ECOLOGY, BEHAVIOR AND BIONOMICS

Rate of Soil Egestion by Larvae of *Hylamorpha elegans* (Burm.) and *Phytoloema hermanni* Germ. (Coleoptera: Scarabaeidae)

Paz Millas[1], Roberto Carrillo[2]

[1]*Graduate School, Faculty of Agricultural Sciences;* [2]*Lab of Entomology, Institute of Production and Plant Protection. Univ Austral de Chile, PO BOX 567 Valdivia, Chile; pazmillas@yahoo.com; rcarrill@uach.cl*

Edited by Antônio R Panizzi – EMBRAPA

Neotropical Entomology 39(5):697-702 (2010)

ABSTRACT - Larvae of *Hylamorpha elegans* (Burm.) and *Phytoloema herrmanni* Germ. feed on roots, although many Scarabaeidae species are able to feed and survive on soil without living roots. In this study we evaluated the potential of *H. elegans* and *P. herrmanni* to ingest soil by estimating the rate of soil egestion. In the laboratory, the rate of soil egestion was determined from gut content and gut transit time of 3rd-instar larvae feeding on soil without roots. The soil egestion rate was 14-21 mg g^{-1} d^{-1} for *H. elegans* and 11-16 mg g^{-1} d^{-1} for *P. herrmanni*. The gut transit time (time of soil passage from mouth to anus) was 2-3 d for both species and the gut content was 41 ± 2 mg g^{-1} for *H. elegans* and 32 ± 2 mg g^{-1} for *P. herrmanni*. The quantitative importance of feeding activity on soil depends upon the population density of larvae in the field, which ranges from 1 to 25 larvae m^{-2}, but in severe outbreaks can reach 300 larvae m^{-2}. High population densities could result in soil egestion rates reaching 20 kg d^{-1} ha^{-1} for *P. herrmanni* and 30 kg d^{-1} ha^{-1} for *H. elegans*.

KEY WORDS: Ingest, compaction, white grub

Scarabaeidae larvae have diverse feeding habits, feeding on carrion, dung, fresh or decomposing plant matter on or in the soil (Ritcher 1958). They constitute part of the soil-macrofauna worldwide, exploiting the rhizosphere of pastures and crops causing severe damage (Kuniata & Young 1992, Harari *et al* 2001, Oyafuso *et al* 2002, Potter & Held 2002).

In southern Chile the indigenous scarabaeid larvae *Hylamorpha elegans* (Burm.) (Rutelinae) and *Phytoloema herrmanni* Germ. (Melolonthinae) are the white grubs which cause the most economic damage to grasses and small grain cereals (Artigas 1994, Cisternas & Carrillo 2001). These larvae feed on a wide range of materials in the soil, including roots of a diversity of plant families, decomposing wood (Artigas 1994) and wheat straw (Durán 1952).

Scarab grubs ingest a mixture of inorganic and organic soil components, including plant roots and microorganisms adhered to soil particles (McQuillan & Webb 1994). Analysis of guts homogenates has revealed that the scarabaeid larvae do not simply consume bulk soil, but feed preferentially on the organic soil constituents (Wensler 1971, Risdill-Smith 1975, McQuillan & Webb 1994). Feeding activity of scarabeid larvae and other macro-fauna can affect the soil organic matter dynamic by different processes: transit through the intestine, shaping the microbial community through transport or selective reduction in the viability of microorganisms, release of labile nutrients from fresh faecal material, protection of non-degraded organic matter in aging cast or faecal pellets, and redistribution and turnover of soil organic matter in whole profiles (Lavelle *et al* 1997). These processes significantly influence the stability and degradation of soil organic matter and modify the soil environment. However, the quantitative importance of this impact depends on the quantity of substrate affected by the feeding activity. Extensive studies on soil consumption rates (estimated from egestion or cast production rates) have been carried out with earthworms, with rate values that depend on species, organic matter content and soil quality (Curry & Schmidt 2007).

