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Notes on the biology of *Nysius natalensis* Evans (*Hemiptera*: *Orsillidae*)

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Four of the more than 100 *Nysius* species known worldwide, occur in South Africa, *viz*. *Nysius natalensis* Evans, *Nysius binotatus* (Germar), *Nysius pallidus* Evans and *Nysius stali* Evans. Although, *N. natalensis* is a sporadic pest of sunflower in South Africa, no description of the different life stages has been published. Such information would facilitate monitoring of the pest and contribute to the improvement of management practices. This study showed that *N. natalensis* oviposits mainly in flowers and attach eggs to the ovaries in the flowers or to the pappus in the case of the Asteracea. Eggs were also laid next to fruits of host plants. The majority of these plant species are weeds occurring in natural vegetation, with eggs most frequently observed in the seed heads of the Asteraceae. Differences in head width and pronotum width of *N. natalensis* can be used to distinguish between the five nymphal instars and adult females, which were significantly larger than adult males and can easily be distinguished by the ovipositor cleft visible on the underside of the abdomen.

Key words: Eggs, false chinch bug, instars, oviposition sites, South Africa.

INTRODUCTION

More than 100 *Nysius* species are known in this cosmopolitan genus (Slater, 1964) of which different species are confined to specific regions in the world. Slater (1964) listed four South African *Nysius* species. These are *Nysius natalensis* Evans, *Nysius binotatus* (Germar), *Nysius pallidus* Evans and *Nysius stali* Evans. *N. natalensis* is the most abundant of the four species in South Africa (Slater, 1964).

N. binotatus destroys vegetable crops and flowers and attack peaches in South Africa (Evans, 1936), it occurs sporadically numerous on turnips and attack sunflower seed heads (Annecke and Moran, 1982). Some of these records may, however, refer to *N. natalensis* since *N. binotatus* is less abundant than *N. natalensis* (Slater, 1964). *Nysius stali* was reported in Nigeria to be a pest on sunflower during flowering and head formation (Misari, 1990), but no reports on damage inflicted to crops could be found for *N. pallidus* in the literature.

N. natalensis is an occasional pest of wheat in South Africa and also attacks onions, leeks, garlic, alfalfa and sunflower (Annecke and Moran, 1982). It also causes significant crop loss to pistachio nuts by feeding and by consequent fungal transmission through lesion formation on nuts (Swart, 2002). Although, large numbers of *N. natalensis* can be found on sunflower [*Helianthus annuus* L. (Asteraceae)] in South Africa, their occurrence is sporadic and unpredictable (Du Plessis et al., 2007). During the seedling stage, the insects feed on vascular

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tissues of young sunflower plants, causing wilting and dying (Du Plessis et al., 2005). Sunflower crops can also be attacked by *N*. *natalensis* from the budding period onwards. It was reported by farmers, but not scientifically confirmed that damage inflicted to seeds during the seed filling period causes a reduction in yield, oil content and germination. *N. natalensis* also occurs on numerous wild host plants in the sunflower production area of South Africa and it is highly polyphagous (Du Plessis et al., 2007). These wild host plants are widespread and their reproductive periods overlap extensively in space and time, which requires consideration in the management of the insect on sunflower (Du Plessis et al., 2007).

Little is however, known about the biology of *N*. *natalensis* (Annecke and Moran, 1982) and information published on it is limited to reports by Matthee (1971; 1974) and publications on its biological control (Haddad et al., 2004), chemical control (Du Plessis et al., 2005) and distribution and the host plant range (Du Plessis et al., 2007). Information on the biology of the pest, as well as its distribution, host plants and impact on sunflower could be used in the development of an Integrated Pest Management program for this pest in South Africa. No information on oviposition sites and descriptions of the immature stages of *N*. *natalensis* are currently available. This is however, essential knowledge needed to monitor *N*. *natalensis* in headlands and sunflower fields to make decisions about management interventions. It can also be used in determining development rates of the different life stages which could be used in prediction models. The objectives of this study were therefore to determine where (in the soil or on host plants) *N*. *natalensis* females lay their eggs, to describe morphological characteristics of the eggs, to describe the immature stages and determine the head and pronotum widths by which each instar can be identified.

