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# Evidences of induced maternal molt inhibition by *Gonatopus chilensis* (Insecta: Hymenoptera: Dryinidae) in *Peregrinus maidis* (Insecta: Hemiptera: Delphacidae) nymphs

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Evidencia de inhibición inducida por la muda de las hembras de *Gonatopus chilensis* (Insecta: Hymenoptera: Dryinidae) en ninfas de *Peregrinus maidis* (Insecta: Hemiptera: Delphacidae). En los Dryinidae (Hymenoptera) la oviposición es acompañada por la emisión de una sustancia paralizante. Los driínidos son parasitoides koinobiontes, por lo cual el hospedador permanece vivo pero en general es incapaz de mudar. El mecanismo por el cual se detiene o bloquea el desarrollo del hospedador es desconocido; se estima que podría deberse a sustancias inyectadas por las hembras durante la oviposición y/o a sustancias producidas por la misma larva del driínido en desarrollo. Se estudió el efecto del parasitoidismo de *Gonatopus chilensis* (Olmi) (Hymenoptera: Dryinidae) sobre los últimos tres estadios ninfales de la chicharrita del maíz *Peregrinus maidis* (Ashmead) (Hemiptera: Delphacidae) a fin de detectar el posible origen de la fuente de inhibición del desarrollo de los juveniles y los períodos que son críticos para que ocurra el fenómeno de inhibición de la muda. La evidencia obtenida permite asegurar que la hembra, en el momento de oviposición, es al menos una de las fuentes de origen del factor de regulación del hospedador.

**Palabras claves: Dryinidae, inhibición de la muda, chicharrita.**

Dryinidae (Hymenoptera) oviposition is accompanied by the emission of a paralyzing substance. They are koinobionts, so the host remains alive but it usually does not molt. The mechanism by which the development of the host is slowed and/or blocked is ignored; it could be due to substances injected by the female during the oviposition and/or to substances produced by the developing dryinid larvae. The effect of parasitism by *Gonatopus chilensis* (Olmi) (Hymenoptera: Dryinidae) was tested in the last three nymphal instars of the corn planthopper *Peregrinus maidis* (Ashmead) (Hemiptera: Delphacidae) to detect the possible source of arresting development and the periods that are critical to molt inhibition phenomena. The obtained evidence permits to ensure that the ovipositing female is at least one of the sources of the host regulatory factor.

**Key words: Dryinidae, molt inhibition, planthopper.**

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## Introduction

Parasitoids, through coevolution with their hosts, have developed numerous mechanisms to maximize their chances of successful development and posterior reproduction. Some parasitic Hymenoptera (i.e.: Pimplinae, Braconidae, Pteromalidae, Eulophidae, Bethylidae, Dryinidae, Sphecidae) are known to inject glandular secretions (venoms) into their hosts during oviposition (Strand, 1986; Coudron *et al.*, 1990; Parkinson and Weaver, 1999). The interactions that have been observed between host and parasitoids include: temporary or permanent paralysis, alterations of the instar length, induction of supernumerary instars, induction of precocious development, highly synchronized development of host and parasite, and/or disturbance of host reproduction (Strand, 1986; Kelly and Coudron, 1990).

Almost all the hymenopteran ectoparasites, except for some species of *Euplectrus* (Eulophidae) and Dryinidae, permanently paralyze their host (i.e.: Braconidae, Ichneumonidae, Eulophidae, and Bethylidae). In *Euplectrus* species a temporal paralysis occurs when the female injects venom into the host either before or at the time of oviposition. This paralysis arrests the host development preventing shedding of the cuticle, and thus helping the attachment of parasite eggs to the host. They inhibit host molting once stinging (= puncture with the ovipositor), oviposition (= egg deposition), or parasitism (= stinging and oviposition) (Coudron and Puttler, 1988).

