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Influence of the moisture content of the substrate on the emerging ability of adults of *Muscidifurax zaraptor* Kogan & Legner, *Nasonia vitripennis* (Walker) (Hymenoptera Pteromalidae) and *Musca domestica* L. (Diptera Muscidae)

Abstract - The ability to move up to the surface of *Muscidifurax zaraptor* Kogan & Legner, *Nasonia vitripennis* (Walker) and *Musca domestica* L. from puparia placed at different depths in substrates with different moisture content was verified. Tests were carried out at 27±1°C and 70±5% R.H.; as substrate was used bran with three different initial moisture contents (15, 60 and 75%). The number of adults of *M. zaraptor* and *N. vitripennis* able to move up to the surface is more influenced than *M. domestica* by depth at which puparia are placed. In the substrate with 15% m.c. *M. zaraptor* and *N. vitripennis* are able to emerge from puparia placed at 3 cm depth; the ability of the first species is limited to 2 cm with 60% m.c., while for the second species it remains the same. In the substrate with 75% m.c. adults of the two parasitoids are not able to reach the surface from puparia placed at 1 cm. In the substrate with 15% m.c. *M. domestica* emerging is not influenced till 6 cm depth; adults are not able to emerge from puparia placed at 20 cm depth. Only 5% of the adults is able to move up to the surface from puparia placed at 1 cm depth in the substrate with 75% m.c..

Riassunto - *Influenza dell'umidità del substrato sulla capacità di sfarfallamento di* Muscidifurax zaraptor *Kogan & Legner,* Nasonia vitripennis *(Walker) (Hymenoptera Pteromalidae) e* Musca domestica *L. (Diptera Muscidae).*

E' stato verificato, a 27±1°C e 70±5% U.R., se individui di *Muscidifurax zaraptor* Kogan & Legner, *Nasonia vitripennis* (Walker) e *Musca domestica* L., sfarfallati da pupari posti a crescenti profondità (da 1 a 20 cm) in substrati con diversa umidità, siano in grado di raggiungere la superficie. Il substrato di sfarfallamento è costituito da crusca con tre diverse umidità iniziali (15, 60 e 75%). Il numero di individui di *M. zaraptor* e *N. vitripennis* in grado di raggiungere la superficie è maggiormente influenzato dalla profondità a cui sono posti i pupari, rispetto a quanto osservato per *M. domestica*. Con il 15% di umidità del substrato *M. zaraptor* e *N. vitripennis* sono in grado di emergere da pupari posti a 3 cm di profondità, con il 60% la capacità della prima specie si riduce a 2 cm mentre rimane invariata per la seconda e con il 75% nessun adulto di entrambe le specie raggiunge la superficie da 1 cm. *M. domestica*, con il 15% di umidità del substrato, non è influenzata dalla profondità fino a 6 cm; non si osservano adulti con

pupari posti a 20 cm. Solo il 5% degli individui è in grado di uscire dal substrato con il 75% di umidità da 1 cm di profondità.

Key words: bran, moisture content, parasitoids, pupae.

INTRODUCTION

The parasitoids *Muscidifurax zaraptor* Kogan & Legner and *Nasonia vitripennis* (Walker) (Hymenoptera Pteromalidae) prefer as host *Stomoxys calcitrans* L. and *Musca domestica* L. (Diptera Muscidae) (Mandeville & Mullens, 1990); *N. vitripennis* also parasitizes Diptera Calliphoridae (Cornell & Pimentel, 1978; Morgan *et al.*, 1979; Petersen & Meyer, 1983; Mandeville, 1988).

Several Authors tested the ability of these species to parasitize pupae of *M. domestica* placed at different depths. Field surveys showed that puparia parasitized by *Muscidifurax* spp. are mainly on the surface of the manure (Legner & Brydon, 1966; Legner, 1967; Legner & Olton, 1971). Legner (1977), in laboratory tests, confirmed that *Muscidifurax* spp. parasitize pupae placed near the surface, recording a higher parasitizing ability on dry substrate and during the colder season.

In laboratory tests, carried out at 27±1 °C and 70±5% U.R. in a substrate with starting moisture content of 48%, *M. zaraptor* showed a greater ability than *N. vitripennis* to find and to parasitize pupae of *M. domestica* (Limonta & Locatelli, 2004). Legner (1977) found pupae parasitized by *M. zaraptor* till to 2 cm depth in wheat flakes, while Rueda & Axtell (1985) to 3 cm depth in poultry manure. As for other Pteromalidae, *Spalangia* species can parasitize puparia placed at 10 cm depth (Rueda & Axtell, 1985). The same Authors didn't find parasitoids in pupae collected at 15 cm depth.

