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Preference and performance of *Polymorphomyia basilica* on different phenotypes of *Chromolaena odorata* and other Asteraceae in the laboratory

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Chromolaena odorata, a weed of neotropical origin, remains insufficiently controlled by biological means in South Africa. The stem-galling fly *Polymorphomyia basilica* was introduced as a potential agent and previously shown to be largely host-specific under no-choice conditions. This study conducted multichoice and no-choice trials to test five nontarget plant species previously selected by *P. basilica* in no-choice trials, and to assess the fly's preference and performance on various *C. odorata* phenotypes, including the southern African biotype (SAB) and the Asian/West African biotype (AWAB). Survival and development rates of *P. basilica* were highest on *C. odorata* (SAB) and *Ageratum conyzoides*. Only a few galls produced adult flies on *Stomatanthes africanus* and *Campuloclinium macrocephalum*, and these adults showed low longevity. *P. basilica* displayed a strong preference for and high performance on *C. odorata* (SAB) in both trial types, with over 90% of progeny surviving to adulthood. Many larvae also developed successfully on the Taiwan 129/130 (AWAB) and Jamaican 117 phenotypes, whereas development was poorer on other phenotypes. Although the cause of variation among phenotypes remains unclear, the results indicate that *P. basilica* is a suitable biocontrol agent for *C. odorata* in South Africa and can sustain populations on the AWAB biotype where it is invasive.

KEYWORDS

biocontrol, host-range, offspring performance, preference, stem-galling fly

Introduction

Interactions between plants and herbivorous insects have been widely studied due to their significant implications for agriculture and invasive species management (Naranjo et al., 2015; Giron et al., 2018). Herbivorous insects can drastically alter the growth and reproductive capacity of their host plants, often reducing overall vigour (Dube et al., 2019). In particular, insects with highly specific host ranges are excellent candidates for biological control programs targeting invasive alien plants (Dube et al., 2019). Classical biological control involves introducing these host-specific natural enemies from the native range of an invasive plant to reduce its populations in the introduced range (Uyi and Igbinosa, 2013; Schwarzländer et al., 2018).

Chromolaena odorata (L.) R.M. King & H. Rob. (Asteraceae) is among the most problematic invasive alien plants in South Africa and worldwide (Gautier, 1992). It is a semi-woody perennial shrub native to the Americas. The biotype invading southern Africa (SAB) exhibits morphological and genetic differences from *C. odorata* invading other parts of the Old World humid tropics and subtropics, referred to as the Asian/West African biotype (AWAB) (Paterson and Zachariades, 2013; Uyi et al., 2017). The SAB has a Jamaican/Cuban origin (Zachariades et al., 1999; Paterson and Zachariades, 2013; Shao et al., 2018), whereas the AWAB likely originates from Trinidad and Tobago (Yu et al., 2014; Shao et al., 2018).

Biological control efforts against *C. odorata* in South Africa began in 1988 (Zachariades et al., 2007). Initially, the performance of some control agents was suboptimal due to phenotypic and genetic differences between the SAB and other *C. odorata* biotypes (Yu et al., 2014; Zachariades, 2021). While two agents, *viz. Calycomyza eupatorivora* Spencer (Diptera: Agromyzidae) and *Pareuchaetes insulata* Walker (Lepidoptera: Erebidae: Arctiinae), have established and are now widely distributed, they remain largely restricted to more moist habitats (Zachariades et al., 2016, 2021). Consequently, *C. odorata* remains a persistent threat in South Africa, prompting the consideration of additional biocontrol agents. This includes the stem-galling fly *Polymorphomyia basilica* Snow (Diptera: Tephritidae), which was imported from Jamaica in 2012 (Dube et al., 2020).

Gall-inducing insects such as *P. basilica* are considered promising biocontrol agents because of their typically narrow host ranges and their adverse effects on the growth and reproduction of host plants (e.g., Hoffmann et al., 2002; Aigbedion-Atalor et al., 2019). Preliminary host-specificity tests indicated that *P. basilica* is highly specific to *C. odorata*, although a few other plant species supported limited feeding and development under no-choice conditions (Dube et al., 2020).

Adult progeny were obtained from *Campuloclinium macrocephalum* (Less.) DC., *Ageratina riparia* (Regel) R.M. King and H. Rob., *Stomatanthes africanus* (Oliv. and Hiern) R.M. King and H. Rob., *Ageratum conyzoides* L., and *Felicia amelloides* (L.) Voss, whereas galls were initiated, but larvae did not complete development on *Adenostemma viscosum* J.R. Forst. and G. Forst (Dube et al., 2020). Except for *F. amelloides*, all these species belong to the same asteraceous tribe (Eupatorieae) as *C. odorata*. The development of galls and production of adult progeny on *F. amelloides* was of particular concern, as it belongs to the tribe Astereae, which is more distantly related. The complete development of *P. basilica* on *S. africanus* was also worrisome, since this species is indigenous and has a restricted range in South Africa. Consequently, these five plant species were selected for further testing.

The current study, therefore, focused on the preference (determined by adult oviposition, i.e., the number of galled shoots) and performance (larval survival and development times) of *P. basilica* on the five asteraceous plants selected during earlier adult no-choice trials. Additionally, the study aimed to examine the specificity of *P. basilica* on several phenotypes of *C. odorata* from both its native range and other parts of its invasive range. The objective was to assess its suitability and safety for release as a biological control agent in South Africa, as well as its potential for the biological control of AWAB *C. odorata*.

Materials and methods

Rearing insects and growing plants

Methods for rearing *P. basilica* and conducting trials on *C. odorata* were adopted from Mahlobo et al. (2023). Trials were carried out in the glasshouse and quarantine laboratory at the Agricultural Research Council, Plant Health and Protection (ARC-PHP), Cedara, KwaZulu-Natal Province, South Africa. The facilities were maintained at 25°C ± 3°C with a relative humidity of 40% to 70%. Six standard insect cages (0.5 m × 0.5 m × 0.9 m, with steel frames and gauze panels), each with a transparent plastic curtain covering the entrance, were used as breeding cages.

As described in Mahlobo et al. (2023), four potted SAB *C. odorata* plants were placed in each cage, along with 10 pairs of *P. basilica* and a small container of enzymatic yeast hydrolase mixed with sugar at a ratio of 1:3. This nutrient mixture supported ovule development and increased female fecundity. Females oviposited in *C. odorata* shoot tips, inducing gall formation. After 2 weeks, plants with galls were transferred to a large walk-in culturing cage (2 m × 4 m × 2 m) for further larval development and pupation. Eclosing adults were captured in glass vials and used for further culturing and experiments. These methods were considered efficient and have been successfully applied in previous studies (see Wang et al., 2018; Dube et al., 2020).

