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THE ESTABLISHMENT OF *RHYZOBIUS FORESTIERI* [*COL. : COCCINELLIDAE*] IN GREECE AND ITS EFFICIENCY AS AN AUXILIARY CONTROL AGENT AGAINST A HEAVY INFESTATION OF *SAISSETIA OLEAE* [*HOM. : COCCIDAE*]

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Introduction releases of the exotic coccinellid predator Rhyzobius forestieri Mulsant were made in 22 localities throughout Greece. The releases were made on olive, citrus, fig and almond trees infested with different Coccidae scales. In 9 of these localities, R. forestieri was recovered 2-5 months later. The most important factor for the successful establishment of R. forestieri seems to be an abundance of scales on the trees.

To study the ability of R. forestieri to enhance overall predation, 3 releases were made in an olive grove heavily infested with Saissetia oleae Olivier on Chios island during 1982 and 1983. The experiment began in June 1982. By April 1983, the S. oleae infestation was clearly in the process of being controlled by the coccinellid.

A native coccinellid, *Exochomus quadripustulatus* L., contributed little during this period because of its obligatory estival diapause. The other main coccinellid, *Chilocorus bipustulatus* L., made an appreciable contribution toward achieving control during the summers of 1982 and 1983, but it was affected from July onward by increasing parasitism of its larvae. Both species are quiescent during the winter months.

The introduced species R. forestieri was readily established in the olive grove and multiplied quickly. One month after the initial release, R. forestieri larvae made up most of the coccinellid larval population in the field. Thereafter until April 1983, R. forestieri larvae and adults comprised the dominant coccinellid population in the olive grove. During the winter months, R. forestieri remains active as long as temperature exceeds 8 °C.

The important increase in *R. forestieri's* population size between July 1982 and April 1983 coincided with the increased rate of predation on *S. oleae*. This suggests that *R. forestieri* played a key role in bringing the *S. oleae* infestation under control during this period. After April 1983, however, the *R. forestieri* population diminished rapidly and *C. bipustulatus* became the dominant coccinellid in the olive grove. It is believed that scarcity of prey was the cause of this reversal : by April 1983, the density of *S. oleae* in the olive grove had already been reduced by about 100 times.

In the Mediterranean zone, serious problems are caused by scales of the family *Coccidae* on several economically-important tree crops. The black scale *Saissetia oleae* Olivier for example is a serious pest in olive groves; *S. oleae, Coccus hesperidum* L. and occasionally *Coccus pseudomagnoliarum* Kuwana, often become serious pests in citrus culture ; and *Ceroplastes rusci* L. often causes serious damage to fig trees.

On all these trees, coccinellid predators are important control agents of the above scales, the most important native coccinellids predators of *Coccidae* scales in Greece being *Exochomus quadripustulatus* L. and *Chilocorus bipustulatus* L. (Argyriou & Katsoyannos, 1976). *E. quadripustulatus* is a typically univoltine species. Oviposition begins in late February. The young adults, 10 to 20 days after they emerge in June, enter an obligatory estival diapause which lasts until the end of September (Katsoyannos, 1976). *C. bipustulatus* has 2 to 3 generations per year depending on the abundance of prey.

The overall effectiveness of the native coccinellids diminishes during summer, firstly because E. quadripustulatus has an adult diapause and secondly because the active population of C. bipustulatus is disadvantaged by high parasitism on the larvae of its 2nd and 3rd generations. With the intention of enhancing the action of the native complex, the originally Australian ladybeetle Rhyzobious forestieri Mulsant was introduced from California into Greece in 1981.

MATERIALS AND METHODS

Two types of releases were carried out. The 1st type attempted to establish R. forestieri in as wide a network of localities as possible. The 2nd type was made in order to study the efficiency of the newly-introduced predator as a biological control agent at spots heavily infested with S. oleae. Except for a pilot release made at Delfi during the summer of 1981, all releases were made during Spring 1982 and Spring 1983 in the 22 localities shown in figure 1 and table 1. In each area, 30 to 40 R. forestieri adults were released on each of 1, 2 or more trees, according to the abundance of prey and the number of R. forestieri available.

