Boll. Ist. Ent. "G. Grandi" Univ. Bologna, 50: 243-252, 1996.

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Comparative Study of Strains of *Phytoseiulus persimilis* Athias-Henriot (Acarina Phytoseiidae). II. Influence of Mass-Rearing on Population Growth. (*)

Introduction

The predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acarina Phytoseiidae) is an indigenous species in the Mediterranean area and Galazzi and Nicoli (1996) reported a list of findings in several countries, including Algeria (Athias-Henriot, 1957) and Sicily in Italy (Lombardini, 1959). *P. persimilis* is now the most widely used beneficial agent for the biocontrol of the red spider mite *Tetranychus urticae* Koch (Acarina Tetranychidae). Small-scale applications were started in 1968, and in 1990 mass-reared *P. persimilis* was used in ca. 7,000 hectares of protected crops world-wide (van Lenteren *et al.*, 1992). In Italy, the initial releases of *P. persimilis* for biological control took place in Sicily using indigenous predators (Nucifora *et al.*, 1983; Vacante and Nucifora, 1987) while the first releases of predatory mites mass-reared in a northern European biofactory date to 1985 in the Po Valley (northern Italy) (Celli *et al.*, 1987).

The opportunity of mass-rearing and releasing strains collected in the Mediterranean area and showing good performances both in greenhouse and in open field crops could provide a spin-off for the biological control of the red spider mite (Benuzzi and Nicoli, 1991). Owing to the results of the first part of the comparative study carried out in the laboratory (Galazzi and Nicoli, 1996), a strain field-collected in Sicily initially appeared more suitable for biocontrol compared to two strains mass-reared in two different biofactories. Nevertheless, the same strain appeared to be negatively affected by ca. 10 generations of laboratory rearing, conditions in which a limited stock of 500-1,000 predators was maintained; this prompted the investigation of the extent to which the decrease in the performances can be imputable to the selection pressure due to the rearing procedures. Therefore, the second part of the comparative study was designed to evaluate if the continuous mass-rearing in a biofactory can negatively influence

(*) Accepted for publication: September 18, 1996.

the population growth indexes of *P. persimilis*, therefore indicating the opportunity of periodically substituting the mass-reared strains with newly field-collected predators.

Among the demographic indexes, the value of r_m characterises the intrinsic capacity of population increase: a crucial factor to the success of Phytoseiidae predators in the biological control of the red spider mite. This index must be regarded as the best available single description of the population growth potential of a species under given conditions (Southwood, 1966). Sabelis (1985) reported demographic indexes for *P. persimilis* calculated by Takafuji and Chant (1976) and by Badii and McMurtry (1984), underlining the differences in the r_m recorded in these studies. Concerning the definitions of r_m and other calculated indexes (R_o, T_c, r_c, λ and T) we followed Southwood (1966): r_m (intrinsic rate of natural increase) is the instantaneous growth coefficient expressed when the population is growing in an unlimited environment and the age structure has become stable; R_o (net reproductive rate) describes the number of times a population multiplies per generation; T_c (cohort generation time) is the mean age of the mothers in a cohort at the birth of the female offspring; r_c (capacity for increase) is the rate of increase calculated by the approximate method and is a valuable description of the rate of animal multiplication; λ (finite rate of natural increase) is the number of times the population increases per unit of time. When the generations are clearly distinct, the species capacity for increase (r_c) closely approximates its intrinsic rate of natural increase (r_m), but when generations overlap, as in *P. persimilis*, and the reproductive period is long or R_o high, the intrinsic rate of increase (r_m) will be higher than the capacity for increase (r_c) (Southwood, 1966).

Materials and Methods

Origin of the strains. The experiment was divided into three laboratory tests carried out from December 1993 to October 1994 using strains of *P. persimilis* originally field-collected in Siracusa, Sicily (37°N latitude), in a farm where mass-reared predatory mites were never released (Antonio Amore, pers. comm.) (tab. 1), and then mass-produced in climatic-controlled glasshouses at the Italian biofactory "Biolab-Centrale Ortofrutticola" in Cesena, using French bean, *Phaseolus vulgaris* L., as host plant of *T. urticae*. At the beginning of each production cycle in winter, the "new" strain was obtained collecting several hundred predatory mites in field, pre-multiplying them separately for a few generations to get the *inoculum* necessary to start the mass-production, and then completely replacing the "old" strain. After the pre-multiplication phase, several thousand *P. persimilis* were continuously maintained during the whole production cycle (from March to October).

