The host range of the moth plant fruit fly, *Anastrepha australis*, a biological control agent for moth plant, *Araujia hortorum*

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SUMMARY

- The host range of the moth plant fruit fly (*Anastrepha australis*) was determined in laboratory tests. *Anastrepha australis* was sourced from Montevideo, Uruguay in South America.
- Experiments conducted in the Manaaki Whenua - Landcare Research containment facility in Auckland, New Zealand, tested eight plant species related to moth plant in the family Apocynaceae.
- Female *Anastrepha australis* lay eggs in the fruit (also known as pods or follicles) of their host and the larvae feed on the seeds within. For the testing, adults were placed in cages and exposed to fruits of the test plants to assess development.
- Eggs laid in moth plant and *O. coeruleum* pods successfully developed to pupae and the larvae fed significantly on both species. No pupae were recovered from any other plant species.
- *Oxypetalum coeruleum* is closely related to moth plant (both belong to the sub-tribe Oxypetalinae). Tests indicate that *O. coeruleum* is within the fundamental host range of the moth plant fruit fly population from Uruguay, and could be at risk of attack in New Zealand if the fruit fly is introduced.
- The testing indicated that plants belonging to the Apocynaceae outside the sub-tribe Oxypetalinae will not be used by *A. australis* in New Zealand.

CONTENTS

INTRODUCTION

This report outlines the history of the biological control programme against moth plant in New Zealand and presents the results of research to determine the host range of *Anastrepha australis*, and hence the direct risk it would pose to valued plants if introduced to New Zealand.

The biological control programme against moth plant

Moth plant, *Araujia hortorum* E. Fourn. is an increasingly important environmental weed in northern regions of New Zealand. Note that *A. hortorum* is regarded as a synonym of *A. sericifera* Brot. by most authorities (e.g. https://www.gbif.org/species/3579928). Nevertheless, we continue to refer to it as *A. hortorum* as this name is in common use in New Zealand. A biological control programme was initiated in 2000 to mitigate the effects of the weed and to reduce the rate of spread, because conventional control methods such as herbicide application and physical removal are expensive, damaging to non-target plants, and often impractical.

Suitability of moth plant as a target for biological control

The programme began with an assessment of the prospects for biological control as a management tool (Winks and Fowler, 2000). A survey was conducted to determine what resident insects and diseases were present on moth plant in New Zealand, and to assess their role in moth plant ecology. Fifty-three invertebrate species were collected and 46 were identified. No specialist moth plant

natural enemies were found, and any damage that could be attributed to invertebrate herbivory was minimal (Winks et al., 2004). The polyphagous sap-feeding passionvine hopper (*Scolypopa australis*) was the only abundant invertebrate found on moth plant during the survey. The oleander aphid (*Aphis nerii*) occasionally produced 'outbreaks' that led, temporarily, to very large populations.

Overall, none of the herbivore niches on moth plant were well utilised in New Zealand (Winks et al. 2004).   Most disease symptoms observed were superficial, non-systemic, minor leaf spots that did not impact severely on the aggressive vegetative growth or flower/fruit/seed production (Winks et al., 2004; Waipara et al., 2006). It was concluded that moth plant was not subject to any significant biotic pressures, and there were no existing relationships that could influence a biological control programme.

Selection of potential control agents

The programme takes two approaches to management of moth plant. Manaaki Whenua - Landcare Research (MW - LR) is seeking to:

1. limit the survival and/or biomass (pest status) of moth plant by the introduction of a range of invertebrates and diseases to attack the roots and foliage.

2. limit the rate at which moth plant populations establish and grow by introducing invertebrates that reduce seed production by attacking reproductive structures such as fruits.

Surveys of plants, pathogens and insects were conducted in the native range of moth plant in the 2004-2006 seasons to locate and identify potential agents for classical biocontrol. In all, 48 plant populations were examined (Waipara et al., 2006). A range of disease-forming organisms and 23 invertebrate species were collected during this survey (Carpintero 2006, 2009; Waipara et al., 2006). Waipara et al. (2006) discuss the merits of the recorded diseases as biological control agents. Carpintero (2006 and personal communication) listed 46 insect species that had been recorded attacking moth plant and related species and suggested that 13 insect species were worthy of further research. MW - LR staff analysed which had the best characteristics to be control agents in New Zealand and selected four. One of these species was a fruit fly *Anastrepha (Toxotrypana) australis* (Q. Paynter, MW - LR, personal communication). A further survey was conducted in Uruguay in 2019. A population of *A. australis* was found in Montevideo and was returned to New Zealand for assessment in containment.