MATERIALS AND METHODS

Stock colony

A stock colony of *N*. *natalensis* was reared from adults initially collected from a wild host plant species, *Portulaca oleracea* L., at Potchefstroom, South Africa (26°44'S 27°05'E), to obtain eggs and nymphs. The stock colony was reared and maintained in Petri dishes (140 mm diameter) at 26 ± 1°C and a 14L:10D photoperiod in an incubator. Sunflower seeds were cut in half and provided *ad libitum* with *P*. *oleracea* seed and stems as food. Water was provided by means of moist filter paper squares (200 mm^2) placed at the bottom of the Petri dishes. A piece of pipe cleaner (15 mm long) was provided as an oviposition substrate. All of the eggs used during this study were collected from second generation laboratory-reared insects.

Oviposition sites on wild host plants

The oviposition sites of *N*. *natalensis* were determined through a roadside survey in each quarter-degree grid in the main sunflower production area of South Africa. The area is 116 881 km^2 and is bounded by Derdepoort (24°65'S 26°40'E) (North West province), Thabazimbi (24°35'S 27°24'E) (Limpopo province) and Bronkhorstspruit (25°78'S 28°76'E) (Mpumalanga province) at the northern borders and Kimberley (28°73S 24°76'E) (Northern Cape province) and Tweespruit (29°20'S 27°06'E) (Free State province) at the Southern borders. Sampling of *Nysius* spp. was conducted and plant species were inspected and collected once in a 3 x 3 m^2 area at the point of arrival in each quarter degree grid. Oviposition sites on plants, inflorescences of grasses, as well as flowers and capitula of these weed species were determined. Top-soil samples were also taken with a spade within a radius of 30 cm around each plant and to a depth of approximately 0.5 cm in these 9 m^2 areas. Pieces of each plant with its reproductive parts e.g. inflorescences, flowers, capitula, or seed were sampled, labelled and put in paper bags individually. Labelled soil samples (point of sampling and plant species under which it was sampled) were transported in plastic bags.

All samples were transported in cooler bags to a laboratory and examined under a stereomicroscope (Wild MZ8) for the presence of *N*. *natalensis* eggs within 24 h of sampling. All eggs found were kept in Petri dishes at 26 ± 1°C and a 14L:10D photoperiod with moist cotton wool to maintain high humidity. Nymphs that hatched from the eggs were reared to maturity in the laboratory to confirm the species identification.

Measurement and description of eggs

A stereomicroscope (Wild MZ8) fitted with a measuring eyepiece was used to measure eggs laterally from the anterior to the posterior pole to an accuracy of 0.01 mm (n = 30). A detailed study of the morphological characteristics of the eggs was done using a scanning electron microscope (SEM) (SEI ESEM Quanta 200). Basic preparation techniques for a SEM were followed. The eggs were fixed in 70% ethanol for 2 to 8 h, after which the ethanol was exchanged with 70% acetone for 15 min. They were then dehydrated in an acetone series (80%, 90% and 2 x 100% for 15 min each without exposing the samples to air and critical point dried (CPD). The eggs were then mounted by means of double-sided carbon tape on SEM stubs and coated with a 25 nm layer of gold/palladium in a sputter coater. Photographs were taken with a FEI Quanta 200 ESEM with an Oxford Inca 200 EDS System.

Body measurements

Forty male-female pairs from the stock colony were kept in Petri dishes (140 mm diameter) in an incubator at 26 ± 1°C and a 14L:10D photoperiod, and provided with sunflower seeds and pieces of *Portulaca oleracea* stem *ad libitum*. Water was provided by means of moist filter paper squares (200 mm^2) placed at the bottom of the Petri dishes to prevent females laying eggs in wet cotton wool. Pieces of pipe cleaner used as an oviposition substrate were replaced daily, and kept separately in the same incubator until the eggs hatched. First instar nymphs were collected within 24 h of hatching and transferred to Petri dishes (90 mm in diameter) in groups of ten. A thin layer of sand covered the bottom of all Petri dishes to allow nymphs to right themselves. Water contained in moist cotton balls was provided daily. Fresh pieces of *P*. *oleracea* capitula and sunflower seed were provided once a week. After moulting to the second instar, nymphs were kept individually in small plastic containers (35 mm diameter and 40 mm high), closed with cling wrap (GLAD wrap, GLAD). Water and food was supplied as indicated above.

Daily observations of the insects were made and exuviae of moulted insects removed. The number of instars was determined by measuring the head width across and including the widest part of the eyes, the pronotum width at the posterior margin and the total

Figure 1. *Nysius natalensis* egg attached to the bristles of the fruit of *Conyza albida.*

body length measured from the apex of the head to the tip of the abdomen. Measurements were done within 24 h after each moulting with a stereomicroscope (Wild MZ8) fitted with a measuring eyepiece. There were 30 replicates for each instar.

