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Possibilities of biological control of the horse chestnut scale insect, *Pulvinaria regalis* Canard (Homoptera: Coccidae), on ornamental trees by releasing its natural enemies

Möglichkeiten zur biologischen Bekämpfung der Wolligen Napfschildlaus, *Pulvinaria regalis* Canard (Homoptera: Coccidae), an Park- und Alleebäumen durch den Einsatz natürlicher Feinde

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Summary

The aim of this study was to investigate possibilities of biological control of the horse chestnut scale insect, *Pulvinaria regalis* Canard (Homoptera: Coccidae), on ornamental trees by releasing its natural enemies. For this purpose, single releases of *Exochomus quadripustulatus* larvae and *Coccophagus semicircularis* as well as *Microterys flavus* adult females and combined release of *E. quadripustulatus* as well as *C. semicircularis* were carried out on lime trees in the centre of Duisburg in 1999 and 2000, respectively. All the three beneficials were reared under defined climatic conditions in the Institute of Phytopathology, University of Bonn.

The release of *E. quadripustulatus* larvae lead to a significant reduction in numbers of *P. regalis* nymphs measured per m^2 leaf area. The release of 20 *E. quadripustulatus* larvae resulted in 2644.3 *P. regalis* nymphs per m^2 while 30 *E. quadripustulatus* larvae resulted in 2250.3 nymphs per m^2. The number of pest was significantly higher in the control treatment with 4298.3 nymphs per m^2. The number of parasitized *P. regalis* nymphs was significantly higher in the *C. semicircularis* females-released plots where it was 9.5 % with 20 and 12.9 % with 30 *C. semicircularis* released females.

A combined release of 20 *E. quadripustulatus* larvae in early summer and 20 *C. semicircularis* females in autumn resulted in a reduction of 1985.8 (46.2 %) *P. regalis* nymphs per m^2 leaf area and an increase in numbers of parasitized nymphs to 610.4 (14.2 %) per m^2 leaf area compared with the control.

After two repetitive releases of *M. flavus* females in spring 2000, a mean parasitization of 53.9 (19.6 %) of *P. regalis* females per tree was observed. *M. flavus* females deposited 1–7 eggs into the adult scale insects, depending on their respective body size. The number of eggs laid by parasitized *P. regalis* females was reduced to a mean of 217.9 eggs per female as compared to 1272.4 eggs laid per unparasitized females.

Key words: *Pulvinaria regalis, Exochomus quadripustulatus, Coccophagus semicircularis, Coccophagus lycimnia, Microterys flavus,* release, predation, parasitization, horse chestnut scale, biological control

Zusammenfassung

Ziel der vorliegenden Arbeit war es, die Möglichkeiten einer erfolgreichen biologischen Bekämpfung der Wolligen Napfschildlaus, *Pulvinaria regalis* Canard (Homoptera: Coccidae), an Park- und Allee-

bäumen durch die alleinige oder kombinierte Freilassung von Larven des Vierfleckigen Kugelmarienkäfers, *Exochomus quadripustulatus* L. (Coleoptera: Coccinellidae), sowie von Weibchen der Schlupfwespenarten *Coccophagus semicircularis* (Förster) (Hymenoptera: Aphelinidae) und *Microterys flavus* (Howard) (Hymenoptera: Encyrtidae) im Freiland zu untersuchen. Hierfür erfolgte die Zucht aller drei Nützlingsarten im Institut für Pflanzenkrankheiten der Universität Bonn unter definierten klimatischen Bedingungen. Anschließend wurden sowohl alleinige Freilassungen aller untersuchten Nützlinge als auch ein kombinierter Einsatz von *E. quadripustulatus* und *C. semicircularis* an Lindenbäumen im Stadtzentrum von Duisburg in den Jahren 1999 und 2000 durchgeführt.

Die Freilassung von 20 bzw. 30 *E. quadripustulatus*-Larven an Lindenbäumen im Stadtzentrum von Duisburg im Jahr 1999 erbrachte eine Reduktion der Anzahl von *P. regalis*-Nymphen pro m^2 Blattfläche auf 2644,3 in der Variante mit 20 *E. quadripustulatus*-Larven und 2250,3 in der Variante mit 30 *E. quadripustulatus*-Larven gegenüber 4298,3 in der unbehandelten Kontrolle. Durch die Freilassung der *C. semicircularis*-Weibchen konnte eine signifikante Steigerung der Anzahl parasitierter *P. regalis*-Nymphen erreicht werden. Es wurden maximale Werte von 9,5 % in der Variante mit 20 und von 12,9 % in der Variante mit 30 freigelassenen *C. semicircularis*-Weibchen ermittelt.