BIOLOGY AND ECOLOGY OF *ANASTREPHA AUSTRALIS*

Taxonomy

Order	Diptera
Family	Tephritidae
Subfamily	Trypetinae
Genus	*Anastrepha*
Species	*A. australis* (Blanchard)

Seven species recognized as the *curvicauda* species group previously formed a genus *Toxotrypana* including the fly of concern in this report. Species of the *curvicauda* group are large bodied and commonly brightly coloured and are thought to mimic wasps. The larvae feed on seed tissues of latex producing plant species.

Although clear differences in external appearance exist between the *curvicauda* species group and *Anastrepha*, there remained similarities in genitalic characters and a clear relationship demonstrated by molecular studies (Mengual et al., 2017). As a result, *Toxotrypana* was synonymised with *Anastrepha* in 2018. *Anastrepha* took precedence due to species within the *Anastrepha* group at the time having far greater economic importance in comparison to species of the *curvicauda* group (Norrbom et al., 2018). *Anastrepha* is a diverse genus of over 300 described species distributed throughout the American tropics and subtropics.

There remains some taxonomic uncertainty to be resolved within the *curvicauda* species group. The *Anastrepha* flies imported into containment for host specificity testing were identified as *Anastrepha australis* (Blanchard) despite some variability in colouration (with some individuals resembling *A. nigra*, see below). This is because investigation of the DNA of the imported population indicated that they all had nearly identical sequenced parts of the 16S rRNA gene (around 500 bp) and CO1 Mitochondrial gene (around 700 bp) regions. The 16S sequences of 16 flies had 1 and 3 bp mismatches with the two *Toxotrypana (Anastrepha) australis* sequences on GenBank and 5 bp mismatches with the single sequence of *T. nigra*. For the CO1 gene region (around 700 bp), all sequences were identical and showed only a 1 bp mismatch with *T. australis.* Therefore, we shall continue to refer to the flies as *A. australis* in this report, although the name is likely to change following the result of ongoing taxonomic studies: Three other *Anastrepha* species: *Anastrepha nigra* (Blanchard), *Anastrepha picciola* (Blanchard), and *Anastrepha proseni* (Blanchard) differ in colouration but may prove to be conspecific with *A. australis,* and a fly described as *Vespomima nigrotaenia* Enderlein appears to be identical to *A. nigra* (A. Norrbom, USDA, pers. comm.) Resolution of these taxonomic issues may result in species being grouped as one species, and nomenclatural changes.

We found native range host records for *A. australis*, *A. nigra* and *A. proseni* in the literature and the only reported host plants of these species are *Araujia* and *Morrenia* spp. (Norrbom 2004; Calvo et al. 2019; Silveira-Guido & Habeck 1978). Moreover, *Morrenia* is now considered to be a synonym of *Araujia* (Rapini et al. 2011) indicating that field host records are confined to a single genus.

The *Anastrepha australis* population imported to containment in New Zealand was collected as larvae developing in *Araujia hortorum* pods growing in the Montevideo neighbourhood of Punta de Rieles in Uruguay in January 2020. All testing in containment included individuals or progeny from this population. If any application is approved to release *Anastrepha australis*, the fruit flies collected in Uruguay will be the parents of any *A. australis* individuals released.

Description

Adult *Anastrepha australis* may be mistaken for wasps due to their size, shape and colour. The body is typically 1.5 cm long. Adults are black and yellow. Two colour morphs exist with some being predominantly yellow and others mainly black. Females have long ovipositors the length of their body.

Distribution

The extent of the native range in South America is uncertain, but it has been recorded in Argentina (Corrientes, Buenos Aires, Santa Fe, Santiago del Estero, Tucumán), Bolivia (Tarija), Brazil (Rio Grande do Sul) (Norrbom et al., 2018), Paraguay (Boquerón) (Clavijo et al., 2020) and Uruguay (Montevideo) (Calvo et al., 2020). The winter temperatures in some of these regions resemble those of northern New Zealand (Table 1). *Anastrepha australis* is expected to establish wherever moth plant is abundant in New Zealand.

