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Influence of the Humidity on Mortality Rate and Embryonic Development Time of Two Strains of *Phytoseiulus persimilis* Athias-Henriot (Acarina Phytoseiidae) (*)

Introduction

The predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acarina Phytoseiidae) has been proved to be a beneficial arthropod extremely efficient in keeping the phytophagous red spider mite *Tetranychus urticae* Koch (Acarina Tetranychidae) under control, and it is widely used on increasing surfaces of protected crops in the IPM and biological control programs. Its efficacy in the Mediterranean region is highly influenced by relative humidity, which can be a limiting factor to take into account before the inoculative release. In fact, while for the phytophagous *T. urticae* life parameters are favoured by hot and dry climatic conditions, for the predatory mite *P. persimilis* long periods of exposition to low relative humidity (under 70%) negatively affect its development, causing high mortality during the moults (Pralavorio and Almaguel-Rojas, 1980). Sabelis (1981) found that the minimum humidity rate for a profitable application of *P. persimilis* is 50%. The most sensitive instar to low relative humidity is the egg, which progressively dries out till collapse. Stenseth (1979) found that at 27°C and 40% RH only 7.5% of the eggs hatched, while at the same temperature but with 80% RH the percentage of egg hatching increased to 99.7%. Perring and Lackey studied (1989) the mortality rate and the pre-imaginal developmental time of an Israelian strain of *P. persimilis* compared to a Californian one at six different thermohygrometric conditions. They found out that 32.2°C was a limiting temperature for both strains at every RH level. At lower temperatures, statistically significant differences were found in the mortality, developmental time and percentage of egg hatching between the two strains.

A preliminary investigation carried out in our laboratory evidenced the difficulty in defining when the egg of *P. persimilis* has really dried out an is not vital anymore, as we found that eggs, which appeared completely collapsed

(*) Lavoro accettato il 18 gennaio 2000.

and opaque after a period of exposition to low RH, could turn turgid again and normally hatch, if exposed to high RH conditions.

In this study the resistance to low RH regimes of a wild Mediterranean strain and of a North-European mass-reared strain were compared. Our aim was, at the same time, to develop a simple methodology to apply for the quality control of mass-reared *P. persimilis* populations.

Materials and Methods

The two strains of *P. persimilis* under investigation were characterized as follows:

1) The NE-strain, original from Northern Europe, was supplied by the british biofactory Ciba-Bunting Biological Control, which developed it since 1992 from a population reared at the Glasshouse Crop Research Institute (UK) and then supplemented it with wild individuals collected in California.

2) The SI-strain, original from Sicily, was collected in the Siracuse province, where it has never been released before.

The two strains were reared in separate rooms. The rearing unit, placed inside a climatized cell, consisted of a plastic basket filled with water to prevent the mite from escaping; it contained a plastic tray as support for bean leaves, infested by *T. urticae* (Fig. I) (Osakabe *et al.*, 1988). The climatic conditions of both rearings were 20-24°C and 16:8 L:D. At the beginning of the experiments both strains had nearly completed two generations.

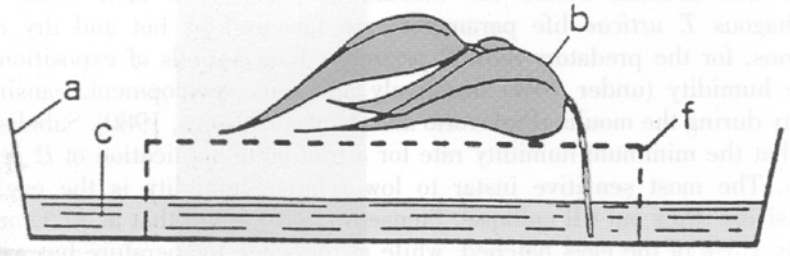

Fig. I. - Plastic basket for *P. persimilis* rearing. a: plastic basket; b: bean leaf previously infested by *T. urticae*; c: water; d: plastic tray.

Preparation of the experimental material - For each strain, four cylinders (∅ 2.5 cm, height 4.0 cm) made of polymethyl-methacrylate, featuring a cap fitted with a stainless steel mesh disk (∅ 2.5 cm, 201 mesh, 36% of air permeability), were prepared with a bean leaf highly infested by *T. urticae*. 100 *P. persimilis* ovipositing females were put in the 4 cylinders and 1 hour was let them for laying their eggs. Females were then removed and the eggs necessary for the experiment were collected. Each egg was separately allocated in a small cylindric capsule.

