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Quality Control of Cold Stored *Phytoseiulus persimilis* Athias-Henriot (Acarina Phytoseiidae). (*)

INTRODUCTION

The predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acarina Phytoseiidae) is the beneficial agent most widely used for biological control of the red spider mite *Tetranychus urticae* Koch (Acarina Tetranychidae). Small-scale applications were started in 1968 in northern Europe, and in 1990 mass-reared *P. persimilis* was released in about 7,000 hectares of protected crops world-wide (van Lenteren *et al.*, 1992).

P. persimilis can be considered endemic in the Mediterranean basin: Athias-Henriot (1957) described the new species collecting specimens in Algeria and many other Authors recorded this species in several places of the Mediterranean basin, before the trade of mass-reared predatory mites started in southern Europe (Galazzi and Nicoli, 1996a).

In Italy, biological control with *P. persimilis* started in Sicily, using native populations (Nucifora *et al.*, 1983; Vacante and Nucifora, 1987). In the following years, predatory mites produced by northern European biofactories were released in many areas, beginning in 1985 in the Po valley (Celli *et al.*, 1987), generally reaching satisfactory results. To obtain a high reliability in the application of predatory mites, Quality Control (QC) of commercial products soon appeared necessary, particularly to measure the performances of the predatory mites submitted to cold storage during the shipment from the production facilities to the application area.

Leppla (1994) has recently discussed the principles of QC of mass-reared arthropods and stated that the application of QC theory to arthropods production has advanced to the point of general implementation. Van Lenteren *et al.* (1994) and van Lenteren (1996) have underlined the importance of QC programmes and described the objectives of the Concerted Action PL 921076 supported by the European Commission 'Designing and implementing Quality control of beneficial insects: towards more reliable biological pest control' (EC-Concerted Action).

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Guidelines have been proposed with the aim of designing simple laboratory tests to measure some parameters related to field performances of the natural enemies. About *P. persimilis*, van Lenteren *et al.* (1994) supported the need to investigate whether there is any significant difference between the performances of the mass-produced predatory mites at 22 °C or 25 °C, being the same biological product distributed and used under very different climatic conditions from northern to southern Europe.

Concerning QC of *P. persimilis*, Morewood (1992) reported the results on the influence of cold storage; Steiner (1993) evaluated shipments from commercial producers; Steinberg and Cain (1994) tested and evaluated the criteria for product QC.

Owing to the results of previous studies (Galazzi and Nicoli, 1996a; 1996b), particularly as regards to the high longevity and fecundity of females, strains of the predatory mite collected in Sicily appeared particularly suitable for the seasonal inoculative release method and they are currently mass-reared at the Italian biofactory Biolab-Centrale Ortofrutticola in Cesena. The aims of this study were: 1. to evaluate the effects of cold storage on the activity of a mass-reared Sicilian strain; 2. to define standards and procedures for the product control guidelines, in particular investigating if there is any difference in the predatory mite response between tests carried out at 22 °C or 25 °C. The temperature of 25 °C has been chosen because it is more appropriate for evaluating the activity of predatory mites to be released in the Mediterranean basin, while the temperature of 22 °C appears more indicative of northern European conditions.

MATERIALS AND METHODS

Origin of the predatory mites. The research consisted of two laboratory experiments carried out on samples of a *P. persimilis* strain originally collected in Sicily and mass-reared at Biolab-Centrale Ortofrutticola (Cesena, Italy) on bean plants, *Phaseolus vulgaris* L. (Leguminosae).

Experimental procedure. The predatory mites were harvested in a mass-production glasshouse. Generally, the personnel starts harvesting the predatory mites when the *T. urticae* population is declining and, particularly, when adult females of *P. persimilis* move to supports placed over the bean plants. The predators can be easily collected at the top of these supports a few times per day for some days (harvesting period).