The Dryinidae (Hymenoptera: Chrysidoidea) is a widespread family composed of small wasps, with approximately 1400 species. All of them are parasitoids and usually also predators of Hemiptera Auchenorrhyncha. Olmi (1984, 1994, 1999) has revised the biology of this family. Although the Dryinidae are classified in the Aculeata, the ovipositor is used for egg laying, and the oviposition is accompanied by the emission of a paralyzing substance or venom. The paralysis does not last long, and as soon as the host recovers, it lives normally until the dryinid larva finishes consuming its haemolymph. Dryinids are koinobionts: host remains alive and feeds on the plant after being parasitized, but it does not molt (except for Aphelopinae and a few Gonatopodinae). The larval development occurs usually totally at the oviposition site; they can be endoparasitoids in the early instars and ectoparasitoids in the last instars, or completely ectoparasitoids, or rarely totally endoparasitoids (*Crovettia*) (Olmi, 1994, 1999).

In Dryinidae, the mechanism by which the development of the host is slowed and/or blocked is still ignored. It could be due either to substances injected by the female during the oviposition (paralyzing it for some minutes), to substances produced by the developing dryinid larvae, or alternatively, a synergic action of both.

Most studies pointed out that after oviposition, nymphs are not able to molt and cannot reach the adult stage (Lindberg, 1950; Pillault, 1951; Subba-Rao, 1957; Ponomarenko, 1975; Chandra, 1980; Virla, 1995a, b; Olmi, 1999). On the other hand, Kitamura (1988) reported that when *Sogatella furcifera* Horvath (Delphacidae) nymphs are attacked by *Haplogonatopus apicalis* R.C.L. Perkins (Gonatopodinae), ecdysis is possible after the parasitoid larval sac emergence. Giri and Freytag (1988) proposed that there might be a critical age for the host to be susceptible to arrest the development, after parasitization.

Pillault (1951) suggested that the metamorphosis of *Dryinus tarraconensis* Marshall (Dryininae) hosts is stopped by a larval factor. He mentioned that the host could not develop if the parasitoid larva was alive, but it was able to recommence the development, molting,

when the larva died. Raatikainen (1967) noted that nymphs of *Javesella pellucida* (F.) (Delphacidae) are able to molt after being parasitized by *Gonatopus bicolor* (Haliday) (Gonatopodinae); but soon after the parasitoid eggs hatched, ecdysis is not longer possible. Lindberg (1950) observed that nymphs attacked by *G. bicolor* are able to molt soon after the oviposition and before the larval sac formation; Ponomarenko (1975) and Virla (1995a, b) made similar findings.

Virla and Mangione (2000), by studying the larval morphology of *Gonatopus chilensis* (Olmi) (Dryinidae: Gonatopodinae), hypothesized that the cephalic lobes present in the larvae could be implicated in the development arrest of the host. The relationship between the presence of cephalic lobes and molt inhibition can be excluded taking into account that nymphs attacked by diverse species of *Anteon* (Anteoninae) are not able to molt. Anteoninae larvae have two conic processes in the head, very different to the Gonatopodinae cephalic lobes (Olmi, 1999; Olmi, pers. comm.).

The aim of the present study was to determine if in the parasitoid *G. chilensis* there is either a mother, a larval factor or both, implicated in the molt inhibition of the corn planthopper *Peregrinus maidis* (Ashmead) (Hemiptera: Delphacidae) nymphs.

## Material and methods

**Insect colonies.** Both *P. maidis* and *G. chilensis* were collected in maize crops (*Zea mays* L.) near Leales (Tucumán province - Argentina), 27° 11' S - 65° 15' W, from December 2000 to January 2001.

Individuals were kept under controlled conditions (25 ± 2 °C; 70-75 % RH; and 14L:10D) in a rearing chamber. *G. chilensis* stocks were kept following the methodology described by Virla (1995b). *P. maidis* breading was made using PET (Polyethylen-Terephtalathe) cylindrical cages (35 cm high x 18 cm diam). For aeration, the top was closed by nylon mesh cloth, and one hole was made on a lateral side. The hole was fitted with a cotton plug. Each cage was placed erect on pots containing four plants of maize as host plant and six adult couples. Adult planthoppers were transferred to a new cage twice a week. Newly emerged nymphs were placed into 5-ml glass vessels with pieces of fresh corn leaves.

In order to perform the experiments, special attention was paid to the colonies to ensure the provision of synchronized nymphal stages of *P. maidis* to be exposed to *G. chilensis*. Data concerning the molting dates were registered.