Table 1 Comparative climates

City	Latitude	Temperature (°C)		
		Summer mean maximum	Winter mean maximum	Annual average temperature
Buenos Aires, Argentina	34° 34'	28.5	14.9	16.6
Montevideo, Uruguay	34° 74'	26.6	18.3	19.3
Auckland	37° 01'	23.0	14.0	15.1

Lifecycle

Females use their long ovipositors to pierce the fruit follicle laying eggs in this space. Females can lay more than 40 eggs per fruit. Larvae hatch and burrow into the fruit, feeding on the seed tissues, typically destroying all seeds causing the fruit to eventually decay from fungus and secondary organisms (Silviera-Guido & Habeck, 1978). Damage by larvae can cause the plants to prematurely drop fruit. After around 30 days, the mature larvae leave the fruit and pupate in the soil, although sometimes pupation takes place within the fruit. Larvae which pupate in late summer overwinter for 7 months and emerge in summer, corresponding with the development of fruit on moth plant. The pupation stage may also last as little as 20 days if larvae mature and pupate earlier in summer suggesting two generations a year in subtropical climates such as Northern New Zealand and Montevideo, Uruguay. Adults live for around a month and in the field may feed on sources such as honeydews, fruit juices and pulp and bird droppings (Aluja et al., 1999). In the lab adults, feed readily on a mixture of brewer's yeast, vegetable protein, sugar and water.

Predicted impact in New Zealand

Little is known about the effect of *Anastrepha australis* on the overall fitness of moth plant in South America although observations have documented the destruction of whole moth plant fruits from larval feeding (Silviera-Guido & Habeck, 1978, Q. Paynter, Z. McGrath, MW - LR, personal observation). *Anastrepha* includes nine major pest species with fruit feeding larvae including the closely related papaya fruit fly (*Anastrepha curvicauda*). This fruit fly is a significant pest of papaya in Florida, Central America and parts of South America. Flies within this genus can build large enough populations to adversely affect their host plants.

METHODS

Origin of the fly population that was tested

The flies used in 2019-2021 tests were collected from the Punta del Rieles neighbourhood of Montevideo, Uruguay (~34°49'15.6"S, 56°06'00"W). Populations were shipped to New Zealand and maintained in the MWLR containment facility at Tamaki, Auckland. Host range experiments were conducted in containment.

Test plant selection

As noted above, *Anastrepha australis* and closely related species that are potentially conspecific colour forms (*A. nigra*, *A. proseni*) have only been recorded attacking species in the genus *Araujia* in South America.

Moth plant is the only representative of the genus *Araujia* in New Zealand and belongs to the family Apocynaceae, tribe Asclepiadeae, subtribe Oxypetalinae. Only three native representatives of the family Apocynaceae occur in New Zealand. Fifteen non-native species belonging to 7 genera of this family are of varying commercial significance to the horticultural industry in New Zealand as ornamentals and house plants. None have major economic significance. Eight test plant species representing 4 tribes and 2 sub-families of the Apocynaceae were selected using the centrifugal phylogenetic method (e.g. Briese 2002; Figure 1), including two representatives of the only native genus (*Parsonsia*) and six ornamental species. This was considered adequate coverage to delimit the host range of the insect in New Zealand. The species tested were:

Oxypetalum coeruleum (also known as tweedia) belongs to the subfamily Asclepiadoideae and is the most closely related species to moth plant grown in New Zealand (belonging to the same subtribe as moth plant: the Oxypetalinae). It is a minor ornamental (Hill, 2018) and foliage of this species can be harvested to feed starving monarch butterfly larvae (*Danaus plexippus*), although it is a poor host. Ramsay (1964) noted that mature and penultimate monarch larvae have been reared on *Oxypetalum*, but usually can only be induced to feed upon this when supplies of swan plant have been exhausted.

Swan plants (*Gomphocarpus fruticosus* and *G. physocarpus*) and milkweed (*Asclepias curassavica*) are the next most closely related plants to moth plant found in New Zealand, belonging to a different subtribe of the subfamily Asclepiadoideae (the Asclepiadinae). These are the common hosts of monarch butterflies in New Zealand (http://www.monarch.org.nz/monarch). Plants that resembled both *Gomphocarpus fruticosus* and *G. physocarpus* as well as likely hybrids were included in testing (treated as *Gomphocarpus* spp. in the analyses). *Asclepias curassavica* was also included in the host range tests.