Preparation of the experimental unit - The temperatures and the relative humidity decided for the experiments were obtained through the method

of the sature saline solutions in isolated environment (Wiston and Bates, 1960). Several experimental units were prepared, each one consisting of a PVC box with a circular hole on the top wall, through which the sature saline solution was introduced in the box. In the cover the probe of an electronic thermohygrometer was inserted. In the anterior wall of the PVC box, a drawer with a grill-made ground, perfectly closed by a gasket, contained the experimental material, keeping the eggs few centimeters above the sature saline solution. A magnetic device, placed under the experimental unit, ensured a soft movement of the solution, preventing salt deposition. Everything was then placed inside the climatic cell.

Experiment 1 - Percentage of eggs hatching at different RH regimes.

To study the influence of the humidity level on the embryonic development of *P. persimilis*, the following constant relative humidities were compared: 50±2%, 73±2%, 90±2%. The resistance of the two strains to a variable RH, was studied as follows: 3 hours at 90%, 16 hours at 50%, 8 hours at 90%, 16 hours at 50%, then at 90% RH till the last control. Trials were run at the constant temperature of 26±1°C, which is considered the optimum one for the rearing of the beneficial mite.

To obtain the exposition of the eggs to the variable RH regime scheduled, the drawer containing the capsules was moved from an experimental unit to another already set at the fixed RH.

The salts used and the concentrations applied to obtain the different humidity levels are summarized in table 1.

Tab. 1. - Salts used and relative concentrations applied to obtain the RH desired for the experiment*.

Chemical formulation	Name	Concentration g/l	RH obtained at 26±1°C
K_2CO_3	potassium carbonate	1750	50±2%
Na_2NO_2	sodium nitrate	1500	73±2%
$Na_2CO_3.10H_20$	sodium carbonate decahydrated	750	90±5%

* The salts were diluted in distilled water.

For each RH regime considered, 30 eggs for each *P. persimilis* strain, separately allocated in cylindric capsules, were put in the drawer of the experimental unit. After 48 hours, the drawer was taken out and each egg was checked for hatching or collapsing under the binocular stereoscope; following checks were done at 72 and 96 hours. Hatched eggs, easily identified by the translucent chorion and by the presence of a slightly mobile larva, were removed. Non-hatched eggs were put again in the climatic cell. The experiment was repeated four times. For each *P. persimilis* strain, the percentage of eggs hatching at the different RH was analyzed though 1 - way ANOVA (results are reported in table 2). The behaviour of the two strains was also compared at each RH considered, through 1 - way ANOVA (results are reported in figure II).

Experiment 2 - Influence of the duration of the exposition at low RH on egg survival and development time.

For each *P. persimilis* strain, groups of 30 eggs, obtained as described above, were exposed to increasing periods of stress at 50±2% RH, at the constant temperature of 26±1°C. The tested exposition periods were 24, 28, 32, 36 hours. after the treatment, eggs were put in an experimental unit with 90±5% RH till hatching or collapsing. A fifth group of eggs was continuously maintained at the optimal RH (90±5% RH) thus acting as a control group. The experiment was repeated 4 times.

For three groups of eggs the controls for hatching or collapsing were conducted after 48, 72, 96 hours; for the fourth group and for the control group controls were conducted every 8 hours to better compare the developmental step. Data were analyzed through a 2 - way ANOVA, considering the strain and the RH treatment as main factors. A linear regression analysis was run between the duration of the low RH stress and the percentage of egg hatching for both strains. The developmental time of the embryonal phase related to the duration of the stressing period was also studied separately for each strain through a linear regression analysis.

Results

Experiment 1 - Percentage of egg hatching at different RH.

In table 2 the mean percentages of hatched eggs for both strains at each RH considered are reported. For the SI strain, data evidenced that egg survival was near to 0% when eggs were exposed at the lower level of relative humidity; when increasing the humidity level, the hatching percentage increased, reaching 38% at the intermediate RH level, and nearly 100% with 90% RH, which proved to be the optimal value in this developmental stage. The groups exposed to the variable RH obtained quite good results, even if the percentage of egg hatching was significantly lower with respect to that one obtained with a constant 90% RH.