Im kombinierten Einsatz erbrachten *E. quadripustulatus*-Larven eine Reduktion des Befalls um 1985,8 *P. regalis*-Nymphen (46,2 %) pro m^2 Blattfläche im Frühsommer, die Freilassung von 20 *C. semicircularis*-Weibchen im Herbst führte zu einer Parasitierung von durchschnittlich 610,4 Nymphen (14,2 %) pro m^2 Blattfläche, verglichen mit der unbehandelten Kontrolle.

Durch die zweimalige Freilassung von *M. flavus*-Weibchen im Frühjahr 2000 wurden durchschnittlich 53,9 (19,6 %) *P. regalis*-Weibchen pro Baum parasitiert. Die *M. flavus*-Weibchen legten 1–7 Eier in die Schildläuse abhängig von deren Größe ab. Die Anzahl der von parasitierten *P. regalis*-Weibchen abgelegten Eier war mit durchschnittlich 217,9 Eiern deutlich geringer als in die von unparasitierten *P. regalis*-Weibchen (1272,4).

Stichwörter: *Pulvinaria regalis, Exochomus quadripustulatus, Coccophagus semicircularis, Coccophagus lycimnia, Microterys flavus,* Freilassung, Predation, Parasitierung, Wollige Napfschildlaus, Horse Chestnut Scale

1 Introduction

Since its introduction into England in the late 1960s of the last century (Harris 1970), the horse chestnut scale, *Pulvinaria regalis* Canard (Homoptera: Coccidae), has spread over vast areas in western and central Europe (Canard 1968, DeMeirleire 1984, Moraal 1988, Kozar et al. 1994). It has been observed in Germany, especially in the Rhineland area for more than 13 years (Sengonca and Faber 1995). Since it's first occurrence, *P. regalis* has spread all over Germany, southwards down to Basel, northwards up to Hamburg and in the east as far as Berlin (Sengonca and Arnold 1999).

Within its new habitat, *P. regalis* thrives mainly on horse chestnut, maple and lime trees in urban areas (Sengonca and Faber 1995, Faber and Sengonca 1996). Massive outbreaks of this noxious pest lead to an overall weakness of the host plants (Hodgson 1994, Sengonca and Faber 1995, Arnold and Sengonca 2001a) and a reduction in plant growth (Speight 1991). Furthermore, a severe aesthetical damage, caused by the egg-laying females, of the host plants occurs. A further spread of *P. regalis* in the next years is most likely due its broad host range and high fecundity (Schmitz 1997, Sengonca and Arnold 1999) as well as based on the observations of the last years (Faber and Sengonca 1996, Sengonca and Arnold 1999, Arnold and Sengonca 2001a).

Since a conventional control of *P. regalis* is not appropriate in urban areas in Germany due to technical reasons and ecological as well as legal constraints, it was aimed, therefore, to develop a biological control strategy by releasing its natural enemies. In this context, the four-spotted ladybeetle *Exochomus quadripustulatus* L. (Coleoptera: Coccinellidae) as a predator (Faber and Sengonca 1995, Arnold and Sengonca 1999, Sengonca and Arnold 2003) and the parasitic wasps *Coccophagus semicircularis* (Förster) [Syn.: *Coccophagus scutellaris* (Dalman)] (Faber and Sengonca 1995, 1997, Arnold and Sengonca 1999, 2001b) and *Coccophagus lycimnia* Walker (Hymenoptera: Aphelinidae) (Arnold and Sengonca 2001b) have already proved to be promising beneficials in laboratory and

field experiments. The parasitic wasp *Microterys flavus* (Howard) (Hymenoptera: Encyrtidae) is already known as an effective antagonist of different pest scale species in various cultures (Ceballos and Hernandez 1991, Argov et al. 1992, Velimirovic 1994, Kim and Morimoto 1995, 1998). Furthermore, it is commercially available, but there is no evidence so far about its efficiency against *P. regalis.*

The aim of this work was, therefore, to evaluate the possibilities of a biological control of *P. regalis* by the release of *E. quadripustulatus* larvae and *C. semicircularis* as well as *M. flavus* adult females in the field. For this purpose, single releases of either of the aforementioned beneficials and a combined release of *E. quadripustulatus* as well as *C. semicircularis* were out carried on lime trees in the city centre of Duisburg in 1999 and 2000, respectively.

