with the egg being deposited directly in the embryo; G, H, I - Isolated egg inside the gallery held by the female; J - Larva consuming the embryo; K - Larva in development consuming the embryo and forming galleries in it; L - Detail of the hole made by *Heilipus draco* to emerge from inside the seed; N - Dorsal face of *H. draco*; O - Lateral face of *H. draco*; P - Detail of the metabolite residues removed by the larvae inside the seed on the vermiculite; Q - *H. draco* emerged and detail of the seed with the orifice of emergence over the vermiculite. (EM: embryo; EN: endocarp; EP: epicarp; ME: mesocarp; PE: pericarp; IN: integument and nucleus; SI: seed integument).

granivorous. During sectioning, only one adult specimen was found inside the seeds during the two consecutive years, which suggests that the development of the curculionid of the adult pupal stage, and its subsequent emergence, occurs when the fruit has already been dispersed from the canopy of the tree, in the soil. As evidenced earlier, the oviposition peak of fruits of *O. puberul*a occurred in October, and in December all fruits were already dispersed. For *H. lauri*, the life cycle from egg to the emergence of an adult is completed in 76.14 ± 7.31 days (Grisales et al., 2017).

When the adult emerges from the seed, it makes a hole that can serve as an indication of predation to discard it and improve the quality of the batch destined for use as seed (Carvalho et al., 2009).

In addition, the elimination of infested seeds prevents the spread of the insect to other regions. Considering that species of the genus *Heilipus* are considered insect pests of fruit crops, especially avocado (Lourenção et al., 1984) being deemed in some countries, such as Colombia, to be pests under official control, especially *H. lauri* and *Heilipus trifasciatus* (Fabricius, 1787) for causing negative economic impacts on the production of *P. americana* (ICA, 2016).

The rate of parasitism of *H. draco* can be considered low, due to the reduced number of parasitoid hymenopterans emerged. However, this information is of paramount importance, since there are few studies that emphasize parasitism in *Heilipus*. In the seeds of *A. rosaeodora,* in which *H. odoratus* causes damage, a male Braconidae (Vanin and Gaiger, 2005) and *Capitonius* sp. (Hymenoptera: Braconidae: Cenocoeliinae) (Nunes et al., 2006) emerged.

During the visual observations of the fruits sectioned, it was found that parasitoid larvae were parasitizing Curculionidae larvae. Thus, these species of parasitoid hymenopterans are probably associated with *H. draco,* a granivorous insect of *O. puberula*. The hymenopterans of the superfamily Ichneumonoidea, family Braconidae, represented by the species *Bracon* sp.1 and *Bracon* sp.2 collected in this study are cosmopolitan, idiobiont ectoparasites related to Coleoptera larvae (Quicke, 1987).

Analyzing Table 1, it appears that in the seeds in which the emergence of insects ocurred, germination was significantly lower, in addition to the fact that, in these seeds, the insects totally consumed the embryo and reserves, leaving only the integument, which shows the damage caused by these insects to the perpetuation of the species. This result proves that the action of the larvae of the Curculionidae can make the seed unfeasible and the greater the occurrence of insects, the lower the germination percentage of the seeds that constitute the batch.

In the fruits stored, the occurrence of ovipositions and larvae that did not complete their development was found in the seeds, not causing the death of the seed. Studying five species of Lauraceae, Carvalho et al. (2009) found that damaged seeds had lower germination percentages compared to seeds without damage. In this same work, the authors found that the damage caused by insect larvae led to the deterioration of the seeds whenever they reached a region close to the embryonic axis and, at least, 50% of the embryo.

This evidence justifies the occurrence of three percent of the germinated seeds, even with the orifice of oviposition made by *H. draco*. Spironello et al. (2004) observed that 35% of the fruits of *A. rosaeodora* infested by insect larvae still germinate when the embryo is not destroyed. However, the authors did not verify the vigor of these seedlings.

As can be seen in the germination test, some control tactics can be used to eliminate damaged seeds. This is because the egg-layings of *H. draco* are performed endophytically, resulting in a small opening in the seed, which can be used as a way of recognizing the seeds with ovipositions for their disposal. Another alternative that indicates the presence of granivorous insects inside the seed, is the visualization of metabolite residues that are removed by the larvae, being retained on the surface or in the container where the seeds are stored.

Some chemical and biological treatments have already been tested on forest seeds, such as *A. rosaeodora* seeds where Torrez et al. (2018) evaluated the efficiency of the insecticide (Karate Zeon 50 CS) and the extract of the entomopathogenic fungus *Beauveria bassiana* Vuill. (1912) in the control of *H. odoratus* larvae in seeds, and found that the treatment of the seeds stopped the damage caused by the larvae, increasing the germination rates, without affecting the initial development of the seedlings.