Data analysis

Mean body measurements were compared by means of analyses of variance followed by Tukey's multiple range test to confirm the number of distinct instars using Statgraphics Plus 4 for Windows (1999).

RESULTS AND DISCUSSION

Oviposition sites on wild host plants

Eggs of *N*. *natalensis* were found on 26 host plant species (Du Plessis et al., 2007), most of which are considered to be weeds and occur in natural vegetation as opposed to cultivated crops. Majority of oviposition hosts were Asteraceae and oviposition sites of *N*. *natalensis* were observed to be in flowers where eggs were attached to the ovaries in the flowers or to the pappus. Eggs are attached with a sticky secretion to the lower half of the pappus or sometimes to the bristles of the fruit while the capitula are still unripe (Figure 1). Eggs of other *Nysius* species, *viz*. *Nysius vinitor* Bergroth (Smith, 1927), *Nysius groenlandicus* (Zett.) (Böcher, 1972) and *Nysius tenellus* Barber (Carrillo, 1967) are also found attached to the pappus and/or pappus bristles of the fruit of Asteraceae. *Nysius groenlandicus* usually oviposits on seeds and fruits equipped with parachute type structures (Böcher, 1972). Eggs and first instar nymphs of *N*. *natalensis* were observed to wind disperse on the fruit of *Conyza albida* Spreng. belonging to the family Asteraceae (H. Du Plessis, Personal observation). Dispersal on wind-borne seeds of Asteraceae was also reported for *N*. *tenellus* in California (Carrillo, 1967).

Böcher (1972) suggested that wind dispersal of fruits of mainly Asteraceae might be responsible for the ubiquity of *N*. *groenlandicus* in Greenland. The chances of survival of the emerging nymphs may, however, be variable, depending on the area where the fruits land and on the ability of the nymphs to find suitable food or host plants (Carillo, 1967). The eggs of *N*. *natalensis* are deposited inside the seed heads of the Asteraceae while they are still young and closed (H. Du Plessis personal observation). Many of the eggs hatch before the seed head opens and dispersal of the fruit takes place. Nymphs are then able to move on the plant or to the soil surface. Fruits do not disperse away from the plants during wind-still days, but fall to the soil surface underneath the plant. When a cluster of eggs is laid on a single seed/fruit, it may be too heavy to be carried over long distances.

In addition, the oviposition host range of *N*. *natalensis* also includes plant species such as sunflower, pig weed (*P. oleracea*) and wild purslane (*P. quadrifida* L.) (*Portulacaceae*) amongst others (Du Plessis et al., 2007) that do not produce windborne seeds. The attachment of eggs to windborne seeds therefore contribute as a means of dispersal, but the widespread occurrence of this

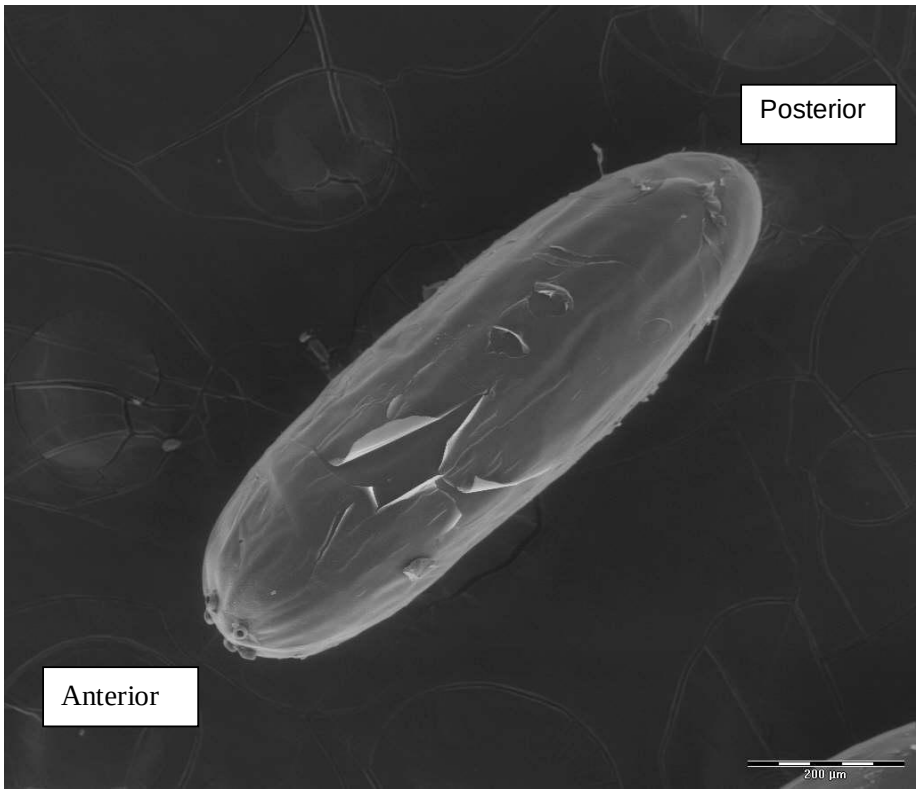

Figure 2. Egg of *Nysius natalensis*.