Plants used were selected from the *C. odorata* "international collection", which includes specimens from both the native and adventive ranges of the species. The collection is maintained at ARC-PHP, Cedara. In November 2019, plants were propagated from *C. odorata* shoot-tip cuttings of SAB (collected from the field in South Africa), four Jamaican varieties (JA 109, 112, 114, and 117), and other phenotypes, including one Venezuelan (VE 126), one Brazilian (BR 27), one Taiwanese (TW 129/130) (representing AWAB), and one Floridian (USA) (FL 108). *Campuloclinium macrocephalum* was also included, as earlier no-choice trials

suggested that *P. basilica* might develop on this species, indicating its potential as a biocontrol agent for this weed (Dube et al., 2020).

Shoot-tip cuttings were rooted with Seradix® No. 1 in a heated mist bed at the University of KwaZulu-Natal, Agricultural Campus, Pietermaritzburg, and later transferred into labelled 18 cm diameter pots (1:1 river sand and Gromor™ potting medium). Plants were watered daily and fertilised using a fertigation dripper system or Osmocote™ for about three months before use in the trials. Prior to trials, pots were placed in a half-filled water bath (0.6 m × 0.4 m × 0.35 m) for 3 h to eliminate ants, and then spray-washed with water to remove spiders. Plants were then moved to the quarantine glasshouse for the trials.

Multichoice trials using adults on asteraceous plants

Propagation of plants used in these trials followed the methods described above. Trial procedures were adapted from previous multichoice tests on *C. connexa* (McFadyen et al., 2003; Day et al., 2016). Six species were used, *viz. C. odorata*, *S. africanus*, *A. riparia*, *C. macrocephalum*, *A. conyzoides*, and *F. amelloides*. These species supported some development in earlier trials (Dube et al., 2020). *Stomatanthes africanus* plants were collected from the field in Mpumalanga (December 2019 and February 2020) and used as soon as they produced new shoots due to propagation difficulty.

Plants with 15–20 actively growing shoot tips were arranged in two rows within the walk-in cage. Plant positions in the cage were randomised using www.random.org/integers/ to minimise spatial bias. For each trial, a vial containing five pairs of fertile *P. basilica* adults was placed at the centre of the cage. Two individuals of each plant species and a control were exposed to adults for 4 days (Monday to Friday). At 24-h intervals, *P. basilica* positions were recorded (on plants, cage floors, or walls), plant positions were re-randomised, and any missing or dead adults were replaced to maintain five pairs. On Fridays, adults were recorded, and the plants were transferred to another culture cage. They were inspected on Mondays, Wednesdays, and Fridays for indicators of *P. basilica* performance, including oviposition, gall formation, larval development, and adult eclosion.

The width and length of galls from which adults had eclosed were measured, and developmental times were recorded. Each trial was replicated five times. Due to limited space, galls from *A. conyzoides* and *C. macrocephalum* were removed and placed in labelled Petri dishes once pupation was confirmed (via visible "windows" on the galls). Resulting adults from all the species were used in continuation tests (Dube et al., 2020).

Continuation test

Emerging adults from the test species were used to assess F1 viability, longevity, and fecundity (measured by the number of galls formed by the F1 adults). Adults were tested on the same species from which they had emerged. Up to four pairs of *P. basilica* were introduced per plant. Plants were replaced after gall formation or if ants were present. Trials were replicated three times, and each trial was terminated 2 weeks after the *P. basilica* pre-oviposition period or when all adults had died. Replication was not possible for *C. macrocephalum* and *S. africanus* because F1 adults died shortly after emergence, preventing gender synchronisation.

All adults were frozen and weighed, and excess adults from *C. odorata* were returned to the culture. Unfortunately, a *Technomyrmex pallipes* Smith (Hymenoptera: Formicidae) ant infestation occurred during the trial, causing premature adult mortality, which was recorded.

Risk analysis

A risk analysis (sensu Wan and Harris, 1997; as applied by Olckers, 2000 and McConnachie, 2015) was conducted using data from the multichoice trials for three parameters: survival to adulthood, gall size, and development rate. Survival was defined as the number of adults that eclosed per species, and gall size was measured as volume. Development rate was assessed by summing the median larval and pupal development times (in days). As shorter development indicates higher fitness, the inverse of total development times (1/number of days from egg to adults) was calculated for each plant species. Values for *C. odorata* were standardised to one, and those of the other test plants were expressed as proportions (e.g., two adults from a test plant vs. four from *C. odorata* = 0.5). These proportional values were then multiplied to obtain a single, final comparative measure of risk.

Multichoice and no trials on *C. odorata* phenotypes and *C. macrocephalum*

In February 2020, eclosed *P. basilica* adults were collected from the culturing walk-in cage and introduced into two standard breeding cages containing four SAB plants and enzymatic yeast hydrolase for 1 week to ensure sexual maturity (Dube et al., 2020). Subsequent trials tested the propagated *C. odorata* phenotypes and *C. macrocephalum*, each with 12–20 growing shoots. In each trial, two plants from each accession and *C. macrocephalum* were randomly placed in a walk-in cage (1.2 m × 2.2 m × 1.9 m), along with 10 pairs of *P. basilica* adults, for 4 days (Monday to Friday). Plants were watered daily, and their positions were rotated. Missing or dead adults were recorded and replaced to maintain 10 pairs. Each trial was replicated five times.

Campuloclinium macrocephalum was included in these trials because adult progeny had emerged from this species during earlier host-range tests (Dube et al., 2020). Since *C. macrocephalum* is invasive in South Africa, it was hypothesised that *P. basilica* could serve as a biological control agent for this species.

On Fridays, plants were transferred to a separate 2 m × 4 m × 2 m walk-in cage for larval development and inspected from day three. The number of galls per phenotype was recorded. However, due to the coronavirus disease (COVID-19; Nidovirales:

Coronaviridae) lockdown (27 March–16 April 2020), detailed development data could not be collected. After the lockdown, plants were inspected three times per week for survival to adulthood, which was assessed by the number of galls showing a "window" for pupation and galls with an open window for adult eclosion.

Gall lengths were measured, including those with open windows, which indicated adult emergence. The remaining galls with windows were removed from the plants and placed in labelled Petri dishes to track survival to eclosion. Adults in the walk-in cage were captured and transferred to the general culture maintenance walk-in cage.

In no-choice trials, two plants from each phenotype and two *C. macrocephalum* plants were placed separately in breeding cages, each provided with enzymatic yeast hydrolase and three pairs of *P. basilica* for 4 days (Monday to Friday). Missing or dead adults were replaced daily. Plants were then transferred to a walk-in cage for larval development and inspected for gall formation. Development times were recorded daily, except on weekends. Upon pupation, plants were covered with gauze sleeves (0.95 m × 0.4 m) to capture adults. Upon emergence, gall dimensions (width and length) were measured, adults were frozen and weighed (using an electronic balance), and their wing length was measured (using an ocular micrometre on a dissecting microscope). Wing length and mass of flies are typically correlated, allowing estimation of body size (Navarro-Campos et al., 2011). The trials were replicated three times.