Strain BIO93 was tested at the end of the production cycle (test 1); BIO94 was tested twice: immediately after the pre-multiplication phase (test 2) and at the end of the cycle (test 3); SIN94 was tested using the progeny of the predatory mites immediately after the collection in field (test 3).

Laboratory rearing. Predators supplied by Biolab were reared for one generation (ca. 10 days) in a climatic chamber at 20-24°C, RH=80±10% and

Table 1 - *Phytoseiulus persimilis* strains (all originally collected in the same farm in Sicily) and phase of the mass-production in which predators were tested.

Strain	History of the strain	Test number	Test period	Phase of mass-production
BIO93	Mass-reared at Biolab in 1993	1	December 1993	End cycle
BIO94	Mass-reared at Biolab in 1994 (BIO94A)	2	March 1994	Start cycle (after pre-multiplication)
	Mass-reared at Biolab in 1994 (BIO94B)	3	October 1994	End cycle
SIN94	First generation after collection in field	3	October 1994	Field collection (before pre-multiplication)

photoperiod L:D=16:8. The rearing unit was a plastic basket, upside-down in a plastic tray, containing bean leaves infested by *T. urticae*; water in the tray prevented the mites from escaping and kept the leaves fresh (Osakabe *et al.*, 1988).

Experimental procedure. The tests were run in a climatic chamber at 25±1°C, RH=80±5% and L:D=16:8 photoperiod on pairs of newly-moulted adults collected during mating in a uniform age rearing. Each pair was isolated in a Plexiglas cylindrical cage (4.0 cm height x 4.0 cm diameter x 0.2 cm thickness), featuring a cap fitted with a stainless steel mesh disk (2.5 cm diameter, 201 mesh, 36% air permeability). A disk of bean leaf, abaxial surface upwards, was placed on a 0.6 cm layer of agar gel at the bottom of each cage and was infested *ad libitum* by *T. urticae*. The male was kept only during the initial three days in the cage. The number of eggs laid per day was recorded throughout the lifetime of each female, the progeny of all females was reared until the end of the development, in order to determine the sex of the new adults.

Calculation of indexes. The capacity for increase (r_c) and the intrinsic rate of natural increase (r_m) were calculated following Southwood (1966). To calculate the growth rates, the data on the developmental time (109.5±14.5 hours; mean±sd) and the pre-imaginal survival (94.4%) of females were obtained from the previous part of the study, when another strain collected in the same area in Sicily was tested at 25±1°C, RH=80±5% and photoperiod L:D=16:8 (Galazzi and Nicoli, 1996). Therefore, day 5 from egg-hatching was considered the first day of adult life.

Statistical analysis. Analysis of variance, followed by Tuckey's test, was applied to the parameters. The percentages were compared by the Chi-square test.

Results

Biological parameters. Figure I shows the oviposition trends of the strains recorded during the three tests and tables 2 and 3 report the data recorded for the females' adult life. All strains showed similar trends of oviposition and in

some females the egg-laying activity lasted over two weeks. Concerning the oviposition activity (tab. 2) and the biological parameters related to sex-ratio (tab. 3), significant differences were found between strains:

1. BIO94A showed significantly lower longevity, total oviposition, and percentage of females in the vital progeny compared to BIO93;

2. BIO94B and SIN94 showed a longer pre-oviposition period compared to BIO94A and BIO93.

3. SIN94 showed a higher number of eggs laid per day of oviposition compared to BIO94A.

No significant differences were found in the percentage of egg-laying females, the duration of oviposition, the number of eggs laid per fertile female, the number of eggs laid per day of life, the vital progeny per fertile female, and the female progeny per fertile female.

These data indicate that the strain pre-multiplied for few generations in the biofactory (BIO94A) showed lower performances compared to the other strain mass-reared for one year (BIO93) and the just field-collected strain (SIN94).

