An improved method to produce adults of *Costelytra zealandica* White (Coleoptera: Melolothinae) from field-collected larvae

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Abstract

Rearing techniques provide a unique opportunity to study aspects of insect ecology, behaviour and physiology. Both the larval and adult stages in Melolonthinae scarabs have important impacts on crop and pasture yields worldwide. Rearing techniques for this group of phytophagous beetles usually results in a low survival rate from larva to adult, varying from 10% to 50%. Here, the current rearing method used for the New Zealand grass grub (*Costelytra zealandica*) was improved by increasing the pupation weight threshold, as well as by changing the container type used to rear the larvae. This improved method produced an 83% increase in the survival rate from larva to adult, and the technique developed here may help increase the laboratory survival rate of other Melolonthinae species worldwide.

Keywords: White grubs, laboratory-rearing methods, mass-rearing.

Introduction

The "art" of insect rearing has a history of more than 3000 years beginning in ancient China when silkworms (*Bombyx mori* L.) were reared to produce fine fabrics (Kurin 2002). Rearing techniques also provide a unique opportunity to study the biology, life cycle and physiology of insects. Some scarab species in the subfamily Melolonthinae, commonly known as white grubs, are important pests worldwide (Jackson & Klein 2006), reducing crop and pasture yield at the larval stage by feeding on roots (e.g., Hata et al. 2014), and in some cases by consuming plant foliage as adults (e.g., East et al. 1983; Oliveira et al. 2007). However, detailed methods of producing laboratory cultures of these animals for scientific research are rare (Romero-López et al. 2011). Amongst those few, the most frequently found rearing technique is to confine the larvae in a plastic container filled with soil and feed them with slices or cubes of plant material (e.g., Burakowski 1993; Hata et al. 2014) or directly with living roots (e.g., Miner 1948; Rodrigues et al. 2010). These containers are kept at optimal temperatures and conditions for adult production.

This rearing approach has been used for different melolonthid species, with survival rates from larva to adult varying generally from 10% to 50% (Burakowski 1993; Rodrigues et al. 2008; Rodrigues et al. 2010; Romero-López et al. 2011; de Souza et al. 2015). Although, an exception for this general trend was found in one Mexican species (*Phyllophaga vetula* Horn), which had a higher survival rate (85%) when compared with other melolonthids (with survival rates from 20% to 50%), all collected in the same area (Aragón-García et al. 2005). This suggests that the success of the method is partly species-dependent.

In New Zealand, the larvae of *Costelytra zealandica* White (Scarabaeidae: Melolonthinae), are endemic pests of pastures, and adults of *C. zealandica* have been found feeding on the foliage of horticultural crops such as blueberries, kiwifruits and vines (East et al. 1983; New Zealand

Winegrowers 2013). Despite the economic impact of such defoliation, laboratory experiments with adults are rare, mainly because of the reduced availability of adults during the flight season (i.e., from late spring to early summer), the short life-span of the adults (i.e., 3-4 weeks) and the difficulty of rearing this species under laboratory conditions (Wigley & Dhana 1992; Lefort et al. 2015a). In 1992, Wigley and Dhana suggested a technique to overcome some of these issues. They proposed the use of varying rearing temperatures throughout the developmental stages in order to accelerate or delay *C. zealandica* larval development, and consequently, adult emergence. This method extended access to adults from July to late-January. In addition, the larval weight necessary to enter the pupation phase, called hereafter, "pupation threshold" is critical for inducing morphological changes from larva to pupa, previously recorded for this species to be 120 mg (Wightman 1974). Despite the noticeable improvements of this *C. zealandica* rearing method in terms of adult availability and in the establishment of temperature thresholds necessary to reach each developmental stage, the highest adult emergence rate never exceeded 25% (Wigley & Dhana 1992). In the current note, we report a modification of the method used by Wigley and Dhana (1992), which greatly improved the survival rate from larvae to adults up to 83%.

Methods

Third-instar *C. zealandica* larvae were collected from Lincoln University's Field Service Centre arable land (Canterbury, NZ, 43°38'S; 172°27'E) from late April to mid-May 2014. Larvae were weighed and individually placed in either: (1) cylindrical plastic containers (CPCs) (2.5 x 7 cm) filled with 50 g of gamma-irradiated (Schering-Plough Animal Health, Wellington, NZ) (Lefort et al. 2015b) moist soil (20% w/w); (2) in 14-well ice cube trays (ICTs) (24.5 x 9 x 2.5 cm); or (3) in 24-well tissue culture plates (TCPs) (Biofil ®, 12.5 x 8.3 x 2.3 cm). CPCs were closed with a plastic lid with holes (Figure 1). A piece of organically-cultivated carrot (*c.* 1 cm^3) was put in each of the three container types, which were

then stored in a dark climate-controlled room at 15°C to increase larval weight until 180 mg, based on the results of Wightman (1974). ICTs were wrapped with moist tissue paper and grouped in pairs inside a plastic bag, to reduce larval and carrot desiccation. In the ICT and TCP treatments, carrot was supplied every 4 to 7 days, while in CPCs it was supplied every two weeks, as larval consumption rate was lower under the latter treatment. During this feeding step, all diseased larvae were removed and the tissue paper was changed and rewetted (ICT only).

