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Contenido de materia orgánica y textura del suelo en el desempeño biológico de larvas de *P. herrmanni* Germ.

Soil organic matter content and soil texture on the biological performance of *P. herrmanni* Germ. larvae

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ABSTRACT

Glasshouse studies were carried out to determine the influence of soil texture and soil organic matter content (OM) on the weight and survival of the root-feeding scarabaeid larvae *Phytholoema herrmanni* Germ. (Coleoptera:Scarabaeidae:Melolonthinae). Survival and weight of third instar larvae were examined in three soil textures (silty loam, sandy loam and sand). Soil texture had a significant influence on larval survival, which was higher in soils rich in clay and silt, than in soils with a high presence of sand particles, however, soil texture did not affect larval weight. On the other hand, the increases in OM percentage and OM type (lability) in a volcanic ash derived soil of different horizons increase the larval weight and survival. This OM effect only occurs on larval survival when soils of horizon A of different origins and texture were tested. The differences observed in the response to the OM could be linked to the type of organic matter because horizon A soils present a larger proportion of labile organic matter than horizon B soils and this could be related to the feeding behaviour of Melolonthinae species (root feeders), however, further studies are needed to confirm this finding.

RESUMEN

Se llevaron a cabo estudios en invernadero para determinar la influencia de la textura del suelo y el contenido de materia orgánica (MO), sobre el peso y la supervivencia de larvas de *Phytholoema herrmanni* Germ. (Coleoptera: Scarabaeidae: Melolonthinae). Se examinó la supervivencia y el peso de las larvas del tercer estadio en tres texturas de suelo (franco limoso, franco arenoso y arena). La textura del suelo tuvo una influencia significativa en la supervivencia de las larvas, siendo mayor en suelos ricos en arcilla y limo, que en aquellos con alta presencia de partículas de arena, sin embargo la textura del suelo no afectó el peso de las larvas. Por otro lado, en un suelo derivado de cenizas volcánicas de diferentes horizontes, incrementos en el porcentaje de MO y tipo de MO (labilidad), aumentan el peso y la supervivencia larvaria. No obstante, este efecto de la MO sólo ocurre sobre la supervivencia de las larvas, cuando se probaron suelos del horizonte A de diferentes orígenes y texturas. Las diferencias observadas en la respuesta al MO podrían ser atribuibles al tipo de materia orgánica, por lo que los suelos del horizonte A presentan una mayor proporción de materia orgánica lábil, que los suelos del horizonte B y esto podría estar relacionado con el comportamiento de alimentación preferentemente de raices que presentan los Melolonthinae, sin embargo, se necesitan más estudios para confirmar este hallazgo.

Palabras clave: Scarabaeidae larva, insectos del suelo, textura del suelo, materia orgánica del suelo.

INTRODUCTION

Soil OM is important for the sustainability of natural ecosystems and the agroecosystems derived from them, with life within the soil being vital for maintaining life on Earth (Gardi *et al.*, 2013). Plant debris suffers the action of decomposers and stabilizers to be transformed in OM, a process where invertebrates play a key role, directly and indirectly (Wolters, 2000), acting over the production of ecological services (Lavelle, 1988) such as a decrease of surface runoff, erosion, water infiltration, nutrient cycling, primary production and climate regulation (Lavelle *et al.*, 2006). Soil characteristics such as texture and OM have an effect on the population dynamic of soil invertebrates including scarabaeid larvae, however, few studies consider this aspect (Macdonald and Ellis, 1990; Simelane, 2007).