2 Material and methods

The rearing of all the three beneficials was carried out at the Institute of Phytopathology under controlled climatic conditions. *E. quadripustulatus* was reared in climatic chambers at a constant temperature of 20 ± 1 °C, a relative humidity of 70 ± 10 %, artificial light of 1800 lx intensity for a period of 16 : 8 h (L : D) while feeding exclusively with *Acyrtosiphon pisum* (Harris) (Homoptera: Aphididae) on broad beans (*Vicia faba* L.). The parasitic wasps *C. semicircularis* and *M. flavus* were reared in controlled climatic rooms at a constant temperature of 20 ± 2 °C, a relative humidity of 60 ± 20 % and artificial light of 2300 lx intensity for a period of 16 : 8 h (L : D). *P. regalis* nymphs reared on potted small horse chestnut trees (*Aesculus hippocastanum* L.) were provided as hosts for *C. semicircularis* while *M. flavus* developed from parasitized nymphs and adults of *Saissetia coffeae* (Walker) (Homoptera: Coccidae) reared on ferns (*Asplenium nigra* L.).

The releases of *E. quadripustulatus* larvae and *C. semicircularis* females were carried out in the vegetation period of 1999, whereas *M. flavus* females were released in the spring of 2000 on lime trees (*Tilia americana* L.) located in the Königstraße in the city centre of Duisburg.

Field experiments with *E. quadripustulatus* started on 31 May 1999 by releasing *E. quadripustulatus* larvae on heavily infested lime trees in Duisburg. Changing numbers of 20 and 30 larvae, about 48 h old, were placed in the close vicinity of egg-laying *P. regalis* females. All the experiments were replicated 12 times. Additionally, an untreated control was carried out. Visual inspections of the stem and the lower canopy area were conducted directly before and on a weekly basis after the release until pupation of the last *E. quadripustulatus* larvae was observed on 19 July. Samples were taken by collecting four twigs per lime tree in a circular pattern. Each twig was approximately 30 cm long with about 20 leaves. The samples were brought into the laboratory where the number of *P. regalis* nymphs was recorded by the use of a binocular. The leaf area was scanned and the number of *P. regalis* nymphs per m^2 leaf area was calculated.

The field experiments with *C. semicircularis* started on 2 September 1999 by the release of 20 and 30 *C. semicircularis* females, about 24 h old, and lasted until 31 October. The releasing date was chosen according to the size of the *P. regalis* nymphs at which a parasitization by *C. semicircularis* was definitely possible (Arnold and Sengonca 2001b). The size of the *P. regalis* was evaluated prior to the release of *C. semicircularis* by measuring the anal plates according to the method described by Sengonca and Faber (1996). There were 12 replications of each of the experiments and an additional untreated control. Samples of twigs and leaves were taken directly before and on a weekly basis after the release of the beneficials as described above. The total number of *P. regalis* nymphs and the percentage of parasitized nymphs was evaluated in the laboratory after scanning of the leaves and then converted into numbers of nymphs per m^2 leaf area.

The efficiency of a combined release of *E. quadripustulatus* and *C. semicircularis* was evaluated by releasing 20 *C. semicircularis* females per tree on 2 September on those lime trees, where already 20 *E. quadripustulatus* larvae per tree had been released in spring. The experiments with the two beneficials were conducted per method described above.

For the field experiments, two successive releases of 25 *M. flavus* females per lime tree were carried out. All the experiments were replicated 12 times on an additional untreated control. The first release took place on 17 April 2000 while the second on 10 May. Visual observations of the stems and branches in the lower canopy of the trees were carried out before each of the two releases and

additionally on 26 April and 3, 17, 24 as well as 31 May. The appearance of egg-laying *P. regalis* females was recorded each time. Furthermore, scales parasitized by *M. flavus* and an equivalent number of unparasitized female scales were taken into the laboratory and kept under climatically controlled conditions until egg laying ceased. The total number of laid eggs was recorded. Simultaneously, the size of the adult scales was measured and the number of *M. flavus* eggs in the parasitized *P. regalis* females was recorded.