After two weeks, larval weight was recorded and individuals weighing more than 180 mg were placed in a new clean CPC, ICT or TCP. All container types were placed in an incubator chamber at 4°C with 60% relative humidity in complete darkness for eight weeks to induce pupation (Wigley & Dhana 1992). A total of 164 larvae were maintained in CPC, 175 in ICT, and 198 on TCP. After eight weeks, all the containers were stored in a dark climate-controlled room at 20°C to induce adult emergence. Adults started to emerge after two weeks in this controlled environment.

A Chi-Squared test of independency was used to evaluate the effect of the container type (CPC, ICT and TCP) on adult survival. Statistical analysis was performed using R v.3.2.5 (R Core Team, 2016).

Results and Discussion

The proportion of larvae that produced adults was highly dependent upon the rearing treatment (χ^2=130; df=2; $p<0.001$). A total of 38, 102 and 164 adults respectively were produced in the CPC, ICT and TCP treatments, giving survival rates of 23.2, 58.3 and 82.8%, respectively. The low survival rate observed in CPC may have been due to high moisture content, either in the CPC or in the incubator chamber, as fungal hyphae were discovered in several tubes at the end of the pupation phase. Furthermore, it is important to note that because the food was placed in the

top of the CPC, an additional foraging effort was needed for the larva to reach its food, which might have had potential consequences for larval weight gain and further adult development. However, a survival rate of 23.2% is within the general trends found in the literature (10% to 50%).

Higher survival rates were obtained in the ICT (58.3%) and TCP (82.8%) treatments, presumably related to a higher larval weight of 180 mg before entering pupation phase, compared with the low survival rate of 25% when larvae weighing 130 mg were used to produce adults (Wigley & Dhana 1992). It has been previously observed that a direct relationship exists between larval weight and successful pupation, as the highest survival rates were obtained with larvae weighing more than 160 mg (Wightman 1974). This relationship is also suggested by the variations in *C. zealandica* life cycle duration; a one-year life cycle is usually present throughout New Zealand, although a two-year cycle can be found in harsh environments (Perrot & Stockdill 1973). This variation in life cycle has been related to food and temperature restrictions, as larvae cannot gain the necessary weight to reach the pupation threshold in one season (Wightman 1974).

The fact that TCP produced 24% more adults than ICT was unexpected, as the methods are similar. Speculatively speaking, this result could be explained by the conditions inside the wells offered by the plate's shape (TCP), with a potential effect on gas exchange from the well with its surrounding atmosphere. When O_2 decreases due to larval respiration, the O_2/CO_2 ratio present in the plate well could change, which might trigger metabolic responses in the larva, as low O_2 levels had been linked to a temporary inhibition of ecdysteroid secretion, with a latter stimulation of the prothoracic glands that secrete ecdysone, promoting metamorphosis (DeLalio et al. 2015).

It has been noted that *C. zealandica* third instar larvae produce a pupation chamber made of soil particles (Pottinger 1968), as described for other

melolonthids (Burakowski 1993; Rodrigues et al. 2010; de Souza et al. 2015), but the implications of this behaviour remains unknown. Therefore, is plausible that this pupation chamber might contribute to triggering metabolic changes at the larval stage by changing the O_2/CO_2 ratio inside it that could trigger larval metamorphosis, increasing adult emergence. Finally, considering the high survival rate found in both the ICT and TCP treatments, our study suggests that in *C. zealandica*, the use of soil (or other substrates) to produce adults from field collected larvae is not necessary. Moreover, the non-soil methods used here offer an easy approach to larval care, with less handling effort and better control of food supply, with easy removal of diseased or dead larvae.

Conclusions

By changing the container type and increasing the larval weight before pupation phase used to rear *C. zealandica*, up to 83% of the larvae were successfully reared to adults. Our study suggests that by using ICT or TCP, a potentially valuable increase in adult emergence under laboratory conditions can be obtained from field-collected larvae. This approach, in combination with changes in temperature during the different developmental stages proposed earlier by Wigley and Dhana (1992), could facilitate laboratory studies with adult *C. zealandica* over a large part of the year, increasing our understanding of its biology and behaviour. Furthermore, the advantages provided by both ICT and TCP could eventually enhance the survival rate of other species of white grubs around the world, especially when conventional methods have produced a very restricted number of adults. However, key issues such as food type, temperature and weight thresholds must be considered from species to species, as slightly variations in these factors could affect the outcome of the culture.

Fig. 1. Container types used to rear *C. zealandica* larvae into adults. a) Ice cube trays, ICT; b) cylindrical plastic containers, CPC; and c) tissue culture plates, TCP. Larvae feeding on organically–cultivated carrot can be seen in a) and c). Pupae can be seen in c).

Acknowledgements

We would like to acknowledge Callaghan Innovation scholarship (KNZLP1201) and Kono Beverages for founding M.G.Chang PhD programme.

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