Demographic indexes. Figures II and III show the trends of the female proportion survival (l_x) and the female progeny per fertile female (m_x). The demographic indexes for the *P. persimilis* strains are listed in table 4. The capacity for increase (r_c) and the intrinsic rate of natural increase (r_m) are also reported in figure IV.

Relatively slight differences in the demographic indexes were recorded: BIO93 showed the apparently highest intrinsic rate of natural increase (r_m), while SIN94 showed the apparently highest capacity for increase (r_c) (fig. IV). When tested at the start of the production cycle, the predators of strain BIO94 (BIO94A) showed lower values, compared to the predators of the same strain at the end of the production cycle (BIO94B), for: intrinsic rate of increase (r_m); capacity for increase (r_c); finite rate of natural increase (λ); net reproductive rate (R_o); mean age of the mothers in a cohort at the birth of female offspring (T_c) and generation time (T) (tab. 4).

The just field-collected strain, SIN94, showed higher r_c, r_m, and λ compared to the mass-reared BIO94B at end cycle. The net reproductive rate (R_o) of SIN94 was similar to BIO94B, but apparently higher than the same strain at the start of the production cycle (BIO94A). SIN94 showed intermediate values in the mean age of the mothers in a cohort at the birth of female offspring (T_c) and in the generation time (T) between the mass-reared strain at the start (BIO94A) and at the end (BIO94B) of the production cycle (tab. 4).

The strain tested in 1993 at the end of the mass-production cycle (BIO93) showed, compared to the other strains, the highest net reproductive rate (R_o), intrinsic rate of increase (r_m), cohort generation time (T_c), and finite rate of natural increase (λ), while it showed a lower capacity of increase (r_c) and a shorter generation time (T) compared to (SIN94) and BIO94B, respectively (tab. 4).

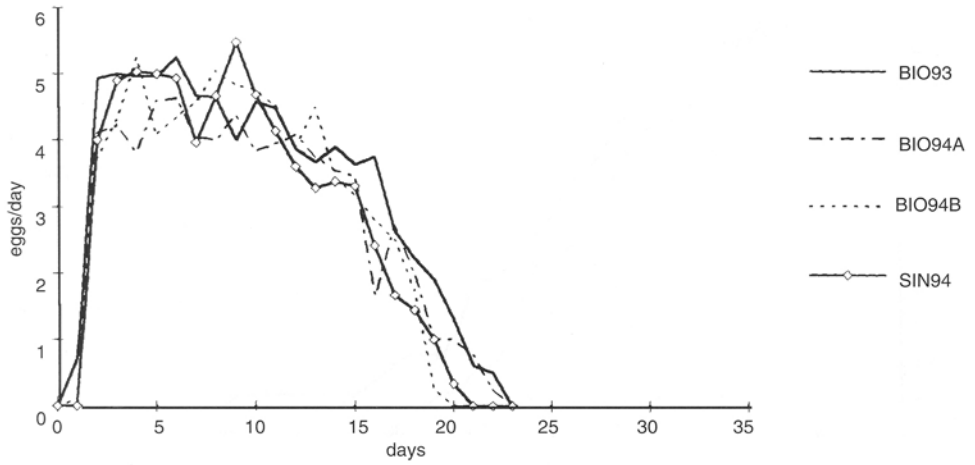

Figure I. Daily oviposition of the *Phytoseiulus persimilis* strains.

Table 2. - Performances of the *Phytoseiulus persimilis* strains originally collected in the same area in Sicily after various periods of mass-rearing (means±sd); different letters indicate significant differences ($p<0.05$).