**Experimental design.** To evaluate the possible source of the factor(s) responsible for the arresting molt, an experiment was set to identify either mother or larval products affecting the host development. The age of the host was also taken into account.

Four situations (treatments) were considered: (1) newly molted stung hosts, without allowed oviposition; (2) 72 h or more molted stung hosts, without allowed oviposition; (3) stung hosts, oviposition allowed, and subsequently eggs punctured; and (4) stung hosts, oviposition allowed, and developing larval sacs killed. Non parasitized hosts were also reared as the experiment control.

For each treatment, female parasitoids (from two to nine days old) were placed into 5-ml plastic vials, and hosts were provided individually in III, IV, or V instars. Wasps were fed with a honey/water solution (1:1 vol/vol).

Dryinids usually catch the host within few minutes after they have

been placed in the vessel. The wasp holds the host for few seconds and inserts the ovipositor in the ventral side of the host thorax paralyzing it (for an accurate description of the Gonatopodinae oviposition behavior see Yamada and Kawamura, 1999).

Thus, to perform treatments "1" and "2", oviposition was interrupted just before, or during the beginning of the egg laying movements. In treatment "3", the parasitoid egg was destroyed within 24 h after oviposition. Treatment "4" was accomplished by killing the parasitoid larval sac (second or third larval stage). The dissections were made under a stereoscope with fine forceps and 000 entomological pins, carefully to prevent wounding the nymphs.

After parasitoid exposure, each host was isolated in glass vials and fed upon with a piece of fresh corn leaf. Vials were daily checked to replace leaves and to register host development by counting exuviae, instar stages, and mortality.

All experiments were carried out in a controlled environment chamber maintained at 25 ± 2 ºC, 70 % RH, and 14L:10 D photoperiod.

For each treatment, dates of oviposition/stinging, host molting, and hopper adult emergence were recorded. *P. maidis* longevity was estimated as the number of days from adult emergence to dead. Supervivence was calculated as the number of days from nymph-dryinid female contact to death.

To detect effects of arrestment factors of *G. chilensis* on *P. maidis*, host survival, percentage of host molt arrestment and the longevity of the hosts in each treatment were recorded.

## Results

**Healthy, unparasitized individuals (control).** In Table 1, the number of individuals that are able to reach the next instar, the age specific mortality and the capacity to reach the adult stage were resumed. The instar duration (in days) is also given.

### "Only stung" individuals

Newly molted individuals. Those stung individuals and with an age of less than 72 h after the last ecdysis were severely affected. The normal process of molting did not take place or this capacity decreased substantially. Some individuals initiated apolysis (molting fluid appeared between the epidermis and the cuticle) but died in the attempt; no cases of exuvium shedding were observed (Table 2). The number of days that the nymphs lived after the exposure to the parasitoid exceeded twice or more the normal instar duration (Table 3).

Nymphs with 72 h or more into the instar. The response of this group of individuals was different. A great proportion, principally between those of fifth instar, was able to molt (Table 4).

In those individuals that are able to molt successfully, times to molt

**Table 1**

| | Instars | | |
|---|---|---|---|
| | IV (n: 20) | III (n: 20) | V (n: 20) |
| Mortality of the instar (in %) | 10 | 15 | 5 |
| % that molts to the next instar | 90 | 85 | 95 |
| % that reaches the adult stage | 80 | 75 | 95 |
| Duration in days (mean ± sd) | 3.47 ±1.12 | 3.18 ± 0,95 | 4.29 ± 0.88 |

**Table 1.** *Peregrinus maidis* age specific mortality and duration of the different instars recorded in the control individuals.

**Table 2**

| | Without molt | | Capable to molt | |
|---|---|---|---|---|
| Instars | Total | % | total | % |
| N III (n: 24) | 20 | 83.33 | 4 | 16.67 |
| N IV (n: 21) | 18 (*) | 85.71 | 3 | 14.29 |
| N V (n: 12) | 7 | 58.33 | 5 | 41.67 |
| Total (n: 57) | 45 | 78.95 | 12 | 21.05 |

**Table 2.** Molt arrestment behavior of the exposed individuals of *Peregrinus maidis* (age less than 72 h after the last ecdysis) only stung by females of *Gonatopus chilensis*.
(*) Two individuals died during the apolysis process.

