Preliminary studies on the morphology and biology of a *Neomargarodes* sp. (Hemiptera: Coccoidea: Margarodidae) from Iran

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Abstract: During the past two years, a species of *Neomargarodes* was collected on bulbous grasses such as *Hordeum bulbosum*, and its biology investigated both in the field and laboratory. These studies showed that much of the biology of this hypogeal margarodid is similar to that of *Porphyrophora* in most aspects of its life cycle. It has one generation a year. The eggs are laid underground at the end of June within a cottony ovisac close to the roots of the host plant. The crawlers emerge in mid-July and settle on the bulbous roots of the host grass. There are two cyst stages, more or less spherical and 2-4.5mm in diameter. The mature cysts occur at the end of May. Sexually mature females and 3rd-instar males emerge from the cysts. The 3rd-instar males somewhat resemble a small adult female as both have powerful fossorial prothoracic claws, bearing a prominent basal heel. The pupa and winged-adult males have distinctive red compound eyes. The adult males and females appear in mid-June to July. This species of *Neomargarodes* differs from *Porphyrophora* spp. in most aspects of its field characters, such as colour, glassy wax and shape of prothoracic claw.

Key words: Graminaeae, *Poa bulbosa.*

Introduction

Neomargarodes is a soil-inhabiting genus of 16 species, mainly restricted to the Palaearctic Region (with 6 species in the USSR) and North Africa (Ben-Dov, 2005; Foldi, 2005). This genus is currently considered to belong to the family Margarodidae (Sternorrhyncha, Coccoidea), subfamily Neomargarodinae, Tribe Neomargarodini (Jakubski, 1965; see Hodgson & Foldi, 2006 for a discussion of the present higher classification within the Margarodidae *sensu* Morrison, 1928). The adult females have well-developed fossorial legs, with a prominent heel on the claw; body with abundant long flagellate setae and loculate derm pores; abdominal spiracles, and an anus lacking a setigerous ring. Characteristically, the structure of the pores in each species is very variable and this is one of the features of the genus (Jakubski, 1965). In addition, abdominal spiracles are also present on the crawlers and on the later immature instars (Hadzibejli, 1966).

As with other margarodids, *Neomargarodes* species are sexually dimorphic; with apterous adult females and smaller alate males. These differences are so distinct that the male and females seem to belong to two different groups of insects.

The life cycle of *Neomargarodes* is similar to that of *Porphyrophora* (see Vahedi & Hodgson, 2007) except that *Neomargarodes* overwinters as a cyst on the roots of the host-plant, whereas *Porphyrophora* overwinters as a diapausing 1st-stage nymph in an ovisac underground. Most species of *Neomargarodes* have a single generation each year (Hadzibejli, 1966; Jakubski, 1965). In other closely related genera, such as *Margarodes*, the development in some species can last even longer - *Margarodes vitis* (Philippi), for instance, can take three years to complete its life cycle, including three cyst instars (Allsopp *et al.*, 2000; Camerino, 2000). With regard to *Eumargarodes laingi* Jakubski in sugarcane fields in Queensland, Hitchcock (1965) indicated that it generally has one life cycle per year but, probably under drought conditions, the nymphal stage may be prolonged to as many as five years.

Hypogeal margarodids are found in all biogeographical regions. In Mediterranean countries, they are represented by about 20 species belonging to four genera: *Dimargarodes*

Silvestri, *Promargarodes* Silvestri, *Neomargarodes* Green and *Porphyrophora* Brandt (Foldi, 2005). *Neomargarodes* species can be separated from those of the other genera by the presence of a prominent heel on the prothoracic claw in adult female and abdominal spiracles on all immature and adult stages.

The present paper describes the economic importance, biology, field morphology and cysts of a *Neomargarodes* species from Iran.

Material and methods

Second-instar nymphs were collected weekly in the field from May onwards by pulling up the host plants. Infested plants were then kept in Petri-dishes under laboratory conditions. Every second day, the plants were checked to determine the duration of each development stage and to note other life-cycle details. When 3rd-instar males or adult females appeared, they were placed into separate Petri-dishes. Whilst quite similar, the sexes can be separated at this stage because the adult females are larger and more setose; and are much more active than the 3rd-instar males. Host plants are unnecessary at this time as neither stage feeds.

