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Research Article

Studies on the fruit feeding weevil, *Paramecops farinosa* (Coleoptera: Curculionidae) in Sri Lanka as a prospective weed biological control agent of invasive weed, *Calotropis* spp.

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ABSTRACT: *Calotropis gigantea* is a large shrub or a small tree native to Sri Lanka with an Ayurvedic medicinal value. The plant is considered as an invasive weed in countries where it has been introduced. *Paramecops farinosa* Schoenherr (Aak weevil) is a monophagous pest that feeds on *C. gigantea*. Present study was conducted to elucidate the life history and damage potential of *P. farinosa*, in order to assess its potential as a biological control agent against *C. procera* and *C. gigantea* in countries where the plants are invasive. The field sampling was done throughout Sri Lanka covering 120 sampling sites from December 2014 to October 2015, and *C. gigantea* fruits were examined for the incidence and intensity of damage by *P. farinosa*. It lays yellowish, oval and mostly one-clustered eggs in the inner-pericarp fibrous layer of the *Calotropis* fruit. Newly emerged larvae were apodous, pale yellowish-white with brown head capsule whereas developing larvae were creamy-white, curved and stout. *Paramecops farinosa* larvae voraciously feed on all *Calotropis* seeds (100%) and fifth larval instar pupated by forming silky cocoons within the seed chamber. The adults feed on leaves, buds and flowers and its damage is highly correlated with the amount of *P. farinosa* inhabit on trees. *P. farinosa* is a seed predator and highly damage reproductive structures of *C. gigantea* thus directly influences the reproductive ability of the plant. These results provide baseline information needed in adopting *P. farinosa* as potential biological control agent against *C. procera* and *C. gigantea.*

KEY WORDS: Bio control, *Calotropis gigantea*, invasive species, *Paramecops farinosa*

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INTRODUCTION

Calotropis species (Family Apocynaceae) are small trees or shrubs (Kumar *et al*., 2013) which is native to tropical and sub-tropical regions of Asia and Africa (Vitelli *et al*., 2008). Three species of *Calotropis* are recorded worldwide, *viz*., *Calotropis gigantea* (L.) Dryand, *Calotropis procera* (Aiton) W.T. Aiton and *Calotropis acia* Buch-Ham (Bebawi *et al*., 2015) and only *C. gigantea* occurs in Sri Lanka. *Calotropis* species are commonly used in Ayurvedic medicine (Kumar *et al*., 2013) which consist of multiple therapeutic properties including anti-bacterial, anti-diabetic, anti-cancer, anti-inflammatory etc. (Kumar and Kumar, 2015). Also, it acts as a biodiversity indicator (Sharma *et al*., 2011).

Despite its benefits in its native range, *C. procera* is an invasive plant in Australia, Brazil, Hawaii, USA and the Caribbean islands (Dhileepan, 2014). In Australia, currently there are no physical and chemical methods that are economically viable in controlling the weed (Vitelli *et al*. 2008). Hence, classical biological control is regarded as the most cost effective and long-term management option for *C. procera* (Dhileepan, 2014). As both *C. procera* and *C. gigantea* share a common pest community, any host specific insect pests found on *C. gigantea* could be used as a biological control agent for *C. procera* (Dhileepan, 2014). In this study, we explored the prospects of using a monophagous pest of *C. gigantea* in Sri Lanka as a prospective biological control agent for *C. procera*.

There are only a few major pest species of *Calotropis* species in its native range countries, and among them *Paramecops farinosa* Schoenherr (Coleoptera: Curculionidae) is a major destructive pest (Dhileepan, 2014; Saikia, *et al*., 2015; Wijeweera, *et al*.,2020). *Paramecops farinosa* or Aak weevil is distributed widely in India, Pakistan (Dhileepan, 2014) and Sri Lanka (Wijeweera, *et al.,*2020). *P. farinosa* is a monophagous feeder on *C. procera* and *C. gigantea* which feed on leaves and flowers of the plant (Sudan *et al*., 2013). Larval stages act as destructive seed predators (Sharma and Amriphale, 2007) which directly influence on the reproductive output of the *Calotropis* plant. The main objective of the present study was to determine the potential use of *P. farinosa* as a biological control agent for *C. procera* in introduced countries. In addition, the study is aimed to

record the distribution and some behavioral patterns of *P. farinosa* in Sri Lanka.

