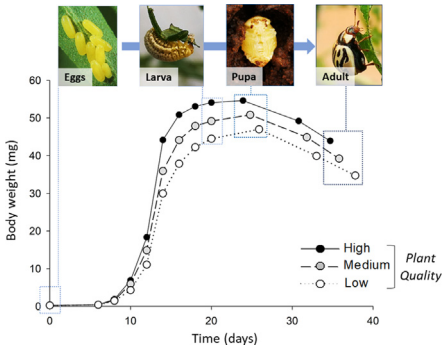
GRAPHICAL ABSTRACT



ABSTRACT

In South Africa, the leaf-feeding beetle, *Zygogramma bicolorata* Pallister (Coleoptera: Chyrsomelidae), was released in 2013 against the invasive annual herb *Parthenium hysterophorus* L. (Asteraceae: Heliantheae). Poor field establishment and variable incidence of the beetle, during the first few years after release, have led to concerns surrounding potential constraints, including host plant quality. This study assessed the influence of high, medium and low host plant quality, as influenced by fertiliser application, on the survival, development and fecundity of *Z. bicolorata*. Although egg to adult survival was high (~80%) and did not differ between plant quality treatments, larvae developed fastest on plants of high and medium quality. Larval feeding was highly damaging, reducing the growth, reproduction and biomass of *P. hysterophorus* in all three plant quality treatments. Fecundity was associated with plant quality, with larger, more fecund females developing on higher quality plants. Overall, this research may aid current mass-rearing efforts, further field research to verify these findings and direct the selection of more suitable release sites for *Z. bicolorata* in South Africa.

1. Introduction

The annual herb, *Parthenium hysterophorus* L. (Asteraceae: Heliantheae), is a noxious invasive plant and recognised as one of the world's most threatening terrestrial weeds (Adkins and Shabbir, 2014). Originating from Central and South America, specifically regions

surrounding the Gulf of Mexico, *P. hysterophorus* is now prevalent in at least 92 countries in the tropics and sub-tropics, of which at least 46 are beyond its natural distribution (Dhileepan and Strathie, 2009; McConnachie et al., 2010; Shabbir et al., 2019). In South Africa, *P. hysterophorus* has invaded large swathes of land in the KwaZulu-Natal, Mpumalanga, North West and Limpopo Provinces for several decades and continues to expand its range (Terblanche et al., 2016). *Parthenium hysterophorus* readily invades a wide variety of habitats, ranging from high fertility agricultural lands to degraded and disturbed low nutrient systems (Henderson, 2001). Plants mature rapidly (in approximately six weeks), producing as many as 30 000 seeds per plant, from which extensive, long-lasting seedbanks are established (Navie et al., 2004). The weed is generally unpalatable to wild and domestic herbivores and displays high levels of allelopathy, allowing it to form dense stands which outcompete and displace native species (Adkins and Shabbir, 2014). Major concerns surround the negative socio-economic impacts of *P. hysterophorus* invasions in agricultural, subsistence, communal rangeland, conservation and human habitation settings (Strathie et al., 2011).

Recognising these threats, Africa's first biological control programme against *P. hysterophorus* was initiated in South Africa during 2003, with a total of four biocontrol agents having been released since then. Among these was the leaf-feeding beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), first released in 2013 (McConnachie, 2015). Releases of *Z. bicolorata* were highly anticipated in South Africa, given substantial damage displayed by the agent in regions of India and Australia (Dhileepan and Strathie, 2009; Dhileepan and Wilmot Senaratne, 2009; Strathie et al., 2011). As adults, the beetles may survive for up to two years, producing as many as four generations in a single summer season (Dhileepan et al. 2000a). Females are highly fecund, laying as many as 900 eggs, singly or in small batches, on the leaves, stems and flowers of *P. hysterophorus* (McClay, 1985; Strathie et al., 2011). Eggs hatch within about five days, with larvae feeding voraciously on leaves as they develop through four larval instars prior to pupation. Pupation occurs in the soil, lasting approximately two weeks, after which adult beetles emerge and begin feeding and copulation (Dhileepan and McFayden, 2012; Dhileepan et al., 2000a). During autumn, adult beetles enter a soil diapause in response to decreasing rainfall, temperature and food availability, which persists through winter (Dhileepan and McFayden, 2012). These beetles then re-emerge during mid-spring, typically three to four weeks after *P. hysterophorus* has begun germinating, and resume feeding (Abels, 2018).