**Table 3**

| | | Capable to molt | |
|---|---|---|---|
| Instars | Without molt | to first molt | to dead |
| N III (n: 24) | n: 20; x: 8.4 ± 4.07 | n: 4; x: 14 ± 2.94 | n: 4; x: 22.25 ± 2.99 |
| N IV (n: 21) | n: 18; x: 14.39 ± 7.12 | n: 3; x: 12.33 ± 1.53 | n: 3; x: 27 ± 3.60 |
| N V (n: 12) | n: 7; x: 15.28 ± 5.34 | n: 5; x: 6 ± 0.71 | n: 5; x: 10 ± 2.55 |
| Total (n: 57) | n: 45; x: 11.86 ± 6.37 | n: 12; x: 10.25 ± 4.18 | n: 12; x: 18.33 ± 8.05 |

**Table 3.** Supervivence of the exposed individuals of *Peregrinus maidis* (age less than 72 h after the last ecdysis) only stung by females of *Gonatopus chilensis*. (x: mean days ± sd)
(*) Five individuals reach the adult stage.

**Table 4**

| | Without molt | | Capable to molt | |
|---|---|---|---|---|
| Instars | Total | % | total | % |
| N III (n: 6) | 2 (*) | 33.33 | 4 | 66.67 |
| N IV (n: 7) | 4 (**) | 57.14 | 3 | 42.86 |
| N V (n: 20) | 4 (***) | 20.00 | 16 | 80.00 |
| Total (n: 33) | 10 | 30.30 | 23 | 69.70 |

**Table 4.** Molt arrestment behavior of the exposed individuals of *Peregrinus maidis* (age 72 h or more after last ecdysis) only stung by females of *Gonatopus chilensis*.
(*) (**) (***) One, two, and three individuals died during the apolysis process respectively.

**Table 5**

| | | Capable to molt | |
|---|---|---|---|
| instars | Without molt | to first molt | to dead |
| N III (n: 6) | n: 2; x: 4.5 +/- 0.71 | n: 4; x: 2 +/- 0.82 | nn: 4; x: 18.25 +/- 2.5 |
| N IV (n: 7) (*) | n: 4; x: 6.25 +/- 7.18 | n: 3; x: 1.66 +/- 0.58 | n: 3; x: 13 +/- 1 |
| N V (n: 20) (**) | n: 4; x: 3 +/- 0.82 | n: 16; x: 1.87 +/- 0.81 | n: 16; x: 5.44 +/- 1.82 |
| Total (n: 33) | n: 10; x: 4.6 +/- 4.45 | n: 23; x: 1.87 +/- 0.76 | n: 23; x: 8.65 +/- 5.48 |

**Table 5.** Supervivence of the exposed individuals of *Peregrinus maidis* (age 72 h or more after last ecdysis) only stung by females of *Gonatopus chilensis*. (x: mean days ± sd).
(*) Three individuals reached the adult stage.
(**) 16 individuals reached the adult stage.

**Table 6**

| | Healthy individuals (*) | from newly molted nymphs | | | from "next to molt" nymphs | | |
|---|---|---|---|---|---|---|---|
| | female | total | female | fmale | total | ffemale | male |
| n | 21 | 5 | 3 | 2 | 19 | 13 | 6 |
| range | ¿? | 2 - 7 | 4 - 7 | 2 | 2 - 7 | 2 - 7 | 2 - 5 |
| mean | 36.16 | 4 | 5.33 | 2 | 3.37 | 3.38 | 3.33 |
| sd | 15.34 | 2.12 | 1.53 | - | 1.42 | 1.50 | 1.37 |

**Table 6.** Longevity of *Peregrinus maidis* individuals that were able to reach the adult stage after being stinged and paralyzed by *Gonatopus chilensis* female.
(*) Data from Fenández-Badillo and Clavijo (1990).

were approximately the expected for healthy individuals. In nymphs that could not molt, the supervivence was more extended but not as it was recorded in the "newly molted" exposed nymphs (Table 5).

