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## Distribution of the eggs of the mopane psyllid *Retroacizzia mopani* (Hemiptera: Psyllidae) on the mopane tree

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### ABSTRACT

The distribution of the eggs of the African mopane psyllid *Retroacizzia mopani* on the mopane tree *Colophospermum mopane* leaves was studied in the field and in the laboratory by observations and scanning electron microscopy. *R. mopani* eggs were observed and collected from the host plant, on which the nymphs live and feed on the leaf phloem. The distribution of eggs and nymphs on the leaflets was observed. Eggs were deposited mostly on the abaxial surface and most eggs (76 %) were found along veins. Contrary to the literature, eggs were not attached to the leaflet surface by a pedicel but were glued to the surface. Eggs were laid mostly in clusters and were often camouflaged by black fungal spots that were common on the leaflets. To avoid desiccation, *C. mopane* leaflets usually fold together to reduce the surface area exposed to sunlight, thus reducing the rate of water loss.

KEY WORDS: South Africa, *Retroacizzia mopani*, *Colophospermum mopane*, oviposition, fecundity, pests.

### INTRODUCTION

*Colophospermum mopane* (J. Kirk ex. Benth.) J. Léonard, commonly known as mopane, is one of the best known and valuable tree species indigenous to southern Africa, often being the dominant in species-poor woodlands. It is especially adapted to drier regions of southern Africa, and is thus an important tree during periods of drought (Palmer & Pitman 1972). It is a host plant for a few endemic insect species, among them being the African mopane psyllid *Retroacizzia mopani* (Petty, 1925) (Ernst & Sekhwela 1987). The biology of *C. mopane* and its relationship with the fauna that utilize it are intricate, suggesting a long history of co-evolution of this tree species with its mammalian and insect fauna (Styles 1994).

Nymphs of *R. mopani* emerge from the eggs when the leaves are turning senescent, which normally starts from June or July depending on climatic conditions. They feed exclusively on the leaf phloem sap of *C. mopane* (Ernst & Sekhwela 1987). In addition to direct feeding damage, honeydew inside the white waxy secretions produced by the nymphs promotes the growth of sooty mould (Dreistadt *et al.* 2004), which can reduce the effective leaf area for photosynthesis.

Many psyllids usually lay their eggs in masses, either superficially or embedded. Eggs are in clusters or solitary, and when in cluster the eggs are typically found in rows, circles or scattered (Dreistadt *et al.* 1999). Eggs can be laid on leaves, on buds or in the leaf axils. Eggs laid in areas such as leaf axils are likely to be protected from biotic and abiotic threats (Urquhart & Stone 1995). A female can lay up to 500 eggs, and at high infestations hundreds and occasionally thousands of eggs may be laid on the same leaf. (Clark 1962). The eggs have a basal pedicle which is inserted into the host plant tissue. In some psyllid species, the pedicel is used to absorb water from the plant to keep the

egg moist. It has been reported that when the source of water is removed the eggs quickly dry up (Hodkinson 1974).

Very little has been published on the biology of *R. mopani*, perhaps because it is not a serious pest of agricultural crops (Hodkinson 1974) and is known to cause less damage to the host plant *C. mopane*. Our knowledge of *R. mopani* is limited to the studies of Pettey (1925) and Ernst and Sekhwela (1987). Pettey (1925) described six males and six females collected on *C. mopane* trees in the Musina area of the Limpopo Province, and Ernst and Sekhwela (1987) studied the life cycle of *R. mopani* as well as the chemical composition of the lerp in Botswana. However, there is no detailed description of the ovipositional biology of *R. mopani*. The objectives of the study reported here were to document various aspects of the ovipositional biology of *R. mopani*, including fecundity, egg distribution, and ovipositional response to physical characters of the leaflets.