species cannot be attributed to this factor alone.*N. natalensis* eggs were also found in the axils of plants of *Pseudognaphalium oligandrum* (DC.) Hilliard and Burtt. and were recorded in soil under *P*. *oleracea* only. Adults and immature insects occurred both on the fruit heads of these plants, and underneath the plants on the soil surface. Eggs recovered from the soil may have fallen from the plants. However, oviposition into the soil could be possible, since this has been observed in *Nysius huttoni* White (Gurr, 1957) and *N*. *niger* Baker (Sweet, 2000).

Despite the abundance of many grass species among the herbaceous host plants, eggs were only found once in spikelets of a single species *viz*. *Melinis repens* (Willd.) Zizka subsp. *grandiflora* (Hochst.) Zizka. The wild host plants of *N. natalensis* are however, so numerous and widespread that their reproductive periods overlap extensively in space and time. *N. natalensis* populations are therefore not constrained by the availability of suitable host plants (Du Plessis et al., 2007).

Measurement and description of eggs

Eggs are longer than broad, slightly concave ventrally and convex dorsally as well as laterally and more pointed posteriorly than anteriorly (Figure 2). The mean length of eggs is 0.86 mm and the mean width 0.29 mm (n = 30). Four to six papilliform micropylar processes surround the anterior pole (Figure 3). Micropyles are explained by (Chapman, 1998) as funnel shaped pores passing through the chorion usually near the anterior pole of an insect egg that allows entry of sperm. The pores are 1 to 2 µm in diameter, often with a wider funnel at the surface of the chorion. The micropylar canal passes through the middle of the process and through the chorion and is surrounded by an open reticulum enclosing airspaces. The number of micropyles in Heteroptera varies from 0 to 70 (Chapman, 1998). Eggs of *N*. *natalensis* are of similar size and appearance and have the same the number of papilliform micropylar processes than that of *N*. *huttoni* (Eyles, 1960). The eggs are however, smaller than that of *N*. *groenlandicus* and the number of papilliform micropylar processes different from the six micropylar processes noted by Böcher (1975) for *N*. *groenlandicus*. The external chorionic surface is sculptured in the anterior third with longitudinal grooves, almost converging at the posterior pole, which is similar to that of *N*. *huttoni* (Eyles, 1960).

The colour of eggs is straw yellow soon after oviposition changing to deep orange before hatching. A pair of red spots indicating the eyes of the first instar nymph is visible prior to hatching at the posterior pole. Eggs are laid singly or in clusters and are attached to the plant as well as to each other with a sticky secretion, which deteriorates with age. Eggs of different colour