MATERIALS AND METHODS

Sampling of *P. farinosa* throughout Sri Lanka

The field sampling was done during December 2014 to October 2015, throughout the island in order to find-out the distribution pattern of *P. farinosa*. During field studies, 120 sites of *C. gigantea* plants were sampled and the presence or the absence of *P. farinosa* on the plants was recorded.

Sampling of *P. farinosa* in Southern Province

Eight sites of Southern province were selected for regular sampling (monthly) in order to study the reproductive biology of *P. farinosa*. Three sites from Matara district, four sites from Hambantota and four sites from Galle districts were selected. The sites were Kalametiya (6^0 6' N; 80^0 55' E), Medilla (6^0 2'N;80^0 48' E), Tangalle (6^0 1'N; 80^0 47'E), Dadalla (6^0 2' N; 80^0 11' E), Thalpe (5^0 59'N; 80^0 16' E), Kamburugamuwa (05^0 56' N; 80^0 29'E), Habaraduwa (5^0 59' N; 80^0 18' E) and Palena (5^0 56' N; 80^0 29' E). The sites were selected randomly and they were located more than 3 km from each other. We studied a minimum of six mature (maximum eight plants) plants. Selected *C. gigantea* plants within the sites were approximately similar in size (1-1.5m in height) assuming similar size plants belonged to same age. To identify the selected plants easily, the stems of the plants were numbered with a white paint.

Life cycle studies of *P. farinosa*

In parallel to field studies, *P. farinosa* were reared in the laboratory to study the mating behavior, oviposition, larval development and pupation. Male and female adults of the species were collected from the study sites directly by hand picking and put them into small plastic vials. Collected *P. farinosa* were reared in 30 × 30 × 30cm sized cages with slide-open doors under laboratory conditions (27 ± 1 ℃, natural photoperiod). They were fed with fresh leaves, flowers and twigs of *C. gigantea*.

Mating and oviposition of *P. farinosa*

In order to study the oviposition behavior of *P. farinosa*, fresh *C. gigantea* fruits of different maturity stages were placed in rearing cages. As *P. farinosa* is nocturnal, observations of pre-mating, mating and post mating were recorded under laboratory conditions during night. Similarly, pre- oviposition, oviposition and post-oviposition behavior of the weevil was observed and recorded.

Eggs of *P. farinosa*

Immature fruits (n = 250) (close to maturity) of *C. gigantea* were collected monthly from selected sites. Egg clusters of *P. farinosa* were extracted from infected fruits under laboratory conditions. Before extraction, the maximum width and the maximum length of the *C. gigantea* fruits were measured using a measuring tape. The number of egg clusters per fruit and eggs per cluster was counted. In order to find any correlation between the volume of oviposited *C. gigantea* fruits and the number of laid eggs per fruit, an equation was used- $4/3\pi ab^2$ where,

a = Maximum length of *Calotropis* fruit/2

b = Maximum width of *Calotropis* fruit/2

Paramecops farinosa laid eggs in immature, developing oval shaped *C. gigantea* fruits. Therefore, an assumption was made as all the studied fruits were prolate ellipsoid in shape and the above equation was applied to gain the volume of *C. gigantea* fruit.

Larval development of *P. farinosa*

Some of the extracted eggs, larval stage and pupae were reared under laboratory conditions at 27± 1 C to study the further development. During high moisture conditions, the mature fruits were rotten with the live larvae. In such conditions, the larvae were transferred to fresh fruits and allowed them to pupate.

Calotropis gigantea fruits (200 in number) oviposited by *P. farinosa* were kept in laboratory conditions and different larval stages (until final larval instar) were extracted periodically by opening the fruits. To determine the number of larval instars, larval stages were placed on 70% alcohol. The maximum length of the head capsule of each larva was measured using a calibrated ocular micrometer in a binocular dissecting microscope.