#### MATERIAL AND METHODS

The study site was on abandoned farmland dominated by mopane trees near Tzaneen (23°49'S:30°10'E, 508 m above sea level). Field visits to the study site were undertaken weekly from May to November 2004. Thirty leaves infested with *Retroacizzia mopani* (Fig. 1) were collected from each of ten randomly selected *C. mopane* trees per visit. The same trees were used throughout the study. The leaves collected were selected from the upper, middle and lower canopies of the selected trees. The distribution and number of eggs were recorded from each leaf sample collected. The length and width

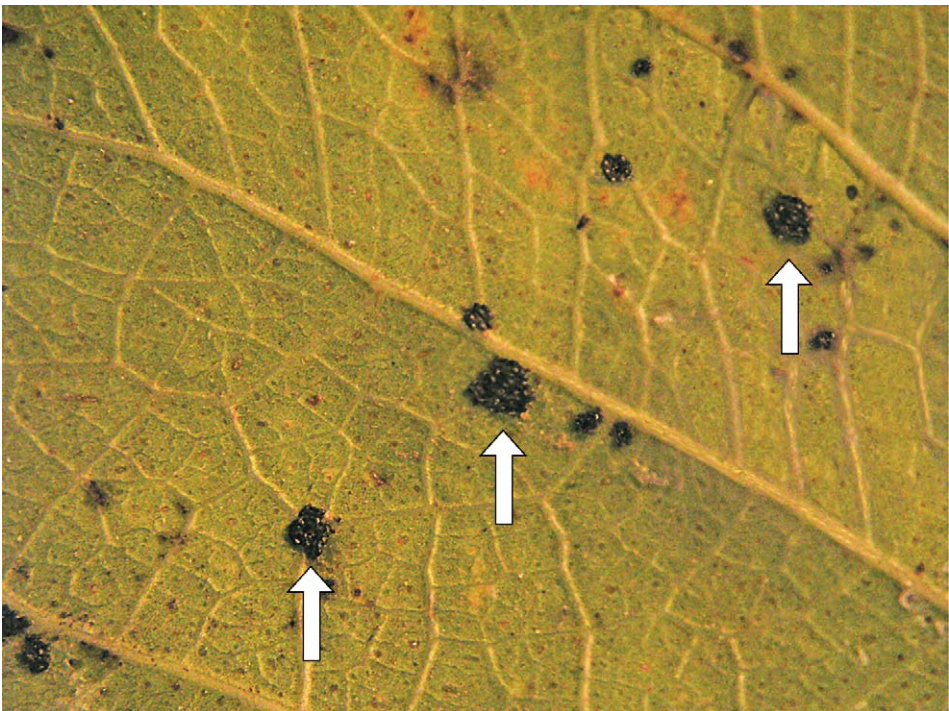


Fig. 1. Egg clusters of *Retroacizzia mopani* (arrowed) on mopane leaves.

of 500 eggs were measured. The calculated mean number of eggs per leaf was used to determine the number per tree.

To determine the number of eggs laid per female, 25 adult females were sampled from the field and sent to the laboratory. Each female was placed in a transparent plastic bottle. Foliage of *C. mopane* was provided as food. Shoots were kept fresh by adding water to the substrate as needed, and the bottles were covered with stockings. Shoots were replaced as foliage became depleted during the experiment. The experimental containers were held at standard rearing conditions. The date on which eggs were first laid by each female was recorded, and the total eggs laid by each female were recorded. Eggs were carefully separated from the egg masses using a dissecting needle and were counted under a stereomicroscope. The data collected for each female included time to first oviposition, total oviposition, total number of egg masses and size.

Twenty eggs from the field and twenty laboratory-laid eggs were ultrasonically cleaned for 10–12 seconds. They were dehydrated through a graded ethanol series (70 %, 80 %, 96 % and 100 %) over a period of 3 hours, critical point dried with CO<sub>2</sub> in absolute ethanol, mounted on aluminium stubs and sputter coated with gold–palladium. Specimens were viewed and micrographed in a Jeol 6100 Scanning Electron Microscope at 7 kV.