An analogous trend was registered also for the BU strain, the only difference being that the percentage of hatched eggs for the groups exposed to the variable RH regime obtained quite a good result, which did not differ from that one obtained at the optimal RH (Tab. 2).

Tab.2. - Percentage of *P. persimilis* egg hatching at different RH*.

	RH at 26±1°C			
STRAIN	50±2%	73±2%	90±5%	Variable 50±2%-90±5%
SI	0.0 d	38.3 c	99.2 a	66.7 b
BU	0.8 C	55.0 B	97.5 A	87.5 A

* Different letters indicate significant statistical differences.

Comparing the two strains, no differences were found between the percentages of survival of the eggs at the lower and the higher constant RH tested, while at the intermediate RH level and at the variable RH level, a higher percentage of hatched eggs was registered for the BU strain, which proved to be more resistant to the stress caused by insufficient RH than the SI strain (Fig. II).

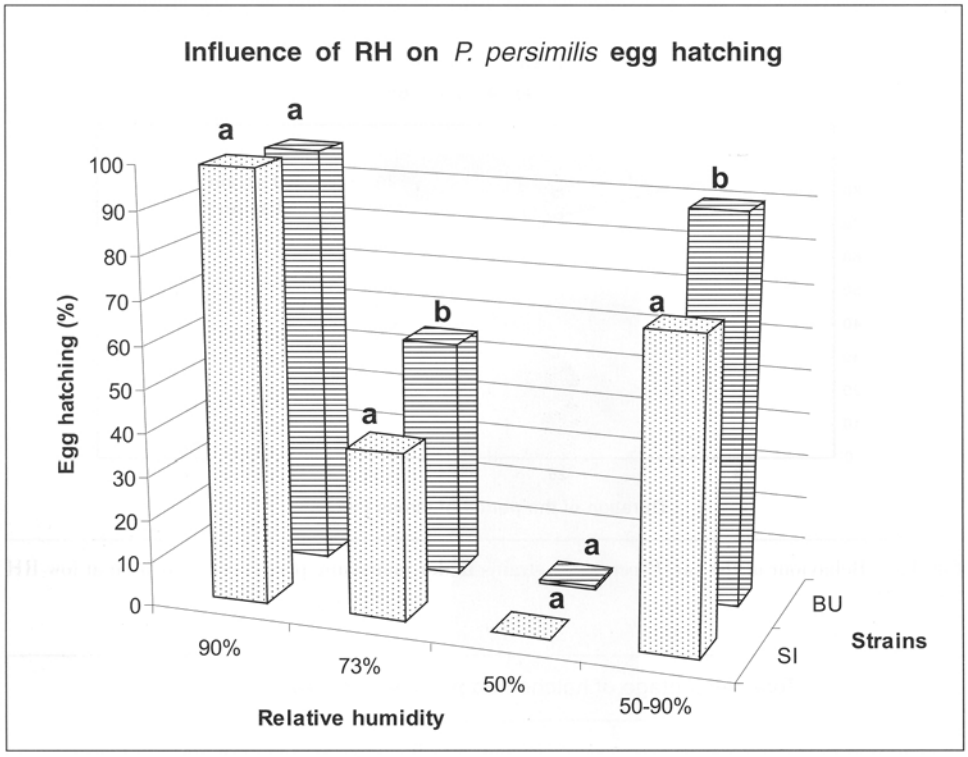

Fig. II. - Percentage of egg hatching of two *P. persimilis* strains at different relative humidity conditions. Different letters indicate statistically significant differences between the two strains.

Experiment 2 - Influence of the duration of the exposition at low RH on egg survival and development time.

As reported in figure III, the percentage of normally hatched eggs was decreasing along with the increasing of the duration of the stress; no significant difference in the survival of the eggs was found between the strains, whose behaviour was similar under all the period of stress considered, showing no interaction between the two main variables studied (the strain and the hours of exposition at low RH).

Only when comparing the two strains on the basis of the total number of hatched eggs for all the RH considered, a significant difference between them emerged, and the survival registered for the BU strain was higher than for the SI strain (Fig. IV).