In experiment 1, the harvesting period lasted one week: at days 1, 3, 5, and 7, groups of ca. 500 newly-harvested adult females were placed in 16 plastic vials (8 cm³ each) containing moist vermiculite and closed with a plastic cap. The plastic vials were divided into 4 groups and cold stored in a dark climatic chamber (8±1 °C; RH=100%) for 0, 3, 7 and 14 days respectively. After storage, survival of predatory mites was checked counting dead and live predators; 30 females were taken from each vial, to test their longevity and fecundity in a climatic chamber at 25±1 °C; RH=80±5% and photoperiod L:D=16:8 (480 females in total). Each female was isolated in a Plexiglas cylindrical cage (4.0 cm height, 4.0 cm diameter, 0.2 cm

thickness), closed by a cap fitted with a stainless steel mesh disk (2.5 cm diameter, 201 mesh, 36% air permeability). At the bottom of each cylindrical cage, lying on approximately 0.6 cm layer of agar gel, was a bean leaf disk, the under blade face-upwards, infested *ad libitum* by *T. urticae*. The cage was set upside-down on a perforated basket so that the leaf disk was on top and the cap mesh on the bottom. The tests were carried out following the QC guidelines reported by van Lenteren *et al.* (1994), with the exceptions of temperature (25 °C instead of 22 °C) and the recording of the eggs laid began immediately after isolation instead of the second day of oviposition.

In experiment 2, the activity of *P. persimilis* at 25±1 °C and 22±1 °C was compared, using in both cases non-stored predatory mites (120 females in total per temperature).

Statistical analysis. Analysis of variance, followed by Tukey's test, was applied to the parameters. The percentages were compared by the Chi-square test. Multiple regression statistics were computed for various parameters.

RESULTS

Experiment 1. Survival. Table 1 reports the survival of *P. persimilis* females collected from the mass-production glasshouse from day 1 to day 7 of the harvesting period and cold stored from 0 to 14 days. Multiple regression in figure I indicates that the survival percentages decreased as the harvesting period advanced and the time of cold storage increased. The computed regression statistics are reported in table 2.

Table 1. Survival (%) of *Phytoseiulus persimilis* females harvested from mass-production and cold stored up to 14 days in vials (ca. 500 predatory mites per vial).

Cold storage (days)	n. vials	Day of harvest from mass-production			
		1	3	5	7
0	4	95.7	97.7	79.4	82.6
3	4	94.4	95.5	78.2	89.3
7	4	90.1	92.1	78.0	78.6
14	4	81.2	85.6	74.2	53.8

Table 2. Regression statistics computed for the survival (%) of *Phytoseiulus persimilis* in the vials, for the regression model: $y = a + b_1x_1 + b_2x_2$.

Parameter	n. vials tested	a ± se	b ₁ ± se	b ₂ ± se	R ²	p
End-storage survival (%)	64	102.54±2.18**	-2.85±0.42**	-1.17±0.18**	0.60	<0.01

** : p < 0.01.

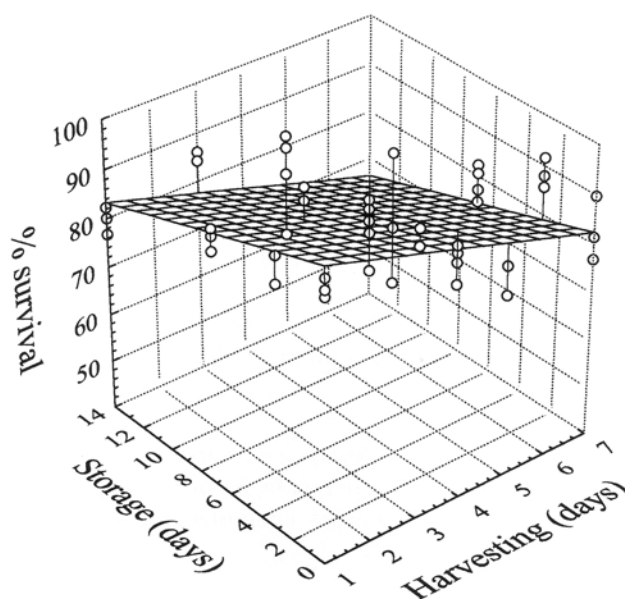


Figure I. End-storage survival (%) of *Phytoseiulus persimilis* females harvested from mass-production and cold stored up to 14 days in vials (ca. 500 predatory mites per vial).