Data analysis

In both multichoice and no-choice trials, female preference (and viability in the continuation tests) was measured by the number of galls developing on each plant, including SAB *C. odorata*, other phenotypes, and the asteraceous plants used. Offspring performance was determined by tracking the survival of the *P. basilica* across life stages, from egg to pupation and adult eclosion, along with developmental time. Gall size was represented as volume, calculated by assuming the gall to be cylindrically shaped, using $V = \pi r^2 h$, where r was the width/2 and h was the length of the gall.

Differences in the number of *P. basilica* galls formed, galls that reached pupation, and galls with adult eclosion were analysed using a Generalised Linear Model (GLM) with a negative binomial distribution and a log-link function. Gall size, adult weights, and wing lengths were analysed using a Generalised Linear Model with a Gaussian distribution and identity link function. *C. odorata* was set as the reference category for comparisons among non-*C. odorata* asteraceous plants, and SAB was the reference category when testing differences between *C. odorata* phenotypes and *C. macrocephalum*. All analyses were performed in Python using the statsmodels package (Seabold and Perktold, 2010). The normality of residuals was assessed using the Shapiro–Wilk test and by visually inspecting Q–Q plots. Data were also checked for clamping by examining residual vs. fitted value plots.

Results

The number of *P. basilica* pairs used was sufficient, and most adults survived throughout the trial period. Healthy, newly eclosed adults emerged from most galls.

Multichoice trials using adults on asteraceous plants

Compared to the other asteraceous plant species, *C. odorata* had the highest number of galls laid on it (coefficient = − 1.741; $p < 0.001$). *Ageratum conyzoides* (coefficient = − 0.018; $p = 0.971$) and *C. macrocephalum* (coefficient = − 0.305; $p = 0.534$) had slightly lower numbers of galls, but these did not differ significantly from *C. odorata* (Figure 1). In contrast, *S. africanus* had a significantly lower number of galls (coefficient = − 1.401; $p = 0.009$), and no galls were recorded on *A. riparia* and *F. amelloides* (Figure 1). These species (*A. riparia* and *F. amelloides*) were excluded from all analyses.

High pupation rates were recorded on *C. odorata* (coefficient = 1.668; $p \leq 0.001$), followed by *A. conyzoides* (coefficient = − 0.233; $p = 0.637$), which did not differ significantly from *C. odorata*. In contrast, both *C. macrocephalum* (coefficient = − 1.668; $p = 0.003$) and *S. africanus* (coefficient = − 2.279; $p \leq 0.001$) had a significantly low number of galls reaching pupation (Figure 1).

The highest number of galls with adults eclosed was recorded on *C. odorata* (coefficient = 1.629; $p < 0.001$), followed by *A. conyzoides* (coefficient = − 0.377; $p = 0.450$), which did not significantly differ from *C. odorata*. In contrast, a significantly low number of adults eclosed from the galls laid on *C. macrocephalum* (coefficient = − 1.735; $p = 0.003$) and *S. africanus* (coefficient = − 2.546; $p < 0.001$) (Figure 1). Notably, adults from *C. macrocephalum* and *S. africanus* were found dead on the first day after eclosion (see continuation tests below).

Campuloclinium macrocephalum (coefficient = 54.963; $p < 0.001$) developed significantly larger galls compared to *C. odorata* (coefficient = 76.351; $p < 0.001$). The galls on *A. conyzoides* (coefficient = − 1.476; $p = 0.869$) did not differ significantly from those on *C. odorata*. Meanwhile, *S. africanus* (coefficient = − 26.509; $p = 0.160$) had the smallest galls, although this difference was also not statistically significant compared to *C. odorata* (Figure 2). Larval development was fastest on *C. odorata*, followed by *A. conyzoides*, then *C. macrocephalum*. *Polymorphomyia basilica* took the longest to develop on *S. africanus* (Table 1).

Continuation tests

A few adults in these trials were found dead prematurely. Although *T. pallipes* was recorded in some cages across all plant species, it is generally not aggressive, so it is unclear whether the ants contributed to the observed *P. basilica* mortalities. For example, five adults were found dead in the *C. odorata* cage the day after the trial was set up. Similarly, on two separate occasions, seven adults were found dead in the *A. conyzoides* cage on the

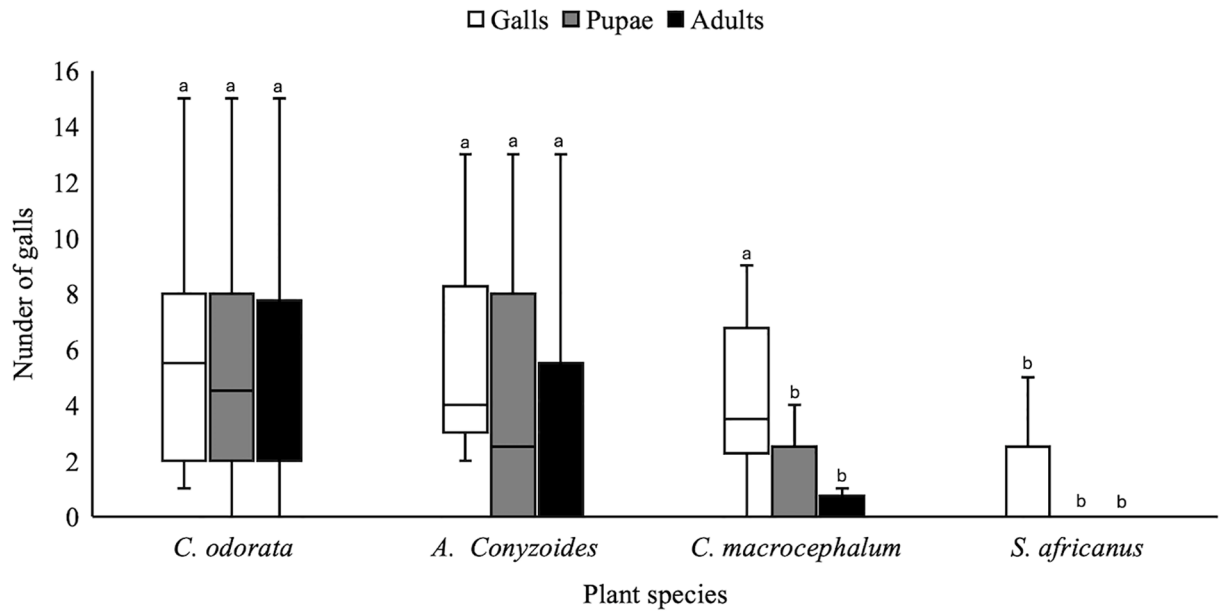

FIGURE 1
Gall parameters of *Polymorphomyia basilica* from multichoice trials on asteraceous plants. Different lowecase letters denote a significant difference between *C. odorata* (SAB) and the other asteraceae/ phenotypes.