Multiple releases of *Z. bicolorata* in South Africa have been undertaken annually since 2013, at numerous sites throughout the most densely invaded areas within KwaZulu-Natal and Mpumalanga provinces (Strathie et al., 2016a). Despite extensive infestations of *P. hysterophorus* and promising initial persistence of *Z. bicolorata* at release sites, < 13% of selected release sites surveyed were found to have established populations of the beetles in 2016 (Strathie et al., 2016b). Furthermore, sites at which *Z. bicolorata* had established, showed high variability in beetle incidence, although some damaging outbreaks were recorded (Chidawanyika et al., 2017). Poor establishment and variable incidence at release sites has prompted concerns surrounding abiotic and biotic constraints on the beetle's efficacy in the field (Strathie et al., 2016b). Initial studies by King (2008) indicated that *Z. bicolorata* would be well suited to South Africa's climate, and that establishment and development of the beetle was unlikely to be climatically constrained. Although egg and larval predation by generalist predators is suspected to hinder *Z. bicolorata*, these are unlikely to entirely prevent the establishment of the beetle (Abels, 2018). Research by Chidawanyika et al. (2017) demonstrated that *Z. bicolorata* may be more susceptible to thermal stress in the field, particularly in beetles emerging from larvae developing on poorer-quality *P. hysterophorus* plants. This suggests that bottom-up drivers, such as host plant quality, may influence the establishment, abundance and effectiveness of *Z. bicolorata* in South Africa.

The availability of high-quality plant resources is a factor often overlooked in weed biocontrol programmes, yet it may have considerable effects on agent performance (Price, 2000; Manrique et al., 2009; Bownes et al., 2013). Host plant quality typically refers to the relative nutritional value of a plant, particularly its nitrogen status (Chown and Nicolson, 2004; Chown and Gaston, 2010). Linked to this is the 'Plant Vigor Hypothesis', which proposes that more vigorous (nutritious) plants are likely to benefit the development of their insect herbivores (Price, 2000; Stiling and Moon, 2005). Plant quality is a key determinant in the development and survival of both neonate and mature phytophagous larvae (Awmack and Leather, 2002; Heisswolf et al., 2005), with increased survival and shortened development times documented on plants of higher quality (Moreau et al., 2006). Larval food quality also influences the size that adult individuals may attain which has consequences for their subsequent reproductive success (Chown and Gaston, 2010; Van Hezewijk et al., 2008). Fecundity is strongly related to larval host plant quality, with females reared on high quality plants being larger in size, and as a result potentially more fecund than females reared on poorer quality plants (Awmack and Leather, 2002). At a population level, plant quality influences insect-plant interactions and may regulate insect abundance, particularly in specialised or host-specific species (Cotesero et al., 2000; Van Hezewijk et al., 2008).

Given the threats posed by *P. hysterophorus* to biodiversity, food security as well as human and animal health, it is imperative that potential constraints to ongoing biocontrol efforts be investigated and agent efficacy is optimised. Variations in plant quality of *P. hysterophorus*, both spatially and temporally, may impact the establishment and performance of *Z. bicolorata* in the field. Therefore, this study aimed to assess the influence of *P. hysterophorus* plant quality on the survival, development and fecundity of the leaf-feeding beetle *Z. bicolorata*. Additionally, larval feeding damage to the growth, reproduction and biomass of *P. hysterophorus* was examined under high, medium and low plant qualities as influenced by applications of nutrients.

2. Materials and methods

All experiments took place within a temperature-controlled glasshouse section of the insect rearing facility (insectary) at the University of the Witwatersrand, Johannesburg. The glasshouse receives full sunlight (~1800 μmol^{-1}: P.A.R) and follows ambient day-night light patterns. Temperatures were set and maintained at 25 ± 2.2 °C during the day (12 h) and 20 ± 1.3 °C at night (12 h), with relative humidity (RH) maintained between 60 and 70%.

Zygogramma bicolorata individuals used in the study were obtained from ~300 eggs supplied from a laboratory-reared culture maintained by the Agricultural Research Council in their Cedara laboratories at Hilton, KwaZulu-Natal. The culture was originally imported from Queensland, Australia in 2005 (McConnachie, 2015). Freshly laid eggs were divided into batches and placed in Petri dishes (~50 eggs per dish) containing sterilised moistened filter paper. The Petri dishes were stored in the insectary glasshouse but kept out of direct sunlight.

2.1. *Parthenium hysterophorus* growth and *Zygogramma bicolorata* inoculation

Sixty *P. hysterophorus* plants were grown from seed collected in the field during July 2017. Plants were grown in 20 cm pots, filled with a nutrient poor loam soil mixture (15% clay: 25% silt: 60% sand) in full sunlight. Upon germination, plants were randomly split into three fertilisation treatments, namely 'high', 'medium' and 'low' (totalling 20 plants per treatment). Plants were watered daily and fertilised using Seagrow® organic fertiliser (N – 53 g/kg, P – 7 g/kg, K – 17 g/kg). Seagrow® fertiliser was mixed with water at concentrations of 5 ml/ℓ, 2.5 ml/ℓ and 0.25 ml/ℓ for the 'high', 'medium' and 'low' treatments,

respectively. Within each of the nutrient treatments, plants received 100 ml of high, medium or low fertiliser mix applied, using a beaker, directly to the soil of each pot every two weeks. These applications were carried out to encompass a spectrum of *P. hysterophorus* nutrient qualities, ranging from low and moderate fertility systems to relatively high nutrient (fertilised) agricultural areas.