Economic importance

The cyst stages of margarodoid species are the main feeding stages and are often known as grass pearls. They have been reported as pests on the roots of several economically important crops around the world, such as grape vines, sugarcane, oil palms and cotton, and on the roots of such perennial grasses as *Diplachne bulgarici, Festuca sulcata, Stipa* sp. and *Aristella bromoides*, and, less frequently, on Compositae and recreational grasses such as golf courses (Hitchcock, 1965; Hadzibejli, 1966; De Klerk *et al.*, 1980; Foldi, 2005). However, the *Neomargarodes* sp. from Iran studied here has not been found on cultivated graminaceous crops but occurs on such bulbous grass species as bulbous barley (*Hordeum bulbosum*) and bulbous meadow-grass (*Poa bulbosum*) on uncultivated hillsides and mountain pastures. The 2nd- and/or 3rd-instar (cyst) stages probably survive through to the next spring either by feeding on the stored nutrients in the nutritive bulb-like basal leaf-bases or by going into diapause if nutrients become scarce.

Life cycle (Fig. 1)

As with other hypogeal margarodoids (see Vahedi, 2002; Vahedi & Hodgson, 2007), all stages of this *Neomargarodes* sp. have each instar modified in various ways for life within the soil and are characterized by having two cyst-form. The life cycle of the males and females diverge after the dispersive first instar [3] has moulted into the cyst-like 2nd-instar [11, 12 & 6]. Generally, the female life cycle involves 3 nymphal instars (i.e. 1st-instar nymph [2, 3 & 10] + 2 cyst-like stages [11, 12 & 6], all of them feeding stages), whereas the male has four immature stages (1st-instar nymph, 2 cyst-like stages (all with stylets and therefore feeding stages), 3rd instar [4] without stylets (the non-feeding prepupal stage, which is mobile and resembles a small adult female), and finally the non-feeding pupal instar) [5]. In both sexes, the first-instar nymphs settle near the roots on the bulbous leaf-bases of the grass host [3] and then, after some initial feeding [10], moult into the cyst-like 2nd-instar [11]. The two cyst stages remain attached to the plant through the winter in both sexes [11 & 12]; the male cyst is smaller than female. In addition, the female cyst has a small pore anterior to the anal aperture, possibly an inactive genital opening (see Fig. 2. [8]). As

in *Porphyrophora* species, the mouthparts are lost at the last moult to the adult female and no further feeding takes place.

In the male life cycle, the 2nd-instar cyst [6] moults into a mobile, non-feeding 3rd-nymphal (prepupal) stage [4], which resembles a small adult female without a vulva. This instar is followed by a non-feeding pupal stage and finally the winged, non-feeding adult male [5 & 7]. Adult males also have modified, fossorial front legs, but without a prominent heel on the claw. There appears to be only one generation a year. In the laboratory, peak oviposition occurred during late June, the eggs hatching in mid July [1], with the incubation period lasting about two week. The eggs are whitish, elongate elliptical, about 600 µm long and 250 µm wide, and each female lays about 80 within a single white ovisac [9]. The 1st-instar nymphs disperse shortly after emergence and search for a suitable host [3], usually settling on the underground swollen leaf bases beside the roots [10]. The life span of virgin males and females was longer than when mated.

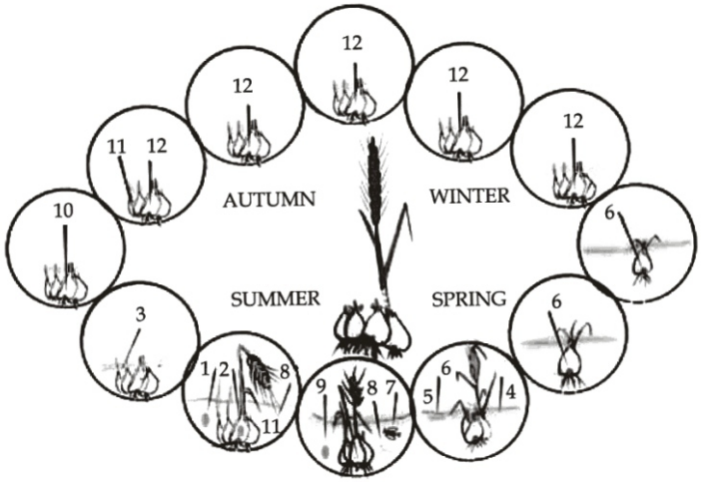

Figure 1. The annual life cycle of *Neomargarodes* sp. in Kermanshah, Iran: 1. Eggs inside ovisac; 2. First-instar nymph inside ovisac; 3. First-instar nymph (infestation); 4. Third male nymph; 5. Pupa; 6. Developed cyst; 7. Adult male; 8. Adult female; 9. female with ovisac; 10. Matured first-instar nymph; 11. First-cyst stage, and 12. Second-cyst stage.