Table 1
Germination and occurrence of insects in *Ocotea puberula* seeds with and without evidence of oviposition by *Heilipus draco*

Treatment	Germination (%)	Insect (%)
Without oviposition orifice	17 a*	0
With oviposition orifice	3 b	10.5

** averages followed by the same letter in the column, do not differ by Tukey test ($p < 0.05$).*

The low percentage of germination in the seeds without oviposition may be the result of the physiological dormancy of the seeds of *O. puberula* which was not overcome in the research because only the integument was removed, consisting of physical dormancy. However, in a study carried out by Vicente (2014), the author concluded that the removal of the fruit integument is satisfactory for overcoming dormancy, without the need to use sulfuric acid, as emphasized by Carvalho (2003). It should be noted, however, that in addition to the germinative (physiological) potential, the seed batch is associated with the genetic, physical and health attributes that qualify the seeds to be used (MAPA, 2013).

In addition to insects, the sanity quality evaluates the presence and degree of occurrence of fungi, bacteria, viruses and nematodes that cause disease or damage to the seeds (Oliveira et al., 2018). In a sanity test using the filter paper method, performed with the same batch of seeds analyzed in the present study, the main fungal genera found associated with the seeds of *Ocotea puberula* were: *Aspergillus* spp., *Cladosporium* spp., *Colletotrichum* spp., *Fusarium* spp., *Penicillium* spp. and *Phoma* spp. (unpublished data). These pathogens possibly also influenced the lower percentage of germination, as according to Oliveira et al. (2018) the association of pathogens with seeds can lead to a reduction in germination capacity, compromising production.

Conclusions

The species *Heilipus draco* is the granivorous insect that causes damage to the seeds of the forest species *Ocotea puberula*.

The ovipositions of *Heilipus draco* are endophytic, being carried out directly on the seed through a hole made by the female.

The seeds with ovipositions of *Heilipus draco* have reduced germination.

The main damages caused to the seeds of *Ocotea puberula* are caused by the larval stage of *Heilipus draco*, which consumes the embryo and the seed reserve.

The larvae of *Heilipus draco* were parasitized by parasitoid hymenoptera of the genera *Bracon*, *Omeganastatus*, *Scambus* and *Triapsis*.

The removal of all seeds with an oviposition orifice to perform the germination test is a control tactic.

Acknowledgments

Thanks to Dr. Prof. Germano Henrique Rosado Neto from the Universidade Federal do Paraná (UFPR) for the identification of the species *Heilipus draco*. This work was supported by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001 [grant number 1669520].

Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

All authors conceived the research. MDFP and ECC wrote the first version of the manuscript and all authors contributed reviewing critically the manuscript for important content. All authors approved the final version of this paper.

References

Almeida, L. P., 2001. Germinação, crescimento inicial e anatomia foliar de plantas jovens de *Cryptocarya aschersoniana* Mez. sob diferentes níveis de radiação. Dissertation, Universidade Federal de Lavras.

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. L. M., Sparovek, G., 2013. Köppen's climate classification map for Brazil. Meteorol. Z. 22, 711-728. https://doi.org/10.1127/0941-2948/2013/0507.

Carvalho, L. R., 2006. Conservação de sementes de espécies dos gêneros *Nectandra*, *Ocotea* e *Persea* (Lauraceae). Thesis, Universidade Federal de Lavras.

Carvalho, L. R., Carvalho, M. L. M., Davide, A. C., 2009. Utilização do teste de raios-X na avaliação da qualidade de sementes de espécies florestais de Lauraceae. Rev. Bras. Sementes 31, 57-66. https://doi.org/10.1590/S0101-31222009000400007.

Carvalho, P. E. R., 2002. Canela guaicá. Embrapa, Colombo. (Circular técnica, 62).

Carvalho, P. E. R., 2003. Espécies arbóreas brasileiras. Embrapa Informação Tecnológica, Brasília.

Castañeda-Vildózola, A., Ángel-Coronel, O. A. D., Cruz-Castillo, J. G., Váldez-Carrasco, J., 2009. *Persea schiedeana* (Lauraceae), Nuevo Hospedero de *Heilipus lauri* Boheman (Coleoptera: Curculionidae) en Veracruz, México. Neotrop. Entomol. 38, 871-872.

Costa, E. C., Cantarelli, E. B., Boscardin, J., Fleck, M. D., 2018. Insetos-praga de sementes e mudas em viveiros florestais. In: Araujo, M.M., Navroski, M.C., Schorn, L.A. (Eds.), Produção de sementes e mudas: um enfoque à silvicultura. UFSM, Santa Maria, pp. 259-282.