Samplings were made before the following winter to check for the establishment of the predator on the release plots. The scale populations were also monitored, by visual observations and laboratory examination of samples taken at the time of the release and at the time of the establishment check. The degree of scale infestation on the trees was defined according to the following categories : very light (1-2 scales/leaf), light (3-8 scales/leaf), medium (9-27 scales/leaf) and heavy ≥ 28 scales/leaf).

Favorable conditions for observing the efficiency of R. forestieri as a biological control agent against a heavy infestation of S. oleae were found in an olive grove at Cambos, Chios island, locality No. 2 of figure 1 and table 1. This olive grove was situated in a plain at sea level. It was rectangular in shape, with the long axis oriented East-West. Three sides were enclosed with 3m-high stone walls ; the north side was open. The grove consisted of 97 7-to-8-year-old olive trees of the Megaritiki variety. The were planted in rows 5 metres apart and were 3 to 4 metres tall. The ground beneath the trees was ploughed almost bare many times during the year and no chemical control measures were used during the experimental period. The trees were irrigated regularly. Citrus orchards surrounded this olive grove on all sides.

R. forestieri adults were released 3 times in this orchard, always in the morning. The 1st release was made on 18 June 1982. 150 *R. forestieri* adults were released on 5 olive trees situated in the centre of the orchard and heavily infested with *S. oleae*. During the 2nd release, on 14 July 1982, 750 adults were released on 31 heavily-infested trees throughout the orchard. The final release took place on 7 April 1983; 900 *R. forestieri* were released on 28 trees with a medium or light *S. oleae* infestation.

Samplings were made periodically to check the development of the scale as well as the population density of the main coccinellid predators. Initially, most of the olive trees were heavily infested with *S. oleae*. The infestation was irregularly distributed, being heavier in the interior and lighter toward the boundaries of the orchard. Sooty mold was present on most of the infested trees. Other species of scales were not found in this orchard.

For sampling the S. oleae population, 4 branches of 20 to 30 cm in length, 1 from each compass direction, were cut at shoulder level from every 2nd olive tree. In the laboratory, the leaves were cut off the branches and mixed together. Twenty five leaves of the 1st sample were examined under stereoscope and a further 225 leaves were visually examined and characterized according to the degree of their infestation. From each subsequent sample, more than 200 leaves were examined under stereoscope. The number and developmental stages of living S. oleae individuals, as well as dead, attacked and parasitized ones, were recorded for each leaf. In addition, 10 leafless twigs 20 to 30 cm in length were examined and the number of scales was recorded. A scale attacked by a predator was recognized by the mark left on its dry body after it had been bitten and partially consumed. Sometimes, an entire portion of the body was missing. Scales completely eaten could not be counted. A parasitized scale was distinguished by the larva or pupa of the parasite found inside it or by the round exit hole found on its empty and dry cuticle.

The coccinellids were sampled by beating 4 branches on each of 10 marked trees with a rubber-covered stick over a 1 m^2 cloth screen, then recording the numbers of adults and larvae of coccinellids thus dislodged.

RESULTS

THE ESTABLISHMENT OF R. FORESTIERI

Of the 18 biotopes which were checked 2-5 months after the introduction releases, R. forestieri was found in 9 (table 1). It multiplied well in the 2 olive orchards (No. 1 and No. 2 of table 1) which were most heavily infested with S. oleae. It reproduced fairly well in a citrus orchard heavily infested with S. oleae in Chios island (No. 17), and it was recovered in another (No. 18), lightly infested with both S. oleae and C. pseudomagnoliarum. The presence of R. forestieri was maintained in 3 other biotopes (No. 3, No. 4 and No. 7) in olive groves with medium to light S. oleae infestations.