3 Results

3.1 Experiments with *Exochomus quadripustulatus*

The release of *E. quadripustulatus* larvae resulted in significantly reduced numbers of *P. regalis* nymphs/m^2 leaf area in both the experiments as compared to the control. On 31 May, at the start of the experiments, some *P. regalis* nymphs had already hatched and established themselves on the leaves. Therefore, a mean of 101.5 *P. regalis* nymphs were recorded per m^2 leaf area in the control. With the release of 20 *E. quadripustulatus* larvae, it was 186.4 nymphs and with 30 larvae, it was 253.3 nymphs per m^2 leaf area (Fig. 1). The number of nymphs increased during the following 3 weeks to a mean number of 815.3 in control, 1206.1 with 20 and 1090.9 with 30 released *E. quadripustulatus* larvae. The nymphs migrated to the leaves and stayed there during the summer months where most of them hatched by the end of June or beginning of July. The hatching period of *P. regalis* ceased by mid July while pupation of the last *E. quadripustulatus* larvae was observed on 19 July. At this time, the number of *P. regalis* nymphs per m^2 leaf area was 4298.3 in the control, 2644.3 in the experiment with 20 and 2250.3 nymphs with 30 released *E. quadripustulatus* larvae.

3.2 Experiments with *Coccophagus semicircularis*

The number of parasitized *P. regalis* nymphs increased due to the release of *C. semicircularis* as compared to the control (Fig. 2). With the release of 20 *C. semicircularis* females, 8.8 % of the *P. regalis* nymphs were found parasitized on 16 September, exactly 14 days after the release of the parasitoids. Parasitization of nymphs was 7.8 % in the experiment with 30 released females while in the control treatment, no parasitization was recorded. Parasitization of nymphs increased until 21 October to a maximum value of 9.5 % with 20 and 12.9 % with 30 *C. semicircularis* released females. An additional parasitization of *P. regalis* nymphs by the native parasitoid *C. lycimnia* was observed during the whole experiment. On 2 September, parasitization was 2.8 % in the control, 3.1 % in the experiment with 20 and 2.5 % with 30 *C. semicircularis* females. Parasitization increased until 21 October to 21.8 % in the control, 19.7 % with 20 and 19.0 % with 30 *C. semicircularis* females. *P. regalis* nymphs parasitized by the two different parasitoid species could be separated simply by their appearance. Nymphs parasitized by *C. lycimnia* turn opaque black, whereas those parasitized by *C. semicircularis* stay translucent with a dark rim consisting of parasitoid faeces. In total, parasitization by both the parasitoids was 29.2 % in the experiment with 20 while 32.8 % with 30 released *C. semicircularis* females as compared to 21.8 % in the control.

3.3 Combined experiments with *Exochomus quadripustulatus* and *Coccophagus semicircularis*

The combined release of *E. quadripustulatus* (20 larvae) in early summer and *C. semicircularis* (20 females) in autumn resulted in a total decrease in *P. regalis* numbers of 60.4 % as compared with the control (Table 1).

The release of 20 *E. quadripustulatus* larvae in early summer resulted in parasitization of 1985.8 *P. regalis* nymphs/m^2 leaf (46.2 %). The parasitization with *C. semicircularis* females was a mean total number of 610.4 (14.2 %) nymphs/m^2 leaf area. Parasitization of *P. regalis* nymphs by *C. semicircularis* in the control was not observed. An additional parasitization by the native parasitoid *C. lycimnia* was recorded in this experiment also. It was 17.1 % (≅ 735,0 *P. regalis* nymphs) per m^2 leaf area in the experiment with *C. semicircularis* and 18.7 % (≅ 803,8 *P. regalis* nymphs) in the control.