Effects of feeding activity of scarab larvae on the transformation of soil organic matter have been seldom investigated (Cazemier *et al* 1997, Li & Brune 2005a,b, 2007). Little is known about the daily mass of soil/substrates that are ingested by the biomass of scarab larvae. In this study we evaluated the potential of *H. elegans* and *P. herrmanni* to ingest soil by estimating the rate of soil egestion by 3rd instars feeding on an andisol soil without living roots. We also determined the weight loss in the gut content caused by digestion and absorption of organic matter (compaction) for *H. elegans.*

Material and Methods

Two andisol soils were used; Valdivia soil series was collected at the Universidad Austral de Chile's Santa Rosa Experimental Station 39º46´ S, 73º14´ W, and Puerto Fonck soil series was collected at the Entre Lagos locality in southern Chile's Osorno province 41º07´ S, 72º44´ W. In both cases, pasture vegetation was removed from the surface, and the top 30 cm was collected. Both soils were sieved through

4 mm mesh and brought to 57% humidity (w/w). Organic matter content (OM%), pH (H_2O 1:2.5), mineral nitrogen (Min N), Olsen phosphorus (P-Olsen), and basis sum were measured (Table 1). Prior to use, soil samples were stored in the dark at 5ºC.

Larvae were obtained at the same location as the Valdivia soil series from a pasture mixed with wild grasses. *Phytoloema herrmanni* larvae were obtained in late April, 2006, and *H. elegans* larvae were collected in mid June, 2006. Larvae were obtained from holes made with a shovel, then transferred to containers with soil, and finally taken to the Universidad Austral de Chile's Entomology laboratory. For both species, 200 3rd instars (L3) were selected in the laboratory according to the width of the head capsule (Cisternas 1986). Larvae were individually transferred to 100 ml plastic containers with 50 g of Valdivia soil at 57% humidity (w/w). Containers were kept in darkness at 14 ± 1ºC. Larvae were left to acclimate to these conditions for 10 days, before further use.

Experiments were carried out in the dark, at 14 ± 1ºC, in modified compact refrigerators. Each plastic container held 50 g of soil (57% w/w) and one larva. Containers were regularly weighed to determine water loss during the experiment, and water was replaced. The soil ingested by larvae passed the gut and was voided as faeces. The rate of soil egestion was calculated by measuring gut content (GC) and estimated gut transit time (GTT).

For each species, 40 L3 were individually placed in 100 cc plastic containers with 50 g of Valdivia soil at 57% humidity (w/w). After 10 days, larvae were removed, weighed, and allowed to void their guts in Petri dishes with moistened absorbent paper for two weeks (Curry & Baker 1998, Fleuren *et al* 2003, Jager *et al* 2003). In order to prevent faeces contact with humidity from the absorbent paper, aluminium foil was placed over the moistened paper. To prevent larvae ingestion, faeces were regularly transferred to small aluminium containers within the Petri dishes. After larvae had voided their guts, they were weighed and frozen at -15ºC. Gut contents from each larva were weighed, dried for 24 hours at 105ºC, and then weighed again (Curry & Baker 1998).

To determinate the gut transit time, 40 larvae of each species were allowed to feed in the red soil (Valdivia series) for 10 days under experimental conditions (14ºC; 0-24L:D). Then they were transferred from the red Valdivia soil to the black Puerto Fonck soil series and kept in the black soil for 3, 6, 9, 12, 15, 18, 21 and 24h. Thereafter, individual larvae were placed on moistened filter paper in clean Petri dishes to measure gut voidance. The time that elapsed between placing the larvae in the black soil and the first black-coloured faeces observed in the dishes was deemed to be GTT (modified methodology from Hartenstein *et al* 1981). GTT was established when 50% or more of the larvae at each feeding interval produced faeces of the second colour.

The experiment was replicated, but this time the order of exposure to the different soil types was inverted; the black soil (Puerto Fonck series) was introduced as the first food for 10 days, and the red soil (Valdivia series) was used as the indicator of GTT. This reversal was performed to see whether soil type affects GTT.