Damage and dispersal ability of *P. farinosa*

The *Calotropis* seedlings were planted (keeping 1m distance from one another) in a new site within the premises of the University of Ruhuna. Two years later *P. farinosa* were introduced to the above site. As there was no *P. farinosa* previously in the site, the following experimental setup was established to observe the damaging ability and the dispersal of *P. farinosa*. The habitat contained 15 *Calotropis* plants having fruits and flowers. Adult *P. farinosa* (four individuals per plant) were introduced only to four plants of this plot and labeled as A, B, C and D (Fig. 1). Establishment, dispersal patterns and the degree of damage caused by *P. farinosa* was observed over a period of a week from the day of introduction. Cumulative number of damaged leaves per plant attacked by the *P. farinosa* and cumulative number of damaged areas per leaf, the number of *P. farinosa* on each labeled *Calotropis* plant was recorded. Similarly, the number

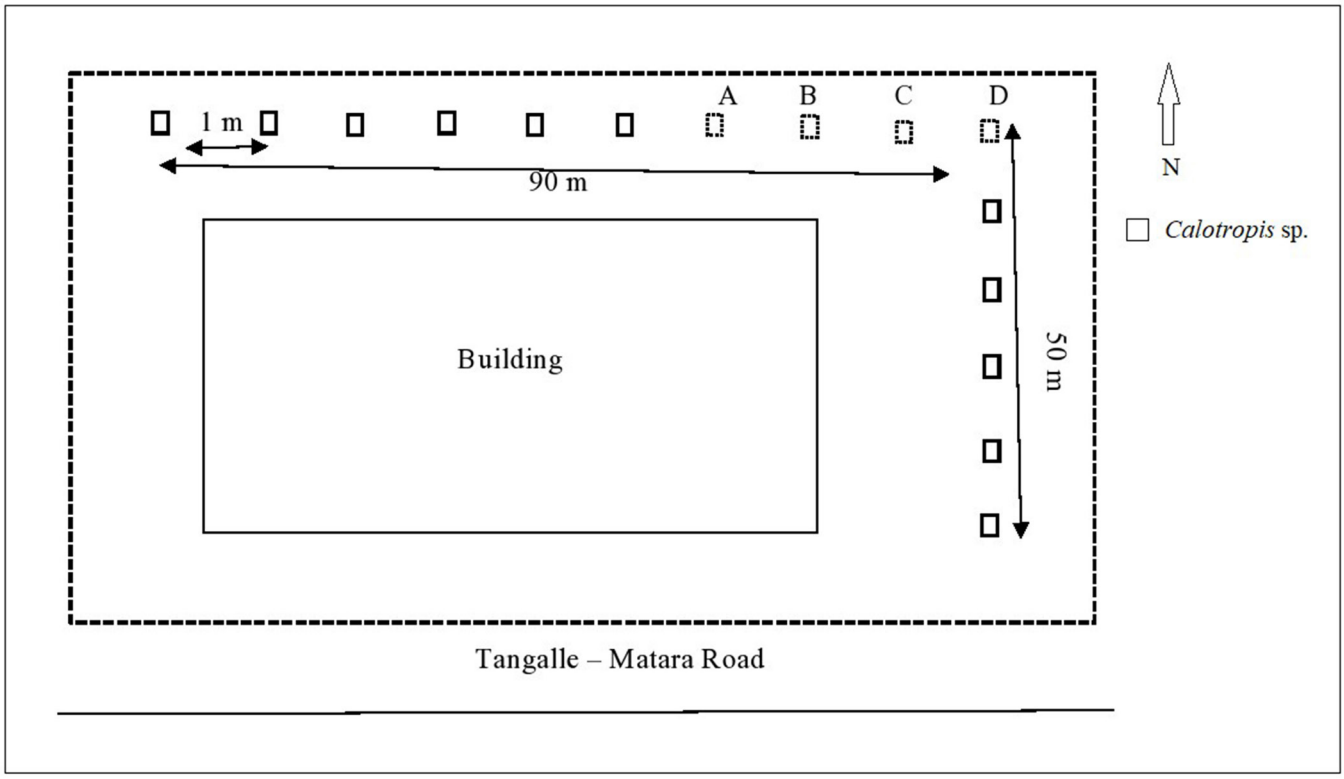


Fig. 1. Map of *Calotropis* trees with introduced *P. farinosa* (Aak weevil) at University premises of Ruhuna

of damaged flower bunches and number of damaged flower buds per bunch and number of *P. farinosa* present on the plant was recorded. The observations were taken in the morning (6.00- 9.00 am) regularly throughout a week in order to get a brief idea on the degree of damage and dispersal ability of *P. farinosa* within a new habitat.

RESULTS

Distribution of *P. farinosa*

The distribution of *Paramecops farinosa* was observed along the distribution of host plant in coastal areas (Southern, Eastern and Northern provinces) as well as inland areas (Northern, North- Central, North- Western and Uva provinces) of Sri Lanka (Fig. 2). It was not recorded in the Central and Western provinces.