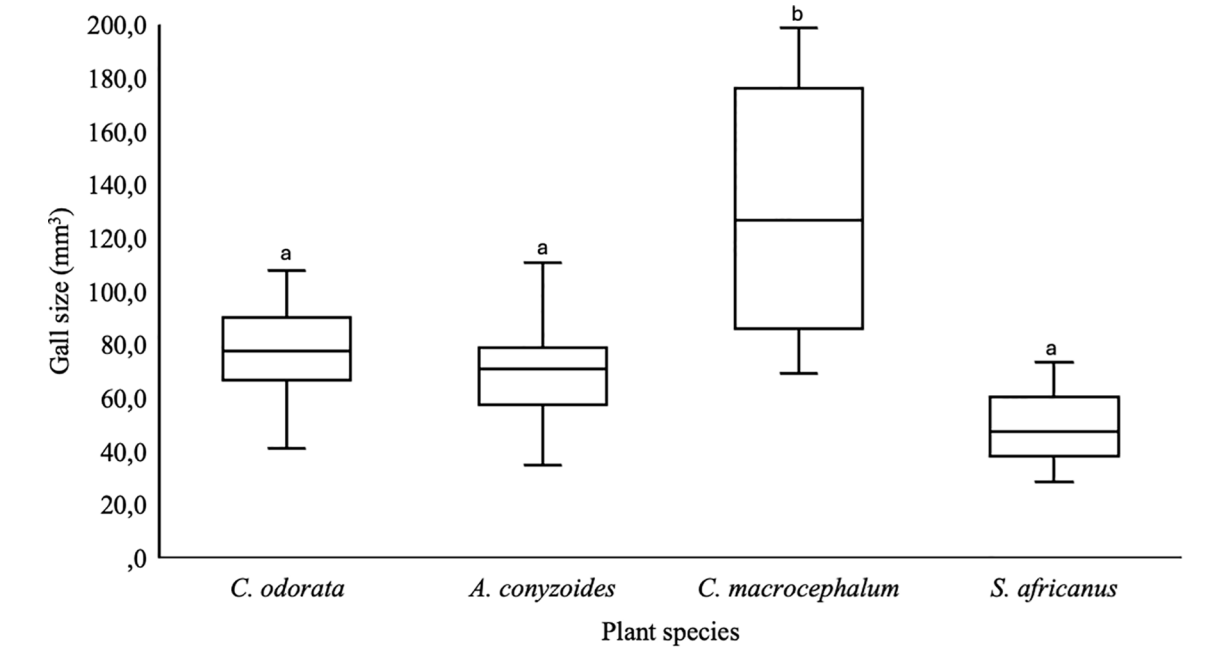

FIGURE 2
Gall size of *Polymorphomyia basilica* on asteraceous plants. Different lowecase letters denote a significant difference between *C. odorata* (SAB) and the other asteraceae/ phenotypes.

second day after setup. Additionally, one adult was found dead in the *C. macrocephalum* cage.

In the *S. africanus* cage, two adults were missing on two occasions, and ants were present. However, ants were also observed in the *C. odorata* and *A. conyzoides* cages on other occasions, yet the adults remained alive and continued laying viable offspring until the trial was terminated.

Most *P. basilica* adults ex *C. odorata* survived for more than 2 weeks after the pre-oviposition period and oviposited, with an average of 11.5 galls forming on *C. odorata*. Adults ex *A.*

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TABLE 1 Developmental time and survival of *Polymorphomyia basilica* in multichoice trials with asteraceous plants.

Plant species	Developmental times		Continuation tests	
	Larvae–pupae	Pupae–adult	Adult weights (mg)	No. of adults
C. odorata	4–6 weeks	14–37 days	1.22 ± 0.170 (n = 12)	11.50 ± 2.88
A. conyzoides	4–8 weeks	16–30 days	1.50 ± 0.284 (n = 11)	13.50 ± 1.50
C. macrocephalum	5–7 weeks	14–26 days	0.90 ± 0.404 (n = 3)	0
S. africanus	6–10 weeks	19–40 days	0.55 ± 0.05 (n = 2)	0

TABLE 2 Risk analysis of host–plant usage by *Polymorphomyia basilica*.

Plant species	P1: No. of adults eclosed	P2: Gall volume	P3: 1/development time	Total (P1 × P2 × P3)
C. odorata	1	1	1	1
S. africanus	0.08	0.67	0.71	0.04
A. conyzoides	0.69	1.04	0.93	0.66
C. macrocephalum	0.18	1.88	0.98	0.32

conyzoides also survived and oviposited, producing an average of 13.5 galls on this species (Table 1). No galls were recorded on *C. macrocephalum* or *S. africanus* (Table 1). Adults from *C. macrocephalum* were either dead or missing, and those from *S. africanus* died soon after the trials were set up, despite the absence of ants in the cages.

There was no significant difference in the weights of F1 adults from *C. odorata* (coefficient = 1.217; $p < 0.001$) and *A. conyzoides* (coefficient = 0.283; $p = 0.360$). Adults from *C. macrocephalum* (coefficient = – 0.317; $p = 0.0508$) and *S. africanus* (coefficient = – 0.717; $p = 0.034$) weighed less, but only adults from *S. africanus* differed significantly from *C. odorata* (Table 1).

Risk analysis

This analysis indicated that, compared to *C. odorata*, *A. conyzoides* posed the highest utilisation risk, followed by *C. macrocephalum*, while *S. africanus* had the lowest risk (Table 2).

Multichoice trial on *C. odorata* phenotypes and *C. macrocephalum*

Galls of *P. basilica* were observed on all *C. odorata* phenotypes used in the trial, as well as on *C. macrocephalum*. The highest number of galls per plant was recorded on the SAB phenotype (coefficient = 2.272; $p < 0.001$), followed by the JA 117 (coefficient = – 0.203; $p = 0.625$) phenotype (Figure 3). Only the JA 109 (coefficient = – 0.991; $p = 0.042$) and JA 114 (coefficient = – 1.174; $p = 0.017$) phenotypes had a significantly lower number of galls compared to the SAB (Figure 4). The number of galls on the TW129/130 phenotype (coefficient = 0.586; $p = 0.221$) and on *C. macrocephalum* (coefficient = – 0.791; $p = 0.102$) was lower but did not differ significantly from the galls on SAB (Figure 4).

On average, over half of the galls formed across the different phenotypes developed pupation windows. Galls on the SAB phenotype had the highest rate, at approximately 94% (coefficient = 2.208; $p < 0.001$). Similarly, galls on *C. macrocephalum* (coefficient = – 0.822; $p = 0.091$) and the TW129/130 (coefficient = – 0.727; $p = 0.133$) also showed high pupation rates, at approximately 91% and 92%, respectively. However, five *C. odorata* phenotypes, *viz.* BR 27 (coefficient = – 0.985; $p = 0.045$), FL 108 (coefficient = – 1.556; $p = 0.002$), JA 109 (coefficient = – 1.252; $p = 0.012$), JA 114 (coefficient = – 1.253; $p = 0.012$), and VE 126 (coefficient = – 1.109; $p = 0.025$) differed significantly from the SAB (Figure 4). Only 45% of the larvae in galls on FL 108 proceeded to pupation, compared to the 94% pupation observed on the SAB.

