Biology of *Ostenia robusta*: observations on life history and behaviour

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Abstract *Ostenia robusta* (Hutton) is an endemic dolichopodid fly that sometimes occurs in the soil under agricultural crops and pastures. Little is known about its biology and the role it may play in agro-ecosystems. A population of these flies near Southbridge, Canterbury, was observed from 2013 to 2015. Adult flies emerged between December and March each year and were monitored with yellow sticky traps placed in wheat, ryegrass, radish and white clover crops. Adults will drink water and feed on 10% honey solution under laboratory conditions but no predatory behaviour was observed and attempts to induce oviposition were unsuccessful. From dissection, maximum female egg load was approximately 60–65 eggs. Very few *O. robusta* larvae were found in 2014 and none were associated with pupae of *Costelytra zealandica* (White), a result contradictory to 2012 and 2013. The diet range of larval *O. robusta* remains unknown, but this predator appears unlikely to play a significant role in the control of *C. zealandica*.

Keywords *Costelytra zealandica*, grass grub, *Ostenia robusta*, Dolichopodidae, predation.

INTRODUCTION

Ostenia robusta (Hutton) (Diptera: Dolichopodidae) is endemic to New Zealand and has been recorded from both the North and South Islands (Bickel 1991; Chynoweth et al. 2013). In 2012 a population of *O. robusta* larvae was discovered at a farm in Southbridge, Canterbury, feeding on pupae of *Costelytra zealandica* (White) (Coleoptera: Scarabaeidae) (Chynoweth et al. 2013). Various pathogens, parasitoids and predators are known to attack grass grub, *C. zealandica* (Cameron & Wigley 1989), but *O. robusta* was not recorded as a predator of *C. zealandica* before 2012. This novel association between two endemic species warranted further

investigation to determine the impact of *O. robusta* on *C. zealandica* populations.

Almost nothing is known about *O. robusta* beyond its original taxonomic description and this only covered the adult stage (Hutton 1900; Bickel 1991). The biology of Dolichopodidae is also poorly described with very few species subjected to detailed study, e.g. *Medetera* spp. that attack bark beetles (Goyer et al. 2004; Hulcr et al. 2005). Adult flies are predatory, usually on soft-bodied insects (Ulrich 2004; Lundgren et al. 2014). The larvae are also thought to be predatory (Dyte 1993; Aukema & Raffa 2004), although the genus *Thrypticus* is adapted to

herbivory on aquatic plants (Dyte 1993; Bickel & Hernandez 2004). Larvae are generally found in moist habitats such as damp soil, leaf litter or rotting wood (Daly et al. 1998) with some species wholly aquatic at the larval stage (Reeves et al. 2004; Hernandez 2008).

Chynoweth et al. (2013) presented the first information about the life history of *O. robusta*. The present study follows directly from that previous work to document adult emergence over two summers and to present observations on feeding and reproductive behaviour. The likely impact of *O. robusta* on *C. zealandica* is considered in light of these observations.

MATERIALS AND METHODS

Adult emergence and larval collections

All *O. robusta* adults and larvae were collected from the same farm at Southbridge, Canterbury (43°49.061S; 172°14.445E), where predation on *C. zealandica* was first observed (Chynoweth et al. 2013). Collections were made across several adjacent paddocks from 2013–2015. These paddocks were irrigated, fertilised and treated with pesticides according to standard agronomic practices each year. The crops were grown for seed and most paddocks followed a clover, cereal, ryegrass rotation over the study period.

Adults were captured on yellow sticky traps (22 × 20 cm) attached to fiberglass canes threaded through pre-made holes punched directly into one side of the trap. Traps were set approximately 2 cm above the ground because *O. robusta* adults were almost always observed on or close to the ground in the field. From 11 December 2013 to 7 March 2014 there were 16 sticky traps placed in one field of clover (2 traps) and two adjacent wheat fields (3 traps in one field, 11 traps in the other). For this first season the trap locations targeted areas where *O. robusta* larvae had been found in 2012 (Chynoweth et al. 2013). From 1 December 2014 to 7 April 2015 there were 16 sticky traps placed across one field of radish (4 traps), one ryegrass field (6 traps) and two adjacent wheat fields (2 traps in one, 4 in the other). For the second season, traps were distributed across the fields rather than clustered in

targeted areas. The two wheat fields were the same for both seasons while the clover field in 2013–14 was sown with ryegrass in 2014–15. The field sown with radish was sampled only in 2014–15. Traps were collected and replaced weekly during both seasons except during the 2-week harvest period in February of each year. The numbers of adult *O. robusta* on both sides of the traps were recorded during both seasons. In 2014–15 the sex of each adult was determined after examination under a stereomicroscope. Adult dolichopodids can be sexed easily from examination of the external genitalia (Borror et al. 1989).