All the remaining test plants belong to the subfamily Apocynoideae, including the only native genus that belong to the family Apocynaceae in New Zealand: *Parsonsia* (native jasmines). There are three representatives of this genus in New Zealand, namely *P. capsularus, P. heterophylla* and *P. praeruptis*. *Parsonsia heterophylla* and *P. capsularis* were considered sufficient to adequately represent this genus in tests. *Parsonsia praeruptis,* was not included as this species is restricted to ultramafic soils of the North Cape, outside the current range of moth plant and far north of the boundary at which Northland Regional Council intends to contain moth plant (Hill, 2018). *Anastrepha australis* is unlikely to ever encounter this species.

Mandevilla laxa and *Stephanotis floribunda* are well-known ornamental species and belong to the tribe Mesechitieae. *Mandevilla* was selected to represent this tribe in tests.

Another commonly cultivated ornamental, *Nerium oleander* (tribe Neriae) was selected for testing.

Fruiting representatives of the subfamily Rauvolfioideae, such as *Vinca major* could not be sourced. *Vinca major* rarely sets seed in New Zealand (Esler 1988).

Other tribes of the family Apocynaceae growing in New Zealand listed by Winks & Fowler (2000) were not represented in tests. The tribe Ceropegiae is represented in New Zealand by a diverse

range of indoor plants. As there are no outdoor representatives that would be exposed to the agent, it was not felt necessary to test species from this tribe.

Similarly, at least seven species of *Hoya* (tribe Marsdeniae) are cultivated in New Zealand (Winks and Fowler 2000) but are grown as indoor ornamentals and are not at significant risk from biological control agents and were not tested.

The tribe Alyxieae (subfamily Rauvolfioideae) is represented in the New Zealand flora by the non-native *Alyxia ruscifolia*, which is casually naturalised and not sold as an ornamental. It was not deemed necessary to test this species.

Oviposition on fruit and survival of larvae

No-choice tests were performed using potted plants containing fruit or fruit on excised stems exposed to *Anastrepha australis* adults at least seven days old in mesh cages over a range of different periods and number of adults. At least one male and one female were included in each replicate and length of replicates ranged from two to 14 days (preliminary analysis indicated that the duration of the test did not influence test results). Adults were regularly fed a mixture of yeast, vegetable protein, sugar and water. A water mist was also sprayed in cages.

The following test plants were included in testing, with replicates set up between March 2020 and August 2021: *Araujia hortorum* (62 replicates); *Oxypetalum coeruleum* (11 replicates); *Asclepias curassavica* (7 replicates); *Gomphocarpus* spp. (20 replicates); *Mandevilla laxa* (4 replicates); *Nerium oleander* (10 replicates); *Parsonsia capsularis* (12 replicates); *Parsonsia heterophylla* (20 replicates).

After exposure to adults, fruits were moved to plastic containers with 2 cm soil substate beneath to allow for pupation. Due to the long nature of the development process before adult emergence, it was decided that it would be best to sift through the soil to quantify development to pupa, rather than wait for adults to develop. Replicates were sampled c. 30 days after inoculation to allow any larvae enough time to pupate. If pupae could not be found, pods were dissected to look for signs of attack to determine if some larval feeding occurred even if larvae did not complete development.

Analysis

Survival to pupation within a replicate was defined as a binary dependent variable where 0= no development to pupation and development to pupation and 1= development to pupation occurred. Test plant species was included as a factor so that the influence of plant species on survival to pupation could be tested.

Pod dissections revealed that larvae were present in some pods but did not develop to pupation, probably due to pods becoming heavily infested so that larvae ran out of food and starved, so a second similar analysis was performed where larval presence was defined as a binary dependent variable where 0=not a host (i.e., no larvae or pupae were found) and 1= host (pupae developed, or dead larvae were found in pods).

Due to quasi-complete separation of the dependent variable, Firth's bias-Reduced penalized-likelihood logistic regression (Heinze and Schemper, 2002) was performed using the logistf package (Heinze et al., 2018) in R (version 4.1.0; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Development to pupa occurred in approximately 60% of replicates on *Araujia hortorum* and 40% of replicates on *Oxypetalum coeruleum*. There was no pupal development on any other test plant species. The plant treatment effect (Test plant species) was highly significant (Likelihood ratio test=60.40 on 7 df, p=1.254e-10, n=146). Survival was significantly higher on *Araujia hortorum*, compared to all other test plant species except *Oxypetalum coeruleum*, (Table 2; Figs 1,2).