Distribution of *C. gigantea* and percentage occurrence of *P. farinosa* in different provinces is presented in Table 1. The highest abundance of *P. farinosa* was recorded in Northern Province of Sri Lanka. Comparatively higher abundance (more sites with *P. farinosa* and more *P. farinosa* per site) was recorded in Eastern and North- Central Provinces of Sri Lanka, where dry climatic conditions prevailed. *Paramecops farinosa* was absent in Western province even though *Calotropis* plants were present. As *P. farinosa* is a monophagous feeder of *C. gigantea* in Sri Lanka, there were no records of *P. farinosa* in Central Province due to the absence of its host plant.

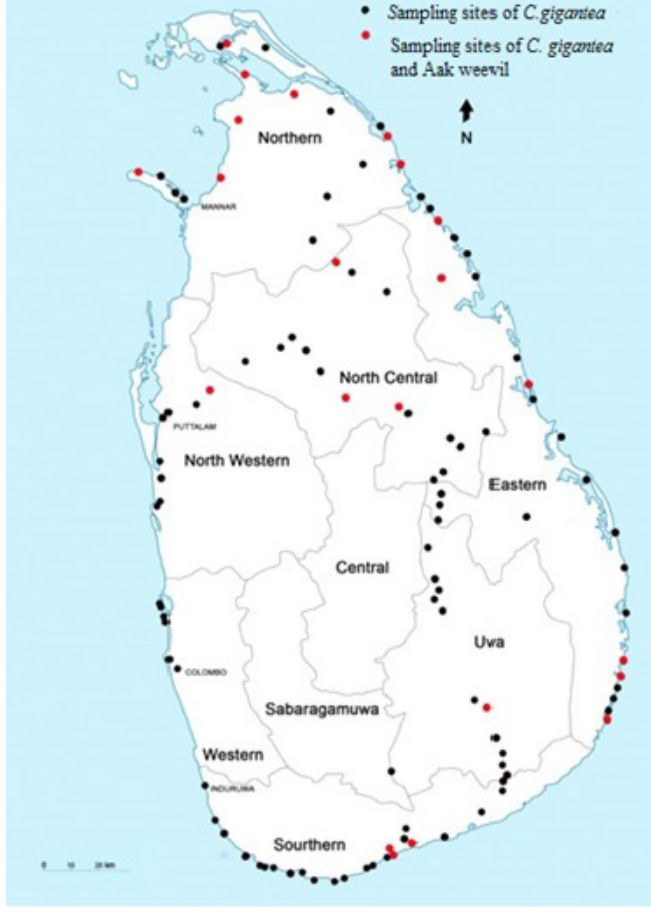


Fig. 2. Sampling sites of *Calotropis gigantea* and *Paramecops farinosa* in Sri Lanka

Table 1. Distribution of *Calotropis gigantea* and the occurrence of *Paramecops farinosa* with respect to provinces in Sri Lanka

Province	Number of sites sampled for *C. gigantea*	Number of sites *P. farinosa* recorded	% of occurrence according to province
Northern	18	08	44.44%
Eastern	25	06	24.00%
North Central	16	03	18.75%
North Western	08	01	12.50%
Southern	24	03	12.50%
Uva	11	01	9.09%
Western	06	00	0.00%
Central	00	00	0.00%
Sabaraga-muwa	01	01	NE*

NE*- Not estimated due to less area coverage of Sabaragamuwa during field visits.

Table 2. Reproductive biology data and morphometric data of *Paramecops farinosa*

Index	Sample size	Mean ± SE
Life history data		
Mating period	15 pairs	30.1 (± 11.0) minutes
Oviposition period	20 females	38.0 (±6.6) minutes
Post oviposition period	10 females	24.2 (±1.1) minutes
Incubation period	35 eggs	5.5 (±0.1) days
Number of eggs per cluster	22 egg clusters	6.8(±2.52)
Larval duration	35 larvae	26.1 (±0.5) days
Pupal duration	25 cocoons	11.5 (±1.3) days
Morphometric data		
Egg - maximum length egg	35 eggs	2.0 (± 0.1) mm
Egg - maximum width	35 eggs	1.1 (±0.1) mm
First instar larva - head capsule width	28 larvae	0.5 (±0.04) mm
Second instar larva - Head capsule width	8 larvae	0.9(±0.07) mm
Third instar larva - head capsule width	8 larvae	1.1 (± 0.06) mm
Fourth instar larva - head capsule length	20 larvae	1.8 (±0.1) mm
Fifth instar larva - head capsule length	13 larvae	2.0 (±0.05) mm
Pupa - maximum length	20 cocoons	15.5 (±0.96) mm
Pupa - maximum width	20 cocoons	9.5 (±2.04) mm