Larval *O. robusta* were collected from soil samples dug from the wheat fields in late 2013 and 2014 (n > 9 samples per year). Areas of up to approximately 1 m² and at least 0.5 m deep were dug out using spades and the soil broken apart by hand to collect *O. robusta* larvae. Samples were taken within a 200 m radius of 43°48.940S, 172°14.424E. Sampling was targeted rather than randomised to minimise disturbance to the crop and aimed for points where *O. robusta* larvae had been found previously, where large numbers of adult flies were found, or where there was evidence of *C. zealandica* activity.

Adult behaviour

Adult *O. robusta* were collected by hand and sweep net in the wheat and ryegrass fields at Southbridge once or twice per week from 13 January to 20 February 2015. Adults were easily seen walking on bare patches of ground between the rows of wheat and could be captured by hand without damaging the crop. A sweep net was used along the edges of the ryegrass paddock to increase the numbers of flies captured. All adults captured were taken back to AgResearch at Lincoln to observe their feeding and reproductive behaviour. From the third collection on 26 January onwards, the adults were gassed and immobilised briefly with CO₂ so that they could be examined using a stereomicroscope to determine their sex before being assigned to cages.

Adults from the first three collections were split between two fine cloth mesh cages (45 × 45 × 60 cm)

and two clear Perspex cages ($45 \times 45 \times 45$ cm). A grass turf (approximately $40 \times 40 \times 10$ cm) in a plastic tray was placed in the bottom of each mesh cage and one of the Perspex cages. The second Perspex cage had a pot containing clover infected with aphids as a possible food source. Other small insects (flies, aphids, etc.) captured in the sweep net when searching for *O. robusta* were released into the mesh cages as potential prey items. In each cage, water and 10% honey solution were provided in separate 10 ml plastic cups with a cotton wick poked through the lid. These water and honey sources were replaced as needed to keep them fresh. From 28 January onwards subgroups of females and males were placed in either one of the Perspex cages (Table 1) or in 2-litre plastic containers (Sistema™) with about 3 cm loose packed Wakanui silt loam and containing water and honey, as above, in an attempt to induce oviposition. Males were marked on the thorax with a white dot of correction fluid so the sexes could be distinguished easily. Second instar *C. zealandica* larvae ($n = 4$ or 5) were added to two of the 2-litre containers to see if their presence would encourage oviposition.

Any *O. robusta* not assigned to the oviposition cages were placed in the mesh cages with the adults remaining from previous collections. Cages and containers were checked every 1–2 days to observe responses to the food sources and substrates provided. When all adults had died the plants and soil were removed from the cages/containers and searched for evidence of oviposition. Some females ($n = 12$) were dissected after death under a stereomicroscope to determine egg load.

Statistical analysis

Trap numbers were not spread equally across wheat and clover crops in 2013–14 so no statistical analysis was conducted on these data. In 2014–15 equal numbers of traps were placed in ryegrass and wheat so the proportion of flies emerging from these two crops was compared using a z-test. The proportion of males and females captured on sticky traps or by hand and sweepnet were also compared using z-tests. All analyses were conducted in SigmaPlot version 13.

RESULTS

Adult emergence and larval collections

1092 *O. robusta* were collected from traps in the wheat fields and 10 *O. robusta* from the clover in 2013–14 with the majority of adults collected in January and February 2014 (Figure 1a). Only 144 *O. robusta* were collected in 2014–15 again with the majority of adults collected during January and February (Figure 1b). 64 flies were collected from traps placed in wheat, 80 from ryegrass and none from radish. Similar proportions of flies were collected from wheat and ryegrass ($z = 1.16$, $P=0.25$). No flies were caught from ryegrass for 2 weeks after harvest in 2015 whereas some flies were caught from the wheat fields straight after harvest in both 2014 and 2015. The trap catches were strongly male biased in 2014–15 with 126 males and 16 females captured ($z = 6.97$, $P<0.001$, Figure 2a). The sex of two specimens could not be determined.

In 2013–14, 48 *O. robusta* larvae were collected and 32 of these (67%) were associated with *C. zealandica* pupae. The remaining larvae were not associated with any prey. Very few *O. robusta*

Table 1 Number of male (M) and female (F) *Ostenia robusta* assigned to different cage treatments intended to induce oviposition.

Collection date	Flies	Cage type	Replicates	Treatment
28 January	2F	2-litre plastic	4	Soil only
2 February	2M + 2F	2-litre plastic	2	Soil only
	2M + 2F	Perspex	1	Grass turf
	2F	Mesh	1	Clover + aphids
9 February	2M + 2F	2-litre plastic	2	Soil + grass grub

Figure 1 Total numbers of adult *Ostenia robusta* captured on sticky traps in wheat, clover and ryegrass at Southbridge, Canterbury, during (a) 2013-14 and (b) 2014-15. Note the different Y-axis scale between the two seasons.

Figure 2 Total numbers of male and female *Ostenia robusta* captured at Southbridge, Canterbury, in 2014-15 (a) on sticky traps and (b) by hand or sweep net.

larvae were collected in 2014 ($n < 5$) and none was associated with *C. zealandica* pupae or any other prey.

Adult behaviour

162 adults were collected by hand and sweep net from the wheat and ryegrass paddocks with most collected in January and February (Figure 2b).