The individuals of *P. maidis* that reached the adult stage had shorter longevity than the given by other authors for healthy individuals (Fernández-Badillo and Clavijo, 1990) (Table 6).

**Parasitized nymphs in which the eggs or larval sacs were punctured/killed.** All parasitized nymphs in which the eggs or larval sac were punctured/killed, died into a period no longer than a week (Table 7), and no one was able to molt. Ten of the nymphs where the

**Table 7**

| *P. maidis* instar | Puncturing the eggs | Killing the dryinid larval sac |
|---|---|---|
| III | n: 24; x: 2.50 ± 0.80 | n: 14; x: 4.43 ± 2.44 |
| IV | n: 26; x: 2.69 ± 0.85 | n: 14; x: 4.86 ± 2.73 |
| V | n: 36; x: 4.05 ± 2.15 | n: 18; x: 4.55 ± 2.51 |

**Table 7.** Supervivence (in days) of the parasitized individuals of *Peregrinus maidis* in which the eggs or larval sacs were punctured/killed. (x: mean days ± sd)

dryinid egg was punctured survived 7 days (all of them in the V instar). In those individuals where the larval sacs were killed, a total of eight individuals lived more than 7 days and a maximum of 9 days (four V, two IV and two III nymphal instar individuals).

The abdomen of most of them, after two days, presented a noticeable change in the internal coloration turning to dark brown. In some individuals the presence of fungus in the remaining dryinid larval sac was observed.

## Discussion and conclusions

The immune system of the host represents a barrier to parasitoids development so, in order to defend the developing progeny, some female are known to inject factors into their host which are able to neutralize host immune responses. Factors identified in secretions from adult female parasitoids, and which have been shown to mediate suppression of the host immune system, include polydnaviruses, virus-like particles, and ovarian proteins (Parkinson and Weaver, 1999). Thus, it could be the reason because no parasitized nymphs, in which the eggs or larval sac were punctured/killed, had made an attempt to begin the molting process and died in few days, mostly affected by fungal development.

The observations made on the exposed and "only stung" nymphs give evidence that in *G. chilensis,* and possibly in all the Gonatopodinae, the molting arrestment is a consequence of substances injected by the female during the paralysis of the host and/or in the first step of the oviposition behavior. So, it could be discarded that arresting development is due only to the action of the developing dryinid larva.

It was observed that some nymphs that attempted to molt after female stung died during the apolysis. In some cases, an attempt to shed the exuvium was made. However, during each instar there exists a period which is critical to molt arrest. Molt arrest occurred in a high proportion (next to 79 %) of the nymphs that were stung within 70-h after ecdysis. On the other hand, most of the exposed nymphs aged 72-h or more into the instar, initiated apolysis and were able to reach the next instar/stage. In Eulophidae the arrestment factor is transferred to the host at the time of stinging, which inhibits host molting apparently by disrupting hormone titres and events normally under endocrine control (Coudron *et al.*, 1990).

It is advantageous for the dryinid to disrupt the molting process of the nymph since ecdysis in the host would result in the shedding of the exuvium to which the parasitoid eggs are attached. This would cause either separation of the immature parasitoid and its food source, or the host may die as a result of entanglement with the exuvium before the dryinid larva develops.

The results of this study offer a new and interesting research line on the regulation of nymph's development and host-parasitoid (hopper-dryinid) interactions, taking in mind that any knowledge on this particular parasitoid-host interaction would be relevant to be directly applied in control of insects, including the use of these regulatory factors as insecticide substances.

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## Literature cited

Chandra, G., 1980. Dryinid parasitoids of rice leafhoppers and planthoppers in the Philippines. I. Taxonomy and bionomics. *Acta oecologica,* 1: 161-172.

Coudron, A. C. & B. Puttler, 1988. Response of natural and factitious hosts to the ectoparasite *Euplectrus plathypenae* (Hym.: Eulophidae). *Ann. Entomol. Soc. Amer.,* 81: 931-937.

Coudron, A. C., T. J. Kelly & B. Puttler, 1990. Developmental responses of *Trichoplusia ni* (Lep.: Noctuidae) to parasitism by the ectoparasite *Euplectrus plathypenae* (Hym.: Eulophidae). *Arch. Insect Biochem. Physiol.,* 13: 83-94.