Gut weight (full) was determined using 40 pre-treated *H. elegans* larvae (L3), which were individually placed in separate containers and maintained under experimental conditions for 10 days. Larvae were then removed, weighed, and killed by freezing at -20ºC. Larval guts were dissected, and guts and bodies were weighed, and frozen. Guts and bodies were dried at 80ºC for 48h and then weighed. Weight decrease in gut content was calculated using a comparison of weight intestinal percentage of full larvae vs. empty larvae. *Hylamorpha elegans* larvae from the gut content experiment (with voided guts) were defrosted, dissected, dried, and weighed in the same manner as the full larvae. Because the dissected larvae with full guts were not the same individuals as dissected larvae with empty guts, a Student's t-test was used to compare the initial live weights of larvae in both categories, showing no significant difference ($P > 0.05$). Compaction was calculated from the difference between the weight decrease in gut and weight of the egested gut contents. Means comparison of gut content and rate of soil egestion were carried out using Student's t-test ($P < 0.05$). In order to determine whether the amount of faeces egested is influenced by the larvae size, a correlation analysis between larval live weight and faecal dry weight was run.

Results and Discussion

Larval live weight after pre-treatment was significantly higher for *H. elegans* ($P < 0.05$) than for *P. hermanni* (Table 2). Three days was the average time required for the ingested red soil to be egested for both species studied (Fig 1a). The period of time necessary for egestion in these species was considerably longer if compared to those for annelids (2.5-7h) (Hartenstein *et al* 1981, Curry & Baker 1998). Therefore, the feeding intervals used for observing the faeces' color changes (3, 6, 9, 12, 15, 18, 21, 24h) are appropriate for earthworms, but very short for the larvae studied here. Although it was not possible to find GTTs values for other scarabeid larvae, the faeces production of *Pachnoda ephippiata* Gerstaecker (two full gut equivalents of faeces per day) (Lemke *et al* 2003), *Sericesthis nigrolineata* Boisduval (2.7 pellet faecal per day) (Risdill Smith 1975)

Table 1 Chemical characteristics of the experimental soils.

Soil (series)	Water pH (1:2.5)	OM (%)	Min N (mg kg^{-1})	C : N ratio	P-Olsen (mg kg^{-1})	Basis sum ($cmol_c$ kg^{-1})
Valdivia	5.5	12.3	14.0	13.8	2.0	1.43
Puerto Fonck	5.1	19.3	26.6	17.0	8.0	1.95

Table 2 Live weight, weight of dry faeces, gut content, gut transit time, and rate of soil egestion for 3rd instars of *Hylamorpha elegans* and *Phytoloema herrmanni*. Means and standard errors are given.

Species	Live weight (mg)	Dry weight faeces (mg)	GC (mg dry weight · g larva biomass^{-1})	GTT (d)	Rate of soil egestion (mg g^{-1} d^{-1})	n
H. elegans	539.5 ± 8.7 a	21.8 ± 1.1 a	40.7 ± 2.0 a	2 - 3	13.6 ± 0.7 – 20.3 ± 1.0	35
P. herrmanni	427.7 ± 8.0 b	13.6 ± 1.0 b	31.9 ± 2.3 b	2 - 3	10.6 ± 0.8 – 16.0 ± 1.2	39

Data from the same column with different letters are significantly different at $P < 0.05$ as tested with LSD Multiple Range test.

and *Sericesthis geminata* Boisduval (6.9 pellet faecal per day) (Wensler 1971), show that GTTs for these species are < 24h. This means that the gut transit for *H. elegans* and *P. herrmanni* is relatively slow, which could make these larvae more efficient in breaking down soil organic matter. However, this would depend upon temperature (Hartenstein *et al* 1981) and associated gut microbiota.