Six weeks after germination, *P. hysterophorus* plants within each of the three nutrient treatments were equally sub-divided into 10 control and 10 beetle treatments. All plants were caged in large mesh cages (0.5 m × 0.5 m × 1.0 m in height) with five plants per cage and four cages per nutrient treatment (i.e. two control cages and two beetle cages). Each of the beetle cages had all plants inoculated with newly-hatched (1st instar) *Z. bicolorata* larvae and set apart within the cages, using clear perspex dividers, to prevent plants from touching and any larval cross-exchange. Control plants were caged and set apart but were not exposed to herbivory by *Z. bicolorata*. To standardise herbivory levels and prevent resource limitation, each of the beetle-treated plants, in the high, medium and low nutrient treatments, received a single larva for every three cm^2 of leaf area calculated at the start of week six (see Equation (1); $n = 100$; $R^2 = 0.95$; Cowie unpublished data), which coincided with egg hatch and larval inoculation. Based on the total *P. hysterophorus* leaf area calculated at week six, the high, medium and low beetle treatments received a total of 42 (4.2 larvae/plant), 33 (3.3 larvae/plant) and 23 (2.3 larvae/plant) neonate larvae, respectively.

$$Leaf\ Area\ (cm^2) = \sum (0.4104[L_1 \times W_1]) + (0.4104[L_2 \times W_2]) + \cdots (0.4104[L_n \times W_n]) \quad (1)$$

L = leaf length (cm); W = leaf width (cm); n = total leaves/plant.

2.2. Development and survival of Zygogramma bicolorata

After inoculation, larval development was assessed daily, with instars classified by head capsule width, with 1st instars = 0.41 ± 0.001 mm, 2nd instars = 0.65 ± 0.016 mm, 3rd instars = 0.98 ± 0.017 mm and 4th (final) instars = 1.44 ± 0.012 mm (Cowie unpublished data). Head capsule widths were photographed under a light microscope (100x magnification) and measured using ImageJ (Ver. 1.5, Wayne Rasband, National Institute of Health, USA). All larvae were weighed (g), using a five-place balance (KERN ABT 100), on alternating days. Developing larvae were collected, using a fine paintbrush, from each plant, weighed and returned to the same plant. Larval body weight measures were concluded when larvae reached their final (4th) instar in each of the treatments. Pre-pupal weights were taken in each of the treatments when 4th instars were observed to attempt burrowing.

Larval burrows were marked using small coloured pins, so pupae could be located easily for later exhumation. Pupae were carefully exhumed from their soil chambers and weighed seven days after the mean first burrowing date of larvae in each of the high, medium and low plant quality treatments. Pupae were then returned to their respective soil chambers and allowed to complete pupation. Newly emerged adult beetles were collected, weighed and measured (total length, width and height – using digital callipers (mm)). All adults collected, from the high, medium and low nutrient treatments, were placed into a single mesh cage (0.5 m × 0.5 m × 1.0 m) containing either three high, medium or low-quality *P. hysterophorus* plants (~7 weeks old), respectively. Pupae/adults which failed to emerge more than a week after the first adults emerged were exhumed and assessed for mortality/survival. Beetle survival to adulthood (%) was calculated as the total number of adult beetles that emerged / total number of larvae inoculated for each of the high, medium and low-quality plants. Once all adults had emerged from their respective treatments, they were used for fecundity assessments (see Section 2.4).

2.3. The effects of larval feeding on Parthenium hysterophorus

To assess the effects of *Z. bicolorata* larval feeding on *P. hysterophorus*, plants in all treatments/cages were measured weekly for a period of six weeks (starting at week 6). Plant height (cm), leaf number, leaf area (cm^2) and flower and seed production were assessed. In addition to morphological and reproductive plant parameters, leaf nitrogen status and chlorophyll content were measured. Leaf nitrogen status was measured, on all leaves, using a single photon avalanche diode (SPAD 502 Plus) (Minolta, Osaka 542, Japan) on two spots per leaf, for all leaves present on the plant. SPAD is frequently used in studies, including the Asteraceae and Heliantheae, as a non-destructive measure of leaf nutrient / nitrogen status (Costa et al., 2001). Similarly, chlorophyll content for all leaves, per plant, was measured using a CCM-300 Chlorophyll Content Meter (Opti-Sciences) on two random spots per leaf.

At the end of the six week period, all plants were harvested for biomass. Each plant was carefully uprooted and washed to remove soil and debris, particularly from the roots. Plants were then sectioned into roots, stems (including floral material) and leaves, and oven dried at 70 °C for five days. Dry biomass for each plant component was then weighed, to three decimal places (g), and recorded. Leaf, stem and root biomass (dry weight) were summed to attain total biomass per plant.

2.4. Zygogramma bicolorata body metrics and fecundity assessments

Beetle surface area was used as a proxy for overall adult body size and was calculated using the formula for an ellipsoid (Eq. (2)). All adult beetles from each of the treatments were then sexed according to the shape of their last abdominal segment (ventrite), with males presenting a narrowed and serrated tip, and females a smooth and entire margin (McClay 1980). Sex ratios were calculated as the proportion of total emerged males / total emerged beetles (see Hasan and Ansari, 2015) in each of the high, medium and low treatments.

$$Beetle\ Area\ (mm^2) = 4\pi\left(\frac{(L \times W)^{1.6} + (L \times H)^{1.6} + (W \times H)^{1.6}}{3}\right)^{0.625} \quad (2)$$

L = half beetle length (mm); W = half beetle width (mm); H = half beetle height (mm).