As both strains showed the same trend under every stress condition considered, the cumulative percentage of hatched egg of both strains was analyzed and related to the number of hours of stress. The result of the linear regression is reported in figure V and shows that the decrease of egg survival depended on the increase

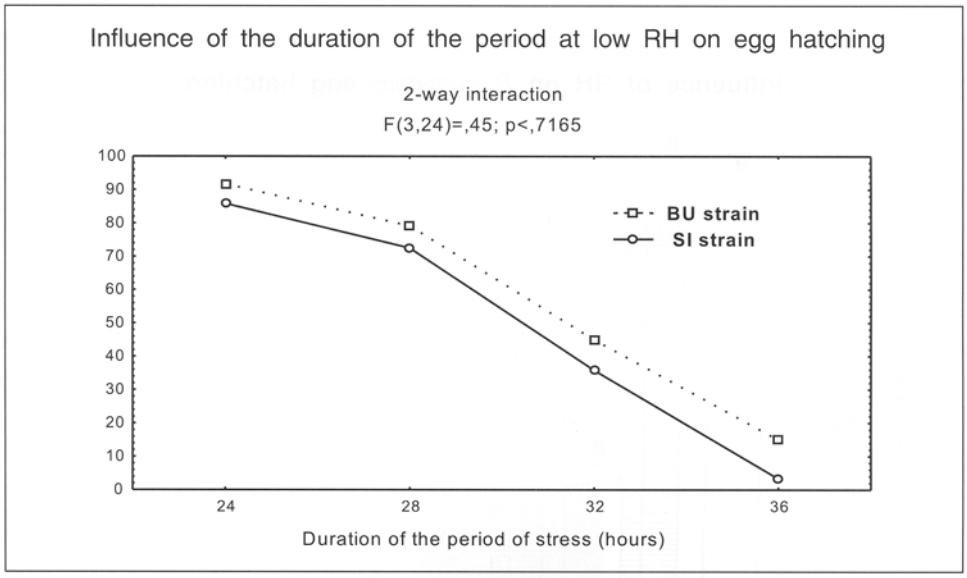

Fig. III. - Behaviour of the two *P. persimilis* strains under increasing periods of exposition at low RH level (50%).

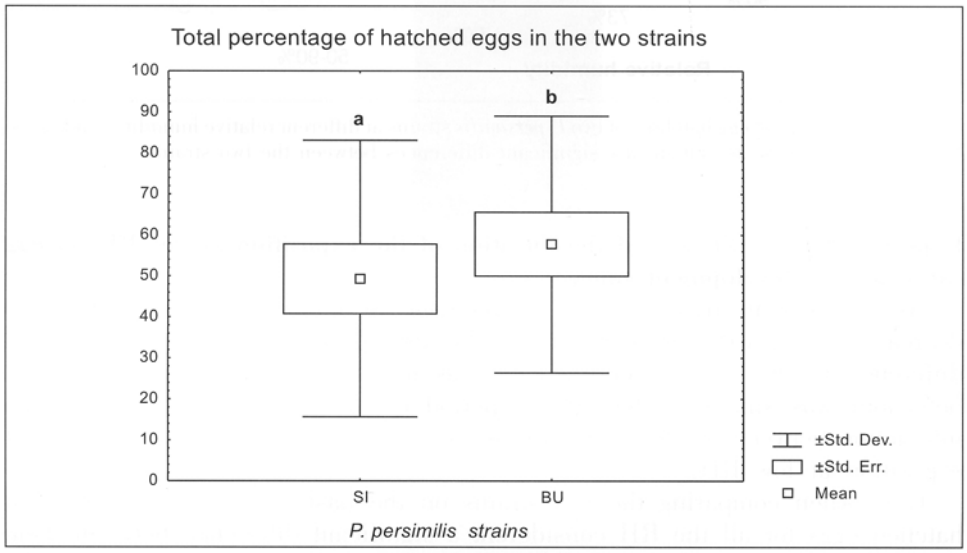

Fig. IV. - Percentage of hatched eggs for each *P. persimilis* strain, calculated on the total number of eggs employed in all the trials on the resistance to increasing duration of the exposition at low RH.