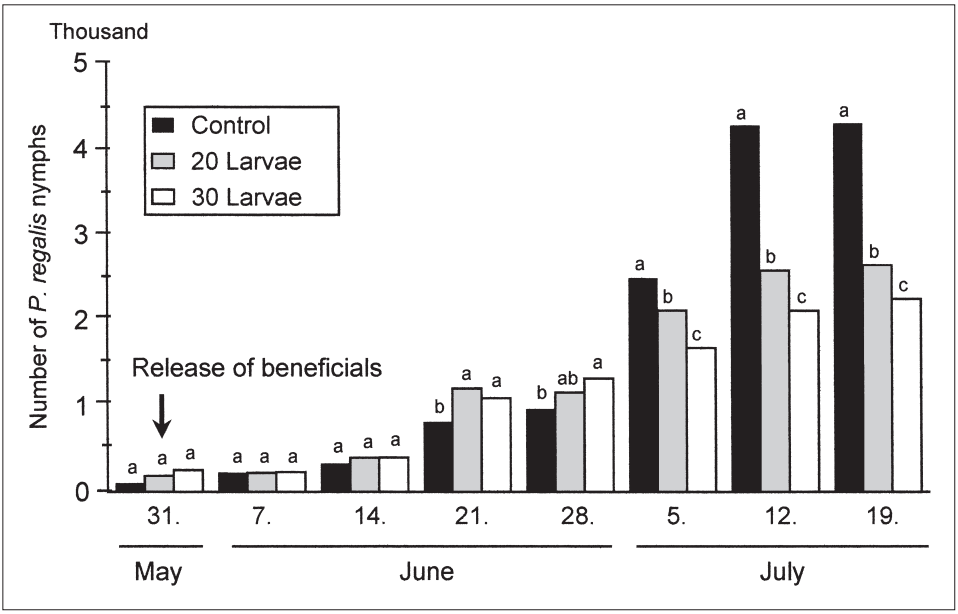

Fig. 1. Mean number of *Pulvinaria regalis* nymphs per m^2 leaf area after releasing different numbers of *Exochomus quadripustulatus* larvae on lime trees in the city centre of Duisburg in 1999. [Values of one evaluation date followed by different letters are significantly different at 5 % level (Tukey's HSD Multiple Range Test).]

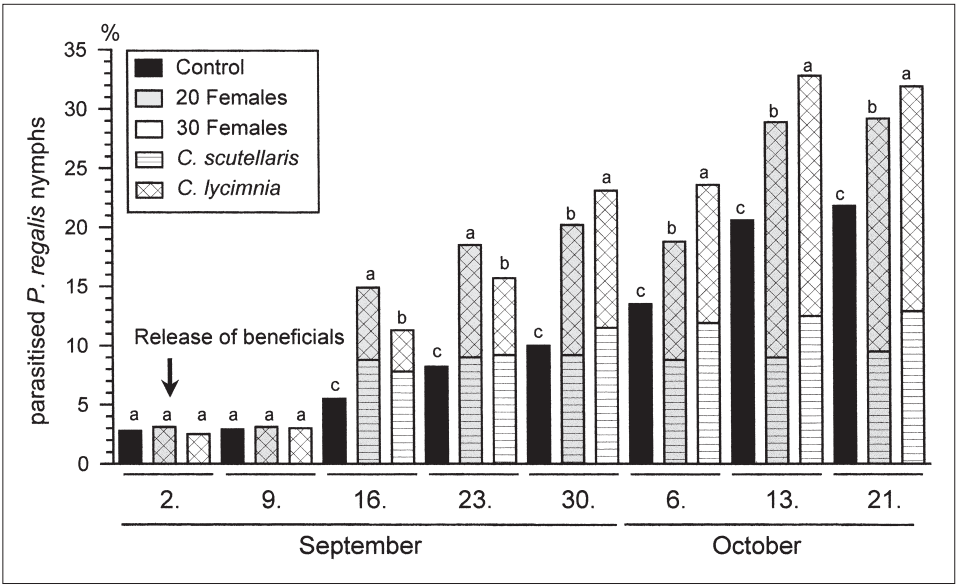

Fig. 2. Percentage parasitization of *Pulvinaria regalis* nymphs caused by releasing different numbers of *Coccophagus semicircularis* females as well as by the occurrence of the native species *Coccophagus lycimnia* on lime trees in the city centre of Duisburg in 1999. [Values of one evaluation date followed by different letters are significantly different at 5 % level (Tukey's HSD Multiple Range Test).]

Table 1. Mean number of consumed and percentage of parasitized *Pulvinaria regalis* nymphs after the combined releases of *Exochomus quadripustulatus* as well as *Coccophagus semicircularis* and by the occurrence of the native species *Coccophagus lycimnia* per m^2 of leaf area on lime trees in the city centre of Duisburg in 1999

Beneficial species	Release of beneficials			Control		
	n (Trees)	Mean number of consumed and percentage of parasitized *P. regalis*-nymphs		n (Trees)	Mean number of consumed and percentage of parasitized *P. regalis*-nymphs	
		Number	%		Number	%
E. quadripustulatus (20 Larvae)	12	1985.8	46.2	12	–	–
C. semicircularis (20 Females)	12	610.4	14.2	12	–	–
Subtotal	12	2596.2	60.4	12	–	–
C. lycimnia	12	735.0	17.1	12	803.8	18.7
Total	12	3331.2	77.5	12	803.8	18.7

3.4 Experiments with *Microterys flavus*

The results of this experiment showed that the released *M. flavus* females accepted adult *P. regalis* as host organism and that a complete development of this parasitoid is possible within this host.