After sexing, each of the high, medium and low-quality cages had eight female and eight male beetles selected for fecundity analyses. Each of the beetle batches were placed into a single new mesh cage (0.5 m × 0.5 m × 1.0 m) containing either eight high, medium or low-quality *P. hysterophorus* plants (leafy ~7 weeks of age). Beetles were monitored daily for copulation in each of the cages and copulating pairs were transferred onto a single *P. hysterophorus* leaf contained in a large, fine-mesh bag, to allow mating and track initiation of egg laying. Females were monitored daily until initial oviposition which was then recorded as the time from mean adult emergence to the date of first observed egg lay. Once all females within each of the treatment cages were observed to have initiated laying eggs, the mesh bags were removed. All eggs laid per plant, were then collected, using a fine paintbrush, from the high, medium and low-quality cages and counted each week for a period of four weeks. Neonate larvae present on the plants during weekly egg collections were removed to limit defoliation but were included in the egg counts. Eggs found on cage walls were included in egg counts of the nearest plant.

From the eggs collected each week in the high, medium and low cages, 20 were sub-sampled and placed into two Petri dishes (10 eggs per dish) to assess egg hatch (%). Additionally, seven newly laid (yellow) eggs per week were selected from each of the cages and weighed (g), to five decimal places, to investigate the influence of plant quality on egg weight.

2.5. Statistical analysis

Zygogramma bicolorata body weight, developmental time and survival were each analysed using Linear mixed effects regression (LMER) within the 'lme4' and 'nlme' packages in R (R Core Team, 2018). To generate F_{stats} and *p-values*, LMERs were assessed using an ANOVA along with a holm adjusted Tukey multiple comparisons test, within the 'car' and 'multcomp' R packages, respectively. Beetle weight was set as the response variable with nutrient treatment as the fixed effect, time set as a continuous predictor and plant number nested within cage set as the random effect. LMER's for both *Z. bicolorata* developmental time and survival followed the same format but did not incorporate time as a continuous predictor.

The influence of *Z. bicolorata* larval feeding damage on *P. hysterophorus* height, leaf number, leaf area, seed production, nitrogen (SPAD) and chlorophyll content were each examined using LMER along with an ANOVA and a Tukey multiple comparisons test. LMER's had height, leaf number, leaf area, seed production, SPAD or chlorophyll set as the response variable with nutrient-herbivory treatment set as the fixed effect, time as a continuous predictor and plant number nested within cage set as a random effect. Total plant biomass was similarly assessed using LMER with biomass set as the response variable, nutrient-herbivory treatment set as the fixed effect and plant number nested within cage set as the random effect.

The effects of plant quality on adult *Z. bicolorata* body size as well as male and female weight were assessed using LMER with body size / weight set as the response variable, nutrient treatment set as the fixed effect and plant number nested within cage set as the random effect. A Pearson's Chi-square test (χ^2) was used to assess the sex ratios of emerging *Z. bicolorata* adults in relation to plant quality treatment. Female fecundity parameters, namely oviposition, eggs laid per plant, and egg weight were all assessed using LMERs with oviposition, eggs laid, and egg weight set as response variables, nutrient treatment set as the fixed effect, time as a continuous predictor (excluding oviposition) and plant number set as the random effect. Lastly, to assess the effects of nutrients on the egg hatch (%), a repeated measures ANOVA with a Tukey HSD *post-hoc* was used.

3. Results

3.1. Development and survival of Zygogramma bicolorata

Overall, *Z. bicolorata* survival, from egg to adult, was relatively high (~80%), and did not differ significantly ($F_{2,18} = 0.3$; $P > 0.05$) between the high (83.3 ± 4.7%), medium (81.7 ± 5.6%) and low (78.4 ± 7.1%) plant quality treatments (Table 1). Larvae successfully progressed through all four larval instars and the pupal stage on the high, medium and low-quality *P. hysterophorus* plants. However, individuals reared on low quality plants spent significantly longer periods as 4th instars and pupae (Table 1). Mean *Z. bicolorata* development time from egg to adult differed significantly ($F_{2,70} = 60.5$; $P < 0.001$), with larvae reared on high (34.6 ± 1.2 days) quality plants developing fastest, followed by larvae reared on medium (35.5 ± 1.3 days) quality and lastly by those reared on low (37.9 ± 1.5 days) quality plants (Table 1; Fig. 1). Larvae reared on high quality plants produced the heaviest adult beetles (43.9 ± 2 mg) followed by those reared on medium (39.2 ± 1.5 mg) and lastly by those reared on low quality (34.7 ± 1.4 mg) plants ($F_{2,1047} = 221.9$; $P < 0.001$; Fig. 1).