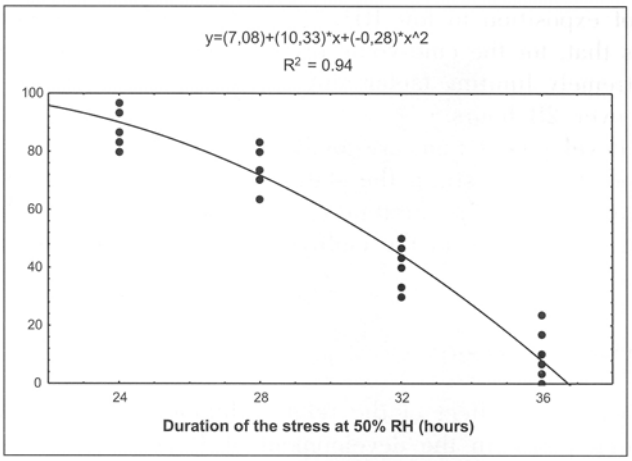

Fig.V. - Percentage of hatched eggs for the increasing periods of stress at low RH, considering both *P. persimilis* strains together.

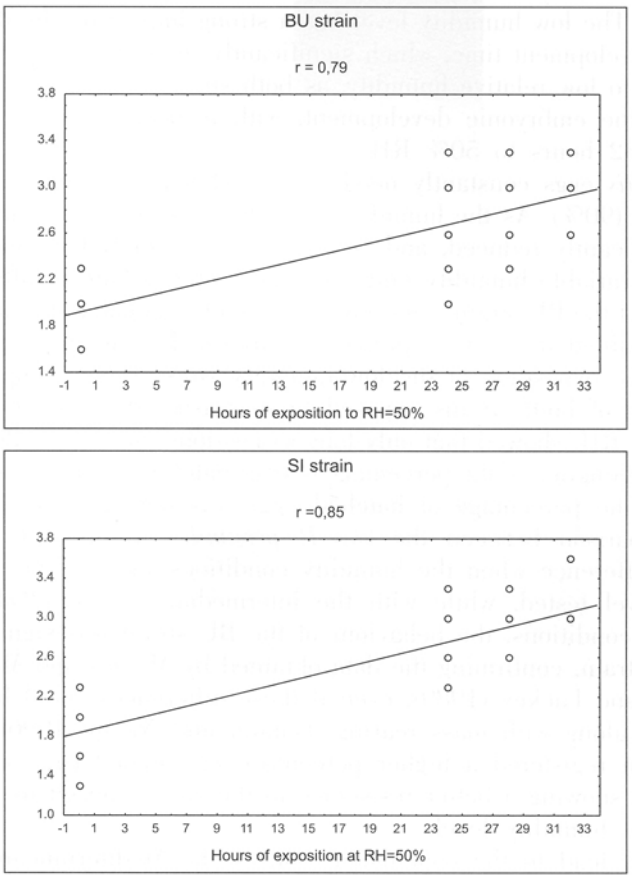

Fig. VI. - Linear regression of the embryonic development time of the two strains of *P. persimilis* depending on increasing period of exposition to low RH conditions.

of the hours of exposition to low RH, following a polynomial curve (R^2=0.94).

This means that, for the embryonic development of *P. persimilis*, the low RH can be an extremely limiting factor only if the exposition of the eggs lasts for long periods, over 28 hours.

When the development time are analyzed in relation to the duration of the stressing period, for each strain the statistical analysis evidenced a significant linear regression between the duration of the exposition to low relative humidity conditions and the increase of the embryonic development time, as reported in figure VI.

Discussion and Conclusions

In this research the effect of the relative humidity on egg hatching, which is a very crucial phase in the development of *P. persimilis*, was studied. Two strains of different geographic origin were compared: the SI strain (collected in Sicily) and the BU strain (supplied by the biofactory Ciba - Bunting).

Relative humidity confirmed to be a crucial parameter for *P. persimilis* development. The low humidity level has a strong impact on the duration of the embryonic development time, which significantly increases along with the hours of exposition to low relative humidity, as both strains took nearly one day more to complete the embryonic development, with respect to the control group, if exposed for 32 hours to 50% RH.

P. persimilis eggs constantly need, for a full hatching success, a very high humidity rate (90%). As the humidity level decrease, the percentage of hatched eggs is significantly reduced, and is near to zero with RH around 50%. Trials run within a variable humidity range obtained intermediate results, with a good performance of the BU strain, whose percentage of eggs hatching was not different from that obtained under the optimal conditions. This let us suppose that the duration of the exposition at the low humidity stress is very important. In fact the behaviour of both strains exposed to increasing duration of the period of stress at 50% RH, showed that only long expositions (more than 28 hours) cause a significant reduction of the percentage of eggs hatching, as for exposition inferior to 28 hours the percentage of hatched eggs was higher than 70%.