First-instar nymph:

Live appearance: body white, slim and elongate, with two long caudal setae, each about 2 times body length, and with very red eyes and rather stout forelegs. Having settled and commenced feeding, the nymph becomes stationary, the body slowly swells and a waxy coat is secreted. The legs and antennae do not grow. This stage lasts about a month.

Mounted specimens: body membranous, elongate, up 1 mm long and 0.5 mm wide; body segmentation clearly visible ventrally; forelegs short and strong, with stout cylindrical femora and a claw modified for digging. Thoracic and 7 (rarely 6) abdominal spiracles present. Long caudal setae each about 2 mm long. Stylets very long, about 2.5 times body length.

Cyst stages (2nd- and 3rd-nymphal stages):
2nd-instar: unmounted: both cyst stages have: (i) six to seven pairs of abdominal spiracles; (ii) a similar number and pattern of abdominal pores; (iii) derm without setae; (iv) long stylets (about 2-5 times width of cyst), (v) no legs, and (vi) rudimentary antennae. The body of the 2nd-instar (1st-cyst stage) is oval, 2.5 mm long and 2 mm wide. It differs from the 2nd cyst stage in having (characters of 2nd-cyst stage (3rd instar) in brackets): (i) body pale in colour (dark); (ii) derm membranous, soft and flexible (chitinous and hard); (iii) body segmentation clearly visible (not obvious); (iv) thoracic and abdominal spiracles obvious (not obvious) and (v) pattern of abdominal pores clear (not obvious).

The 1st-cyst stage is the same colour as the 3rd-instar male (pale) but differs from it in having (characters on 3rd-instar male (prepupa) in brackets): (i) body without flagellate setae (present); (ii) antennae reduced (present, as a normal antennae); (iii) legs absent (present); (iv) abdominal pores present (absent), and (v) stylets present (absent).

The 1st-cyst stage secretes white filamentous secretions from both thoracic and abdominal spiracle orifices.

3rd-instar (2nd-cyst stage): live appearance (Fig. 2): as indicated above, these are somewhat similar to the previous instar but are often more spherical and 2-4.5 mm in diameter. All have a conical mouth opening [6] and long stylets [7]. Both male and female cysts are dark brown, shiny and metallic, covered by one thick, rough and granulated waxy layer. When this is removed, the underlying cyst wall has a pearly sheen, from which the common name "ground pearls" is derived. Rudimentary antennae are present dorsally. Male cysts are usually smaller than the female cysts and have no trace of possible genital opening.

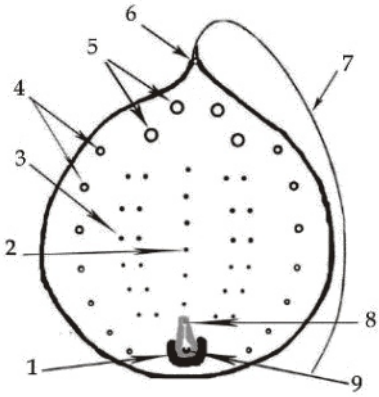

Figure 2. Ventral view of unmounted 2nd-instar (mature cyst stage) of *Neomargarodes* sp. 1. Sclerotised band around anal opening; 2. Median row of pores; 3. Two submedial rows of pores; 4. Abdominal spiracles; 5. Thoracic spiracles; 6. Conical mouthparts; 7. Stylets; 8. Possible undeveloped genital opening, and 9. Anal aperture.

Body without segmentation, but posterior five abdominal segments usually indicated by presence of small, orange, cylindrical micropores, each about 1 µm diameter, in a row of 5 pores across width of each segment, with two pairs submedially [3] and 1 medially[2]. Each cyst with two larger thoracic and seven (rarely 6) indistinct smaller abdominal spiracles [4 & 5] (Hadzibejli (1966) indicated that the cysts of *Neomargarodes festucae* (Hadzibejli) Archangelskaya had 8 abdominal spiracles and those of *N. setosus* Borchsenius

had 7 for female and 6 for male). Each thoracic spiracle with a small peritreme with 3 obscure perispiracular sensilla on lateral margin of peritreme [not shown in the figure]. Abdominal spiracles becoming smaller posteriorly. The anal aperture [9] is surrounded by a U-shaped sclerotisation [1].

Rarely, an intermediate-sized cyst occur which differs from the other cyst stages in having a unique waxy "moustache" close to mouth, composed of a group of 8-12 silky white filaments.

Male: live appearance

Light-brown, with large, bright red compound eyes; fore-wings transparent, each with three rudimentary veins; with 2 lots of long, fine white tail-filaments, arising dorsally from abdominal segments VI and VII, each filament about twice body length. Antennae long and filiform, 7-segmented, each segment with short fine setae; fore-legs large and stout; claw without distinct heel. Penial sheath rather square, with a long eversible endophallus.