3.2. The effects of larval feeding on Parthenium hysterophorus

Zygogramma bicolorata larval feeding significantly reduced the height ($F_{5,19} = 44.1$; $P < 0.001$) of *P. hysterophorus* in only the high and medium quality treatments by 27% and 24%, respectively (Fig. 2A). Similarly, *Z. bicolorata* reduced the leaf production ($F_{5,19} = 86.6$; $P < 0.001$) of high and medium quality *P. hysterophorus*

Table 1
Mean ± SE (*n*) larval and pupal development time (days) and survival for *Zygogramma bicolorata* reared on *Parthenium hysterophorus* plants of high, medium and low quality. Different lower-case letters indicate significant differences between *Z. bicolorata* development stages, overall development time and survival on plants of differing qualities ($P < 0.05$).

	Plant Quality		
Development stage (days)	High (*n*)	Medium (*n*)	Low (*n*)
Egg	5.9 ± 0.3^a (42)	5.9 ± 0.3^a (33)	5.9 ± 0.3^a (23)
1st Instar	5.7 ± 0.4^a (42)	5.7 ± 0.4^a (33)	6.0 ± 0.6^a (22)
2nd Instar	4.1 ± 0.5^a (41)	4.2 ± 0.6^a (31)	4.6 ± 0.6^a (21)
3rd Instar	2.8 ± 0.4^a (39)	3.0 ± 0.3^a (31)	3.3 ± 0.5^a (20)
4th Instar	4.0 ± 0.5^a (38)	4.2 ± 0.5^a (30)	4.7 ± 0.7^b (20)
Pupa	12.1 ± 0.4^a (37)	12.5 ± 0.4^a (29)	13.4 ± 0.5^b (18)
Overall (n)			
Development time (days)	34.6 ± 1.2^a (35)	35.5 ± 1.3^b (27)	37.9 ± 1.5^c (18)
Survival (%)	83.3 ± 4.7^a (35)	81.7 ± 5.6^a (27)	78.4 ± 7.1^a (18)

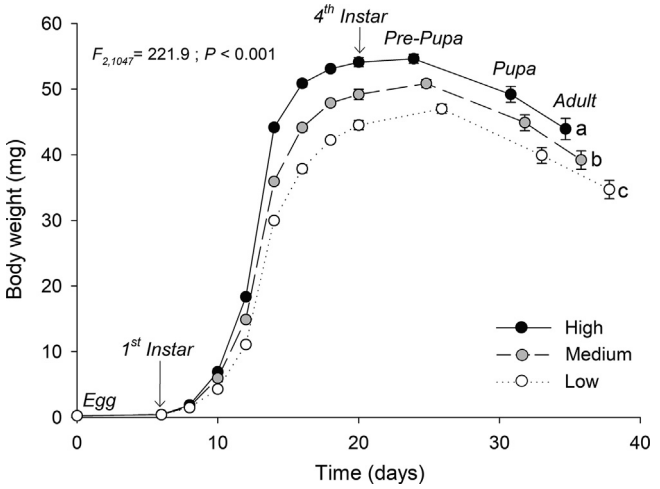


Fig. 1. Mean (± SE) body weight of developing *Zygogramma bicolorata* eggs through to emerging adults reared on high, medium and low-quality treatments. F_{stat} and *P-values* indicate overall difference in *Z. bicolorata* body weights between plant quality treatments using a linear mixed model. Different lowercase letters indicate significant differences between treatment body weights ($P < 0.05$).

by 26% and 34% (Fig. 2B). *Parthenium hysterophorus* suffered significant reductions in leaf area ($F_{5,19} = 49.7$; $P < 0.001$), arising from larval feeding, across all three plant quality treatments, with the greatest reduction occurring in the low nutrient treatment (66%) (Fig. 2C). Larval feeding was found to significantly reduce the reproductive output (seeds) ($F_{5,18} = 13.9$; $P < 0.001$) of *P. hysterophorus* plants in the high, medium and low nutrient treatments by 61, 64 and 68% respectively (Fig. 2D).

Leaf nitrogen (SPAD) and chlorophyll content differed between the plant quality treatments, but not between beetle and control plants, with the high-quality plants displaying the greatest leaf nitrogen (SPAD) (39.5 ± 0.9 units) and chlorophyll contents (469 ± 20.2 mg.m^2) (Fig. 3A-B). *Zygogramma bicolorata* larvae significantly reduced the total biomass of *P. hysterophorus* in all three plant quality treatments ($F_{5,13} = 29.1$; $P < 0.001$, Fig. 4). Biomass reductions were inversely proportional to plant quality, with lowest quality plants experiencing the greatest reduction in biomass (38.2%), followed by the medium (28.1%) and lastly the high-quality plants (24%) (Fig. 4).