Experiment 1. Survival and fecundity. Table 3 reports the survival of *P. persimilis* females isolated at 25 °C after cold storage, compared with the QC standard (>80% living females after 5 days at 22 °C) included in the guideline proposed by the experts' group working within the EC-Concerted Action. The predatory mites survival was not significantly lower than 80% up to day 3 after isolation (except for 1 sample), but several samples showed a survival lower than 80% particularly at day 5 after isolation. Table 4 reports the reproductive activity of *P. persimilis* females isolated at 25 °C after cold storage.

Multiple regression statistics, increasing the cold storage days and the harvesting period, were computed for: female survival after 5 days from isolation; pre-oviposition period; female fertility and number of eggs laid during the 6 days after isolation (tab. 5). The oviposition trend was plotted using the data of all the fertile females together (n=253). The multiple regressions pointed out that survival after 5 days from isolation (fig. II), percentage of fertile females (fig. III) and number of eggs laid from day 1 to day 6 after isolation (fig. IV) increased as the harvest period advanced and decreased with cold storage duration. On the contrary, the pre-oviposition period (fig. V) increased, both scaling up the storage days and advancing the harvesting. On the first day after isolation, 43.5% of the fertile females (110 out of 253) laid 6.3% of the total amount of eggs laid during the period in which fecundity was recorded (6 days) (fig. VI).

Table 3. Survival (%) of *Phytoseiulus persimilis* females after isolation at 25°C. Different letters indicate significant differences in comparison with the QC standard proposed by the EC-Concerted Action guideline (χ^2 test).

Day of harvest from mass-production	Cold storage (days)	n. females	Survival (%) after isolation					QC standard
			day 1	day 2	day 3	day 4	day 5	
1	0	30	100	100	73 ^a	50 ^b	37 ^b	80 ^a
3	0	30	100	97	90	70 ^a	43 ^b	
5	0	30	97	97	87	83	63 ^a	
7	0	30	97	97	97	97	77 ^a	
1	3	30	100	97	63 ^a	50 ^b	37 ^b	
3	3	30	100	93	73 ^a	43 ^b	23 ^b	
5	3	30	100	100	93	67 ^a	63 ^a	
7	3	30	100	97	90	80	77 ^a	
1	7	30	100	100	90	67 ^a	50 ^b	
3	7	30	100	90	83	67 ^a	53 ^b	
5	7	30	97	90	87	57 ^b	47 ^b	
7	7	30	100	100	93	73 ^a	57 ^b	
1	14	30	100	87	57 ^b	33 ^b	23 ^b	
3	14	30	100	100	83	70 ^a	33 ^b	
5	14	30	100	100	67 ^a	53 ^b	30 ^b	
7	14	30	100	97	83	80	50 ^b	

Table 4. Reproductive activity of *Phytoseiulus persimilis* females isolated at 25°C after cold storage.

Day of harvest from mass-production	Cold storage (days)	n. females	Egg-laying females (%)	Pre-oviposition (days)	n. eggs laid/fertile female up to day 6
1	0	30	53.3	0.6 ± 0.5	9.4 ± 9.5
3	0	30	56.7	0.5 ± 0.5	7.8 ± 6.2
5	0	30	66.7	0.9 ± 0.9	14.0 ± 8.5
7	0	30	76.7	1.1 ± 0.8	14.8 ± 8.2
1	3	30	43.3	0.2 ± 0.6	10.0 ± 8.7
3	3	30	43.3	0.5 ± 1.1	5.0 ± 6.4
5	3	30	60.0	0.6 ± 1.0	16.8 ± 8.5
7	3	30	73.3	0.8 ± 0.8	12.7 ± 7.8
1	7	30	50.0	0.3 ± 0.5	9.0 ± 7.3
3	7	30	40.0	0.3 ± 0.5	10.0 ± 6.4
5	7	30	46.7	0.8 ± 0.9	9.6 ± 7.8
7	7	30	63.3	1.1 ± 0.6	13.0 ± 8.6
1	14	30	33.3	0.6 ± 0.8	5.4 ± 7.8
3	14	30	50.0	1.0 ± 0.9	7.3 ± 8.4
5	14	30	40.0	0.8 ± 0.4	9.5 ± 6.8
7	14	30	46.7	1.4 ± 0.9	11.7 ± 7.7