In 2 biotopes (no. 19 and No. 20) in Calamata, where *R. forestieri* was released on fig trees heavily infested with *C. rusci*, samplings made 2 months later yielded only a small number of adults. Neither larvae nor eggs were found. In an almond orchard (No. 22) in Veria with a light infestation of *Sphaerolecanium prunastri* Fonscolombe, no *R. forestieri* individuals were found during the check conducted 2 months after the release.

In 7 biotopes where *R. forestieri* was released on olive trees bearing medium to light or very light infestations of *S. oleae* (Nos. 5, 6, 8, 9, 10, 11, 16), no *R. forestieri* was found during the establishment checks. In the north of Greece (Chalchidiki), 4 olive tree biotopes (Nos. 12, 13, 14, 15) with light infestations of *S. oleae* on the release date were not checked later for establishment.

THE EFFICIENCY OF R. FORESTIERI AS AN AUXILIARY CONTROL AGENT

The 1st S. oleae samples from Chios olive grove (No. 2 of figure 1 and table 1) were collected on 14 July 1982, almost 1 month after the initial R. forestieri release there. Laboratory examination of these showed that all of the leaves were still infested with scales (table 2). By

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Introduction and establishment of R. forestieri in Greece

TABLE 1

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Fig. 1. The distribution of the introduction-release sites in Greece. The numbers of the localities correspond to those given in table 1 and in the text.

November 1982, only 5 months after the initial release, the *S. oleae* infestation was clearly in the process of being controlled and 30 % of the leaves were free of scales. The average number of scales per infested leaf had diminished by 18.7 times smaller than initially. In April of the following year, about half of the leaves were free of scales. Compared with the initial infestation, the average number of scales per infested leaf had now decreased by 97.6 times and the maximum number of scales per leaf was 19.7 times smaller. The reproductive phase of *S. oleae*, which occurs in June and July, did not change the overall regression of the scale population, so that by September 1983 the olive trees were practically scale-free. Among the few leaves which were still infested, most had only 1 or 2 living scales, 23.7 % had 3 to 8 scales, 4.0 % had 9 to 26 scales and only 0.6 % had 50 to 76 living scales.

In addition to count of scales, visual observations were made to estimate the degree of S. oleae infestation on each olive tree and in the orchard as a whole. Generally, these observations agree with the trend shown by the sampling results presented in table 2. At the time of the 1st and 2nd R. forestieri releases, 54 % of the trees were bearing a heavy or medium infestation with the crowns and branches more or less covered with sooty mold. The remaining 46 % were bearing a light infestation. Ten months later, in April 1983, this situation had changed completely. Only 1 % of the trees had a heavy infestation and 6 % a medium infestation. The remaining 93 % of the trees were bearing a light or very light living infestation, with no evidence of fresh sooty mold on the leaves, although some residual black marks were present on the branches.

TABLE 2

Release (a) (made after sampling)	Adults released No.	Date of sampl	No. of leaves	Infested leaves	Infested leaves %	Living scales per infested leaf				
			examined	No.		x	SD	Min.	Max.	
2nd	750	14 July 82	250 (25 under) stereoscope)	250	100	312.3	124.3	129	533	
-	_	24 Nov. 82	211	150	71.1	16.7	20.8	1	93	
3rd	900	7 Apr. 83	390	200	51.3	3.2	3.4	1	27	
_	-	17 July 83	250	1 21	48.4	9.9	11.6	1	89	
		9 Sept. 83	1002	295	29.4	0.8	3.4	1	76	

The S. oleae infestation in relation to R. forestieri releases in the Cambos-Chios olive grove (locality No. 2)

(a) 1st release, 150 R. forestieri adults, 18 June 1982.

The reduction of the S. oleae infestation in the olive grove at Cambos - Chios can be further analyzed by comparing the mortality of fixed 1st, 2nd and 3rd instar larvae (table 3). Recorded dead individuals were sorted into 3 categories : attacked by predators, parasitized, and dead from unknown causes.