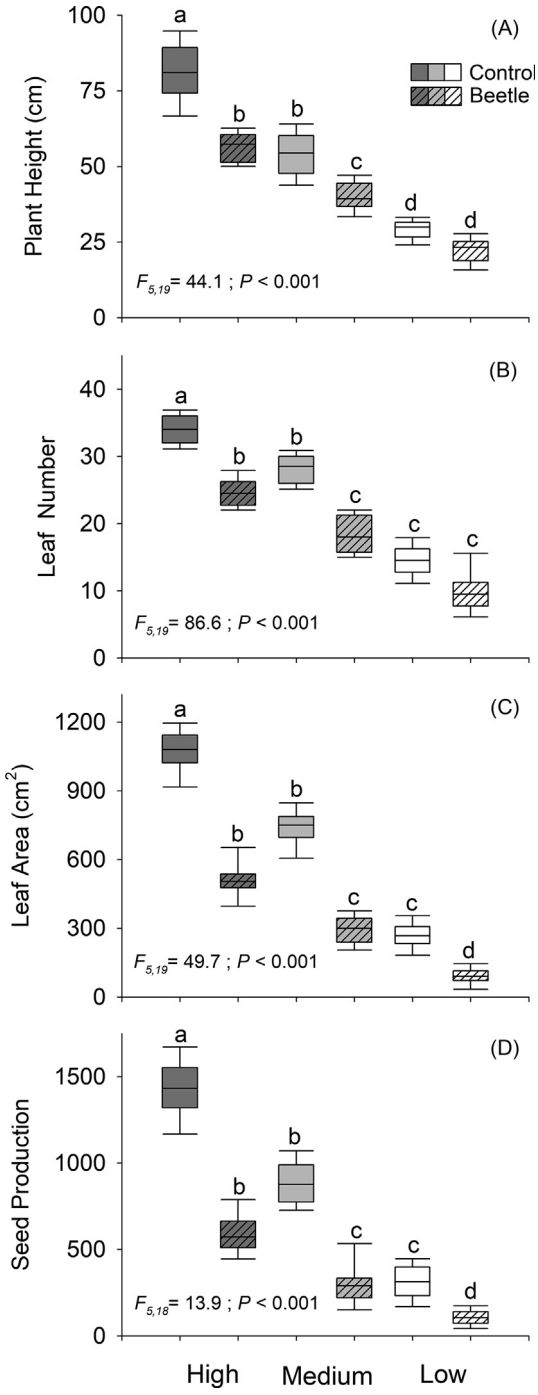


Fig. 2. *Parthenium hysterophorus* (A) height, (B) number of leaves, (C) leaf area and (D) seed production for high, medium and low quality plants after six weeks of herbivory (beetle) or no-herbivory (control) by *Zygogramma bicolorata* larvae. F_{stat} and *P-values* indicate overall difference in *P. hysterophorus* parameters between treatments using linear mixed models. Boxplots show median, 10th, 25th, 75th and 90th percentiles, and differing lowercase letters indicate significant differences between treatments ($P < 0.05$).

3.3. *Zygogramma bicolorata* body metrics and fecundity assessments

Zygogramma bicolorata reared on high quality plants emerged as significantly larger adult beetles (70.4 ± 2.5 mm^2) when compared to beetles reared on low quality plants (63.2 ± 2.3 mm^2) ($F_{2,69} = 21.6$; $P < 0.001$). Emerging males were also found to be significantly heavier as plant quality increased ($F_{2,28} = 25.4$; $P < 0.001$), whereas the heaviest females developed on high and medium quality plants

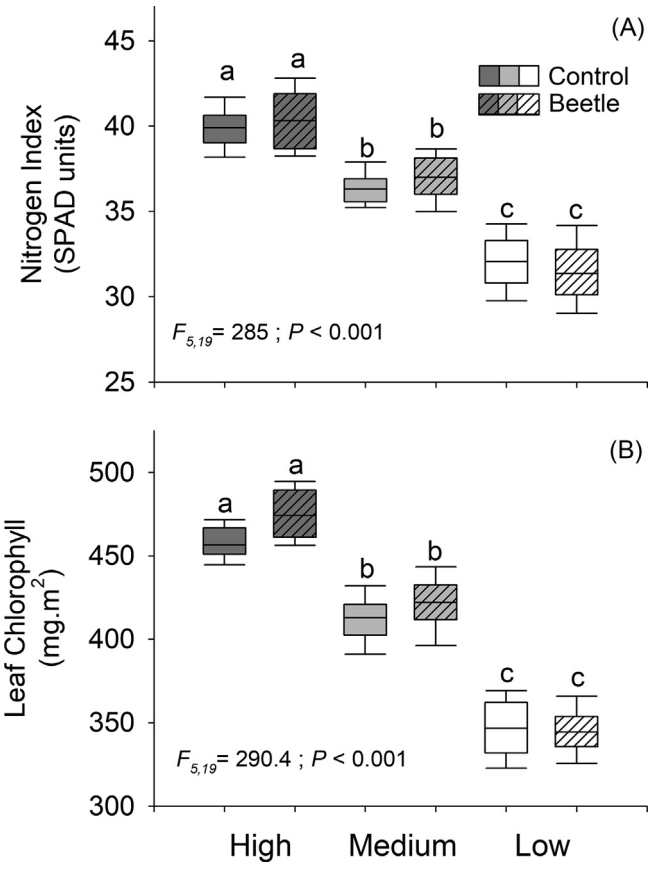


Fig. 3. *Parthenium hysterophorus* (A) leaf nitrogen (SPAD) and (B) chlorophyll context for high, medium and low quality plants after six weeks of herbivory (beetle) or no herbivory (control) by *Zygogramma bicolorata* larvae. F_{stat} and *P-values* indicate overall difference in *P. hysterophorus* leaf nitrogen / chlorophyll between treatments using linear mixed model. Boxplots show median, 10th, 25th, 75th and 90th percentiles, and differing lowercase letters indicate significant differences between treatments ($P < 0.05$).