Reproductive behavior and life cycle of *P. farinosa*

Copulation took place during nights or early morning hiding among twigs, flower buds and leaf axils. The male-female recognition was facilitated by antennal movements. After several mounting attempts, male succeeded in the copulation process. Copulation lasted for 15- 45 minutes and sometimes lasted more than an hour.

Paramecops farinosa laid eggs inside the immature fruits by cutting a hole (approximately 1 mm in diameter) of pericarp using its mandibles. After laying eggs, she covered the hole with emerged latex of the fruit. The complete oviposition process lasted 30-45 minutes, then female defecated closer to the oviposited spot and left the fruit.

Eggs were laid in the fibrous tissue located between outer and inner pericarp of the fruit. The eggs were shiny, delicate and oval and round at both ends. Creamy – yellowish eggs were laid in clusters and glued together. Mostly (82 %; n =18) an infected fruit consisted of only one cluster per fruit but occasionally (18 %; n=4) contained two clusters per fruit. However, there was no correlation ($p = 0.285$, $r = -0.238$) between the number of egg clusters per fruit and the volume of *Calotropis* fruit which used for egg laying. Similarly, there was no correlation ($p = 0.493$, $r = -0.154$) between the number of *P. farinosa* eggs in a *Calotropis* fruit and the volume of *Calotropis* fruit. The number of eggs per cluster varied from 3 to 11.

Pale yellowish-white bodied, delicate, apodous larvae with brownish head capsule emerged from eggs. Emerged larvae penetrated the fibrous layer as well as the inner pericarp and enter into the seed chamber. Developing larvae fed on maturing seeds and underwent five larval instars. Larval instars not morphologically deviated greatly except the size. Larval instars were creamy white having curved, stout bodies with jumping movements. The complete larval development took place in 25-31 (26 ± 2.9) days.

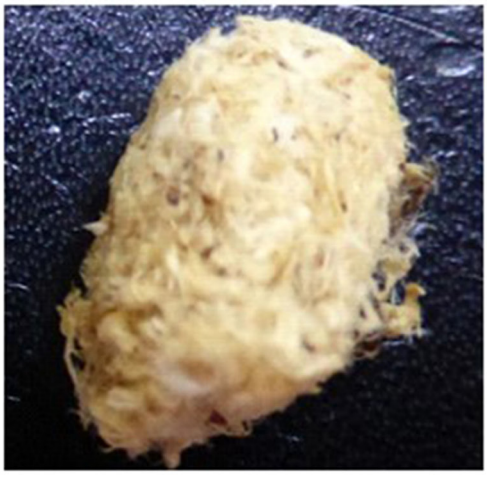
Pupation occurred within the fruits using silky material of seeds. Larvae about to pupate chew and break- down silky materials into small pieces and wrapped them perfectly as a ball around their body and encapsulated. Cocoons were oval shape and rounded at both ends. Outer layers of the cocoon were loosely arranged while inner layers were firmly wrapped. The pupation lasted for 9-15 days. *P. farinosa* adult emerged during dusk or at night by cutting a hole in the cocoon wall. Emerged weevils were pale brown in color and later turned into grayish black.