P. herrmanni is one of the most important belowground pests affecting pasture, berries and small grain cereals in southern Chile (Durán, 1954; Prado, 1991; Cisternas et al., 2020). During the larval stage, this species consumes a mixture of inorganic and organic soil components, together with plant roots and microorganisms (Risdill-Smith, 1975; McQuillan and Webb, 1994; Millas and Carrillo, 2011). Several analyses have shown that scarabaeid larvae do not consume just bulk soil but also organic soil constituents such as OM and especially particulate organic matter (POM) (Mac Quilan and Webb, 1994; Millas and Carrillo, 2011). Therefore, it is reasonable to think that the larval development could be altered by the content and type of OM. Physical and chemical characteristics of soils, such as OM, pH, soil water content and temperature, as well as soil structure, texture, water holding capacity and aeration, have shown influence on below-ground insect larvae (Turpin and Peters, 1971; Régniere et al., 1981; Mac Donald and Ellis, 1990; Simenale, 2007). This study aimed to determine how the OM content and type and variety of soils affect the performance of *P. herrmanni* larvae. We hypothesised that 1) high OM content in the soil improves the survival of third instar larvae, 2) the larvae in soils with higher content of OM present better condition (higher weight), 3) high content of sand particles in the soil constituents will reduce the third instar larvae survival and 4) a decrease in soil OM content induces an increase in root consumption.

MATERIAL AND METHODS

The experiments were carried out in a glasshouse located at the Faculty of Agricultural and Food Sciences, Campus Isla Teja, Universidad Austral de Chile.

Biological material. Third instar larvae of *P. herrmanni* were collected during the first week of May from degraded pastures at the Austral Agriculture Research Station belonging to the Universidad Austral de Chile (39°55′S, 74°14′W). The identification of the larvae and determination of the instar were performed according to Cisternas (1986). The plant material consisted of wheat plants (*Triticum aestivum* L cv. Kumpa) sowed at the rate of 160Kg per ha⁻¹ immediately after incorporating the larvae. The plant material was included to provide a complementary food source for third instar larvae because they mainly feed on roots (Millas and Carrillo, 2011).

Soil. The first experiment was performed using Duric Hapludand (volcanic ash soil from Valdivia series, identified as Trumao) collected from the Austral Agriculture Research Station at two different depths: 2-10 cm (14.0% OM), horizon Ap, silt loam texture (clay 25.1%, silt 67.2%, sand 7.7%) and 30-40 cm depth (3.2% OM), horizon B, silt clay loam texture (clay, 32.8%, silt, 62.9%, sand, 4.5%). A detailed description of the soil can be found in Haas *et al.*, 2018. The same Valdivia series soil at 2-10 cm depth was used for the second experiment, as well as alluvial Misceláneo Pantano (MP-1) collected from the Experimental Station in an alluvial plain, horizon Ap sandy loam (clay, 6.1%, silt, 46.5%, sand 47.5%, (4.8% OM) obtained at the same depth and fine river sand, sand (clay 2.0%, silt, 0.5%, 97.5% sand), (0.1% MO), were tested. The OM, texture and soil chemistry analysis were carried out at the Institute of Agricultural and Soil Engineering. All soils were air dried and sieved at 4 °C until analyses (Table 1).

Two glasshouse experiments were carried out, the first one compared the response of the third instar scarabaeid larvae in Valdivia soil series (Trumao) collected at different depths horizon Ap (14.2% MO) and B (3.2% MO). The second experiment also measured the response of third instar larvae in three different soils with different contents of OM: silty loam (Trumao), sandy loam (Alluvial soil) and river sand (Sand). Each treatment had 8 replicates.

The plots consisted of plastic containers of 44.5 cm high and 31 cm diameter containing 35 L of soil. Soil moisture was kept at 60% of soil water holding capacity. To obtain this condition, the soil was dried at 108 °C for 48 h, then the same soil was saturated with water and weighed 24 and 48 h later to determine the water holding capacity of the soil. Containers with soil were weighed twice a week and refilled with water to obtain 60% of the holding water content of each container. Plots were filled with sieved soil, without structure. Seeds were sown manually at 160 kg ha⁻¹ and disinfected with fluquincezanole and prochloraz (Galmano®) 24 h before sowing. Fertilization was similar for all the soils: triple superphosphate (200 U of P_20_5 per ha⁻¹) and potassium nitrate (168 U of K₂0 and 180 U of N per ha⁻¹).