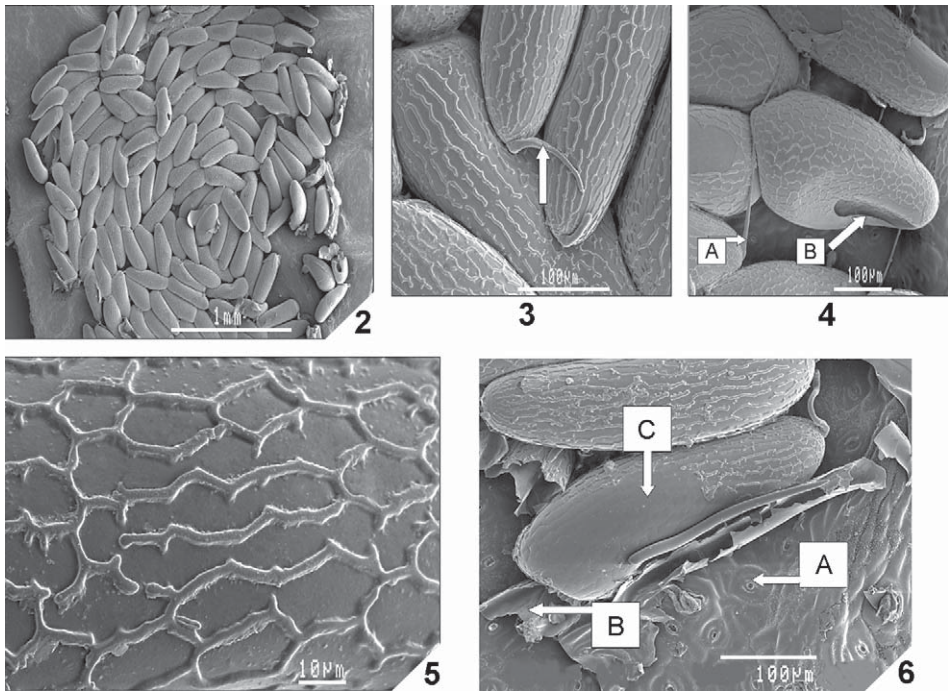
Statistical analyses were performed using the Mann-Whitney Rank Sum Test and results are presented as mean  $\pm$  Standard Deviation.

## RESULTS

From visual observations, eggs occurred randomly in the lower, middle and upper strata of the tree canopy. They were randomly laid on both the adaxial and abaxial surfaces of mature green and senescent leaves. The mean number of eggs per field collected leaf was 1340. However, some field collected leaves had more than 2000 eggs per leaf. The calculated number of eggs per tree was 4,581,460 based on a tree with a mean of 3419 leaves ( $n=5$ ).

The majority (76 %) of eggs on the abaxial surface were found along veins, with the remainder found between veins (14 %), along leaf margins (6.62 %) and on the pulvinus (3.31 %). The trend was not very different on the adaxial surface, with 72.88 % of eggs deposited along veins, 21.87 % between veins, 2.33 % along leaf margins and 2.92 % on the pulvinus. A few eggs were occasionally found on the leaf petiole. Egg clusters were semi-spirally arranged and touched one another (Fig. 2). The mean number of egg clusters on abaxial leaflet surfaces was significantly higher than on adaxial leaflet surfaces ( $7.73 \pm 5.64$  and  $3.56 \pm 4.61$  respectively) ( $p < 0.01$ ). In the laboratory, the number of eggs laid by females ranged from 30 to 320, with a mean of  $235 \pm 17$ .

Egg clusters appeared to the naked eye as black spots on the leaflet. However, under the light microscope, the eggs were black with smooth shiny grey spots closely spaced near the basal end of the egg. Eggs were conical in shape with a basal pedicel and an apical filament (Figs 3, 4). The size ranged from 245–360  $\mu\text{m}$  in length and 100–125  $\mu\text{m}$  in width at the broadest part. There was no difference in size between field-collected and laboratory-laid eggs. The thickness of the eggshell varied from 1.0  $\mu\text{m}$  to 1.34  $\mu\text{m}$  ( $n=30$ ). Scanning electron microscopy showed the egg to be surrounded by a membrane 0.30  $\mu\text{m}$  thick, which had a reticulate sculpture 1.5  $\mu\text{m}$  thick and 4.0  $\mu\text{m}$  wide (Fig. 5). The black surface of the egg below the membrane was smooth (Fig. 6).



Figs 2–6. (2) Scanning electron micrograph showing the semi-spiral arrangement of eggs; (3, 4) *Retroacizzia mopani* eggs: (3) Pedicel (arrowed), (4) Egg filament (A) and pedicel groove (B); (5) Egg membrane showing the reticulate sculpture in the form of ridges; (6) Peeled egg membrane exposing the smooth egg surface: (A) Stomatal opening, (B) Membrane, (C) Smooth egg surface.