When larvae ate the second colour soil for < 3h, there

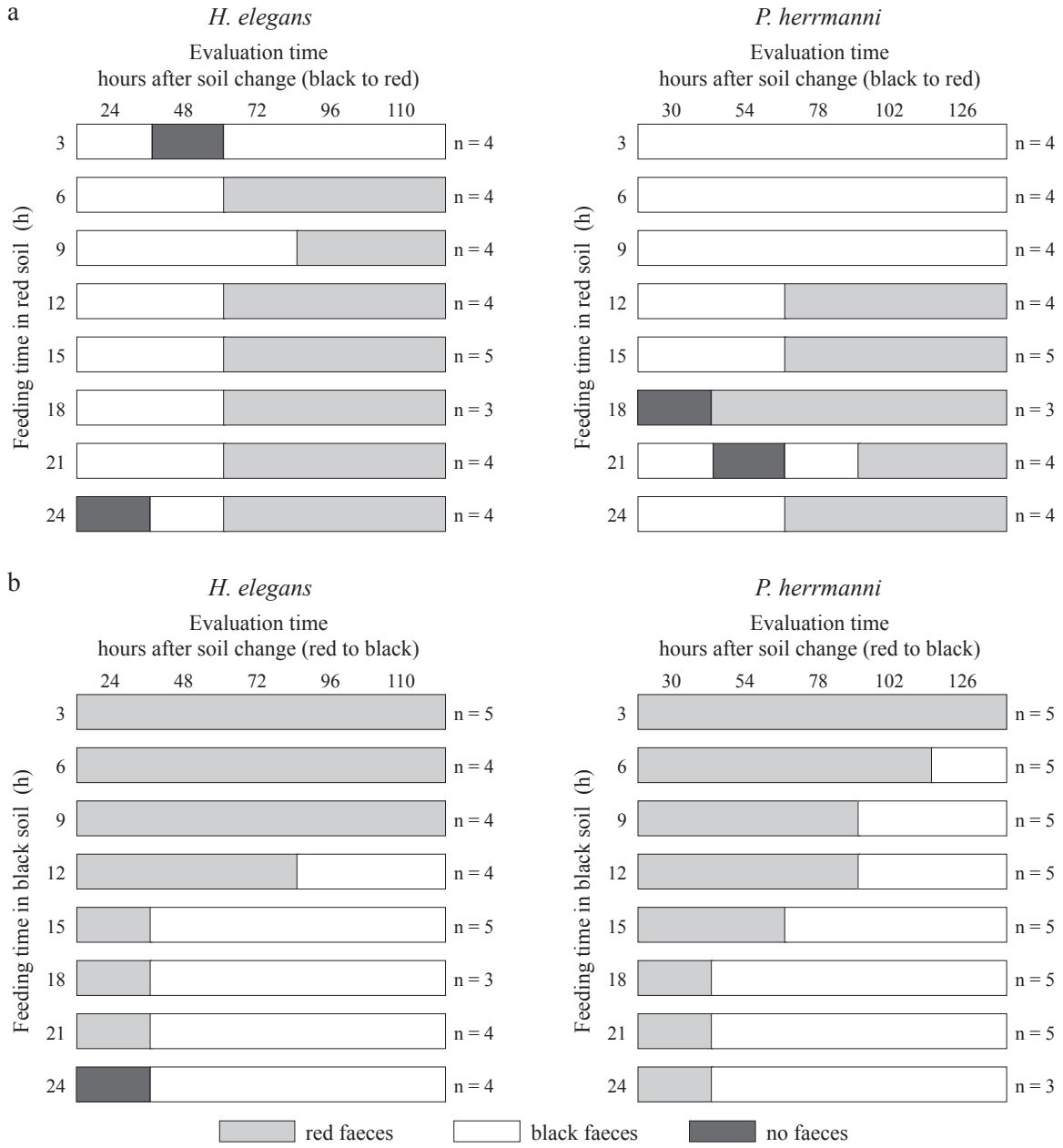

Fig 1 Gut transit time (h) for *Hylamorpha elegans* and *Phytoloema herrmanni* larvae when feeding on a) red soil or b) black soil.

were no visible faeces of the second colour at any of the evaluation time points (Fig 1), which could be explained by the fact that both larvae ingested too little soil for a change to be detected, or because there was a lag time in feeding after the introduction of larvae into the second type of soil.

In both studied species, the GTT was 24h lesser in the black soil than in the red soil (Fig 1b). This difference can be explained because the higher content of organic matter in the black soil (Puerto Fonk) (Table 1) could increase the feeding rate and thus increase the gut transit speed. This is consistent with Wensler (1971), who found that mid-gut volume of *S. geminata* remained constant when larvae were under *Lolium perenne* turf or in soil without plants, while the rate of defecation was significantly greater in the former medium. This result suggests that the substrate type influences the rate of passage of ingested material through the gut, with faster passage of soil with higher organic matter content due to the presence of roots.