Strain	Test 1 BIO93 (end cycle)	Test 2 BIO94A (start cycle)	Test 3 BIO94B (end cycle)	Test 3 SIN94 (just field-collected)
N. females	30	30	30	30
Egg-laying females (%)	100[a]	90.0[a]	100[a]	100[a]
Longevity (days)	16.6±6.9[a]	11.8±7.3[b]	15.7±6.9[ab]	13.6±5.2[ab]
Pre-oviposition period (days)	0.5±0.5[a]	0.5±0.5[a]	1.0±0.4[b]	1.0±0.0[b]
Oviposition period (days)	13.5±5.2[a]	11.7±7.3[a]	11.9±4.8[a]	11.0±4.9[a]
Total eggs laid / female	61.1±23.9[a]	39.5±29.4[b]	53.5±23.8[ab]	51.3±22.6[ab]
Total eggs laid / fertile female	61.1±23.9[a]	45.6±29.4[a]	53.5±23.8[a]	51.3±22.6[a]
Eggs / day of life / fertile female	3.7±0.6[a]	3.3±1.1[a]	3.3±0.8[a]	3.6±0.8[a]
Eggs / day of oviposition	4.5±0.5[ab]	4.1±0.7[a]	4.3±0.6[ab]	4.6±0.6[b]

Table 3. - Traits (means±sd) of the progeny of the *Phytoseiulus persimilis* strains; different letters indicate significant differences ($p<0.05$).

Strain	Test 1 BIO93 (end cycle)	Test 2 BIO94A (start cycle)	Test 3 BIO94B (end cycle)	Test 3 SIN94 (just field-collected)
Egg-laying females (%)	100[a]	90[a]	100[a]	100[a]
Vital progeny / fertile female	56.4±23.3[a]	42.8±25.3[a]	51.0±23.3[a]	49.4±21.8[a]
Female progeny / fertile female	49.4±21.1[a]	36.7±22.7[a]	42.9±19.9[a]	41.6±19.0[a]
Females in the vital progeny (%)*	87.4±6.6[a]	81.4±11.5[b]	83.4±4.2[ab]	83.3±4.7[ab]
Progeny sex-ratio (females/males)	7.1	6.0	5.3	5.3

* Only females with a vital progeny ≥10 were considered.

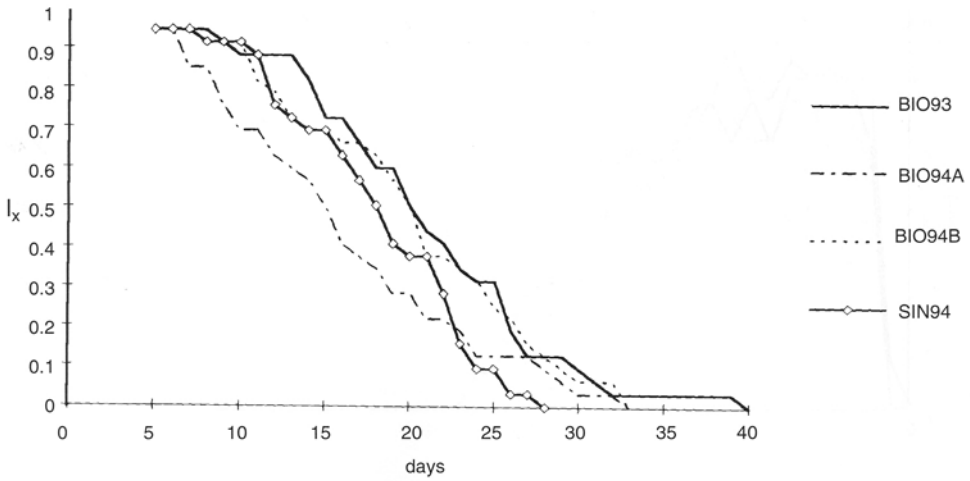

Figure II. Female survival rate (l_x) of the *Phytoseiulus persimilis* strains tested.