Mean pedicel length was  $84 \pm 9.5 \mu\text{m}$  and that of the filament  $266 \pm 23 \mu\text{m}$  ( $n=30$ ). No pedicel puncture marks were observed on the leaflet surface to indicate that the egg was attached to the leaf surface by the pedicel. Rather, eggs appeared to be glued tightly to the leaflet surface so that they could not be removed without breaking them. *Colophospermum mopane* leaflets fold together to reduce the surface area of the leaves exposed to the heat of the sun, thereby reducing the rate of water loss.

#### DISCUSSION

The eggs of *R. mopani* were laid on both mature green and senescent *C. mopane* leaves between July and September, when temperatures are between  $11.4^\circ\text{C}$  and  $23.5^\circ\text{C}$  and monthly rainfall is as little as 10.8 mm. It thus seems that climatic conditions determine *R. mopani* emergence and subsequent oviposition. In contrast, *Arytaina genistae* (Latreille), which is found in temperate regions, is known to oviposit at any time of the year (Watmough 1968a).

The eggs were mainly laid in clusters and arranged in a semi-spiral. The cluster of eggs is an indication that different females either avoid mixing their eggs with other females' or a single female oviposits at different times at different sites on the same leaflet. The presence of several egg clusters on leaflets also suggests the possibility of more than one female using the same leaflets for oviposition. This is in support of Clark's (1962) findings for *Cardiaspina albitextura* Taylor that, when infestation is

high, hundreds and occasionally thousands, of eggs may be placed on the same leaf. The spiral arrangement of *R. mopani* eggs in a cluster is in contrast to that of Australian psyllids, which generally lay their eggs in rows or circles (Urquhart & Stone 1995). Perhaps eggs in a cluster assist to maintain the optimum temperature for hatching.

The black eggs of *R. mopani* conform to the statement by Urquhart and Stone (1995) that psyllids generally lay yellow, brown or black stalked eggs. It is speculated that the black colour of the eggs is an evolutionary development to blend in with the black fungal spots, which are common on leaflets as a camouflage, or to absorb enough heat to enhance hatching. Egg hatching in *Arytainilla spartiophila* (Förster), for example, is known to be delayed at low temperatures (Watmough 1968b).

According to White (1968), water in the form of cell sap is absorbed through the pedicel to keep the eggs from drying up. This was not the case with *R. mopani*, as the pedicel does not penetrate the tissue to absorb water. To avoid desiccation, *C. mopane* leaflets fold together to reduce the surface area exposed to the heat of the sun, causing reduction in the rates of water loss. This may also be the reason why the abaxial surface is preferred to the adaxial surface during oviposition.

In this study, no pedicel-punctured marks were observed on the leaflet surface to indicate its attachment to the surface. Rather, a sticky (glue-like) substance found in the place where the pedicel is in contact with the leaves could facilitate egg attachment to the surface. The reticulate sculpture might assist in enlarging the surface area of the egg to which the glue can adhere. The fact that eggs are laid superficially on the leaflet surface of *C. mopane* trees might be an indication that predatory pressure is not very high, while in *Arytaina spartii* (Guérin-Ménéville) for example, the eggs can be deeply embedded in the leaf tissue (Hodkinson 1974). It would seem that embedding the eggs in the leaf tissue is an indication of behavioural adaptation to the very cold conditions of England. In the lowveld region where *R. mopani* occurs, however, the winters are mild and dry. The temperature is between 11.4 °C and 23.5 °C, and mean afternoon humidity about 38.9 %.

*Retroacizzia mopani* occurs and feeds on *C. mopane* leaves probably due to the lack of alternative hosts plants as the emergence of *R. mopani* coincides with the dry winter period of South Africa, when many trees are leafless. Egg masses of *R. mopani* were only observed on *C. mopane* during the study. This link between feeding and oviposition in *R. mopani* probably serves as a mechanism to ensure that eggs are deposited primarily on plants that nymphs and adults have found to be acceptable source of food for developing nymphs. Details of oviposition behaviour and larval habits need further investigation.

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