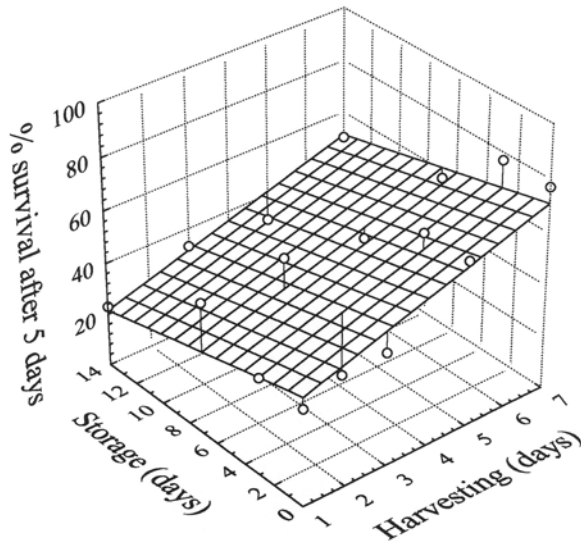


Figure II. Survival (%) of *Phytoseiulus persimilis* females at day 5 after isolation at 25°C.

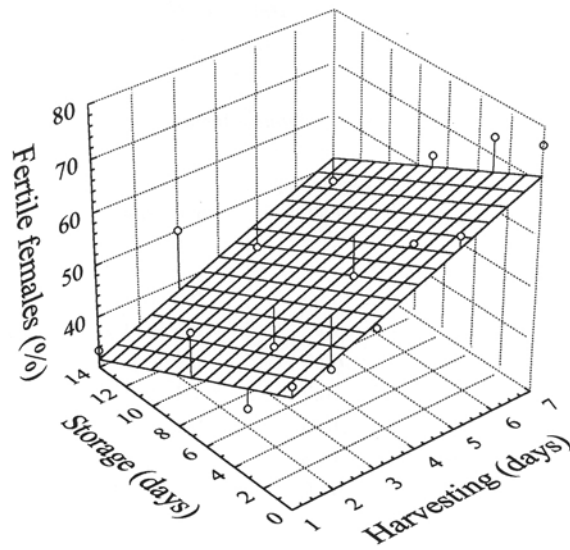


Figure III. Egg-laying females (%) of *Phytoseiulus persimilis* during isolation period at 25°C, after cold storage.

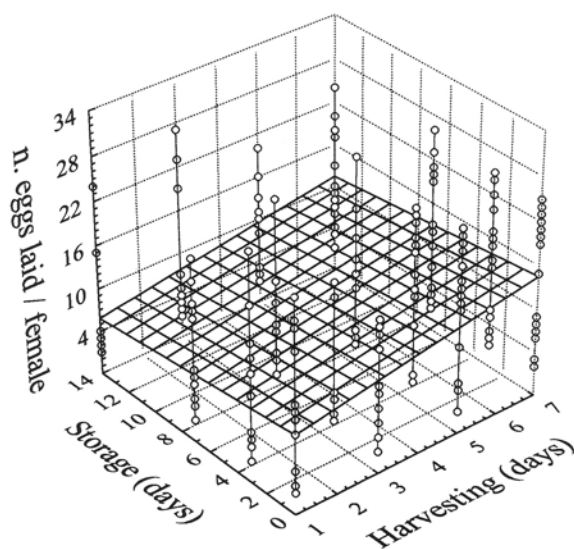


Figure IV. Total oviposition (n. eggs/fertile female) of *Phytoseiulus persimilis* during the isolation period (up to 6 days), after cold storage.