Compared to the SAB, the number of adults emerging from the galls was lower in all phenotypes. Only the TW 129/130 (coefficient = – 0.922; $p = 0.061$) and the JA 117 (coefficient = – 0.612; $p = 0.206$) phenotypes did not differ significantly from the SAB (coefficient = 2.112; $p < 0.001$) (Figure 3). The number of adults emerging from galls on *C. macrocephalum* (coefficient = – 1.200; $p = 0.017$) was significantly lower than that from the SAB. Less than 55% of the galls on *C. macrocephalum* produced adults, and most of those adults were found dead or moving slowly in the Petri dishes. The *C. odorata* phenotypes from Venezuela (VE 126), the USA (FL 108), and JA 109 were the least preferred hosts, with only 14.2% (coefficient = – 2.339; $p < 0.001$), 21.4% (coefficient = – 2.222; $p = 0.014$), and 41.7% (coefficient = – 2.222; $p < 0.001$) of galls producing adults, respectively (Figure 4).

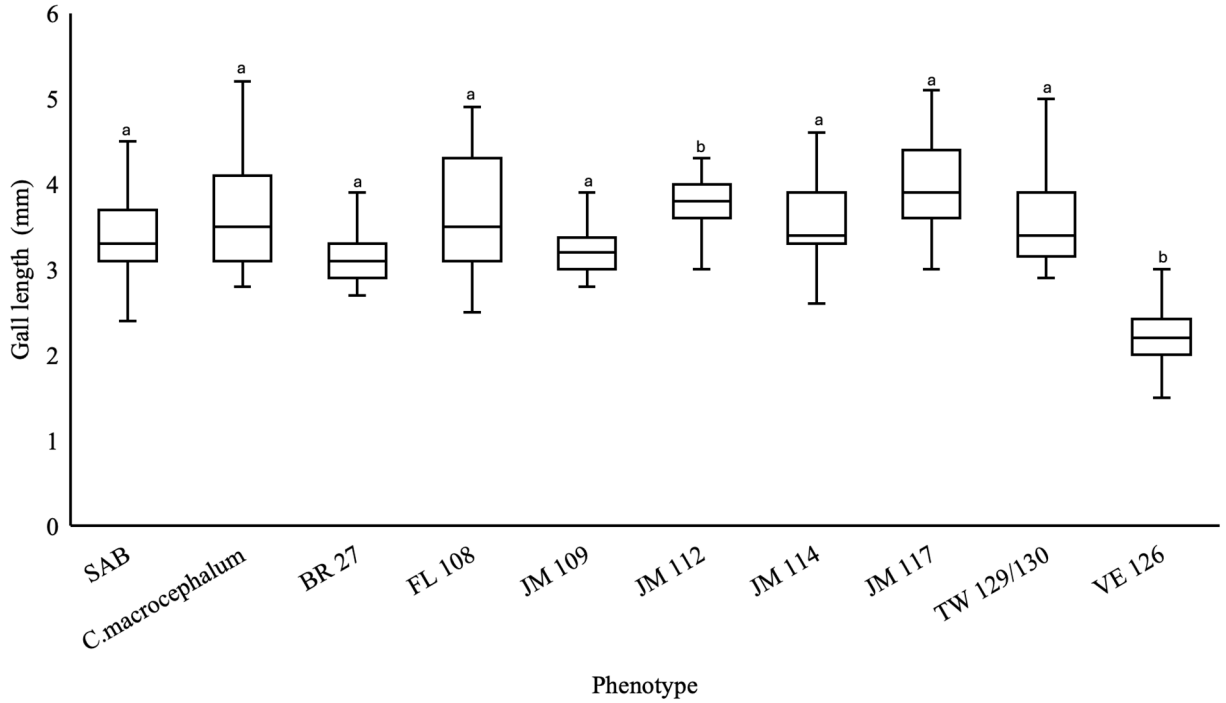

FIGURE 3
Gall length of *Polymorphomyia basilica* in multichoice trials with *C. odorata* phenotypes and *C. macrocephalum*. Different lowecase letters denote a significant difference between *C. odorata* (SAB) and the other asteraceae/ phenotypes.

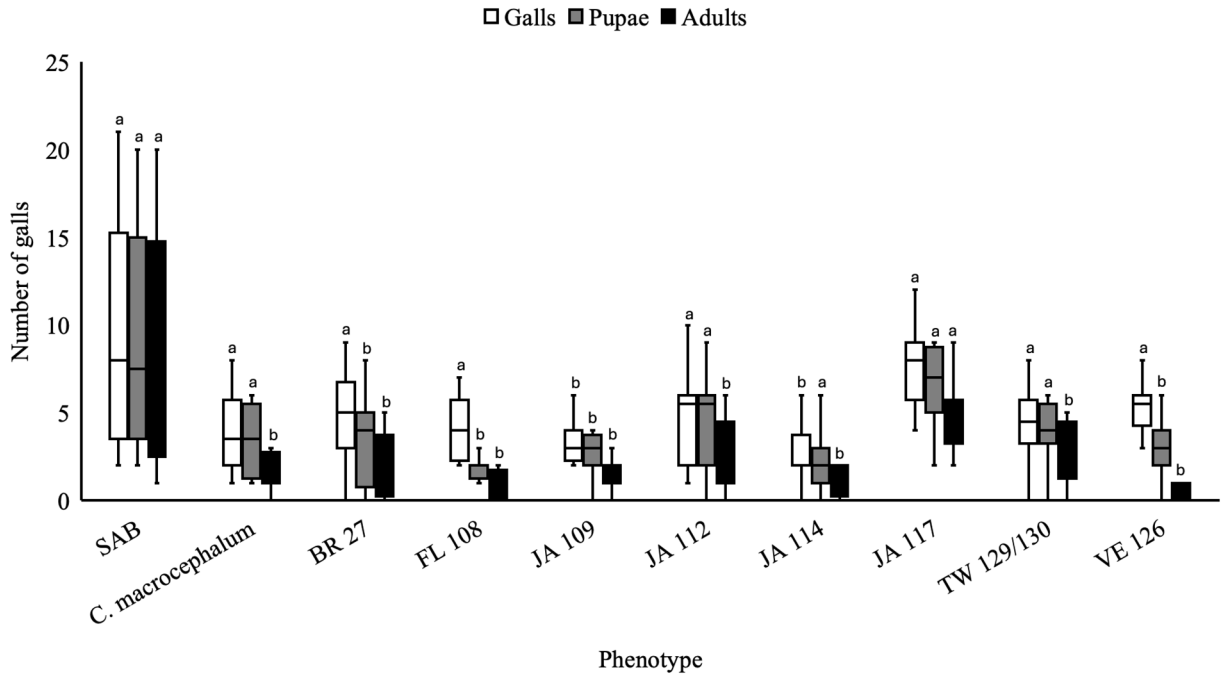

FIGURE 4
Gall parameters of *Polymorphomyia basilica* in multichoice trials with *C. odorata* phenotypes and *C. macrocephalum*. Different lowecase letters denote a significant difference between *C. odorata* (SAB) and the other asteraceae/ phenotypes.