The voided gut content was significantly greater (21.8 ± 6.3 mg larva^{-1}) for *H. elegans* than *P. herrmanni* (13.6 ± 6.2 mg larva^{-1}) ($P < 0.05$). The faeces produced/g live mass was also greater for *H. elegans* (40.7 ± 11.9 mg dry mass g^{-1} of live mass) than *P. herrmanni* (31.9 ± 14.6 mg dry mass g^{-1} of live mass) (Table 2).Therefore, assuming similar density under field conditions, *H. elegans* will have a greater quantitative impact on the soil than *P. hermanni*. There was no correlation between faecal dry weight and larval live weight in either species (Fig 2). This could indicate that within 3rd instars, the production of dry faeces is not influenced by the size of the larvae.

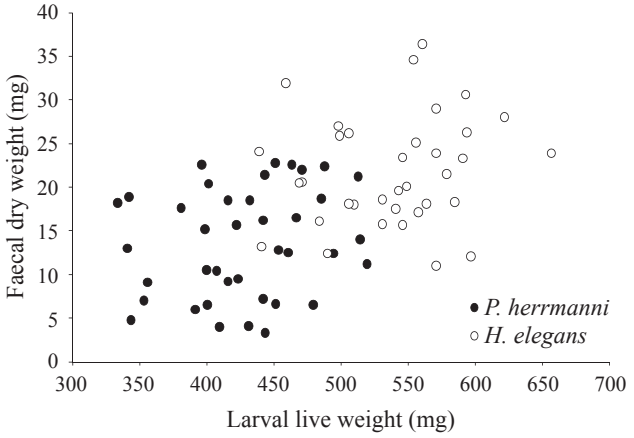

Fig 2 Gut content (dw) in relation to larval weight of *Hylamorpha elegans* and *Phytoloema herrmanni*. The correlation coefficient was not statistically significant for either species ($P < 0.05$) (Pearson r = 0.15 and Pearson r = 0.20 for *H. elegans* and *P. herrmanni*, respectively).

The rate of soil egestion calculated from gut content (GC) and gut transit time (GTT) for 3rd instars of *H. elegans* and *P. herrmanni* (Table 2) was clearly lower than that estimated for the annelids (Curry & Baker 1998). There are several studies on feeding behavior of scarab larvae, mostly focusing on larval weight gain or selective consumption feeding on different substrates. Some compare number of faecal pellet /day (Wensler 1971, King 1977). Considering the variation between pellet weight and larval size, it is not possible to compare the rate of soil egestion in most cases. The rate of soil egestion was calculated using the data of the live weight and weight of daily faecal production of 3rd instars of *Pachnoda. ephippiata* (Lemke *et al* 2003) and *Sericesthis nigrolineata* (Risdill-Smith 1975). *Hylamorpha elegans* and *P. herrmanni* rates of soil egestion were lower than *P. ephippiata*, (85 mg g^{-1} d^{-1}), but higher than *S. nigrolineata* (7.6 mg g^{-1} d^{-1}), indicating a range that may depend on soil type, larval metabolism or specific habits.

When we compared the dry mass of full guts (116.9 ± 3.9 mg g^{-1} of live weight larva) with the dry mass of empty guts (56.1 ± 3.4 mg g^{-1} of live weight larva), we observed that the egesting of faeces produced a decrease in gut weight of 60.8 (mg per g of live weight larvae) (Table 3), which is lower than the weight of faeces produced (40.7 mg g^{-1} of live mass).This difference could be due to compaction, which corresponds to the weight loss in the gut content caused by digestion and absorption of organic matter (Fleuren *et al* 2003). The compaction observed in *H. elegans* larvae fed on Valdivia soil (31%) was remarkably higher than that found for *Eisenia andrei* in different substrates/soils (Fleuren *et al* 2003, Jager *et al* 2003). These authors showed that as the digestion values and/or organic matter content of the substrate increased, the compaction also increased. When compaction is taken into account, the digestion of organic matter during gut passage (Fdig) can be calculated from OM fraction in ingested (Fom-ingesta) and egested (Fom-egesta); and the compaction can be calculated from the Fdig and Fom-ingesta by the following (Jager *et al* 2003):