On the date of release of the first 25 *M. flavus* females per lime tree, the 17 April 2000, some *P. regalis* females had already started to build their ovisac and lay eggs. At this time, the mean number of *P. regalis* females per tree was 9.8 % in the treated experiment while 12.3 in the control (Fig. 3). These numbers increased steadily during the next weeks. At the end of the experiment, on 31 May, 275.4 *P. regalis* females per tree were recorded in the treated variant while 223.5 per tree in the control. The first *P. regalis* females parasitized by *M. flavus* were observed on 3 May. On this day, a mean number of 1.3 parasitized *P. regalis* females per lime tree were recorded. Parasitization increased until the second release of *M. flavus* on 10 May up to 17.3 and by the end of experiment up to 53.9 (19.6 %) *P. regalis* females were parasitized per tree. There were found no parasitized females in the control.

The number of eggs laid by *M. flavus* in *P. regalis* females was dependent on the size of the host (Table 2). Female parasitoids deposited just a single egg in scale insects with a body size less than 3.1 mm while 1.4 eggs (4 eggs at maximum) per female were deposited with a size up to 4.9 mm. The highest number of 3.2 eggs/female (maximum 7 eggs/female) were recorded with a body size bigger than 4.9 mm.

Parasitized *P. regalis* females laid less eggs than unparasitized ones (Table 3). In some cases, even the build-up of the ovisac and, subsequently, the egg-laying process was prevented. The examination of parasitized and unparasitized females revealed a mean number of 1272.4 eggs per female (range 359 to 2412 eggs) in the case of unparasitized females. The parasitized females laid a mean 217.9 eggs. The highest number of eggs recorded was 673 eggs. In some cases, no eggs were laid at all.

4 Discussion

The results obtained in the present experiments clearly indicate that *P. regalis* populations on lime trees can be significantly reduced through releasing its artificially reared native beneficials *E. quadripustulatus, C. semicircularis* and *M. flavus.*

E. quadripustulatus larvae feeding on *P. regalis* eggs contributed the main part in reduction of the scale. With the release of this predator, the number of scales per m^2 leaf area of the lime trees was

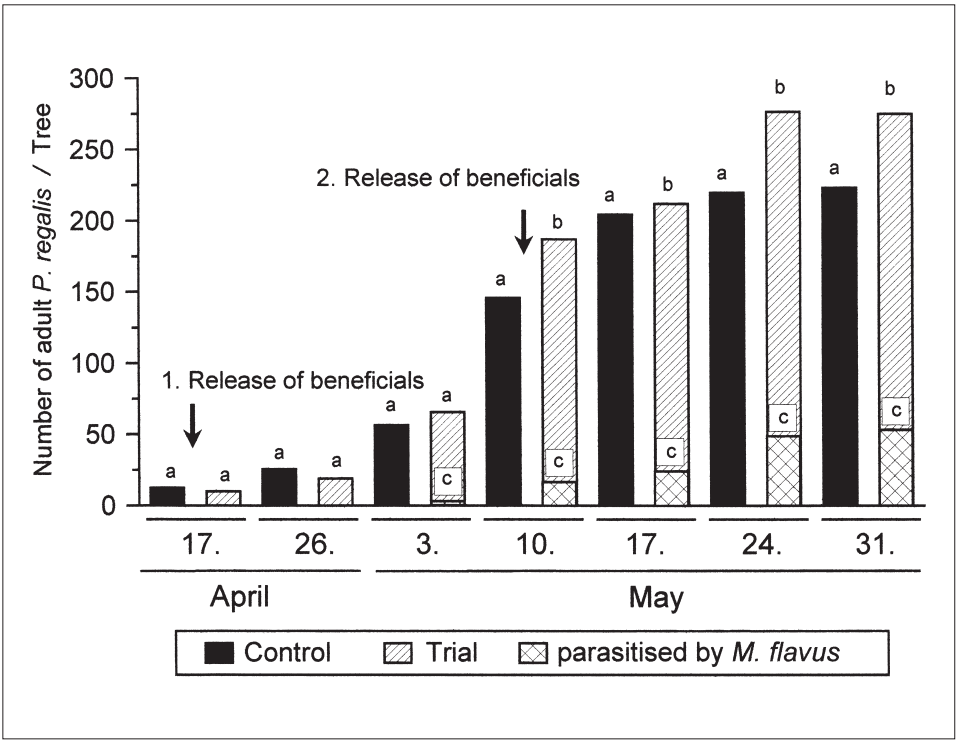

Fig. 3. Mean number of unparasitized and parasitized *Pulvinaria regalis* females after two releases of *Microterys flavus* on lime trees in the city centre of Duisburg in 2000. [Values of one evaluation date followed by different letters are significantly different at 5 % level (T-Test).]