Mating pair of *P. farinosa*

Oviposition of *P. farinosa*

Eggs of *P. farinosa*

Larvae of *P. farinosa*



Cocoons of *P. farinosa*

Newly hatched *P. farinosa*

Fig. 3. Mating, oviposition and lifecycle stages of *Paramecops farinosa*

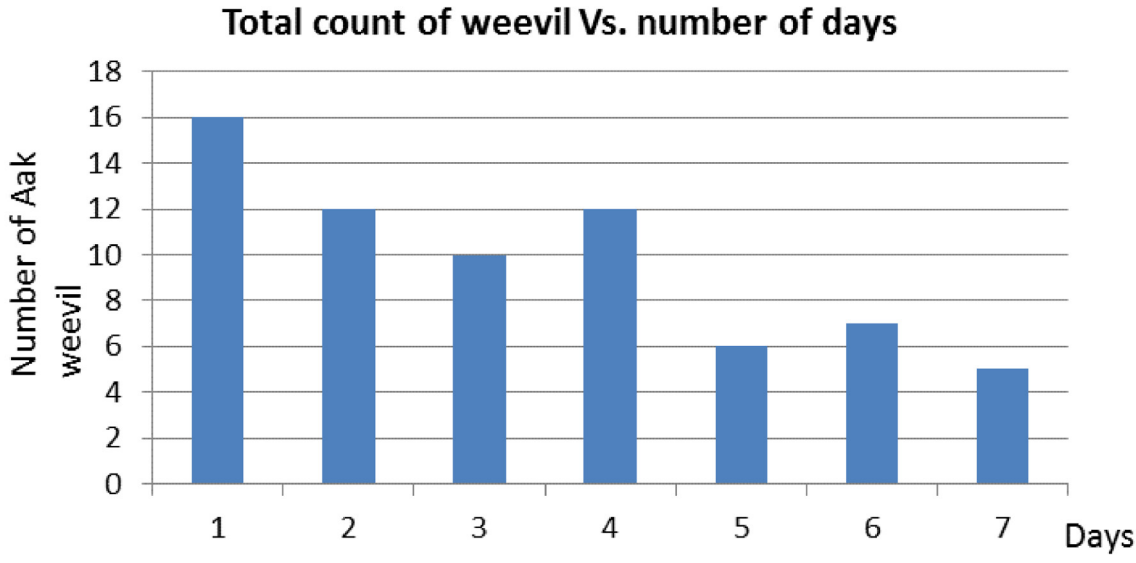


Fig. 4. Cumulative number of *P. farinosa* in the four selected *C. gigantea* plants during a period of one week

Damage caused by *P. farinosa*

Both larval and adult stages of *P. farinosa* fed on different parts of *C. gigantea*. The present study revealed that all larval stages fed on seeds and completely destroyed (100%) the infested fruit. According to field observations of the site in University premises, adult weevils voraciously fed on tender leaves, flowers and flower pods causing great damage. In the site, there was a fluctuation of *P. farinosa* throughout the week (Fig. 4).

The total number of introduced *P. farinosa* (16) was observed only on the first day after the introduction (Fig. 4). Missing weevils were not observed on the rest of the *C. gigantea* plants (11 plants) in the new habitat or on weeds associate with *Calotropis* plants. During the week the cumulative damaged areas per plant has increased except in the plant D (Fig. 5). The cumulative number of damaged areas per leaf was always equal or higher than the cumulative number of damaged leaves. There was a great deviation in plant D, where no *P. farinosa* observed on the plant at day time, except its damage on plant. Also, the amount of damage was less in plant D (Fig. 5) when compared to plant A, B and C.

The number of damaged leaves was not proportionate to the number of *P. farinosa*. Similarly, no correlation ($r = 0.06$, $P = 0.8$) was found between the cumulative number of damaged leaves and the number of *P. farinosa* on *Calotropis* trees. Also, results confirmed no correlation ($r = 0.03$, $p = 0.9$) between the cumulative number of damaged areas on leaves and the number of *P. farinosa*. Aak weevil preferred flower buds than than leaves, causing more damage to the flower buds during the first three days of the week. Later they feed on leaves than flower buds (Fig. 5).

Similarly, the amount of damage on flowers/ flower buds in a particular plant fluctuated during the study period. However, there were correlations between the number of damaged flower bunches and number of *P. farinosa* per plant ($r = 0.53$, $p = 0.006$) as well number of damaged buds and number of *P. farinosa* ($r = 0.59$, $p = 0.001$) on a plant.

DISCUSSION

Life history studies revealed that *P. farinosa* oviposited on developing fruits. This may be due to two major reasons. Developing fruits are easy to penetrate and ensure the placement of eggs between outer and inner pericarp layer. On the other hand, developing fruits having immature seeds were a suitable food source for newly emerged larvae with delicate, developing mouthparts. After oviposition, as Sudan (2013), observed, females deposit fecal matter at the oviposition site to make it aware for other females. Similar observations were recorded in the present study also.