Results

The basic structure of the adult male of this species appears very similar to that of *N. erythrocephalus* Green described by Hodgson & Foldi (2006) apart from one important character – the structure of the antennae. On *N. erythrocephalus*, segments IV, V and VI are branched, whereas no segments are branched on the present species, even though it has only 7 segmented antennae, as on *N. erythrocephalus*.

Adult female: live appearance (Fig. 3)

Adult female rotund, variable in size, 2.5-8 mm long and 1.75-6 mm wide; body white, membranous and setose, setae flagellate, golden-yellow, arranged in bands around each segment. Forelegs large and fossorial; prothoracic claw with a prominent heel [2]; no obvious eye. Antennae thick and conical, with 6 segments [1]. Mesothoracic legs similar in structure to metathoracic legs, both with a long, pointed claw [3].

Test

As with *Porphyrophora* (Vahedi, 2002; Vahedi & Hodgson, 2007), adult female *Neomargarodes* descend into the soil to a maximum depth of about 2-4 cm shortly after mating, where she settles and eventually oviposits. No host plant is needed at this stage. A cottony, flocculent wax is secreted by the multi- and monolocular pores present throughout the derm of the adult female. These wax filaments loosely cover the whole body and, when not compacted by soil (such as when in a Petri-dish), the length of each wax filament is several times that of the body, and can be up to about 2cm long (longer than was noted for *Porphyrophora* (Vahedi, 2004)). However, beneath the soil, the wax filaments become compacted, forming a dense cover that surrounds the entire body and eggs. Whilst 3rd-instar males do secrete some wax, this is mainly from the posterior end of the abdomen. As the eggs and 1st-instar nymphs are believed to be highly sensitive to unfavourable conditions, the main function of the margarodid ovisac is probably mainly to protect the eggs and 1st instar from evaporation during the dry season (June and July in Kermanshah) and from other adverse environmental changes and predators and parasitoids.

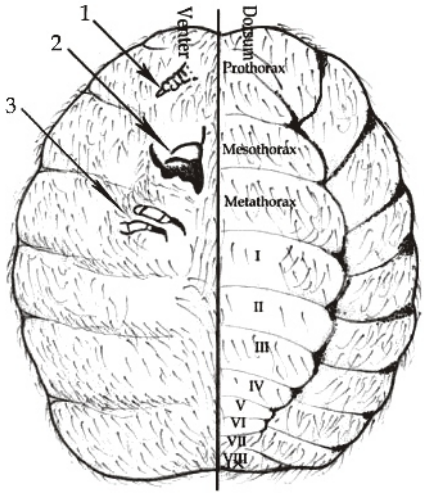

Figure 3. Dorsal (left) and ventral (right) view of unmounted adult female *Neomargarodes* sp.: 1. Conical antennae; 2. Fossorial prothoracic legs; and 3. Meso- and meta thoracic legs.

Emergence from cyst

In all observed cases, a small emergence hole was made in the cyst wall by the foreclaws through which the head and forelegs of the next stage (pupa or adult female) slowly emerged, followed by the rest of the body; thus oviposition took place outside the cyst in the waxy ovisac. Hitchcock (1965) observed two oviposition methods with *Eumargarodes laingi* in Queensland sugarcane: (i) usually, the adult females made an emergence hole using the front claws, but then remained within the cyst, with only their heads and forelegs protruding; the eggs were then laid inside the cyst, with the young nymphs exiting through the hole in the cyst wall, or (ii) more rarely, the adult females did not make an emergence hole, the young nymph then escaping through cracks in the cyst wall.

Discussion

The identity of the present species is unknown. Hadzibejli (1966) studied two species (*N. setosus* Borchsenius and *N. borchsenii* Hadzibejli (now synomysed with *N. festucae* Archangelskaya)) on the roots of perennial grasses and Compositae in the steppe zones in eastern Georgia. Six other species of *Neomargarodes* have been recorded from the Middle East: *N. aristidae* Borchsenius, *N. chondrillae* Archangelskaya, *N. polygonis* Jashenko, *N. ramosus* Jashenko, *N. rutae* Borchsenius and *N. triodonotus* Jashenko, but none of these species have been recorded off *Hordeum* or *Poa* species (Ben-Dov, 2005).

As a result of the extremely slow rate of dispersal of *Margarodes* species, long distance movement is attributed to anthropogenic causes. Examples of possible human induced ground pearl introduction into uninfected sites include movement of infested organic material on tools, farming implements and tractors, or sale and/or introduction of infested plants. In addition, it is likely that the 1st-instar nymphs can be carried passively on the surface of water or on air currents to new locations.

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