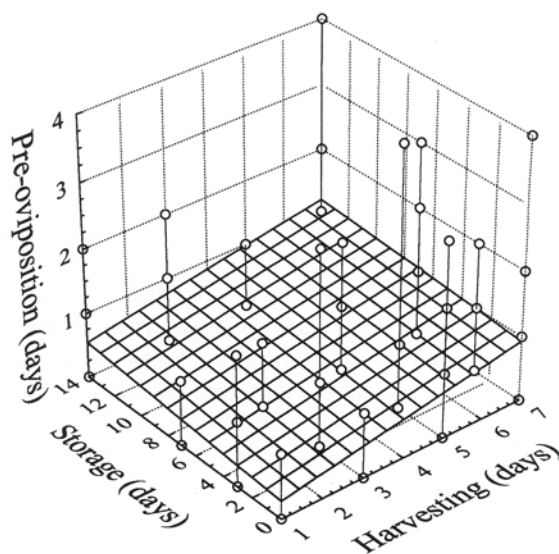


Figure V. Pre-oviposition period of *Phytoseiulus persimilis* females isolated at 25°C, after cold storage.

Table 5. Regression statistics computed for the regression model: $y = a + b_1x_1 + b_2x_2$.

Parameter	$a \pm se$	$b_1 \pm se$	$b_2 \pm se$	R ²	p
Survival at day 5 after isolation (%)	36.56 ± 6.29**	4.91 ± 1.20**	-1.42 ± 0.51*	0.65	< 0.01
Fertile females (%)	47.98 ± 3.98**	3.27 ± 0.76**	-1.41 ± 0.32**	0.74	< 0.01
Pre-oviposition period (days)	0.17 ± 0.12 n.s.	0.11 ± 0.02**	0.02 ± 0.01*	0.10	< 0.01
n. eggs laid/female up to day 6	8.06 ± 1.22**	0.94 ± 0.22**	-0.23 ± 0.10**	0.09	< 0.01

** : $p < 0.01$; * : $p < 0.05$; n.s.; $p > 0.05$.

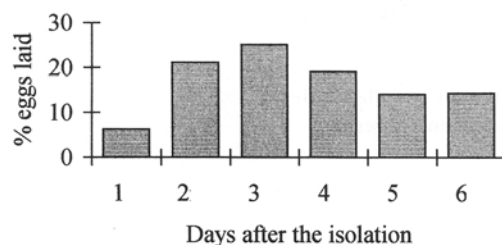


Figure VI. Daily oviposition (% of the total eggs laid) of *Phytoseiulus persimilis* females during the isolation period at 25°C, after cold storage.

Experiment 2. Activity at 25° C and 22° C. Table 6 reports survival of *P. persimilis* females at 25°C and 22°C, from day 1 to day 5 after isolation and table 7 summarizes the oviposition activity of the same females. Unlike at 25°C, survival of females at 22°C was always higher or not significantly different from the QC standard (>80% after 5 days). Considering all the females isolated at the same temperature, the pre-oviposition period was longer and the percentage of fertile females was higher at 22°C compared to 25°C; furthermore, a lower percentage of females laid eggs for more than 1 day, but no significant differences were recorded between the number of eggs laid per female at the two temperatures. As recommended by the EC-Concerted Action guideline for this parameter, the number of eggs laid was calculated from day 2 to day 6 of the oviposition activity at 22°C, while it was calculated from day 1 to day 6 after isolation at 25°C.

Table 6. Survival (%) of *Phytoseiulus persimilis* females after isolation at two temperatures. Different letters indicate significant differences in comparison with the QC standard proposed by the EC-Concerted Action guideline (χ^2 test).

Day from isolation	temperature °C	n. females	Day of harvest from mass-production				QC standard
			1	3	5	7	
1	25	30	100	100	97	97	80 ^a
	22	30	100	100	100	100	
2	25	30	100	97	97	97	
	22	30	97	100	100	100	
3	25	30	73 ^a	90	87	97	
	22	30	90	100	100	97	
4	25	30	50 ^b	70 ^a	83	97	
	22	30	87	93	93	90	
5	25	30	37 ^b	43 ^b	63 ^a	77 ^a	
	22	30	77 ^a	90	87	87	