Fernández-Badillo, A. & S. Clavijo, 1990. Biología de la chicharrita del maíz, *Peregrinus maidis* (Homoptera: Delphacidae), en Venezuela. *Rev. Fac. Agronomía* (Maracay), 16: 35-46.

Giri, M. & P. Freytag, 1988. Biology of *Dicondylus americanus* (Perkins) (Hymenoptera - Dryinidae). *Boll. Lab. Entomol. Agr. "F. Silvestri"* (Portici), 45: 49-58.

Kelly, T. J. & A. C. Coudron, 1990. Total and specific ecdysteroids in the haemolymph of *Trichoplusia ni* (Lep.: Noctuidae) and its parasite, *Euplectrus plathypenae* (Hym.: Eulophidae). *J. Insect Physiol.,* 36: 463-470.

Kitamura, K., 1988. Comparative studies on the biology of dryinid wasps in Japan. 5. Development and reproductive capacity of host attacked by *Haplogonatopus apicalis* (Hymenoptera-Dryinidae) and the development of progenies in the parasites in their host. *Kontyu,* 56 (3): 659-666.

Lindberg, H., 1950. Notes on the biology of dryinids. *Soc. Scient. Fennica Comm. Biol.,* 10: 1-19.

Olmi, M., 1984. A revision of the Dryinidae (Hymenoptera). *Mem. Amer. Entomol. Inst.,* 37 (1-2): 1-1913.

Olmi, M., 1994. The Dryinidae and Embolemidae (Hymenoptera - Chrysidoidea) of Fennoscandia and Denmark. *Fauna Entomol. Scandinavica,* 30: 7-98.

Olmi, M., 1999. *Fauna d'Italia, Hymenoptera. Dryinidae - Embolemidae.* Edizione Calderini, Bologna, Italia, 425 pp.

Parkinson, N. & R. Weaver, 1999. Noxious components of venom from the pupa-specific parasitoid *Pimpla hypochondriaca. J. Invert. Pathol.,* 73: 74-83.

Pillault, R., 1951. Notes sur *Dryinus tarraconensis* (Hym., Dryinidae) préateur d'une cicadelle. *Ann. Soc. Entomol. Fr.,* 120: 67-76.

Ponomarenko, N. G., 1975. The special features of larval development in Dryinidae (Hym.). *Entomol. Obozr.,* 54 (3): 534-540.

Raatikainen, M., 1967. Bionomics, enemies and population dynamics of *Javesella pellucida* (F.) (Homoptera: Delphacidae). *Ann. agric. Fennicae,* 6 (2): 1-149.

Strand, M. R., 1986. The physiological interactions of parasitoids with their hosts and their influence on reproductive strategies, pp. 97-136. In: Waage, J. & D. Greathead (eds.), *Insect Parasitoids.* Academic Press, London.

Subba-Rao, B. R., 1957. The biology and bionomics of *Lestodryinus pyrillae* Kieff. (Hym., Dryinidae) a nymphal parasite of *Pyrilla perpusilla* Walk. and a note on its role in the control of *Pyrilla. J. Bombay Nat. Hist. Soc.,* 54: 741-749.

Virla, E., 1995a. Aspects of the biology of *Gonatopus desantisi* (Hymenoptera - Dryinidae). *Frustula Entomol.,* 17 (30): 29-43.

Virla, E., 1995b. Biología de *Pseudogonatopus chilensis* Olmi 1989 (Hymenoptera - Dryinidae). *Acta Entomol. Chilena,* 19: 123-127.

Virla, E. & S. Mangione, 2000. Morfología de los estados preimaginales de *Gonatopus chilensis* y consideraciones sobre las estructuras relacionadas a la nutrición de sus larvas inmaduras (Insecta: Hymenoptera: Dryinidae). *Neotrópica,* 46: 37-49.

Yamada, Y. & M. Kawamura, 1999. Sex identification of eggs of a dryinid parasitoid, *Haplogonatopus atratus*, based on oviposition behaviour. *Entomol. Exp. et applicata,* 93: 321-324.