The ability of *M. zaraptor* and *N. vitripennis* to move up to the surface from different depths of a substrate was not surveyed. In this study the ability to emerge of parasitoids in puparia placed at different depths in substrate with different moisture content was verified.

MATERIALS AND METHODS

Muscidifurax zaraptor, *Nasonia vitripennis* and *Musca domestica* are reared in laboratory at Istituto di Entomologia agraria dell'Università degli Studi of Milan. Although *N. vitripennis* is cited as a gregarious parasitoid (Smith, 1969), our strain is a solitary one. The three species are reared in conditioned chamber at 27±1°C, 70±5% R.H. and a photoperiod 14:10 (L:D) h.; 10 couples of parasitoids were used to parasitize 100 puparia of *M. domestica*. Parasitized puparia were used after a week, period of time necessary to verify the parasitization, as *M. domestica*, at 27±1° C and 70±5% R.H., emerge within seven days from the pupation.

Tests were carried out in poliethylene jars (Ø 12 cm and 17 cm height) with a cap with a 4x4 cm hole closed by a net (120 mesh). Bran with three different initial moisture content (15, 60 and 75%) was used as substrate. In the middle of each jar 20 puparia of *M. domestica* were placed on a layer of bran (1 cm) and progressively layers of 1 cm were added till adults emerging was observed. Tests were carried out, one by one, with puparia of *Musca domestica* not parasitized and parasitized by *M. zaraptor* or by *N. vitripennis*. In the tests with parasitized puparia, the inner walls of the jar were spread with inert glue, in order to trap parasitoids emerging from the surface and simplify the count of the individuals. The development from egg to adult lasts 18-20 days in *M. zaraptor*, 13-15 days in *N. vitripennis*, 6-8 days in *M. domestica*. Then tests were controlled after 20 days for *M. zaraptor*, after 15 days for *N. vitripennis*, and after 8 days for *M. domestica*. For each test four repetitions were carried out.

Puparia of *M. domestica* and parasitized puparia, kept at the same condition of the tests, were placed in jars without the substrate in order to verify the percentage of emerging of *M. domestica*.

The results were analysed with SPSS 10.0 for Windows (one-way ANOVA and Duncan's test).

RESULTS

A mean of 95% of flies emerged from not parasitized puparia, while a mean of 5% of *Musca domestica* emerged from puparia parasitized by *Muscidifurax zaraptor* and *Nasonia vitripennis*.

Emerging of *M. zaraptor* was not verified already at 1 cm with a moisture content of 75% of the substrate (Table 1); emerging was observed till 2 cm with 60% m.c. (0.5±0.3), while it was observed till 3 cm with 15% m.c. (1.3±0.5).

A similar behaviour was observed for *N. vitripennis* (Table 2) with 75% m.c.,

Table 1 - Mean number (±S.E.) of adults of Muscidifurax zaraptor *Kogan & Legner emerged from 20 pupae placed at different depth in substrates with different initial moisture content (15-60-75%).*

Depth (cm)	Mean number of adults (±S.E.)		
	Substrate m.c.		
	15	60	75
1	8.0±0.7a	5.3±0.6b	0.0±0.0c
2	8.8±1.3a	0.5±0.3b	0.0±0.0b
3	1.3±0.5a	0.0±0.0b	0.0±0.0b
4	0.0±0.0a	0.0±0.0a	0.0±0.0a

Values followed by different letters are significantly different for 95% of confidence interval (Duncan's test).

Table 2 - Mean number (±S.E.) of adults of Nasonia vitripennis *(Walker) emerged from 20 pupae placed at different depth in substrates with different initial moisture content (15-60-75%).*

Depth (cm)	Mean number of adults (±S.E.)		
	Substrate m.c.		
	15	60	75
1	2.0±0.4b	8.5±0.3a	0.0±0.0c
2	1.5±0.3a	1.5±0.3a	0.0±0.0b
3	0.8±0.5a	0.5±0.3a	0.0±0.0a
4	0.0±0.0a	0.0±0.0a	0.0±0.0a

Values followed by different letters are significantly different for 95% of confidence interval (Duncan's test).