Cuaranhua, C. J., 2010. Frutificação, dispersão e predação por insetos de frutos/sementes de imbuia (*Ocotea porosa*). Dissertation, Universidade Federal do Paraná.

Dorneles, D. U., 2014. Caracterização fitossanitária e potencial germinativo de sementes de *Eugenia uniflora* L. Dissertation, Universidade Federal de Santa Maria.

Farago, P. V., Budel, J. M., Duarte, M. R., Nakashima, T., 2005. Análise morfoanatômica de folhas de *Ocotea puberula* (Rich.) Nees, Lauraceae. Rev. Bras. Farmacogn. 15, 250-255. https://doi.org/10.1590/S0102-695X2005000300016.

Ferreira, D. F., 2008. Sisvar: um programa para análises e ensino de estatística. Rev. Symp. 6, 36-41.

Grisales, V. D., Vallejo, A. M. C., Muñoz, A. C., 2017. Ciclo de vida y descripción morfológica de *Heilipus lauri* Boheman (Coleoptera: Curculionidae) en Colombia. Acta Zool. Mex. 33, 231-242.

Hirano, E., 2004. Maturação fisiológica, tolerância à dessecação e conservação de sementes de lauráceas da Mata de Araucária de Santa Catarina. Thesis, Universidade Federal do Paraná.

Instituto Colombiano Agropecuario – ICA, 2016. Resolución No. 00001507. Diario Oficial de Colombia, Bogotá. Available in: http://www.ica.gov.co/getattachment/81591168-ac45-478a-b84bf054d4e5829b/2016R1507.aspx (accessed 20 March 2020).

Janzen, D. H., 1987. Insect diversity of a Costa Rican dry forest: why keep it, and how? Biol. J. Linn. Soc. Lond. 30, 343-356.

Lima, A. C., 1956. Insetos do Brasil: 10° Tomo, Coleópteros. Escola Nacional de Agronomia, Rio de Janeiro. (Série didática, 12).

Lorenzi, H., 1998. Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil. Vol. 1. Plantarum, Nova Odessa.

Lourenção, A. L., Rosseto, C. J., Soares, N. B., 1984. Ocorrência de adultos de *Heilipus catagraphus* Germar, 1824 (Coleoptera: Curculionidae) danificando frutos de abacateiro. Bragantia 43, 249-253. https://doi.org/10.1590/S0006-87051984000100022.

Lourenção, A. L., Soares, N. B., Rosado-Neto, G. H., 2003. Ocorrência e danos de larvas de *Heilipus rufipes* Perty (Coleoptera: Curculionidae) em abacateiro (*Persea americana* Mill.) no Estado do Ceará. Neotrop. Entomol. 32, 363-364. https://doi.org/10.1590/S1519-566X2003000200028.

Marchiori, J. N. C., 1997. Dendrologia das angiospermas: das magnoliáceas às flacurtiáceas. UFSM, Santa Maria

Menalled, F. D., Liebman, M., 2008. Seed predation by insects. In: Capinera, J.L. (Ed.), Encyclopedia of Entomology. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-6359-6_4110.

Mikich, S. B., 2002. A dieta frugívora de *Penelope superciliaris* (Cracidae) em remanescentes de floresta estacional semidecidual no centro-oeste do Paraná, Brasil e sua relação com *Euterpe edulis* (Arecaceae). Ararajuba 10, 207-217.

Milanesi, L.S., 2008. Estudos sobre a biologia de canela-sassafrás [*Ocotea odorifera* (Vell.) Rohwer] na Mata Atlântica do Rio Grande do Sul. Monography, Universidade Federal do Rio Grande do Sul.

Ministério da Agricultura, Pecuária e Abastecimento – MAPA, 2009. Regras para Análise de Sementes. Brasília.

Ministério da Agricultura, Pecuária e Abastecimento – MAPA, 2013. Instruções para análise de espécies florestais. Brasília.

Muxfeldt, R. E., Faria, J. M. R., Tonetti, O. A. O., Silva, E. A. A., 2012. Utilização do teste de raios–x na avaliação dos efeitos da dessecação e infestação em diásporos de canela-batalha - *Cryptocarya aschersoniana* Mez (Lauraceae). Cerne 18, 657-666. https://doi.org/10.1590/S0104-77602012000400016.