Third instar larvae (n = 8) were placed on the soil, allowing the larvae to penetrate the soil by their own means. Those larvae that did not penetrate the soil were replaced until the number of larvae in each plot was reached.

The larvae were harvested on August 9th and the experiments had been set up on the 8th of May, therefore, the experiments lasted 93 days. Water was used to separate the larvae from the soil and plants, then the material was sieved to obtain all the larvae. The number of larvae per plot was counted to estimate survival. The larvae of each plot were weighed individually in an analytical balance Acculab® (200 g, readability 0.01 g). Plants were harvested by cutting at ground level, then the soil was washed off from the foliage and roots before drying them in an oven at 60 °C for 72 h to weigh them.

Statistical analysis STATGRAPHICS 5.1. The data in percentage (survival) were arcsine transformed and normality was tested using the Kolgomorov-Smirnov test. Subsequently, the data were subjected to ANOVA using the F test and the means were separated using LSD and Duncan test when the treatment effect was significant ($p \le 0.05$).

RESULTS

Effect of the organic matter

Table 2 shows that the increase of OM from 3.2% to 14.2% ($p \le 0.000$) in soil of the same origin increases larval survival from 10.93% to 35.93% and larval weight ($p \le 0.000$) from 0.20 to 0.36 g. It is possible that, despite the soil having the same origin, the type of organic matter was diametrally different and this aspect will be analyzed in the discussion. In one of the experiments, there was a statistically significant effect ($p \le 0.000$) of OM and larvae on root dry weight and plant height.

Effect of soil texture

In Table 3, it is possible to observe that the percentage of OM in soils with plants increased the survival of larvae of *P. herrmanni* ($p \le 0.000$), with values of 48.43%, 35.93% and 25.56% in soils with 14.2%, 4.8%

Table 2. Survival and larval weight of *P. herrmanni* larvae in a soil of the same origin with different percentages of organic matter.

Cuadro 2. Supervivencia y peso de larvas de *P. herrmanni* en un suelo del mismo origen con diferentes porcentajes de materia orgánica.

OM and soil texture	Survival (%)	Larval weight (g)
14.2%, silt loam	35.93 a	0.36 a
3.2%, silt clay loam	10.93 b	0.20 b

Numbers followed by different letters within each column differ significantly ($P \le 0.05$, LSD test).

Table 3. Survival and larval weight of *P. herrmanni* in tree soilswhit different organic matter percentage, soil texture and origin.**Tabla 3.** Supervivencia y peso de larvas de *P. herrmanni* en tressuelos con diferente porcentaje de materia organica,textura yorigen.

OM, soil texture and origin	Survival (%)	Larval weight (g)
14.2%, silt loam, volcanic ashes	48.43 a	0.35 b
4.8% sandy loam, alluvial	35.93 b	0.40 a
0.1%, sand, alluvial	25.56 b	0.31 b

Numbers followed by different letters within each column differ significantly ($P \le 0.05$, Duncan Test).

Table 1. Physical and chemical characteristics of the substrate used.

Cuadro 1. Características físicas y químicas del sustrato utilizado.

Characteristics	Trumao 2 – 10 cm	Trumao 30 - 40 cm	Alluvial soil	Sand
pH water (1:2.5)	5.4	5.5	5.4	7.0
pH CaCl (1:2.5)	4.8	5.0	4.5	5.9
Organic matter (OM) (%)	14.2	3.2	4.8	0.1
Mineral nitrogen (N-NH4) (mg kg -1)	16.8	42.0	18.2	14.0
P-Olsen (mg kg ⁻¹)	25.4	2.6	72.9	2.1
Exchangeable potassium (mg kg $^{-1}$)	426	196	55	39
Exchangeable sodium (cmol kg ⁻¹)	0.05	0.15	0.13	0.03
Exchangeable calcium (cmol kg ⁻¹)	2.70	1.93	1.30	1.24
Exchangeable magnesium (cmol kg ⁻¹)	0.46	0.22	0.34	0.44
Bases (cmol kg ⁻¹)	4.30	2.80	1.91	1.81
Exchangeable aluminium (cmol kg ⁻¹)	0.40	0.05	1.42	0.02
CICE (cmol kg ⁻¹)	4.70	2.85	3.33	1.83
Aluminum saturation (%)	8.5	1.8	42.6	1.1

and 0.1% of OM, respectively. However, the weight of the larvae was higher with the intermediate OM concentration ($p \le 0.000$). There was an increase in root consumption in soil with different textures, but the differences were not significant ($p \ge 0.05$).