Fdig = [1 – (Fom-egesta/Fom-ingesta)] [1 / (1- Fom-egesta)]

Fcom = (1 / 1 – Fdig·Fom-ingesta)

Based on the above formulae and data of Risdill-Smith (1975) for *S. nigrolineata* fed with soil alone, we were able to obtain the digestion values of 0.39 and 0.55; and compaction percentages of 30% and 31% when the OM in ingesta were 0.59 and 0.43, respectively. These compaction values are very similar to the ones obtained for *H. elegans* in this study. These

Table 3 Intestinal weight/g initial live mass comparisons between larvae with full and empty guts. Means and standard errors are given.

Larvae with:	Initial live weight (mg)	Live weight before dissection (mg)	Gut dry weight (mg)	Rest of body dry weight (mg)	Relative gut weight (mg dw gut· g live larva^{-1})	n
Full guts	552.8 ± 13.0	552.8 ± 13.0	64.2 ± 2.1	37.0 ± 1.6	116.9 ± 3.9	30
Empty guts	534.3 ± 9.4	411.0 ± 9.2	30.1 ± 2.0	32.5 ± 1.7	56.1 ± 3.4	29
Difference					60.8	

higher compaction values in relation to *E. andrei* could be due to the higher content of OM in ingesta caused by selective consumption of scarab larvae (Wensler 1971, Risdill-Smith 1975, Mc Quillan & Webb 1994), whose digestion values are similar to those of *E. andrei*.

Although the estimated rates of soil egestion for *H. elegans* and *P. herrmanni* are lower than those of most species, population densities at which these larvae are currently found in the field influence their importance in the soil. According to Durán (1952), these species are normally found at densities between 1 and 25 larvae m^{-2}, but 200 and even 300 larvae m^{-2} can be found in severe outbreaks. Considering a normal variation in population density, the rate of soil egestion/unit of surface area would reach 1.7 kg d^{-1} ha^{-1} for *P. herrmanni* and 2.5 kg d^{-1} ha^{-1} for *H. elegans* with normal populations, and 20 kg d^{-1} ha^{-1} and 30 kg d-1 ha-1 with high density populations for *P. herrmanni* and *H. elegans*, respectively.

The potential of *H. elegans* and *P. herrmanni* as soil saprophages is lower than that of annelids and the humivorous scarab larva *P. ephippiata*, probably because they are not soil feeding specialists. Our observations showed that *H. elegans* has a greater potential to be an important element of the saprophagous soil macrofauna than does *P. herrmanni* and *S. nigrolineata*. The former produces higher proportions of faeces/unit of live larval weight when it is feeding on soil alone, probably because Rutelinae are less phytophagous specialists than Melolonthinae (Ritcher 1958).

Acknowledgments

The study was carried out as partial fulfilment for a doctoral degree in Agricultural Science at Universidad Austral de Chile. It was conducted as part of CONICYT programme for postgraduate supported by funds from the Chilean government.

References

Artigas J (1994) Entomología económica, insectos de interés agrícola, forestal, médico y veterinario. Vol II. Universidad de Concepción, Concepción, 943p.

Cazemier A E, Op den Camp J J M, Hackstein J H P, Vogels G D (1997) Fibre digestion in arthropods. Comp Biochem Physiol 118ª: 101-109.

Cisternas E (1986) Descripción de los estados preimaginales de escarabeidos en praderas antropogénicas de la zona sur de Chile. Tesis Lic. Agr., Universidad Austral de Chile, Facultad de Ciencias Agrarias, Valdivia, 119p.

Cisternas E, Carrillo R (2001) Pololo café de las praderas. Informativo Instituto de Investigaciones Agropecuarias INIA Remehue Nº 24.

Curry J P, Baker G (1998) Cast production and soil turnover by earthworms in soil cores from South Australian pastures. Pedobiologia 42: 283-287.