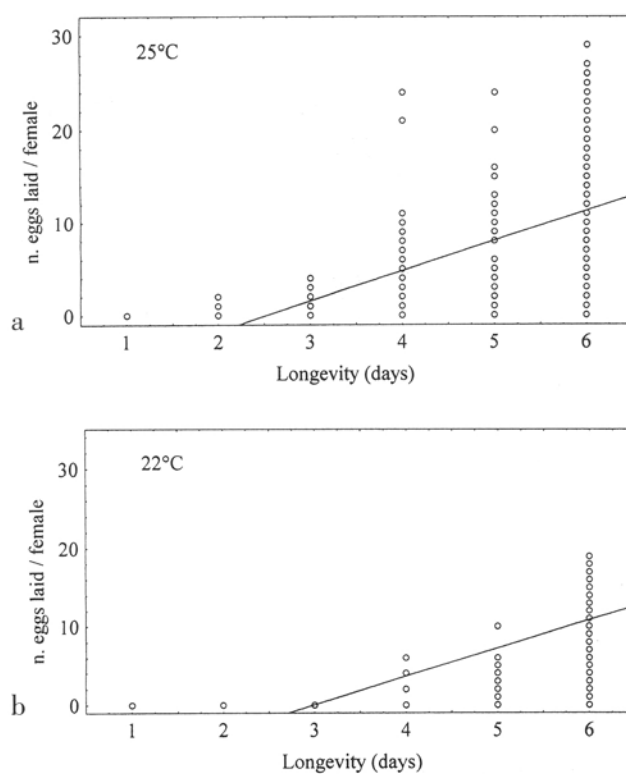


Figure VII. Linear correlations between longevity and number of eggs laid per female of *Phytoseiulus persimilis* at 25°C (a) and 22°C (b).

Table 7. Parameters related with oviposition of *Phytoseiulus persimilis*, at two temperatures. Different letters indicate significant differences (means±sd; ANOVA; χ^2 test) (n. females=30 each temperature tested).

Parameter	temperature °C	Day of harvest from mass-production				
		1	3	5	7	all females
Pre-oviposition period (days)	25	0.6±0.5 ^a	0.5±0.5 ^a	0.9±0.9 ^a	0.9±0.4 ^a	0.6±0.7 ^a
	22	1.1±0.5 ^b	1.4±0.5 ^b	1.4±0.5 ^b	1.3±0.5 ^b	1.3±0.5 ^b
Fertile females (%)	25	53.3 ^a	56.7 ^a	66.7 ^a	76.7 ^a	58.9 ^a
	22	70.0 ^a	73.3 ^a	83.3 ^a	76.7 ^a	75.8 ^b
Females laying eggs min. for 2 days (%)	25	26.7 ^a	40.0 ^a	56.7 ^a	63.3 ^a	62.5 ^a
	22	60.0 ^b	66.7 ^a	76.7 ^a	76.7 ^a	78.3 ^b
n. eggs laid/female	25 *	9.4±9.5 ^a	7.8±6.2 ^a	14.0±8.5 ^a	14.8±8.2 ^a	10.6±8.5 ^a
	22 **	9.3±7.8 ^a	14.4±7.2 ^b	13.2±6.4 ^a	12.2±6.9 ^a	12.4±7.3 ^a

*: from day 1 to day 6 after isolation

**: from day 2 to day 6 of oviposition

Experiments 1 and 2. Correlation between longevity and fecundity. Considering all females tested at 25°C and 22°C, two linear correlations were found between the longevity and the number of eggs laid during the 6 days after isolation for both temperatures (fig. VII; tab. 8).

Table 8. Correlation statistics computed for the longevity and fecundity of all *Phytoseiulus persimilis* females considered in experiments 1 and 2, for the correlation model: $y = a + bx$.

temperature °C	n. females	a ± se	b ± se	R	p
25	480	-8.20 ± 0.84**	3.25 ± 0.18**	0.63	< 0.01
22	120	-10.73 ± 3.08**	3.59 ± 0.56**	0.57	< 0.01

**: p < 0.01

CONCLUSIONS

Survival of predatory mites after cold storage. The predatory mites were sampled 4 times during the harvesting period of 7 days. It has been proved that the duration of storage negatively influenced the survival of *P. persimilis* because low temperature and lack of food probably caused the death of the weaker or older mites. The day of harvest from mass-production appeared to affect mortality similarly to storage duration: survival of predatory

mites harvested at day 1 and stored 14 days appeared to be similar to the non-stored females harvested at days 5 and 7 from mass-production. It is likely that the decrease of prey density on bean plants, as the harvest period increases, causes a loss of fitness of predatory mites and, consequently, their higher mortality during the storage.