Table 2. Mean number of eggs laid by *Microterys flavus* females into *Pulvinaria regalis* females of different body sizes on lime trees in the city centre of Duisburg in 2000

Size of *P. regalis*-(♀♀)	n	Number of *M. flavus* laid eggs		
		Mean	min	max
< 3.1 mm	165	1	1	1
3.1–4.9 mm	327	1.4	1	4
> 4.9 mm	214	3.2	1	7

Table 3. Mean number of eggs laid by unparasitized *Pulvinaria regalis* females and parasitized by *Microterys flavus* on lime trees in the city centre of Duisburg in 2000

P. regalis-(♀♀)	n	Number of eggs laid by *P. regalis*		
		Mean	min	max
Parasitized	706	217.9	0	673
Unparasitized	750	1272.4	359	2412

significantly lower as compared to the untreated control. Furthermore, highest reduction in scale number was observed in the experiment with 30 released *E. quadripustulatus* larvae. Various researchers have reported efficiency of *E. quadripustulatus* larvae against different scale insect species (Schmutterer 1952, Katsoyannos 1976, Iperti et al. 1977, Raspi 1988, Viggiani 1991, Montiel and Santaella 1995, Mols et al. 1996). It is obvious from the results of the present and earlier experiments that major part of predation occurs always in that time of the year, when the *E. quadripustulatus* larvae appear simultaneously to the egg masses of the scale insects, whereas other beneficials exert a higher regulatory pressure on scale insects in other month (Mols et al. 1996). The efficiency of *E. quadripustulatus* larvae against scale insect nymphs is, therefore, clearly lower as Merlin (1993) observed in Brussels while conducting experiments with *Eupulvinaria hydrangeae* on ornamental trees. He recorded a mean predation of 134.5 *E. hydrangeae* nymphs during the whole postembryonic development of *E. quadripustulatus.* He stated that the spatial distribution of the *E. hydrangeae* nymphs on the leaves has been a negative factor within the process of finding the prey by the relatively immobile *E. quadripustulatus* larvae. His judgement is supported by observations made by Schmutterer (1952) who found adult lady beetles feeding on a broad variety of scale insect species in different locations but larvae only in bigger colonies of scale insects. The eggs of *E. quadripustulatus* are obviously deposited only in such places, where the larvae have a fair chance to find sufficient food for their development. The coincidence of *P. regalis* and *E. quadripustulatus* in the Rhineland area (Faber 1997, Sengonca and Arnold 2003) supports also the statement that *P. regalis* eggs have to be considered as the main food source of *E. quadripustulatus* larvae in this environment. The efficiency of adult *E. quadripustulatus* against other scale insect species is also very good as reported by different researchers (Laudeho and Katsoyannos 1977, Merlin 1993, Faber and Sengonca 1995, Sengonca and Arnold 2003). Laudeho and Katsoyannos (1977) recommended the release of artificially reared *E. quadripustulatus* in olive grooves for the control of *Saissetia oleae.* However, the high mobility and dispersal tendencies of the adult beetles have to be considered in this context (Merlin 1993). Therefore, the release of *E. quadripustulatus* larvae instead of adult *E. quadripustulatus* seems to be more effective in controlling *P. regalis* according to the results obtained in these experiments.