Galls of *Polymorphomyia basilica* formed on the VE 126, BR 27, and JA 109 phenotypes were shorter than those on SAB (coefficient = 3.381; $p < 0.001$); however, only the galls on VE 126 were significantly shorter than those on SAB (coefficient = – 1.147; $p < 0.001$). In contrast, galls on other *C. odorata* phenotypes were longer, with only JA 112 (coefficient = 0.445; $p < 0.001$) and JA 117 (coefficient = 0.631; $p < 0.001$) differing significantly from SAB. Galls on *C. macrocephalum* were also significantly longer (coefficient = 0.3064; $p = 0.008$) (Figure 3).

No-choice trials on *C. odorata* phenotypes and *C. macrocephalum*

The no-choice trial showed a trend similar to the multichoice trials regarding *P. basilica* preference. The SAB phenotype had the highest number of galls (coefficient = 2.708; $p < 0.001$), followed by JA 117 (coefficient = – 0.693; $p = 0.252$) and TW 129/130 (coefficient = – 0.762; $p = 0.209$). Significantly fewer galls were recorded on FL 108 (coefficient = – 1.667; $p = 0.009$), JA 112 (coefficient = – 1.408; $p = 0.024$), and VE 126 (coefficient = – 1.935; $p = 0.003$) (Figure 5). The difference in the number of galls formed between the SAB and *C. macrocephalum* was not significant (coefficient = – 1.204; $p = 0.051$) (Figure 5).

Compared to the SAB (coefficient = 2.663; $p < 0.001$), only JA 117 (coefficient = – 0.741; $p = 0.223$) and TW 129/130 (coefficient = – 0.791; $p = 0.194$) had a similar number of galls in which larvae reached pupation (Figure 5). On *C. macrocephalum* (coefficient = – 2.257; $p = 0.001$), only 33% of the galls formed showed pupation windows, compared to 94.3% on the SAB. All other *C. odorata* phenotypes had a significantly lower number of galls developing pupation windows.

Regarding adult emergence, no phenotype had a similar number of adults emerging from the galls as the SAB (coefficient = 2.651; $p < 0.001$). Adult flies eclosed from 90% of the galls on the SAB, followed by 57.1% on JA 117 (coefficient = – 1.265; $p = 0.042$) and 42.5% on TW 129/130 (coefficient = – 1.804; $p = 0.005$), although these phenotypes differed significantly from the SAB. In all other *C. odorata* phenotypes and on *C. macrocephalum* (coefficient = – 2.833; $p < 0.001$), fewer than 40% of the galls produced adults (Figure 5).

Galls formed on *C. odorata* from Brazil (BR 27) (coefficient = – 37.046; $p = 0.016$) were significantly smaller than those formed on the SAB (coefficient = 82.471; $p < 0.001$). In contrast, galls on TW 129/130 (coefficient = 20.241; $p = 0.017$), JA 117 (coefficient = 28.600; $p < 0.001$), and FL 108 (coefficient = 44.516; $p = 0.007$) were significantly larger (Figure 6a). The size of galls laid on *C. macrocephalum* (coefficient = 1.6282; $p = 0.921$) was similar to that on the SAB.

Larval development was fastest on the TW 129/130 phenotype. On average, *P. basilica* completed development in approximately 51 days on the TW 129/130 phenotype, compared to ~ 66 days on the SAB. In contrast, galls on all other *C. odorata* phenotypes and on *C. macrocephalum* took longer than the SAB to progress through the observed life stages and complete development. Galls on the SAB began developing pupation windows as early as 24 days after trial setup, with some adults emerging 7 days later. Galls on phenotypes such as BR 27 and VE 126 only began showing signs of pupation around 40 days posttermination. Similarly, galls on *C. macrocephalum* also took longer than those on the SAB to reach

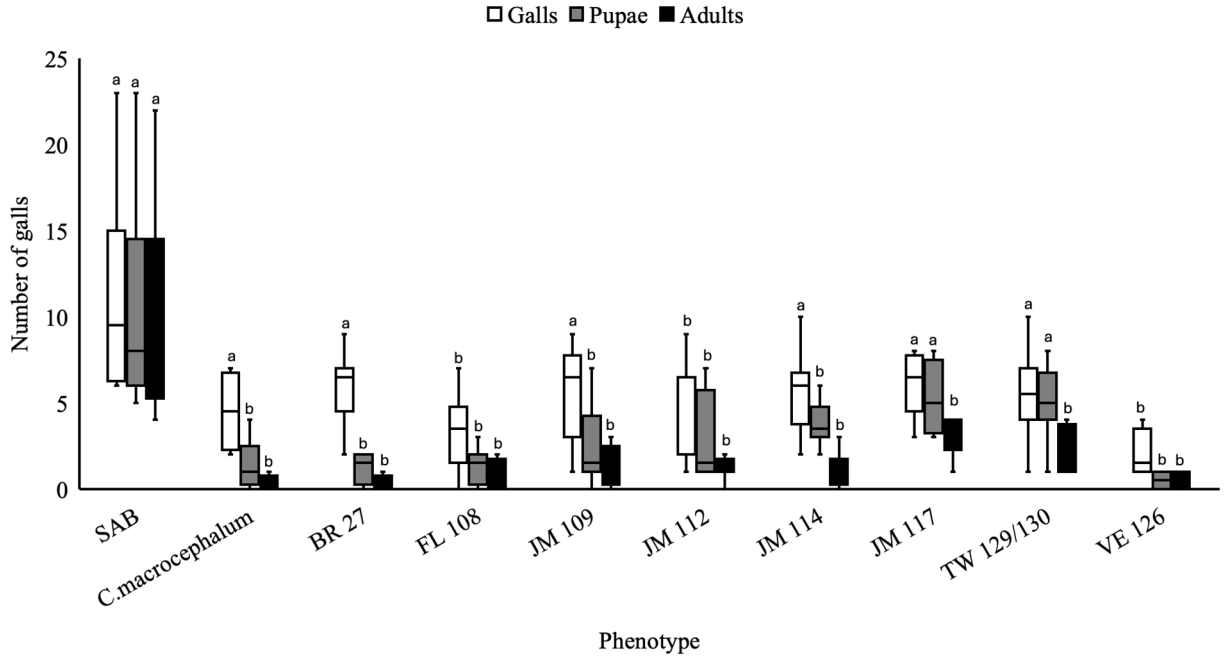

FIGURE 5
Gall parameters of *Polymorphomyia basilica* in no-choice trials with *C. odorata* phenotypes and *C. macrocephalum*. Different lowecase letters denote a significant difference between *C. odorata* (SAB) and the other asteraceae/ phenotypes.