Curry J P, Schmidt O (2007) The feeding ecology of earthworms – a review. Pedobiologia 50: 463-477.

Duran L (1952) Aspectos ecológicos de la biología del San Juan verde, *Hylamorpha elegans* (Burm.) y mención de las demás especies de escarabeidos perjudiciales en Cautín. Agric Téc 12: 24-36.

Fleuren R, Jager T, Roelofs W, Groot A, Baerselman R, Peijnenburg W (2003) Feeding behaviour of *Eisenia andrei* in two different field contaminated soils. Pedobiologia 47: 670-675.

Harari A R, Ben-Yakir D, Chen M, Rose D (2001) Economic injury levels for the scarabaeid *Maladera matrida* infesting peanut fields in Israel. Entomol Exp Appl 98: 79-84.

Hartenstein F, Hartenstein E, Hartenstein R (1981) Gut load and transit time in the earthworm *Eisenia foetida*. Pedobiologia 22: 5-20.

Jager T, Fleuren R, Roelofs W, Groot A (2003) Feeding activity of the earthworm *Eisenia andrei* in artificial soil. Soil Biol Biochem 35: 313-322.

King P D (1977) Effect of plant species and organic matter on feeding behaviour and weight gain of larval black beetle *Heteronychus arator* (Coleoptera: Scarabaeidae). New Zeal J Zool 4: 445-448.

Kuniata L S, Young G R (1992) The biology of *Lepidiota Reuleauxi* Brenske (Coleoptera: Scarabaeidae), a pest of sugarcane in Papua New Guinea. J Aust Entomol Soc 31: 339-334.

Lavelle P, Bignell D, Lepage M, Wolters V, Roger P, Ineson P, Heal OW, Dhillion S (1997) Soil function in a changing world: the role of invertebrate ecosystem engineers. Eur J Soil Biol 33: 159-193.

Lemke T, Stingl U, Egert M, Friedrich M, Brune A (2003) Physicochemical conditions and microbial activities in the highly alkaline gut of the humus-feeding larva of *Pachnoda ephippiata* (Coleoptera: Scarabaeidae). Appl Environ Microbiol 69: 6650-6658.

Li X, Brune A (2005a) Digestion of microbial biomass, structural polysaccharides, and protein by the humivorous larva of *Pachnoda ephippiata* (Coleoptera: Scarabaeidae). Soil Biol Biochem 37: 107-116.

Li X, Brune A (2005b) Selective digestion of the peptide and polysaccharide components of synthetic humic acids by the humivorous larva of *Pachnoda ephippiata* (Coleoptera: Scarabaeidae). Soil Biol Biochem 37: 1476-1483.

Li X, Brune A (2007) Transformation and mineralization of soil organic nitrogen by the humivorous larva of *Pachnoda ephippiata* (Coleoptera: Scarabaeidae). Plant Soil 301: 233-244.

McQuillan P, Webb W (1994) Selective soil organic matter consumption by larvae of *Adoryphorus couloni* (Burmeister) (Coleoptera: Scarabaeidae). J Aust Entomol Soc 33: 49-50.

Oyafuso A, Arakaki N, Sadoyama Y, Kishita M, Kawamura F, Ishimine M, Kinjo M, Hirai Y (2002) Life history of the white grub *Dasylepida sp*. (Coleoptera: Scarabaeidae), a new and severe pest on sugarcane on the Miyako Islands, Okinawa. Appl Entomol Zool 37: 595-601.

Potter D A, Held D W (2002) Biology and management of the Japanese beetle. Annu Rev Entomol 47: 175-205.

Ridsdill-Smith T J (1975) Selection of living grass roots in the soil by larvae of *Sericesthis nigrolineata* (Coleoptera: Scarabaeidae). Entomol Exp Appl 18: 75-86.

Ritcher P (1958) Biology of Scarabaeidae. Annu Rev Entomol 3: 311-334.

Wensler R J (1971) Locomotor and feeding activity of larvae of the scarabaeid *Sericesthis germinata* (Coleoptera). Entomol Exp Appl 14: 270-282.

Received 28/I/09. Accepted 26/X/09.