DISCUSSION

Mixed results were obtained regarding the effect of the amount of OM on the weight of *P herrmanni*, therefore, we rejected that particular hypothesis. We accepted the second hypothesis which states that an increase in soil OM increases larval survival. Also, we found that an increase in the percentage of sand particles reduces survival, so we accepted that hypothesis. Finally, we accepted the hypothesis that a drastic reduction of OM increases root consumption. However, the experiments showed interesting results that require the analysis of 1) the effect of the type of OM and 2) the effect of OM on root consumption.

Influence of OM on larval weight

In the first experiment, which involved the same soil (common parent material) obtained at different depths (horizons A and B), the percentage of OM significantly affected the larval weight. On the other hand, in the second experiment that used different soils (distinct parent material) with diverse textures but all from the same horizon, the effect of the percentage of OM was unclear because it was not statistically significant between soils with high levels of OM (14.2%) and low levels of OM (0.1%). In the first experiment, the higher weight of the larvae in horizon A with a greater percentage of OM could be linked with the type of OM present in the horizon A (first 2-10 cm) when compared to horizon B, rather than with the percentage of OM because the first one has a greater proportion of labile organic matter and the second one has stabilized (recalcitrant) OM (Rovira and Vallejo, 2007; Kleber et al., 2010). Labile OM is formed by the transit between fresh plant residues and humified OM and is defined as particulate organic matter (Gregorich and Janzen, 1996). On the contrary, at 30-40 cm depth the soil organic matter is humified OM, this kind of material has largely covalently bonded humic polymers with unique chemical structures that are different from those of the starting material (Kleber et al., 2010; Li et al., 2019). This situation could affect its use by below-ground insects since only some of them present specific microorganisms associated with their gut (termites) that enable them to use this type of OM (Martin, 1983; Lavelle, 1997; Bignell, 2006). The results suggest that the gut of P. herrmanni did not have the diversity of microorganisms or enzymes required to properly utilize this type of OM (highly humified). Huang et al., (2010)

indicated that the hind gut of the Melolonthinae (root feeding) sub family, to which *P. herrmanni* belongs, is shorter than that of Dynastinae (wood and humus feeding larvae) and with less diverse bacteria, suggesting that it is less specialized in the digestion of stabilized organic matter. On the other hand, when using soils only from horizon A, as in the case of the second experiment, the OM is principally labile and could be easily digested by the larvae, in this case, alluvial soil with inundative periods (anaerobic conditions) could present a high component of vegetable material (Reedy and Patrick, 1975) that can be easily digested by the larvae and could explain their increase of weight, despite the percentage of OM being lower than in the volcanic soil, and would explain the reduced consumption of roots observed in this treatment (Figure 1). Nonetheless, the results show that the role of the soil OM on below-ground insects is a complex field with major knowledge gaps. The complexity of what we know as soil OM (Swift, 1999) could explain the diverse effects of this material on below-ground invertebrates; some researchers have pointed out that the content of OM in the soil improves the condition of below-ground larvae (De Aquino et al., 2008).

In general, there was no increase in root consumption when reducing the percentage of OM in the soil, except in sand soil with a low level of OM (0.1%). Many authors have found a decrease in root damage by white grubs when increasing organic matter and POM (Davidson, 1969; Villalobos, 1994; Millas and Carrillo, 2011). A possible explanation of the absence of response in this experimental work could be related to the feeding behaviour of third instar larvae, which consume preferentially roots and not OM (Mac Quillan and Webb, 1994; Millas and Carrillo, 2011), consequently, the importance of the level of OM on feeding behaviour should be lower; at the same time, the low temperature of the glasshouse with no heating used in this study during late autumn and early winter could explain the low incidence of root damage caused by the larvae.