Table 2 Coefficients (Coef), standard errors of the coefficients (se(coeff)) and lower and upper confidence 95% intervals of the coefficients for the Firth's logistic regression investigating the proportion of replicates in which pupae developed. P-values indicate if differences in coefficients relative to the intercept are significant, where intercept = the first plant species in the data file alphabetically (i.e. *Araujia hortorum*)

	Coef	se(coef)	lower 0.95	upper 0.95	Chisq	p
(Intercept)	0.3856625	0.258737	-0.1120154	0.89951	2.29974	0.129
Asclepias curassavica	-3.0937127	1.582732	-7.9759343	-0.91554	9.297024	0.002
Gomphocarpus spp.	-4.0992345	1.48935	-8.9611021	-2.03582	25.92319	0.000
Mandevilla laxa	-2.5828876	1.686631	-7.4859262	-0.28026	5.037023	0.025
Nerium oleander	-3.4301849	1.540035	-8.3033021	-1.30418	13.42696	0.000
Oxypetalum coeruleum	-0.8964881	0.674406	-2.2479468	0.351924	1.975148	0.160
Parsonsia capsularis	-3.6045383	1.523246	-8.4739826	-1.49919	16.08295	0.000
Parsonsia heterophylla	-4.0992345	1.48935	-8.9611021	-2.03582	25.92319	0.000

The analysis of larval presence indicated that approximately 70% of replicates on *Araujia hortorum* and 64% of replicates on *Oxypetalum coeruleum* were attacked. There was no evidence of larval feeding on any other test plant species. The plant treatment effect (Test plant species) was highly significant (Likelihood ratio test=80.952 on 7 df, p=8.771e-15, n=146). The proportion of replicates with larvae present was significantly higher on *Araujia hortorum*, compared to all other test plant species except *Oxypetalum coeruleum*, (Table 3).

Table 3 Coefficients (Coef), standard errors of the coefficients (se(coeff)) and lower and upper confidence 95% intervals of the coefficients for the Firth's logistic regression investigating the proportion of replicates in which larvae and/or pupae were found. P-values indicate if differences in coefficients relative to the intercept are significant where intercept = the first plant species in the data file alphabetically (i.e. *Araujia hortorum*)