Longevity and fecundity after cold storage. As the harvest period increased, survival of predatory mites during storage decreased, but the surviving females showed a higher longevity, fertility, and fecundity after isolation at 25°C. It can be supposed that cold storage selected the most resistant females (they probably are the youngest predators). On the contrary, as the days of storage increased, longevity, fertility, and fecundity of the females isolated at 25°C decreased, probably owing to low temperature and to lack of food. The pre-oviposition period increased as the storage days and the harvest period increased.

Comparison of activity at 25°C and 22°C. At 25°C, 43.5% of the fertile females started oviposition during the first day after isolation, laying 6.3% of the total eggs during 6 days. Moreover, pre-oviposition period was significantly shorter at 25°C rather than at 22°C (and a lower percentage of females laid eggs at least for 2 days). For the QC tests at 25°C, these findings confirm the opportunity of considering the oviposition activity from the initial day up to day 6 after isolation, rather than counting the eggs laid only from day 2 to day 6 of oviposition at 22°C as advised by the EC-Concerted Action guideline. At 22°C, both longevity (>80% at day 5 after isolation) and fecundity (>10 eggs/female) fitted the QC standards proposed by the EC-Concerted Action guideline. Nevertheless, at 25°C, only fecundity fitted the standard, while the longevity standard appeared too high and might be reduced to '>80% at day 3 after isolation'. Steinberg and Cain (1994) tested the quality of three commercially available natural enemies (product QC). Concerning *P. persimilis* fecundity, at 25°C, the Authors recorded results similar to those found in the present study (ca. 10 eggs/female in 5 days after the beginning of egg-laying), but no mortality was recorded during the test period. This is clearly different from the data recorded in the present study, which showed a generally low survival, often lower than 80% after 5 days from isolation. This discrepancy is apparently difficult to explain, and could be explained with eventual differences in the harvesting methods adopted for mass-production.

Correlation between longevity and fecundity. The existence of a linear correlation between longevity and fecundity (during the 6 days after isolation) suggests it should be possible to carry out the QC tests (at least the routine ones), recording only longevity of *P. persimilis* females, avoiding the counting of eggs. The simplification of procedures cuts the need of work for QC of mass-produced predatory mites which is confirmed to be necessary to guarantee the positive results of biocontrol both in protected crops and in open field.

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of beneficial insects: towards more reliable biological pest control' (PL 921076). The authors wish to express their gratitude to Biolab-Centrale Ortofrutticola, Italy for supplying the predatory mites and the relative information, and to Marcella Arnone for her technical assistance.

SUMMARY

The predatory mite *Phytoseiulus persimilis*, endemic in the Mediterranean basin, is mass-reared by several commercial insectaries for the biological control of the red spider mite *Tetranychus urticae* Koch (Acarina Tetranychidae). The aims of this study were: to study the effects of cold storage on the performances of a *P. persimilis* strain field collected in Sicily and mass-reared in biofactory; to evaluate the activity of females at 22°C and 25°C contributing to the definition of a guideline for a quality control (QC) test, within the Concerted Action supported by the Commission of the European Communities (PL 921076). The predatory mites were collected from a mass rearing unit during the harvesting period (4 collections in 7 days). They were then kept in plastic vials within moist vermiculite (ca. 500 mites per vial) and stored in a dark climatic chamber (8±1°C; RH=100%) for 0, 3, 7 and 14 days (16 vials for each collection subdivided in 4 vials for each storage period; 64 vials in total). At the end of the cold storage, 30 females were isolated from each vial (25±1°C; 80±5% RH; L:D=16:8) to test the pre-oviposition period, the longevity and the fecundity. Furthermore, the activity of non-stored females was compared at 25±1°C and 22±1°C. Both the duration of cold storage and late harvests negatively influenced the *P. persimilis* survival; nevertheless, the surviving females coming from the late harvests showed a higher longevity, fertility and fecundity compared with the females coming from the first ones. Considering all the predators isolated at 25°C, 43.5% of the fertile females started to lay eggs the first day after isolation; moreover, the pre-oviposition period was significantly lower compared with 22°C and a lower percentage of females laid eggs for more than one day. These results suggest the opportunity, for the QC tests conducted at 25°C, of considering the eggs laid from day 1 to day 6 after isolation, rather than the eggs laid from day 2 to day 6 of oviposition (as proposed for tests at 22°C), allowing for a reduction in the time needed for checks. At 22°C, the results fitted the QC standards proposed for longevity (>80% after 5 days) and fecundity (>10 eggs/female); while, at 25°C, the results fitted only the fecundity standard. The existence of a linear correlation between longevity and fecundity during the 6 days after isolation (both at 22°C and 25°C) indicates that the work necessary to carry out the QC tests can be reduced by recording only the longevity of *P. persimilis*.