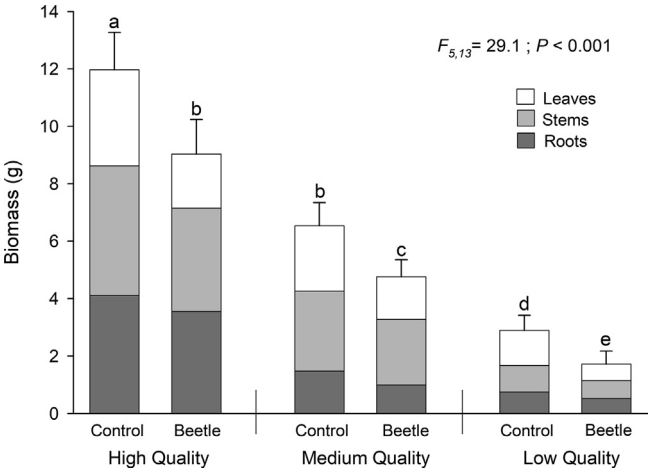


Fig. 4. Mean (± SE) biomass (leaves, stems and roots) for high, medium and low quality *Parthenium hysterophorus* plants exposed (beetle) and unexposed (control) to *Zygogramma bicolorata* larvae. F_{stat} and *P-values* indicate overall difference in total *P. hysterophorus* biomass between treatments using linear mixed model. Different lowercase letters indicate significant differences between treatments ($P < 0.05$).

($F_{2,35} = 19.5$; $P < 0.001$). Sex ratios did not differ between any of the treatments ($\chi^2_2 = 0.02$; $P > 0.05$), but females were more prevalent than males, with an average ratio of 1:1.2 male to females (Table 2).

Table 2
Life history parameters of *Zygogramma bicolorata* (mean ± SE (*n*)) reared on high, medium and low quality *Parthenium hysterophorus* plants. Different lower-case letters per row indicate significant differences between *Z. bicolorata* parameters ($P < 0.05$). Initial oviposition reflects time from mean adult emergence to the date of first egg lay.

	Plant Quality			
Beetle metrics	High (*n*)	Medium (*n*)	Low (*n*)	*LMER/ANOVA/χ^2*
Body size (area mm^2)	70.4 ± 2.5^a (35)	66.4 ± 1.9^b (27)	63.2 ± 2.3^c (18)	$F_{2,69} = 21.6; P < 0.001$
Male weight (mg)	39.7 ± 1.2^a (16)	37.1 ± 0.8^b (12)	32.2 ± 1.0^c (8)	$F_{2,28} = 25.4; P < 0.001$
Female weight (mg)	46.9 ± 1.8^a (19)	43.5 ± 1.6^b (15)	38.3 ± 1.2^c (10)	$F_{2,35} = 19.5; P < 0.001$
Sex ratio (♂:♀)	0.46 (16:19)^a	0.44 (12:15)^a	0.44 (8:10)^a	$\chi^2_2 = 0.02; P > 0.05$
Fecundity parameters				
Initial oviposition (days)	13.5 ± 0.5^b (8)	14.3 ± 0.5^{ab} (8)	15.4 ± 0.6^a (8)	$F_{2,14} = 8.3; P < 0.01$
Eggs laid (per plant)	102 ± 7^a (32)	89 ± 8^{ab} (32)	75 ± 8^b (32)	$F_{2,83} = 22.4; P < 0.001$
Egg weight (mg)	0.25 ± 0.01^a (28)	0.24 ± 0.01^{ab} (28)	0.23 ± 0.01^b (28)	$F_{2,72} = 8.2; P < 0.01$
Egg hatch (%)	68.8 ± 4.0^a (8)	70.0 ± 5.1^a (8)	66.3 ± 5.3^a (8)	$F_{2,21} = 0.1; P > 0.05$

Females from high quality *P. hysterophorus* plants began oviposition significantly earlier than females reared on low quality plants ($F_{2,14} = 8.3; P < 0.01$; Table 2). Additionally, female beetles reared on high quality plants laid significantly more ($F_{2,83} = 22.4; P < 0.001$) and significantly heavier ($F_{2,72} = 8.2; P < 0.01$) eggs than females from the low-quality treatment over a four-week period (Table 2). No difference in egg hatch (%) was found between plant quality treatments ($F_{2,21} = 0.1; P > 0.05$), with an average hatch of ~70% (Table 2).