The comparison between the two *P. persimilis* strains did not show any significant difference when the humidity conditions were at the optimum or at the lower level tested, while with the intermediate level (73%RH) or under variable RH conditions, the behaviour of the BU strain was significantly better than the SI strain, confirming the data obtained by Moraes and McMurty (1985) and Perring and Lackey (1989), even if these differences could be reduced till disappearing along with mass rearing (Galazzi and Nicoli, 1996b). In general, the BU strain registered a higher percentage of hatched eggs with respect to the SI strain, showing a better resistance to the stress caused by the exposition to insufficient humidity level.

This could lead to the conclusion that, in the Mediterranean environment, during the dry summer season, the BU strain should be recommended for the inoculative release in the IPM programs. Anyway, to this proposal, we have to

report here that other studies, conducted within the same ECLAIR project, evidenced, for the SI strain, better results when other important parameters are considered, such as female longevity and fecundity (Galazzi *et al.*, 1993; Galazzi e Nicoli, 1996a; Galazzi e Nicoli, 1998), which make it more suitable than the BU strain for its use in the Mediterranean area, as the crucial predatory activity is essentially performed by the progeny of the released individuals. A precise exam of many different biological parameters is needed to identify which is the most suitable strain of the beneficial for every particular environment.

It would be desirable to verify the results of this study under field conditions, where the microclimate created by the leaves can influence egg hatching despite of the general RH conditions. For the same reasons, it would be convenient to evaluate the resistance of the mass-reared beneficial mites to low humidity conditions through a specific test during the quality control programs, and we suggest that since the methodology applied in this study is sufficiently simple and very precise, it can be adopted for this aim.

Acknowledgments

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SUMMARY

The effect of relative humidity on egg hatching and its impact on embryonic development time were studied. Two strains of different geographic origin were compared: the SI strain, collected in Sicily, and the BU strain, supplied by the Ciba – Bunting biofactory. To produce the eggs necessary for the experiments, the two strains were reared in climatized cells, placed in separate rooms. The temperatures and the relative humidity chosen for the experiments were obtained through the method of the sature saline solutions in isolated environment. Several experimental units, with all the different RH needed, were prepared.

To study the influence of RH on egg hatching, groups of eggs of the two strains were exposed to the following constant relative humidity until they either hatched or collapsed: 50±2%, 73±2%, 90±2%. The effect of the exposition to a variable RH was also tested as follows: 3 hours at 90% RH, 16 hours at 50%, then 8 hours at 90% RH, 16 hours at 50% RH and then at 90% RH, till the last control. To study the effect of prolonged period of stress at low RH on the embryonic development time, for each *P. persimilis* strain, groups of eggs were exposed to increasing periods of stress at 50±2% RH, 24, 28, 32, 36 hour. A fifth group of eggs was continuously maintained at the optimal RH (90±5% RH) thus acting as control. All trials were run at the constant temperature of 26±1°C, which is the optimum one for rearing the beneficial mite.

Relative humidity confirmed to be crucial for *P. persimilis* development, which need, for a full success in egg hatching, constantly a very high humidity rate (90%). As the humidity decreases, the percentage of hatched eggs significantly decreases, and is reduced to zero with RH around 50%. Trials run within a variable humidity range evidenced a good performance of the BU strain, whose percentage of eggs hatching was not different from that obtained in the optimal conditions.

This allowed us to suppose that the duration of the exposition at the low humidity stress is the limiting factor. In fact, only long expositions (more than 28 hours) at 50% RH cause a significant reduction in the percentage of eggs hatching, as for an exposition inferior to 28 hours the percentage of hatched eggs was higher than 70%. The low humidity level has also a strong impact on the duration of the embryonic development time, as both strains took nearly one day more to complete the embryonic development, with respect to the control group, if exposed for 32 hours to 50% RH.

In general we can say that the BU strain registered a higher percentage of hatched eggs with respect to the SI strain, showing a better resistance to insufficient or not optimal humidity level, even if these differences could be reduced till disappearing along with the mass rearing. Anyway, the SI strain, in other researches, obtained better results when other important parameters were considered, such as female fecundity and longevity. A precise exam of many different biological parameters is needed for the identification of the more suitable strain for every particular environment.