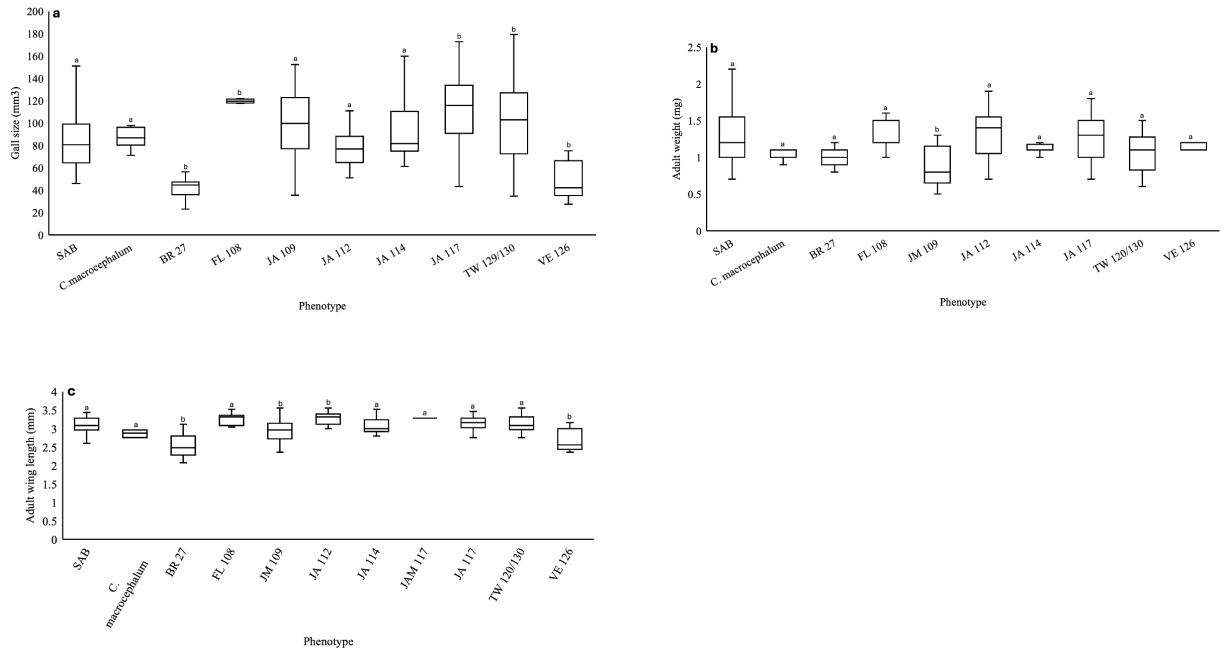

FIGURE 6
Gall size **(a)**, adult weights **(b)**, and wing length **(c)** of *Polymorphomyia basilica* in a no-choice trial with *C. odorata* phenotypes and *C. macrocephalum*. Different lowecase letters denote a significant difference between *C. odorata* (SAB) and the other asteraceae/phenotypes.

pupation and complete development (Table 3). Additionally, adults emerging from *C. macrocephalum* were found dead or moving slowly on the gauze sleeve.

P. basilica adults that eclosed from the JA 109 phenotype (coefficient = − 0.459; $p = 0.030$) weighed significantly less than those emerging from galls on the SAB (coefficient = 1.439; $p < 0.001$). Adults eclosing from all other *C. odorata* morphotypes and *C. macrocephalum* (− 0.359; $p = 0.295$) weighed less but did not differ significantly from those emerging on the SAB (Figure 6b).

Polymorphomyia basilica adults emerging from the BR 27 (coefficient = − 0.539; $p < 0.001$), JM 109 (coefficient = − 0.158; $p = 0.0220$), and VE 126 (coefficient = − 0.396; $p < 0.001$) phenotypes were significantly smaller in wing length than those that emerged from the SAB (coefficient = 3.099; $p < 0.001$). Conversely, significantly larger adults emerged from the JA 112 phenotype (coefficient = 0.185; $p = 0.004$). Adults emerging from *C. macrocephalum* (coefficient = − 0.172; $p = 0.124$) did not differ significantly from those emerging from the SAB (Figure 6c).

TABLE 3 Developmental times of *Polymorphomyia basilica* in no-choice trials with *C. odorata* phenotypes and *C. macrocephalum*.

Phenotype	Developmental times	
	Larvae–pupae	Pupae–adult
SAB	3.5–7 weeks	7–39 days
C. macrocephalum	6–8 weeks	8–26 days
BR 27	6–9 weeks	31–44 days
FL 108	6–8 weeks	8–16 days
JA 109	5–12 weeks	13–43 days
JA 112	4–8 weeks	4–26 days
JA 114	5–8 weeks	13–29 days
JA117	4–9 weeks	6–36 days
TW 129/130	4–8 weeks	5–32 days
VE 126	8–8 weeks	23–32 days

Discussion

This study examined the ability of *A. conyzoides*, *C. macrocephalum*, *S. africanus*, *A. riparia*, and *F. amelloides* to support the development of *P. basilica*, compared to its target weed, *C. odorata*. Galls developed and adults eclosed on *C. odorata*, *A. conyzoides*, *C. macrocephalum*, and *S. africanus*, but no galls formed on *A. riparia* and *F. amelloides*. *Felicia amelloides* is phylogenetically more distantly related (tribe Astereae) to *C. odorata* than the other four test species, which all belong to the Eupatorieae tribe along with *C. odorata* (Panero and Crozier, 2016). Among the Eupatorieae species tested, *A. riparia* was the only one on which galls did not develop.

As previously shown by Dube et al. (2020), oviposition and gall formation on *A. conyzoides*, *C. macrocephalum*, and *S. africanus* may be attributed to the presence of pyrrolizidine alkaloids, which are common in most Eupatorieae and to which the herbivores of *C. odorata* are adapted (Hartmann, 2009). Additionally, several biological control agents of *C. odorata* have been observed selecting *A. conyzoides* during host-specificity tests or in the field (Kluge and Caldwell, 1993; Dube et al., 2017). These include *Conotrachelus reticulatus* (Coleoptera: Curculionidae) (Delgado

et al., 2014), *Dichrorampha odorata* (Lepidoptera: Tortricidae) (Dube et al., 2017), as well as *Pareuchaetes insulata* and *Pareuchaetes pseudoinsulata* Rego Barros (Walker) (Lepidoptera: Arctiidae) (de Chenon et al., 2002, Zachariades et al., 2011).