Nunes, A. B., Ronchi-Teles, B., Spironello, W. R., Pinto, A. A., Sampaio, P. T. B., 2006. Ocorrência de *Capitonius* sp. (Hymenoptera: Braconidae: Cenocoeliinae) parasitando larvas de *Heilipus odoratus* (Coleoptera: Curculionidae), broca da semente de pau rosa em Manaus, AM. In: XXVI Congresso Brasileiro de Zoologia, Londrina. Anais. Londrina: UEL.

O'Brien, C. W., Wibner, G. J., 1982. Annotated checklist of the weevils (Curculionidae sensu lato) of North America, Central America, and the West Indies (Coleoptera: curculionidae). Mem. Am. Entomol. Inst. 34, 1-382.

Oliveira, L. M., Araujo, M. M., Flôres, A. V., Wielewicki, A. P., Poletto, T., Fantinel, V. S., 2018. Análise da qualidade física, fisiológica e sanitária de sementes florestais. In: Araujo, M.M., Navroski, M.C., Schorn, L.A. (Eds.), Produção de sementes e mudas: um enfoque à silvicultura. UFSM, Santa Maria, pp. 121-144.

Pereira, M. V. L., Peixoto, A. L., Maio, F. R., 1995. Plantas utilizadas como recurso alimentar pela fauna silvestre na represa de Ribeirão das Lajes, Rio de Janeiro, Brasil. Rev. Univ. Rural Ser. Cienc. Vida 17, 25-40.

Quicke, D. L. J., 1987. The old world genera of Braconinae wasps (Hymenoptera: braconidae). J. Nat. Hist. 21, 43-157. https://doi.org/10.1080/00222938700770031.

Rosa, L. S., Ohashi, S. T., 1999. Influência do substrato e do grau de maturação dos frutos sobre a germinação do pau-rosa (*Aniba rosaeodora* Ducke). Rev. Cienc. Agrar. 31, 49-55.

Souza, L. A., 2006. Fruto. In: Souza, L.A. (Ed.), Anatomia do fruto e da semente. UEPG, Ponta Grossa, pp. 9-123.

Souza, L. A., Moscheta, I. S., 1999. Morfo-anatomia da flor de *Ocotea puberula* (Rich.) Nees (Lauraceae). Acta Scientiarum 21, 343-348. https://doi.org/10.4025/actascibiolsci.v21i0.4448.

Souza, L. A., Moscheta, I. S., 2000. Morfo-anatomia do desenvolvimento do fruto de *Ocotea puberula* (Rich.) Nees e de *Nectandra megapotamica* (Spreng.) Mez (Lauraceae). Acta Cient. Venez. 51, 84-89.

Spironello, W. R., Sampaio, P. T. B., Ronchi-Teles, B., 2004. Produção e predação de frutos em *Aniba rosaeodora* Ducke var. *amazonica* Ducke (Lauraceae) em sistema de plantio sob floresta de terra firme na Amazônia Central. Acta Bot. Bras. 18, 801-807. https://doi.org/10.1590/S0102-33062004000400010.

Torrez, A. A. A., Chevreuil, L. R., Sousa, K. A., Sampaio, P. T. B., Bruno, F. S., Sales-Campos, C., 2018. Tratamientos químico y biológico en semillas de *Aniba rosaeodora* para el control de *Heilipus odoratus* (Coleoptera, Curculionidae, Molytinae) en viveros, Amazonas, Brasil. Bosque 39, 299-308. https://doi.org/10.4067/S0717-92002018000200299.

Vanin, A. S., Bená, D. C., Chamorro, M. L., Rainho, H. L., 2019. Curculionidae in Catálogo Taxonômico da Fauna do Brasil. PNUD, Rio de Janeiro. Available in: http://fauna.jbrj.gov.br/fauna/faunadobrasil/9406 (accessed 28 January 2020).

Vanin, S. A., Gaiger, F., 2005. A new spermophagous species of *Heilipus* Germar from the Amazonian Region (Coleoptera, Curculionidae, Molytinae). Rev. Bras. Entomol. 49, 240-244. https://doi.org/10.1590/S0085-56262005000200007.

Vernalha, M. M., 1953. "*Heilipus parvulus*" Bohn, 1943, praga da imbuia (*Phoebe porosa*) no horto florestal de Vila Velha. In: Congresso Florestal Brasileiro, 1953, Curitiba. Anais. Curitiba: Instituto Nacional do Pinho, pp. 125-128.

Vicente, D., 2014. Dormência e armazenamento de sementes de *Ocotea puberula*. Dissertation, Universidade do Estado de Santa Catarina.

Wibner, G. J., O'Brien, C. W., 1986. Annotated checklist of the weevils (Curculionidae sensu lato) of South America (Coleoptera: curculionidae). Mem. Am. Entomol. Inst. 39, 1-563.