Finally, we suggest that since the methodology applied in this study is sufficiently simple and very precise, it can be adopted as a specific test in quality control programs.

KEY WORDS: *Phytoseiulus persimilis*, humidity, embryonic development, strains, quality control.

Influenza dell'umidità sulla mortalità e sul tempo di sviluppo embrionale in due ceppi di *Phytoseiulus persimilis* Athias-Henriot (Acarina Phytoseiidae)

RIASSUNTO

Oggetto di questa ricerca è stata l'influenza dell'umidità relativa su alcune fasi cruciali del ciclo biologico di *P. persimilis*: la schiusura delle uova e il tempo di sviluppo embrionale. Lo studio è stato condotto su due ceppi di diversa origine geografica: il ceppo SI (raccolto in Sicilia) e il ceppo BU, fornito dalla biofabbrica Ciba-Bunting. Per produrre le uova necessarie agli esperimenti, i due ceppi sono stati allevati in celle climatizzate poste in ambienti separati. Le temperature e le umidità relative stabilite sono state ottenute con il metodo delle soluzioni saline sature in ambiente chiuso.

Per studiare l'influenza del livello di umidità sulla percentuale di schiusura, gruppi di uova sono stati esposti ai seguenti livelli costanti di UR: 50±2%, 73±2%, 90±2%, fino alla schiusura o al collasso; è stata studiata anche l'influenza di un livello variabile di UR, comprendente periodi di bassa UR, come segue: 3 ore al 90%, 16 ore al 50%, 8 ore al 90%, 16 ore al 50% e poi ancora al 90% fino alla schiusura o al collasso. Per studiare l'effetto di un lungo periodo di stress a bassa UR sui tempi di sviluppo embrionale, per ogni ceppo, gruppi di uova sono stati esposti a 24, 28, 32, 36 ore al 50±2% UR. Un quinto gruppo di uova è stato mantenuto al livello ottimale del 90% UR, come controllo. Tutte le prove sono state condotte alla temperatura di 26±1°C, considerata ottimale per l'allevamento di *P. persimilis*.

L'umidità relativa si è confermata fattore di cruciale importanza per lo sviluppo di *P. persimilis*, che necessita, per un pieno successo nella schiusura delle uova, di un livello costantemente elevato di UR (90%). Al decrescere dell'UR, la percentuale di schiusura delle uova diminuisce significativamente, fino ad azzerarsi se l'umidità resta costantemente al 50%. Le prove condotte al livello variabile di umidità, comprendente periodi di esposizione al 50% UR, hanno evidenzieto una buona resistenza del ceppo BU, la cui percentuale di schiusura non differiva significativamente dal gruppo mantenuto alle condizioni ottimali di UR. La durata del periodo di esposizione a bassa UR è quindi determinante: solo esposizioni continuate per più di 28 ore abbassano la percentuale di schiusura al di sotto del 70%. La bassa UR ha anche un forte impatto sulla durata dello sviluppo embrionale, che aumenta significativamente all'aumentare del periodo di stress, come dimostrato dal fatto che entrambi i ceppi, esposti per 32 ore al 50% UR, hanno impiegato un giorno in più per completare lo sviluppo embrionale, rispetto al gruppo di controllo.

In generale il ceppo BU ha fatto registrare percentuali di schiusura delle uova maggiori rispetto al ceppo SI, dimostrando una migliore resistenza a livelli non ottimali di UR, anche se queste differenze possono ridursi fino a scomparire a seguito di un allevamento massale. Inoltre il ceppo SI, nell'ambito di altre ricerche, ha ottenuto migliori risultati per altri importanti parametri presi in esame, come fecondità e longevità delle femmine. Ciò dimostra l'importanza di esaminare molti e diversi parametri biologici prima di stabilire qual è il ceppo più adatto da impiegarsi in ogni particolare ambiente.

Riteniamo, inoltre, che la metodologia applicata in questo studio possa essere al contempo sufficientemente semplice e molto precisa per essere inserita come test specifico nei controlli di qualità durante i processi di allevamento degli ausiliari messi a punto dalle biofabbriche.

PAROLE CHIAVE: *Phytoseiulus persimilis*, umidità, sviluppo embrionale, ceppo, controllo di qualità.

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