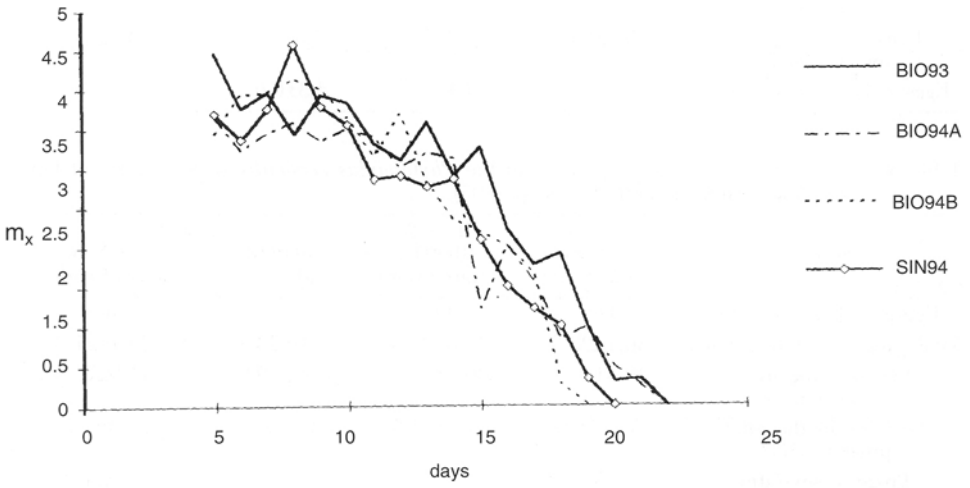

Figure III. Female progeny per fertile female (m_x) of the *Phytoseiulus persimilis* strains tested. Day 5 = first day of the adult life.

Table 4. - Demographic indexes of the strains of *Phytoseiulus persimilis* tested.

Strain	R_o	T_c	r_c	r_m	T	λ
BIO93 (end cycle)	46.7	12.6	0.304	0.395	9.7	1.485
BIO94A (start cycle)	30.1	11.7	0.291	0.363	9.4	1.438
BIO94B (end cycle)	40.5	12.4	0.300	0.376	9.8	1.456
SIN94 (after collection in field)	39.2	11.9	0.309	0.383	9.6	1.466
Badii and McMurtry, 1984*	-			0.374	-	1.453
Takafuji and Chant, 1976*	63.2			0.317	13.1	1.373

* Reported by Sabelis (1985) at ca. 25°C.

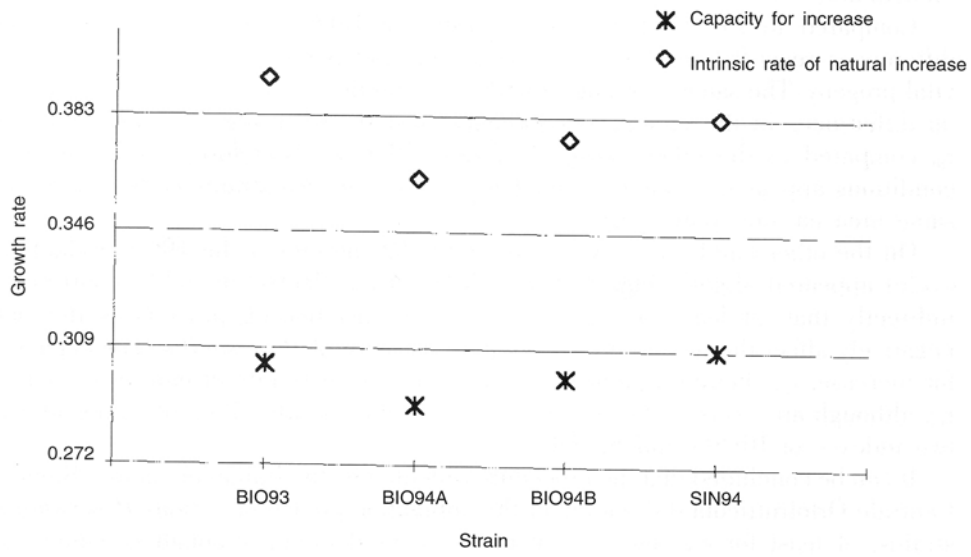

Figure IV. The capacity for increase (r_c) and the intrinsic rate of natural increase (r_m) of the *Phytoseiulus persimilis* strains tested.

Discussion and conclusions

The aim of this experiment was to evaluate the effects of the selection pressure, induced by mass-rearing, on some biological parameters and on the population growth indexes.

For all the Sicilian strains tested, the intrinsic rates of natural increase, r_m, were relatively close to the value calculated by Badii and McMurtry (1984) and higher than that reported by Takafuji and Chant (1976).