Effect of soil OM and texture on larval survival

In our experiments, the survival rate increased along with the increase of the OM but in our case, the results could be an artifact because in our experiments the increase of OM occurred with a reduction in the percentage of sand particles. Soils with an increase of sand particles have shown a reduction in the survival of below-ground Coleoptera, especially first instar larvae (Turpin and Peters, 1971; Lummus *et al.*, 1983; Macdonald and Ellis, 1990; Simenale, 2007), however, the survival decrease is mainly attributed to rapidly drying soils (Simenale, 2007), a situation that in this study is highly improbable since soils were irrigated twice a week at 60% of field water holding capacity. A



Figure 1. Effect of *P. herrmanni* on total dry matter of wheat plants in different types of soil.Figura 1. Efecto de *P. herrmanni*, sobre la materia seca total de plantas de trigo, en distintos tipos de suelo.

second reason for the decrease in survival is the sharp edge of sand particles that could damage larvae tissues and has been reported as the cause of a lower survival rate (Regniere et al., 1981). Therefore, it is likely that the effect of the sand particles could be related to the abrasive effect of the sharp edges of the sand, which has been reported to cause mortality (Simenale, 2007). This effect could be both external and internal, affecting the cuticle and the gut because the larvae move through the soil and ingest soil (Millas and Carrillo, 2011). Alterations in the midgut and hindgut could be important because in these regions the associated microorganisms, enzymes and gut fermentation occur (Lemke et al., 2003; Li and Brune, 2005). Although the peritrophic matrix, a non-cellular sieve like structure that lines the midgut, protects the insect against abrasive food particles (Terra, 2001), it could be insufficient against sand abrasive particles that are coarse, large (0.5-2.0 mm) and more irregular in shape when compared to silt and clay (Simenale, 2007). Furthermore, the effect of sand particles could reduce the protection of this matrix against microorganism infection (Kuraishi et al., 2011). A third explanation could be related to the reduction in larval movement in sand soils which could be important for first instar larvae as reported by Macdonald and Ellis (1990) and Simenale (2007) but could be irrelevant for larvae of larger size like the ones in this study. However, it is necessary to indicate that one of the limitations of this type of experiment is the use of plant pots with tamized soil, because it produces a soil profile with a poor structure that limits the accuracy of the results obtained and might not be representative of nature (Pacchioli and Hower, 2004). Another possible interpretation of the results obtained regarding the negative effect of a high content of sand could be related to lower diversity of microorganisms, especially bacteria, in this type soils (Carson et al., 2009; Seaton et al.,

2020; Xia et al., 2020). Prokaryotic and fungal communities play an important role in food digestion (Egert et al., 2005; Huang et al., 2012), therefore, low gut bacterial diversity could affect larvae survival. Nonetheless, it is necessary to indicate that the microorganisms responsible for the digestion of recalcitrant OM present in the hindgut are more or less independent of soil conditions (Zhang and Jackson, 2008). Unfortunately, in Chile we lack information on the microorganism community in scarabaeid larvae gut, however, this is a new area of research worldwide. This study is an important contribution to the knowledge of scarabaeid larvae behaviour in soils with high content of sand particles since previous studies have been mainly carried out in first instar larvae and insects of the Chrysomelidae family (Lummus et al., 1983; Simenale, 2007).

CONCLUSIONS

The results of this study show that an increase in the percentage of sand particles in soil reduces the survival of *P. herrmanni*, in its third instar larvae. Also, the type of OM (degree of stabilization) has a higher impact on the larval weight of third instar larvae than the percentage of OM, being larger in a soil with higher content of labile OM. Finally, root consumption of *T. aestivum* plants by the scarabaeid larvae *P. herrmanni* increased only in soils with very low levels of OM.

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