Dissected fruits revealed that *P. farinosa* eggs were observed in the fibrous layer which was placed in between the inner and outer pericarp layers of the fruit. As a result, emerged *P. farinosa* larvae should pierce the inner pericarp layer in order to enter into the seed chamber. *P. farinosa* oviposited fruits mostly (81.82%) contained only one cluster it might be due to territory marking of *P. farinosa* females on oviposited fruits. Only a few fruits (18.18 %) contained two clusters per fruit. It might be due to high competition for fruits as well as lack of suitable fruits for oviposition.

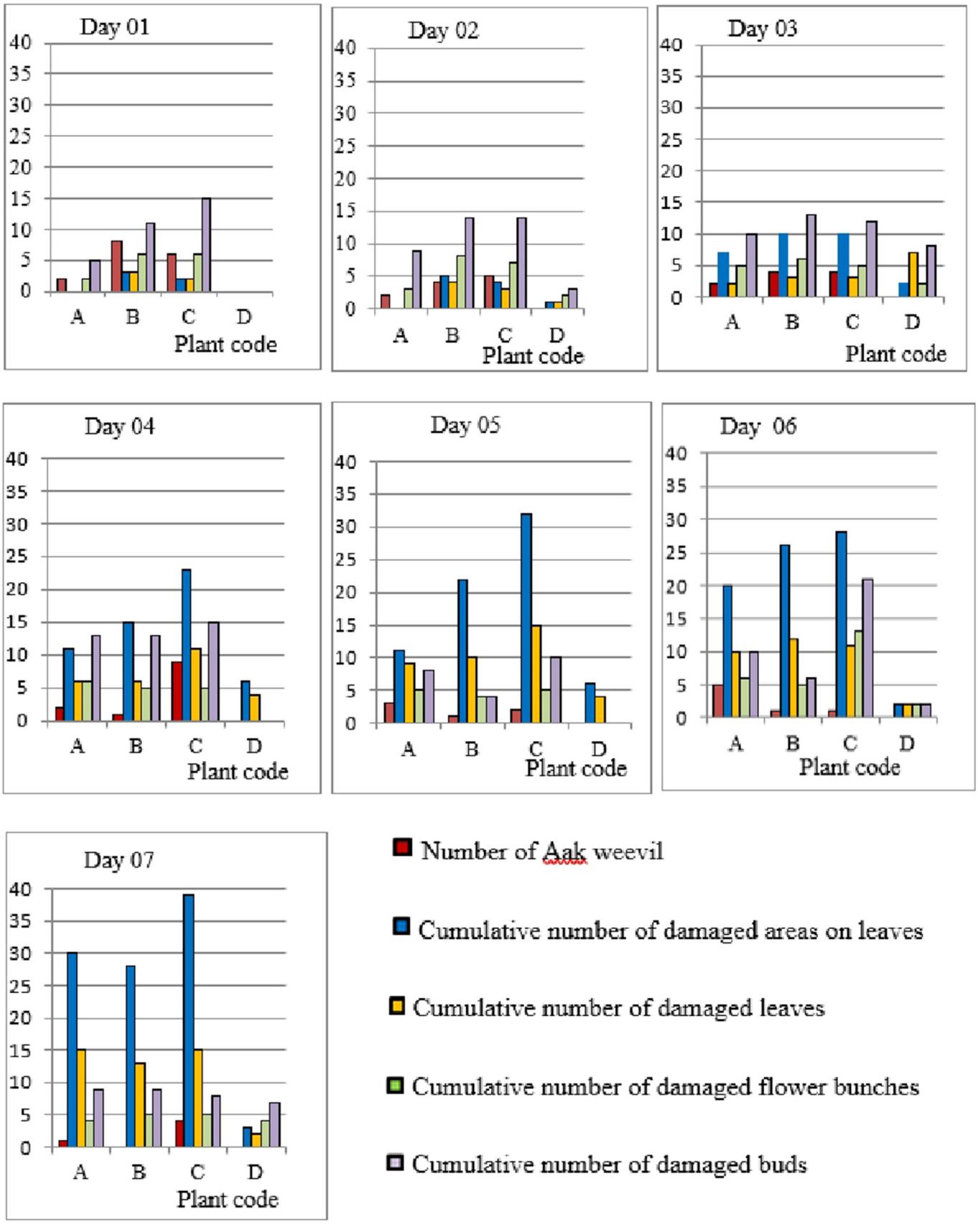


Fig. 5. Damages of *Paramecops farinosa* on different plant parts of *Calotropis gigantea* within a period of one week