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TABLE 3

Date of sample	No. of <i>S. oleae</i> larvae (L1, L2, L3) counted	Living %	Attacked by predators %	Parasitized %	Dead from unknown causes %
14 July 1982	8974	88.2	3.8	0	8.1
24 Nov. 1982	5306	37.6	47.4	1.8	13.2
7 April 1983	1667	41.3	16.4	6.7	35.6
17 July 1983	2790	88.9	5.3	0.4	5.4
9 Sept. 1983	1626	52.2	31.3	0	16.5

Analysis of the fixed larvae of S. oleae at Cambos - Chios locality No. 2 according to the categories : living, attacked by predators, parasitized and dead form unknown causes

In the samples taken on 24 November 1982, 5 months after the 1st release, the mortality of *S. oleae* from unknown causes was rather low (13.2 %), while almost half of the scale population was destroyed by the activity of predators. The rate of mortality from unknown causes was rather high on 7 April 1983. It is likely that a great number of *S. oleae* larvae which had been attacked by predators and remained dried on the leaves throughout the winter, with their marks obscured by weather and the passage of time, lacked the evidence permitting them to be classified as destroyed by predators. In July, the month which is also the peak of the annual reproductive period of *S. oleae*, the larvae of the scale are very young, mostly of the 1st instar, and are often eaten completely without trace when attacked by predators. This may explain the relatively low rates of predation recorded in July of both 1982 and 1983.

The rate of parasitism, mostly due to the introduced encyrtid (Hymenoptera) parasite *Metaphycus helvolus* Compere (Argyriou & DeBach, 1968), was very low throughout the year and generally unimportant as a control factor in this locality.

The sampling results which show the population development of the introduced predator in relation to the native coccinellids are presented in table 4. A rapid increase of the population of *R. forestieri* occurred during the 5 months following the initial release. In July 1982, *R. forestieri* larvae accounted for the majority of the total larval population in the field. From August through November 1982, *R. forestieri* adults and larvae comprised the majority of *Coccinellidae* found. *R. forestieri* larvae were the only larvae recovered in November; they were still the great majority in April 1983.

In late spring and summer 1983, the size of the R. forestieri population decreased, even though it was temporarily enhanced by the final release (900 adults) made on 7 April. This is probably due to scarcity of prey resulting from the *S. oleae* regression; the same limiting factor appears to have acted against the establishment of R. forestieri in 7 olive-tree biotopes during the introduction releases. The shortage of prey did not inhibit the slower-developing *C. bipus-tulatus* to the same extent.

A continuous presence of adults of the 2 main native predators of S. oleae during the experimental period is shown in the same table. But it was observed that the larval population of E. quadripustulatus disappears during the summer. This can be explained by the fact that the adults of E. quadripustulatus are in their summer diapause. Later, the E. quadripustulatus adults pass through a winter quiescence; they do not oviposit until the following February

(Katsoyannos, 1976). In *C. bipustulatus*, an important larval population measured in August 1982 decreases and then disappears by November. The *C. bipustulatus* adults pass through a winter quiescence and do not oviposit until the following April.

TABLE 4

Date	Total	%				Total	%				
of sample	adults No.				Other	r Coccinellid larvae No.	R. fores- tieri	E. quadri- pustulatus		Othe	
18 Jun 82	29	before releases	62.1	34.5	3.5	36	before releases	94.4	5.6	0	
14 Jul 82	83	- 2.4	26.5	32.5	38.6	210	75.2	5.2	16.2	3.3	
20 Au. 82	99	63.6	2.0	16.2	18.2	92	53.3	0	46.7	0	
6 Oc. 82	91	60.4	0	25.3	14.3	22	68.2	0	31.8	0	
24 No. 82	49	71.4	2.0	22.5	4.1	28	100.0	0	0	0	
7 Ap. 83	54	24.1	33.3	35.2	7.4	26	88.5	11.5	0	0	
8May 83	5	60.0	40.0	0	0	16	25.0	75.0	0	0	
17 Jul. 83	20	20.0	15.0	65.0	0	22	18.2	0	81.8	0	
9 Se. 83	14	14.3	0	71.4	14.3	4	50.0	0	50.0	0	