4. Discussion

This research demonstrated that the development of *Z. bicolorata* is strongly influenced by *P. hysterophorus* plant quality. The prolonged larval development time of larvae reared on low quality plants is a common response to sub-optimal quality plants displayed by many phytophagous insects, particularly amongst the chrysomelids (Obermaier and Zwölfer, 1999). Larvae may compensate for lower quality diets through increased consumption and digestion of poorer quality resources (Wheeler, 2001; Cornelissen and Stiling, 2006). This was evidenced by the feeding of *Z. bicolorata* larvae in the low nutrient treatment as well as their slowed accumulation of body mass and extended time periods spent as instars. Promisingly, no difference in larval mortality was found between the high, medium and low plant quality treatments, suggesting that *Z. bicolorata* is tolerant of a wide range of host plant qualities. This tolerance maintains important implications for the feeding behaviour of phytophagous insects (Obermaier and Zwölfer, 1999; Wetzel et al., 2016), and in a biological control context may be reflected by increases or decreases in damage exerted on target weeds (Heard and Winterton, 2000).

The reduced growth and reproduction of *P. hysterophorus* caused by developing larvae in all three plant-quality treatments reaffirmed the potential effectiveness of the agent. Continued feeding by *Z. bicolorata* larvae on leaves and apical meristems is known to reduce the plant height, leaf production, leaf area and biomass of *P. hysterophorus* (Dhileepan et al., 2000a,b). Whereas reductions in flowering and subsequent seed production are likely to have resulted from the indirect effects of herbivory, namely plant resource depletion and re-allocation (Dhileepan et al., 2000b). The greatest reduction in biomass on *P. hysterophorus* plants of lower quality may be explained by the 'Plant Vigor Hypothesis', in which plants of higher quality readily re-allocate additional resources towards mitigating some of the losses incurred by herbivory (Price, 1991; Manrique et al., 2009; Wetzel et al. 2016). *Parthenium hysterophorus* maintains the ability to partially compensate for *Z. bicolorata* herbivory as shown by Cowie et al. (2018). Although not significant, increases in leaf nitrogen (SPAD) and chlorophyll content, in the high and medium quality treatments, suggest photosynthetic up-regulation as seen in similar experiments (Cowie et al., 2018), which may partially account for the reduced levels of damage experienced by *P. hysterophorus* in higher quality treatments.

Parthenium hysterophorus plant quality also affected the size of emerging *Z. bicolorata* adults. Increased larval food quality, particularly nitrogen (SPAD), is strongly associated with the emergence of larger adult insects (Chown and Gaston, 2010). More importantly, larval plant quality and adult body size frequently reflect the reproductive performance and fecundity of numerous insect species (Awmack and Leather, 2002; Moreau et al., 2006), a trend that has also been recorded for Z. *bicolorata*, with larger females being more fecund (Afaq, 2013). Increases in *Z. bicolorata* fecundity at higher plant qualities may be explained by the size and weight of beetles, with greater fat reserves amassed by larger females. Larger energy stores afford females the ability to provision more resources towards reproductive development, allowing for shortened pre-oviposition periods as well as the production of larger eggs and, or, egg batches (Chown and Nicolson, 2004; Ishihara and Ohgushi, 2006). However, male body weight and size should not be discounted when considering fecundity in *Z. bicolorata*, as Afaq (2013) showed that mating between larger males with larger females produced the most viable and vigorous offspring. Ultimately, improved reproductive outputs are sought in biocontrol programmes as they offer the potential for multiple and larger overlapping generations, which enhance damage to target weed populations (Cotesero et al., 2000; Price, 2000; Van Hezewijk et al., 2008).

Although laboratory-based (individual-level) studies offer focused insights towards the effects of host plant quality, caution should be exercised when extrapolating these findings to field- or population-levels (Bownes et al., 2013). The survival, development and fecundity of *Z. bicolorata* suggests that varying host plant quality is unlikely to directly affect the initial establishment of the agent. However, reduced host plant quality may present underlying hinderances for the biocontrol of *P. hysterophorus*, that should be acknowledged. Prolonged larval development increases the susceptibility of vulnerable immature stages to both biotic and abiotic stresses (Awmack and Leather, 2002; Moreau et al., 2006), which is detrimental to ongoing biocontrol efforts (Stiling and Moon, 2005). In the case of *Z. bicolorata*, egg and larval predation and thermal tolerance as influenced by adult age and feeding status are suspected constraints to *Z. bicolorata* populations in South Africa (Strathie et al., 2016a,b; Chidawanyika et al., 2017; Abels, 2018). Poor quality plants may diminish the physiological (thermal) tolerance of adult beetles, exacerbating heat stresses experienced by *Z. bicolorata* in the field (Chidawanyika et al., 2017). A potential mitigation to the adverse effects of low host plant quality, which prevent initial establishment, may be attained through the fertilisation of relatively small patches of *P. hysterophorus* during and after agent release (Price, 2000). Although seemingly counterintuitive, fertilising target weeds may promote, support and even enhance agent establishment, abundance and performance, as seen and suggested in other weed biocontrol programmes (e.g. Room and Thomas, 1985; Heard and Winterton,

2000; Van Hezewijk et al., 2008). More successful establishment of *Z. bicolorata* in fertilised or recently fallow croplands and their margins, and sites with high-nutrient soils may be expected, barring the influence of other factors constraining establishment.

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