|	Coef	se(coef)	lower 0.95	upper 0.95	Chisq	p
(Intercept)	0.8023465	0.274715	0.283113	1.358063	9.377935	0.002
Asclepias curassavica	-3.0937127	1.582732	-7.9759343	-0.91554	12.61739	0.000
Gomphocarpus spp.	-4.0992345	1.48935	-8.9611021	-2.03582	33.5739	0.000
Mandevilla laxa	-2.5828876	1.686631	-7.4859262	-0.28026	7.088337	0.008
Nerium oleander	-3.4301849	1.540035	-8.3033021	-1.30418	17.89453	0.000
Oxypetalum coeruleum	-0.8964881	0.674406	-2.2479468	0.351924	0.194291	0.659
Parsonsia capsularis	-3.6045383	1.523246	-8.4739826	-1.49919	21.25846	0.000
Parsonsia heterophylla	-4.0992345	1.48935	-8.9611021	-2.03582	33.5739	0.000
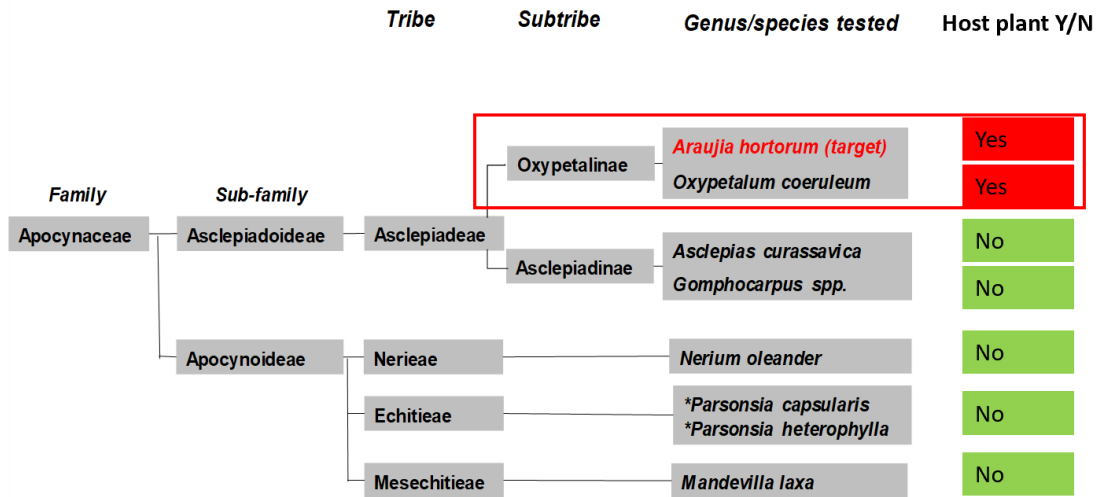
Figure 1 *Anastrepha australis* host and non-host plants in no-choice development tests, in relation to a phylogenetic tree of the test plants based on phylogenies of the Apocynoideae published by Lens et al. (2009) and the Asclepiadoideae published by Keller and Liede-Schumann (2017).
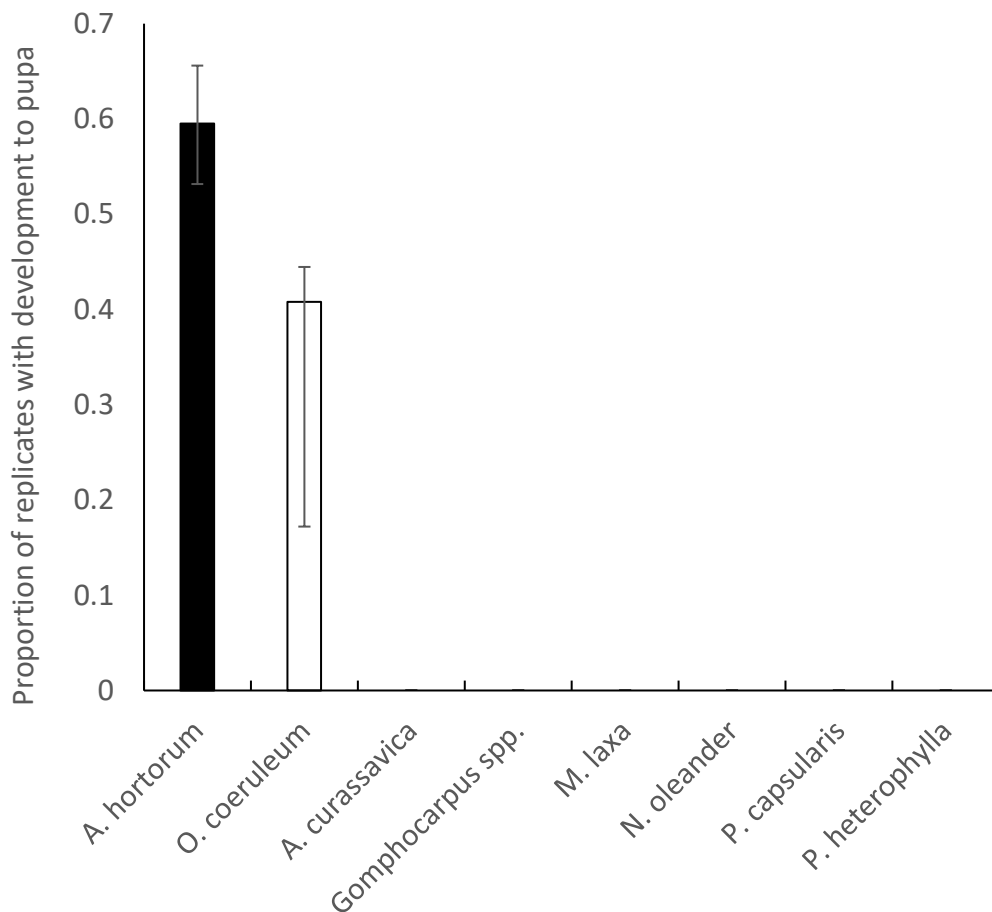

Figure 2 Proportion of replicates with development to pupae.

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