Table 3 - Mean number (±S.E.) of adults of Musca domestica *L. emerged from 20 pupae placed at different depth in substrates with different initial moisture content (15-60-75%) (jar ∅ 12cm).*

Depth (cm)	Mean number of adults (±S.E.)		
	Substrate m.c.		
	15	60	75
1	18.5±0.3a	18.3±0.5a	1.0±0.6b
2	17.8±0.3a	18.0±0.6a	0.0±0.0b
3	16.8±0.3a	17.5±0.6a	0.0±0.0b
4	18.5±0.5a	6.8±0.8b	0.0±0.0c
5	16.8±0.5a	7.0±0.7b	0.0±0.0c
6	15.8±1.4a	6.3±0.9b	0.0±0.0c
7	17.5±0.6a	0.0±0.0b	0.0±0.0b
8	16.8±0.5a	0.0±0.0b	0.0±0.0b

Values followed by different letters are significantly different for 95% of confidence interval (Duncan's test).

while the mean number of emerged adults is considerably lower with 15% m.c. (2.0±0.4 at 1 cm; 1.5±0.3 at 2 cm and 0.8±0.5 at 3 cm). With 60% m.c. of the substrate, *N. vitripennis* is more efficient than *M. zaraptor* (8.5±0.3 at 1 cm; 1.5±0.3 at 2 cm and 0.5±0.3 at 3 cm).

Significant differences were not observed for the adults of *M. domestica* (Table 3) emerged from 1 to 3 cm depths in the substrate with 15 and 60% m.c.. Starting from 4 cm, and increasing the depth, the mean number of emerged adults from the substrate with 60% m.c. considerably decreased. As far as 8 cm, in the substrate with 15% m.c., the mean number of adults of *M. domestica* was between 15.8 and 18.5. In the substrate with 60% m.c., from puparia placed at 6 cm a mean number of 6.3±0.9 individuals moved up to the surface, at 7 cm no adults were observed. Only 1.0±0.6 was able to emerge from the substrate with 75% m.c. from 1 cm depth.

Table 4 - Mean number (±S.E.) of adults of Musca domestica *L. emerged from 20 pupae placed at different depth in substrates with 15% initial moisture content) (cardboard cylinder ∅ 40cm).*

Depth (cm)	Mean number of adults (±S.E.)
8	4.2±1.9a
16	1.2 ±0.5b
20	0.0±0.0b

Values followed by different letters are significantly different for 95% of confidence interval (Duncan's test).

It was observed that bran with 15% m.c. became close-packed, leaving a space of about 1 mm from the walls of the jar. Some of the adults found on the surface could be attracted towards the walls by air and light and used this space to reach the surface. Other tests were carried out in cardboard cylinders with a diameter of 40 cm (Table 4). At these conditions in the test at 8 cm depth, the mean umber of adults that emerge decreased from 16.8±0.5, counted in the jar of little dimension, to 4.2±1.9 in the cardboard cylinder. Only few adults emerged from puparia placed at 16 cm (1.2±0.5), while no adults were observed at 20 cm.

CONCLUSIONS

The number of adults of *Muscidifurax zaraptor* Kogan & Legner and *Nasonia vitripennis* (Walker), able to move up to the surface, is more influenced by depth at which puparia are placed compared to *Musca domestica* L.. Parasitoids emerge with difficult from puparia placed under the surface, in fact we observed several adults trapped in the bran.

The ability to emerge of adults of *M. zaraptor* decreases with an increase of the moisture content, while at a depth of 1 cm emerged adults of *N. vitripennis* increase from 15 to 60% m.c.. Smith & Rutz (1990) observed that parasitization by *N. vitripennis* increases in dry substrates. Smith & Rutz (1991) recorded that *M. zaraptor* parasitize puparia close to the surface, indipendently from the moisture content. As regards to other Pteromalidae, *Spalangia cameroni* Perkins and *S. nigroaenea* Curtis are not significantly influenced by moisture content of the substrate in laboratory test, however they are more common in wet manure (Smith & Rutz, 1991).

In the substrate with 15% m.c. till 8 cm, the increase in depth does not influence the ability of emerging of *M. domestica*, while with 60% m.c. a decrease in the number of emerged adults was observed. Undoubtedly a wet substrate hinders the movement of the adults; in fact mature larvae move towards dry part of the manure in order to pupate.

It was observed that newly emerged adults of *M. domestica* can move in the dry substrate not only vertically but also laterally, if the specimen perceives an easier way.

The ability to emerge from puparia placed in the depth is linked to the substrate. *M. domestica* is able to emerge from puparia placed from 5 to 60 cm in the ground (Hill, 1990) and from 1,2 m depth in clay, sand and loam (Kettle, 1984),

Results prove that parasitoids have difficulty in moving up to the surface especially in substrate with a high moisture content. In livestock breeding accumulation of dung increase the possibility that puparia will be buried by a thick layer that preclude emerging of *M. zaraptor* and *N. vitripennis*. Therefore several releases are necessary where dung continuosly accumulates, as moisture interferes with emerging parasitoids. Tests carried out in Nebraska (Petersen, 1986) prove that release, at the beginning of spring, of pupae of *M. domestica* previously killed by freezing together with pupae parasitized by *M. zaraptor*, helps to build up early season populations of the parasitoid.

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