Composition of the coccinellid population sampled at Cambos - Chios before and after the R. forestieri releases

The rate of parasitism on the larvae of *C. bipustulatus* was important. Thus, on 14 July 1982, one out of 34 (i.e. 3 %) of *C. bipustulatus* larvae were parasitized. Later, however, a considerable increase in the rate of parasitism on the larvae of this species was observed : 46% on 20 August and 91 % on 6 October 1982. In the summer of 1983, the observed rate of parasitism was 18 % on 17 July and 82 % on 9 September. No case of parasitism on *R. forestieri* was recorded during the study period.

DISCUSSION

As the introduction releases showed, R. forestieri was easily established on olive and citrus trees, given an abundant supply of prey. Several bioecological characteristics of R. forestieri indicate that it should be able to build up population rapidly under favorable conditions. These are : its multivoltinism in cages outside the Athens laboratory--6 generations per year, 1 generation per month during the summer (Katsoyannos, 1983) ; its fecundity in laboratory studies-400 to 1000 eggs per female (Katsoyannos, 1983) ; the lack of summer or winter diapause ; the longevity of the adults-3 to 10 months (Katsoyannos, 1983) ; and the lack of parasitism in the localities tested.

The importance of the abundance of prey in the establishment of a released population of R. forestieri became evident from the failure of the predator to establish itself on the 7 checked olive groves bearing from medium to very light S. oleae infestations. Also the importance of

the prey species to the reproductive success of R. forestieri is shown by the failure of the predator to reproduce after it was released in 2 fig orchards heavily infested with C. rusci.

In the absence of any chemical or biological treatment, the regression of a heavy *S. oleae* infestation would normally be expected to take 2, 3 or even more years. Such a regression is usually the result of the combined action of many factors. Parasitism and predation aside, the most important of these factors are the climate (especially the summer heat) and the reduction of available leaf surface resulting from the accumulation of sooty mold and the fall of leaves from the trees (**Orphanidis & Kalmoukos**, 1970). At Cambos-Chios, the climatic conditions were moderate and no extreme heat was recorded during the 2 summers. Also during the 10 months following the 1st *R. forestieri* release, the leaves were gradually cleaned up and the crowns of the trees bore a rich foliage.

The rate of parasitism was always very low. On the other hand, the rate of predation 5 months after the 1st R. forestieri release was relatively high, suggesting that predation was the most important factor in the control of the S. oleae infestation.

The role of E. quadripustulatus was not important during the summer of 1982, because of the obligatory diapause of the adults. The population increased again by April 1983, at which time the S. oleae infestation was already in regression. However E. quadripustulatus certainly contributed to the further reduction of the scale population during the spring of 1983.

The contribution of the tri-voltine C. bipustulatus was appreciable during 1982, but its total population size was always less than 2/3 that of R. forestieri between July 1982 and April 1983, the period during which most of the control of S. oleae occurred. The steadily increasing rate of parasitism on C. bipustulatus larvae from July onward is an important limiting factor for this species. No parasitism was observed on R. forestieri larvae or adults.

During the winter months, both *E. quadripustulatus* and *C. bipustulatus* are in quiescence, while *R. forestieri* remains active as long as the temperature remains above $8 \degree C$ (Katsoyannos, 1983). This was the case at Cambos-Chios during the winter of 1982-1983.

From April 1983 onward, the olive grove was no longer heavily infested with S. oleae and because of the importance of an abundance of prey to R. forestieri, this predator was in a less favorable environment. Although 900 adults were released in April, only a few individuals were recovered from samplings in May 1983 and thereafter. C. bipustulatus seems better able to survive in this condition of prey scarcity than R. forestieri, although it too was affected : in summer 1983, the total population of C. bipustulatus decreased to half or less of its numbers of the previous year.