The survival and development rates of *P. basilica* were highest on *C. odorata* and *A. conyzoides*, lower on *C. macrocephalum*, and lowest on *S. africanus*. This aligns with the results of previous no-choice trials, where *P. basilica* survival was minimal on *S. africanus*, and adults did not live more than 1 day after eclosion from the plants (Dube et al., 2020). These findings indicate that *S. africanus* and *C. macrocephalum* are less preferred hosts. Continuation tests supported multichoice results, as adult progeny from *S. africanus* and *C. macrocephalum* died on the first day after emergence. In contrast, *A. conyzoides* and *C. odorata* successfully supported the complete development of *P. basilica*, even when ants were present.

Risk analysis indicated that, compared to *C. odorata*, only *A. conyzoides* is a reasonably suitable host, while *C. macrocephalum* and *S. africanus* are much less suitable. Since *A. conyzoides* and *C. macrocephalum* are native to the Americas and invasive in South Africa, their attack by *P. basilica* is of no concern. *Stomatanthes africanus* presents minimal risk. Additionally, it occurs in high-altitude, colder grasslands in Mpumalanga and Limpopo provinces (Retief, 2002), with no overlap in distribution with *C. odorata*.

This study also examined the oviposition preference and offspring performance of *P. basilica* across different phenotypes of *C. odorata* and *C. macrocephalum*. In the laboratory, *P. basilica* preferred and performed best on the SAB *C. odorata*, which showed the highest oviposition rates, fastest development, and lowest mortality in both multi- and no-choice trials. *P. basilica* also performed reasonably well on the JA 117 phenotype, which closely resembles the SAB. For some parameters, including the number of galls formed, pupation rates, and developmental times, *P. basilica* performed well on the TW 129/130 = AWAB phenotype.

In contrast, low oviposition, high gall mortality, and slow development rates were observed in the other phenotypes from Jamaica and other regions of the native range of *C. odorata*. While *C. odorata* from Florida and the South American mainland have been shown to differ genetically from the SAB, no genetic differences were detected between the SAB and Jamaican plants (Paterson and Zachariades, 2013; Shao et al., 2018).

The observed increase in preference and performance of *P. basilica* on the SAB, compared to most Jamaican phenotypes, could be explained by the Evolution of Increased Competitive Ability (EICA) hypothesis. The EICA hypothesis proposes that when plants are introduced to new areas without natural enemies, they often reallocate resources from defence to growth and development (Blossey and Notzold, 1995; Handley et al., 2008). Consequently, these plants may become more susceptible to their natural enemies upon reintroduction (Handley et al., 2008). Dube (2019) found some support for this in SAB plants collected from field sites in South Africa with and without prior exposure to *P. insulata*—some plant growth and reproductive metrics were lower in plants with exposure to the moth, indicating that SAB may have regained some of its defence mechanisms since the moth's introduction. Alternatively, the laboratory colony of *P. basilica* may have evolved since its introduction in 2012—as it has been continuously reared on SAB since then. It may have adapted to it, resulting in higher preference and performance metrics than those observed on other *C. odorata* phenotypes.

Polymorphomyia basilica formed galls on all the *C. odorata* phenotypes tested, indicating that these lie within the fly's physiological host range. However, host selection for oviposition may be influenced by encounter rate, which provides limited information about the suitability of a host plant (Singer, 1986). Insects tend to be more discriminating when the likelihood of encountering a previously preferred host is high (Singer, 1986; van Driesche and Reardon, 2004). In the current study, this is reflected by the lower average number of galls formed per plant on the other phenotypes during multichoice trials compared to the means observed in no-choice trials.

The results showed that *P. basilica* larvae exhibited differences in performance across the various *C. odorata* phenotypes and *C. macrocephalum*. Phenotypes that enhanced offspring survival, such as SAB, JA 117, and TW 129/130, were preferred, and larval growth rates were faster on these plants. In contrast, *P. basilica* larvae experienced higher mortality and slower development on *C. odorata* phenotypes from Brazil and Venezuela, making these phenotypes less suitable. Similarly, *C. macrocephalum* proved to be a poor host for *P. basilica*, with larvae taking longer to develop and adults exhibiting low survival rates.

In some fruit flies, adult size is an indicator of fitness, and insects tend to be smaller when developing on low-quality host plants (Beukeboom, 2018; Navarro-Campos et al., 2011). This is consistent with the findings of the present study, where *P. basilica* adults emerging from the BR 27, JA 109, and VE 126 phenotypes were smaller. These phenotypes also had some of the lowest numbers of galls laid and high larval mortality. This supports the "Mother knows best" hypothesis, which posits that females prefer host plants that enhance offspring fitness, resulting in lower gall mortality and shorter developmental time (Santos et al., 2008; Balagawi et al., 2013; de la Masselière et al., 2017). However, this differed for *P. basilica* on the TW 120/130 phenotype, where many galls survived and adults eclosed in the shortest time, yet the emerging adults had low mass.

The results of this study indicate that host use by herbivorous insects differs from host suitability. Although a positive correlation between preference and performance exists, availability and abundance may influence preference. When insects are introduced into a novel habitat, ecological traps are bound to occur as they try to adapt and apply the Mother Knows Best theory (Hale and Swearer, 2016). The Mother Knows Best hypothesis does not consider ecological shifts that occur over time (García-Robledo and Horvitz, 2012). Therefore, future studies should continue to focus on offspring performance on different host plants (Singer, 1986; Balagawi et al., 2013; Hafsi et al., 2016).

Although this was not tested in the current study, it was apparent that host plants influence the life-history characteristics of the larvae and adults. Plant differences, i.e., physical and chemical composition, result in different attraction levels (Heard and Cox,

2009). The variation in attraction among the phenotypes from Jamaica indicates that these differences may involve more than just physical traits.

Conclusion

It is proposed that *P. basilica* is sufficiently host-specific for release as a biocontrol agent of *C. odorata* in South Africa. Although *A. conyzoides* and *C. macrocephalum* may experience some oviposition and damage from *P. basilica*, this is not considered a concern, as both species are declared invaders under NEMBA. Moreover, *C. macrocephalum* will not support the development or sustain populations of *P. basilica* in the field.

The risk of releasing *P. basilica* is minimal compared to its benefits, as demonstrated by its biological performance on *C. odorata* (Mahlobo et al., 2023). The agent also merits consideration in countries where the AWAB is invasive, since *P. basilica* showed substantial development on this biotype.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

TM: Funding acquisition, Writing – original draft, Investigation, Data curation, Formal analysis, Visualization. ND: Validation, Resources, Writing – review & editing, Conceptualization, Project administration, Supervision, Methodology. CZ: Methodology, Conceptualization, Writing – review & editing, Validation, Supervision, Resources, Project administration. TCM: Project administration, Validation, Supervision, Writing – review & editing, Resources, Funding acquisition.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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