Some differences were recorded in the r_m values of the tested strains: the mass-reared BIO93 showed the highest r_m, followed by the just field-collected SIN94, the mass-reared BIO94 at end cycle (BIO94B) and the same strain at start cycle (BIO94A). It can be noted that the mass-reared BIO94 showed a higher value of

r_m at the end of the production cycle compared to the same index recorded at start cycle, indicating that 8 months in the mass-rearing (ca. 25 generations) did not affect the performances of the predators. On the contrary, the increase in the r_m value could indicate a selective process of adaptation induced by the mass-rearing conditions. The critical phase of the production cycle appeared to be the pre-multiplication period, when a relatively small number of field-collected predatory mites were initially multiplied to obtain the stock necessary to start the routine mass-rearing procedures; during the pre-multiplication, the risk of bottle-necks induced by uncorrect procedures was probably high. The existence of this critical point is indirectly confirmed by the high r_m recorded for the predators just collected in field (SIN94), compared to the low r_m of BIO94A that was tested immediately after the pre-multiplication phase.

Compared to BIO93, the lower r_m value of BIO94A keeps pace with the differences recorded in longevity, oviposition, and percentage of females in the vital progeny. The same strain at the end of the production cycle (BIO94B) showed no differences in the biological parameters tested and a slightly lower value of r_m compared to the other end-cycle strain (BIO93). Therefore, similar rearing conditions appear to induce similar performances in two strains collected in the same area ca. one year apart.

On the other hand, the r_m value of BIO93 (at the end of the 1993 production cycle) appeared slightly higher than SIN94 (just collected in field), confirming indirectly that, at least for ca. one year, the mass-rearing procedures did not negatively affect the potential of population increase of *P. persimilis*. The capacity for increase, r_c, showed a pattern similar to the intrinsic rate of natural increase, r_m, although an inversion has been recorded in the slightly different values of the two indexes of BIO93 and SIN94.

It can be concluded that the procedures adopted by the Italian biofactory Biolab-Centrale Ortofrutticola did not affect the population growth of various *P. persimilis* strains, at least for ca. one year of the mass-production, although it cannot be excluded that a longer period could have negative consequences, particularly when bottle-necks in the number of predators occur, as reported in the previous part of the study; in fact small laboratory rearings (500-1,000 predators maintained in stock) induced a quick decline in the performances of adult females (Nicoli and Galazzi, 1996). Therefore, after only one year of mass-production the substitution of the strain does not appear to be necessary if a large stock of predators is maintained, also because some risks may occur after the field collection of wild predatory mites, particularly when the pre-multiplication procedures induce bottle-necks in the population. Routine quality control tests appear necessary to evidence any possible decline in the performances of the mass-reared *P. persimilis* strains.

Acknowledgements

This publication is part of a research project supported by the European Union (ECLAIR Program; Contract no. AGRE-CT91-0062). The authors wish to express their gratitude to Antonio Amore and Massimo Benuzzi (Biolab-Centrale Ortofrutticola, Cesena, Italy) for supplying the tested *P. persimilis* strains and the relative information, and Pasquale Romano for his technical assistance.

SUMMARY

The aim of this work was to evaluate the effects of the selection pressure, induced by the mass-rearing procedures, on biological parameters and population growth indexes, to evaluate the opportunity of substituting periodically the mass-reared strain with field-collected predators. Three laboratory trials were carried out on three *P. persimilis* strains, originally collected in Sicily, in the same farm. The strain mass-reared in 1993 at Biolab-Centrale Ortofrutticola (Cesena - Italy) was tested at the end of the annual mass-production cycle; the strain mass-reared at Biolab in 1994 was tested two times, i.e. at the beginning (March) and at the end (October) of the production cycle; the third strain was field-collected in October 1994 and the progeny immediately tested. The tests were run in a climatic chamber at 25±1°C, RH=80±5% and photoperiod L:D=16:8, on pairs of newly emerged adults isolated during mating. The values of the intrinsic rate of natural increase, r_m, showed slight differences among strains: the strain mass-reared in 1993 (end-cycle) showed the highest $r_m$value (0.395), followed by the just field-collected strain (0.383) and then by the 1994 strain at end-cycle (0.376) and start cycle (0.363), respectively. At the end of the production cycle, the 1994 strain showed a r_m value higher than at start cycle, indicating that 8 months of mass-rearing (with ca. 25 generations) did not negatively affect the performances of the predators. This increase in the r_m value between start and end of the production cycle could indicate a selective adaptation of the strain to the mass-rearing conditions. The critical phase was the pre-multiplication period, when a relatively small number of field-collected predatory mites were reared to obtain the stock necessary to start the routine mass-rearing procedures. The main differences in the r_m values keep pace with significant differences in longevity, oviposition, and percentage of females in the vital progeny. The r_m values calculated for the two end-cycle strains showed slight differences compared to the just field-collected one, confirming indirectly that ca. one year of mass-rearing did not affect the potential of population increase of *P. persimilis*. Therefore, it can be concluded that the substitution of the mass-reared strains does not appear to be necessary after only one year of production, if a large stock of predators is maintained; furthermore, the collection in the field of wild predators shows some risks, particularly when the pre-multiplication procedures induce bottle-necks in the population.