Controllo di qualità e conservazione al freddo di *Phytoseiulus persimilis* Athias-Henriot (Acarina Phytoseiidae)

RIASSUNTO

Phytoseiulus persimilis è un predatore originario del bacino del Mediterraneo che viene allevato da varie biofabbriche per la lotta biologica contro *Tetranychus urticae* Koch (Acarina Tetranychidae). Scopi della ricerca sono stati: studiare l'influenza della conservazione al freddo sulla qualità di un ceppo di *P. persimilis* raccolto in Sicilia e allevato in biofabbrica; valutare l'attività delle femmine a 22°C e 25°C per contribuire alla definizione di una linea-guida per un test di controllo di qualità (QC), nell'ambito dell'Azione Concertata finanziata dalla Commissione delle Comunità Europee (PL 921076). I predatori sono stati prelevati a giorni alterni da una serra di produzione massale (4 prelievi nei 7 giorni di raccolta). Quindi sono stati conservati in fiale con vermiculite umida (ca. 500 individui per fiala) e posti in una camera climatica buia a 8±1°C, UR=100% per 0, 3, 7, 14 giorni (16 fiale per ciascuna raccolta suddivise in 4 fiale per ogni durata di stoccaggio; totale 64 fiale). Al termine della conservazione al freddo, 30 femmine per ogni fiala sono state isolate a 25±1°C; UR=80±5% e L:D=16:8 per rilevare il periodo di pre-ovideposizione, la longevità e la fecondità. Inoltre è stata confrontata l'attività di femmine non conservate al freddo a 25±1°C e 22±1°C. La sopravvivenza di *P. persimilis* è stata influenzata negativamente sia dalla durata della

conservazione al freddo che dal progredire delle raccolte; tuttavia, le femmine sopravvissute provenienti dalle ultime raccolte hanno mostrato una maggiore longevità, fertilità e fecondità rispetto a quelle provenienti dalle prime. Considerando tutti i predatori isolati a 25°C, il 43,5% delle femmine fertili ha iniziato a deporre uova già il primo giorno dopo l'isolamento; inoltre il periodo di pre-ovideposizione è risultato più breve rispetto a 22°C, così come è risultata inferiore la percentuale di femmine che ha ovideposto per più di 1 giorno. Per i test di QC a 25°C, appare quindi consigliabile considerare il numero di uova deposte dal giorno 1 al giorno 6 dopo l'isolamento, invece che dal giorno 2 al giorno 6 di ovideposizione (come proposto per i test a 22°C), riducendo la durata delle osservazioni. A 22°C, sono stati rispettati gli standard di qualità proposti per la longevità (>80% dopo 5 giorni) e la fecondità (>10 uova/femmina); mentre a 25°C lo standard è stato rispettato solo per la fecondità. L'esistenza di una correlazione lineare tra la longevità e la fecondità nei 6 giorni seguenti l'isolamento (sia a 22 che a 25°C) indica che il lavoro necessario alla conduzione dei test di QC può essere ridotto registrando la sola longevità di *P. persimilis*.

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