The sex ratio was strongly male biased: 107 male, 25 female, 30 undetermined ($z = 5.82$, $P < 0.001$, Figure 2b). No predation by *O. robusta* was observed in the cages but adults were seen to drink from the water and honey wicks occasionally. Field-collected adults lived for up to 2 weeks after capture. Adults (presumably males) were observed walking and perching on the bottom

Table 2 Egg complement and fat bodies found in field-collected female *Ostenia robusta*.

Collection date	Eggs present	Fat bodies present
21 January	61	None
	65	None
	57	Few
28 January	1	None
	4	None
	5	None
	63	None
2 February	0	None
	66	Many
	20	None
	5	Some

of the Perspex cages in a similar manner to the behaviour seen on bare ground in the wheat fields. The adults appeared to space themselves across the floor of the cage and on four occasions two (presumed) males were observed to wrestle briefly (< 5 s) before separating again. These interactions were not attempts at mating – one such attempt was seen but the female was not receptive. She threw the male off twice in < 5 s then flew away.

Four female *O. robusta* appeared to have a full or near full complement of mature eggs (61.5 ± 1.7 eggs, mean $\pm$ se) upon dissection, based on the abdomen containing large numbers of eggs generally with very few or no fat bodies (Table 2). Six females had probably oviposited some or all eggs prior to capture because they had no fat and less than one-third of the maximum egg complement. Two females had both eggs and fat bodies present so presumably were not yet ready for oviposition. No evidence of oviposition was found in any of potential substrates provided in the cages and there was no evidence of attack on the *C. zealandica* larvae exposed to *O. robusta*.

DISCUSSION

Numbers of adult flies trapped were an order of magnitude higher in 2013–14 compared with 2014–15, which may indicate variation between

seasons or between different crops. The very low numbers emerging from clover suggest this may not be a favoured crop for *O. robusta* when compared with the numbers emerged from the ryegrass in the same field the following season. Radish was also apparently unfavourable in 2014–15 but that field had not been sampled previously for adults and no *O. robusta* larvae were ever found in this field (R.J. Chynoweth, personal observation). Across the two seasons the bulk of adults were captured from wheat crops grown following white clover but sampling effort was not distributed equally across wheat, ryegrass, clover and radish so caution is advisable when interpreting the data.

Peak emergence for adult *O. robusta* occurred from January to February over two consecutive summers at the one location sampled. Of the 36 museum records for *O. robusta* (J.W.M. Marris, Lincoln University, personal communication), 28 have the month of collection recorded and 19 of these records are from January or February. This indicates adults will be easiest to find during those month. However, note that males were easier to find than females. This bias was not related to collection method and a key constraint on the present observations was the low numbers of females captured. A likely explanation for the predominance of males in field samples is that they engage in some sort of courtship display that coincidentally makes them more vulnerable to capture. Dolichopodidae are known to engage in complex courtship rituals involving male displays and active pursuit of the female either in the air or on the ground (Land 1993; Zimmer et al. 2003). The behaviour of *O. robusta* under both field and laboratory conditions supports this hypothesis but more detailed observations are needed to confirm it.

No firm conclusions were able to be drawn about adult prey choice or oviposition preferences. Adults did drink liquids but no predation events were seen. The most likely explanation is the prey items offered were not suitable but it is possible that *O. robusta* does not feed as an adult. There is also no positive evidence about the conditions required for oviposition

by this species. The failure to oviposit probably means suitable food/prey were not provided for early instar *O. robusta*. Another possibility is that despite about one-third of the females carrying mature eggs, they may not have mated prior to capture and the laboratory conditions were unsuitable for successful courtship and mating. Favourable environmental conditions are important for successful mating in captivity by dipteran species with complex mating rituals such as the Tephritidae (Benelli et al. 2013). Equivalent information is not yet available for the Dolichopodidae.

Larval *O. robusta* were found attacking *C. zealandica* pupae for the first time in 2012 (Chynoweth et al. 2013) and again in 2013, but none of the few *O. robusta* larvae found in 2014 were associated with *C. zealandica* and no such association has been detected elsewhere when *C. zealandica* pupae were collected for other purposes (R.J. Townsend, personal observation). It is possible that the association between these *O. robusta* and *C. zealandica* was coincidental and does not reflect the usual diet of *O. robusta*. It is still unclear what *O. robusta* larvae typically eat because few potential prey items were found in the soil samples taken from Southbridge. A few *O. robusta* larvae collected in 2013 were exposed to small earthworms, *Allolobophora calliginosa*, in soil for up to 6 weeks without any evidence of predation (R. J. Townsend, personal observation). If a larger population of *O. robusta* can be found in future then DNA analysis of larval and adult gut contents is probably the most fruitful approach to identify the prey taken by *O. robusta* during both life stages. So far all observations of *O. robusta* feeding on *C. zealandica* are confined to a few fields at one site and only in two seasons. Unless predation by *O. robusta* on *C. zealandica* can be confirmed across more sites and multiple years, it is unlikely that this predator has a significant impact on *C. zealandica* populations.

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