S. oleae, in Greece, completes only 1 generation and occasionally a partial 2nd generation per year (Canard & Laudého, 1977). In July 1983, a new generation of S. oleae appears in the results given in table 2. The stable number of scales between April and July (when a great increase would normally be expected) is an indication of the efficiency of the remaining predators, although their action is not clearly shown in table 3 because totally-consumed 1st instar larvae of S. oleae could not be counted.

The purpose of the work described in this paper was twofold : 1st, to establish in Greece an efficient exotic predator capable of enhancing the action of the existing native coccinellids; 2nd, to attempt the control of a heavy infestation of *S. oleae* by repeated releases of the introduced predator. The results suggest that *R. forestieri* is particularly suitable for use in the latter case. The key factor seems to be *R. forestieri's* requirement for an abundance of prey. This somewhat limits its ability to be easily established everywhere but, at the same time, favors its use as an auxiliary biological control agent against serious outbreaks of *S. oleae*.

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RÉSUMÉ

L'installation de *Rhyzobius forestieri* [*Col. : Coccinellidae*] en Grèce et son efficacité dans la lutte contre Saissetia oleae [Hom. : Coccidae]

Des lâchers de *Rhyzobius forestieri* Mulsant, coccinelle exotique introduite, ont été réalisés dans 22 localités en divers points de la Grèce. On a procédé à ces lâchers sur des oliviers, des agrumes, des figuiers et des amandiers infestés par différents *Coccidae*. *R. forestieri* a été retrouvé 2-5 mois plus tard dans 9 de ces localités. Le facteur le plus important pour la réussite de l'installation de *R. forestieri* paraît être la présence abondante de cochenilles sur les arbres.

Au cours des années 1982 et 1983, 3 lâchers de *R. forestieri* ont été poursuivis dans une oliveraie de l'île de Chios gravement infestée par *Saissetia oleae* Olivier. Le but de cet essai était d'étudier les possibilités de cette coccinelle pour renforcer l'efficacité de la lutte contre la cochenille au moyen du complexe de prédateurs. L'essai a commencé en juin 1982. En avril 1983 l'infestation des arbres par *S. oleae* était nettement réduite grâce à l'activité des prédateurs coccinellides.

Pendant cette période la contribution de la coccinelle indigène Exochomus quadripustulatus L., à la réduction de S. oleae, n'était pas importante, à cause de sa diapause estivale. La contribution de l'autre coccinelle indigène importante, Chilocorus bipustulatus L., durant l'été 1982 et 1983 était notable, malgré son affaiblissement à partir du mois de juillet à cause d'un taux de parasitisme croissant sur ses larves. Ces 2 espèces indigènes passent l'hiver en quiescence.

L'espèce introduite R. forestieri s'est installée aisément sur les oliviers et s'est multipliée rapidement. Un mois après son premier lâcher, les larves de R. forestieri comptées constituaient la majorité de la population larvaire des coccinelles sur le terrain. Au-delà et jusqu'en avril 1983 continuellement, les larves et les adultes de R. forestieri constituaient la population de coccinelles prédominantes dans l'oliveraie. Pendant les mois d'hiver R. forestieri est en activité, lorsque les températures sont supérieures à 8 °C.

Entre juillet 1982 et avril 1983, l'augmentation de la population de *R. forestieri* était parallèle à l'augmentation du taux de prédatisme noté sur *S. oleae*. Cette coïncidence explique le rôle clé de *R. forestieri* dans la réduction de *S. oleae* pendant cette période. Néanmoins, après le mois d'avril 1983 la population de *R. forestieri* a diminué rapidement et *C. bipustulatus* est devenue la coccinelle de lère importance dans l'oliveraie. Il semble que la rareté de la proie était la cause de ce renversement : En avril 1983, la densité de *S. oleae* sur les oliviers était déjà réduite à peu près de 100 fois.

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