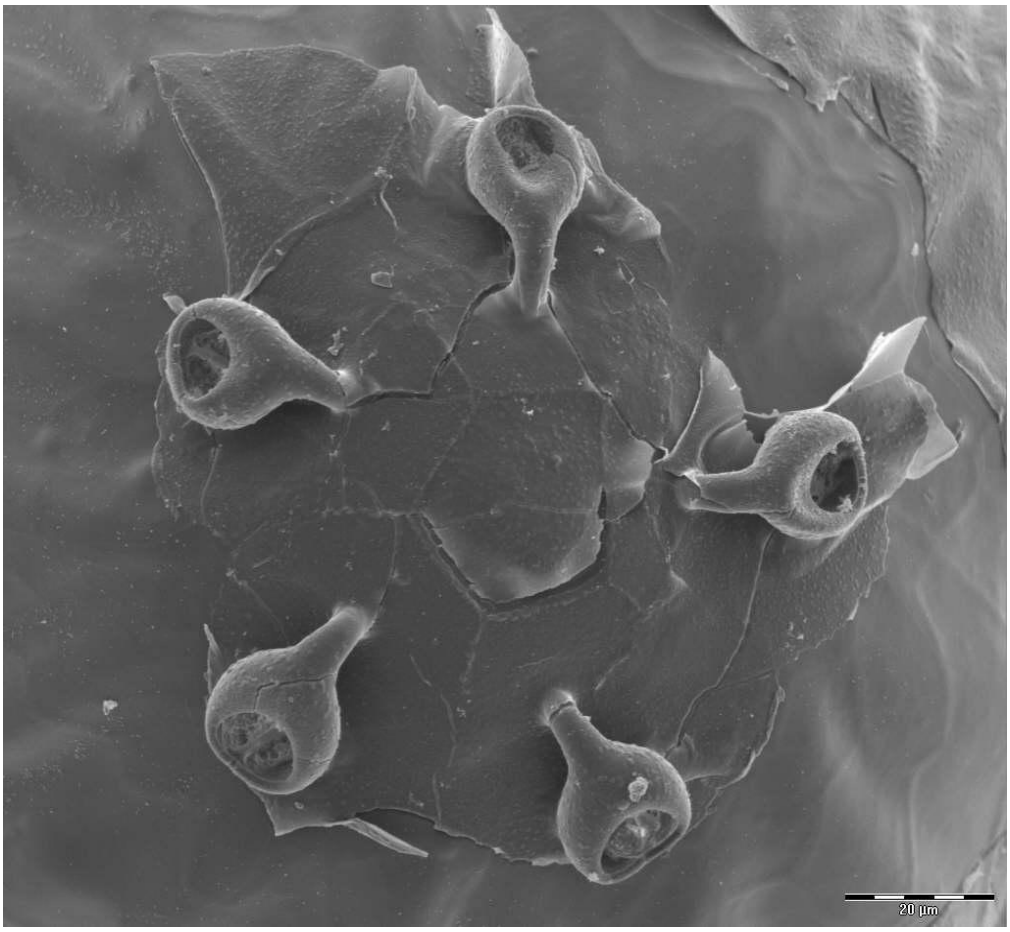

Figure 3. Papilliform micropylar processes surround the cephalic pole of a *Nysius natalensis* egg.

and therefore different ages were found attached to each other in clusters on host plants. It may indicate the presence of an oviposition pheromone that attracts gravid females. Eggs of the same age laid in groups will allow neonate nymphs, which tend to aggregate, to be together directly after hatching. Attachment of eggs in groups and to the substrate may prevent them from becoming dislodged from host plants and protection offered by the oviposition site. The sticky secretion that aids in sticking the eggs to one another and to the substratum when dried are also known for rhyparochromines (Hemiptera: Lygaeidae) (Malipatil, 1979).

Body measurements

The nymphal phase of *N*. *natalensis* consists of five instars. Mean head widths, mean pronotum widths, as well as mean body lengths of juvenile instars differed significantly from each other (P = 0.05) (Table 1). The pronounced differences in head width and pronotum width can therefore be used to distinguish between the five nymphal instars of *N*. *natalensis*. The nymphs of *Nysius* species in general are, however, remarkably similar in appearance, with a brown and white striped dorsal head and thorax, and a mottled reddish brown and white coloured abdomen (Sweet, 2000). Adult females were significantly larger than adult males (P = 0.05) (Table 1) and can easily be distinguished by the ovipositor cleft visible on the underside of the abdomen.

Conclusion

The current description of the life stages and oviposition combined with development rates of *N*. *natalensis* (Du Plessis et al., 2011) can facilitate monitoring of the pest and contribute to the improvement of management practices.

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Table 1. Head and body measurements of *Nysius natalensis* nymphal instars and adults (n = 30).

Instar	Mean head width (mm ± S.E.)	Mean pronotum width (mm ± S.E.)	Mean body length (mm ± S.E.)
1	0.29 ± 0.002[a]	0.31 ± 0.003[a]	0.73 ± 0.007[a]
2	0.38 ± 0.003[b]	0.44 ± 0.005[b]	1.17 ± 0.017[b]
3	0.55 ± 0.004[c]	0.65 ± 0.007[c]	1.66 ± 0.025[c]
4	0.67 ± 0.004[d]	0.79 ± 0.005[d]	2.10 ± 0.024[d]
5	0.88 ± 0.010[e]	1.05 ± 0.016[e]	2.93 ± 0.039[e]
Adult males	0.89 ± 0.008[e]	1.06 ± 0.008[e]	3.70 ± 0.023[f]
Adult females	1.004 ± 0.005[f]	1.27 ± 0.010[f]	4.18 ± 0.035[g]

Means within the same column followed by the same letter do not differ significantly at P = 0.05 (Tukey's HSD).

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