Studio comparativo su ceppi di *Phytoseiulus persimilis* Athias-Henriot (Acarina Phytoseiidae). II. Influenza dell'allevamento massale sull'accrescimento della popolazione.

RIASSUNTO

Lo scopo di questo studio è stato di valutare gli effetti della pressione selettiva, indotta dall'allevamento massale, sui parametri biologici e sugli indici di accrescimento della popolazione, per valutare l'opportunità di sostituire periodicamente il ceppo allevato con predatori raccolti in campo. Sono state effettuate tre prove di laboratorio su tre ceppi di *P. persimilis*, raccolti in Sicilia nella stessa azienda. Il ceppo allevato al Biolab-Centrale Ortofrutticola (Cesena, Italia) nel 1993, è stato studiato alla fine del ciclo annuale di produzione massale; il ceppo allevato al Biolab nel 1994, è stato studiato due volte, all'inizio (marzo) e alla fine (ottobre) del ciclo di produzione; il terzo ceppo è stato raccolto in campo nell'ottobre del 1994 e la progenie è stata studiata immediatamente. Le prove sono state svolte in una camera climatizzata a 25±1°C, UR=80±5% e fotoperiodo L:D=16:8, su coppie di adulti neomutati isolati durante l'accoppiamento. I valori del tasso intrinseco di incremento naturale, r_m, hanno mostrato leggere differenze tra i ceppi: il ceppo proveniente dall'allevamento massale del 1993 (fine ciclo) ha mostrato il più alto valore di r_m (0,395), seguito dal ceppo appena raccolto in campo (0,383) e poi dal ceppo del 1994 a fine ciclo (0,376) e inizio ciclo (0,363) rispettivamente. Alla fine del ciclo produttivo, il ceppo del 1994 ha mostrato un valore di r_m più alto rispetto allo stesso a inizio ciclo; questo suggerisce che 8 mesi di allevamento massale (pari a ca. 25 generazioni) non influenzano negativamente le performance dei predatori, ma che anzi si è probabilmente avuto un adattamento selettivo alle condizioni dell'allevamento massale. La fase critica è risultata il periodo di pre-moltiplicazione, quando un numero relativamente piccolo di acari predatori raccolti in campo è stato allevato per ottenere il quantitativo necessario a iniziare le procedure di routine di allevamento massale. Le più rilevanti differenze nel valore di r_m, sono andate di pari passo con le differenze significative nella longevità, ovideposizione e percentuale di femmine nella progenie vitale. I valori di r_m calcolati per i due ceppi a fine ciclo hanno mostrato leggere

differenze, confrontati con il ceppo appena raccolto in campo, confermando indirettamente che un anno di allevamento massale non influenza il potenziale di incremento della popolazione di *P. persimilis*. Quindi, si può concludere che la sostituzione dei ceppi allevati non sembra necessaria dopo solo un anno di produzione, se è stato mantenuto un elevato numero di predatori; inoltre, la raccolta in campo di predatori selvatici comporta dei rischi, in particolare quando le procedure di pre-moltiplicazione provocano colli di bottiglia nella popolazione.

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