Fig. 6. Nature of damage of *Paramecops farinosa* on *Calotropis gigantea*

According to the present study, *P. farinosa* in Sri Lanka is characterized by its larger eggs in comparison to Indian species. The mean length and width of eggs in Sri Lanka was 2.03 (± 0.09) mm, 1.12 (±0.07) mm, respectively while in India, the egg size was recorded as1.25 (± 0.12) mm in length and 0.80 (±0.11) mm in width. In contrast, Sri Lankan *P. farinosa* has a smaller pupa (length 15.52 ± 0.96 mm and width 9.45 ± 2.035 mm) than the pupa in India (length 25.24 ± 4.06 mm and width 20.05 ±4 .29 mm) (Sudan, 2013).

Paramecops farinosa pupae formation occur at the fifth larval stage and pupae were observed within the seed chamber, in between outer and inner pericarp layer of attached fruits or inside the decaying detached fruits. Under unfavorable conditions (decayed fruit, lack of food for larvae) the larvae pupate earlier. Such pupae were small in size compared to cocoons formed in favorable conditions. The emerged adults from such small pupae were weak, small in size and unable to compete with healthy adults for mating, food, etc.

Newly emerged *P. farinosa* were darker in color (black) than adult which having whitish greyish color. The dusty, mealy powder is secreted by the weevil in later stages of life (Saikia *et al.*, 2015) which might reduce desiccation and aid for camouflage as *Calotropis* plants too contain similar dusty appearance on leaves and twigs.

Both adult and larval stages of *P. farinosa* feed on different parts of *C. gigantea*. Adult *P. farinosa* mostly feed on tender leaves and flower buds while larval stages feed on seeds. Flower buds were highly susceptible to *P. farinosa* attack and a single bite of *P. farinosa* led to drop-down of flower buds. As they cut latex canals of the leaves, flowers and flower buds (Sudan, 2013), it might lead to dehydration and defoliation. Feeding on flowers, flower buds and seeds, directly influence the reproductive output of the plant while leaf consumption leads to a reduction of plant growth. The survival tendency of *P. farinosa* was very high in unfavorable conditions. The present study reveals that they survive up to 2-3 weeks of starvation.

The dispersal behavior of *P. farinosa* in a new habitat reveals that *P. farinosa* can adapt to a new habitat. Numbers of introduced weevils found on the *Calotropis* plants, had been reduced over time. This reduction may be due to the mortality of *P. farinosa* or other unknown factors. However, they had not moved to other *Calotropi*s plants within the same plot which were not included for the survey. The Observations revealed that some individuals adapted to the new habitat successfully and undergone mating and oviposition. Fluctuation in the number of *P. farinosa* on a plant shows, *P. farinosa* dispersed among the *Calotropis* plants during nights. They might move from one *Calotropis* plant to another to find-out a partner

to mate. The Observation of the number of damage patches on leaves was always higher than the number of damaged leaves. It indicates they prefer to consume a selected leaf without moving to several, fresh leaves. On the other hand, they tend to aggregate as two or three individuals and feed on a single leaf. As result, great destruction on certain regions of the plant was observed. Leaf consumption in the plant D was low and *P. farinosa* were not found on that plant during day time. It might be due to various reasons such as slight differences in the chemical composition of the plant, effect of sea breeze where the plant was highly exposed to the wind or an unknown reason. The observations revealed that number of leaf damage was not proportionate to the number of *P. farinosa* on a selected plant during day time. It might due to the behavioral pattern of *P. farinosa*. They might be move from one plant to another plant for leaf consumption and mating but they might not select the same plant to stay during the day time.

According to the observations, *P. farinosa* caused great destruction to the *Calotropis* plants as their both larval and adult stages feed on different parts of plants. The low dispersal ability of *P. farinosa* however limits the destructiveness of *Calotropis* plant in a geographic area. Both factors; the degree of damage and the dispersal ability of *P. farinosa* should be considered thoroughly when it is used as a potential biological control agent. On the other hand, specificity testing should be conducted for the species in order to confirm food sources of them and the nature of *P. farinosa*- plant interactions.

The findings of the present study provide detailed information on distribution, damage to the host plant, mating, oviposition and life cycle stages of *P. farinosa* in Sri Lanka where no known records are previously available. In addition, the present study will provide essential information of *P. farinosa* on *Calotropis* spp. to explore further the possibility of using it as a biological control agent.

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