The release of *C. semicircularis* females resulted in significantly higher numbers of parasitized *P. regalis* nymphs. Also, parasitization is directly depending on the number of parasitoids released. The time of release in late summer proved to be suitable for the parasitoids, because of the bigger size of the *P. regalis* nymphs and their high abundance at that time of the year (Sengonca and Faber 1996, Arnold and Sengonca 2001b). Furthermore, scale insects of bigger size are generally more suitable for the development of parasitoids belonging to the family Aphelinidae than smaller size (Viggiani 1984). However, the observed maximum 12.9 % reduction in the *P. regalis* population seems to be not high enough for a long-term biological control. This might be due to the extremely high fecundity of *P. regalis,* i. e., 3000 eggs per female (Speight 1994). Higher parasitization can normally be found only in specialized host-parasitoid-complexes, i. e., in vineyards (Hoffmann 1999), citrus- (Velimirovic 1994) or olive grooves (Xharo 1989, Civantos and Sanchez 1993). Here, parasitization of up to 97 % of the respective scale species was observed. Since *P. regalis* is an introduced pest insect in Europe, it seems to be that the indigenous parasitoid fauna has not yet adapted themselves enough to this new host insect. But, it was very interesting to find that the native species *C. lycimnia* had already accepted *P. regalis* on the lime trees as hosts and parasitized *P. regalis* nymphs. Parasitization by both *Coccophagus* species reached up to 32.8 % of the *P. regalis* nymphs on the lime trees. Interference between the two parasitoid species might take place due to their preference for *P. regalis* nymphs of the same body size but there is still a lack of knowledge concerning this topic. *C. lycimnia* is generelly known to be the more efficient parasitoid species since the adult female display a behavioural pattern, known as "host feeding" additional to the parasitization of the scales. The females puncture the epidermis of unparasitized scale insects with their ovipositor and feed on the outcoming haemolymph, subseqently killing the scale insect. This behaviour was not observed with *C. semicircularis* in the present or earlier experiments (Jarraya 1975; Faber and Sengonca 1997). Furthermore, it can be stated that *C. semicircularis* displays autoparasitic behaviour when males are not abundant. Autoparasitism means that male parasitoids develop as parasites of female larvae of their own species (Timberlake 1913; Flanders 1936, 1959; Jarraya 1975), so reducing the success of any biological control measure. However, both parasitoid species appear to be promising candidates in the biological control of *P. regalis* since they are

already established in the urban environment and their abundance can be easily increased by providing alternative hosts or by the release of artificially mass-reared specimens. Additional measures, which could enhance the population densities of beneficials is by manipulating the habitats (Landis et al. 2000). One possibility would be the cultivation of nectar and pollen-producing plants, e. g., *Phacelia* spp., in the vicinity of trees and plants infested with *P. regalis,* since a sufficient supply with a carbohydrate-rich diet has a positive influence on the overall performance and life span of the Aphelinidae (Muegge and Lambdin 1989, Viggiani 1991, Faber and Sengonca 1997). Furthermore, both species can easily be reared under artificial conditions in the laboratory.

The combination of *E. quadripustulatus* predator and *C. semicircularis* parasitoid represents a valuable possibility in biological control of *P. regalis* as found in the present experiments. The efficiency of both beneficials added up and resulted in 60.4 % decrease in scale insect numbers, which has to be considered as a promising result and provides a sound basis for further experiments. Negative and interspecific interactions between the two species, i.e., non-additive effects (Ferguson and Stiling 1996), can be excluded due to the different release dates of the respective species during the vegetation period.

The release of *M. flavus* has to be considered as a possible and valuable supplement in biological control of *P. regalis,* since this species attacks the adult females before or even during the egg-laying period of the scale insect. A negative influence on the efficiency of the two other beneficial species *E. quadripustulatus* and *C. semicircularis,* attacking *P. regalis* eggs and nymphs, is not likely due to their different prey strategies. *E. quadripustulatus* larvae feed mainly on the eggs and only seldom on the bodies of adult female *P. regalis.* Furthermore, the release of *M. flavus* should take place before or at the start of the egg-laying period of *P. regalis. E. quadripustulatus* larvae, on the other side, should be released after the *P. regalis* females have deposited their eggs to ensure sufficient food supply for the predator. There is also no danger that *C. semicircularis* or *C. lycimnia* would parasitize adult female *P. regalis,* since these parasitoids prefer smaller scale insect stages. Flanders et al. (1961) stated that the small size of the ovipositor of the female *Coccophagus* might be the reason for this behaviour, making it impossible for the rather small parasitoids to deposit their eggs in the gut of bigger-sized scale insects. In case of egg deposition in the haemolymph, it is most likely that the parasitoid eggs are encapsulated by the host organism (Muegge and Lambdin 1989). It has to be considered also, that 19.6 % parasitization of *P. regalis* females observed in this experiment, is much lower for a long-term biological control of *P. regalis.* However, this number could be increased by accurate and timely releases of artificially reared beneficials according to the prevailing climatic conditions in the respective habitat and by an optimized beneficial/pest ratio. The aim should be to establish a permanent *M. flavus* population on the infested trees.

The present study clearly indicated that the release of beneficials could be a valuable tool in biological control measures against the horse chestnut scale insect *P. regalis.* At present, the predator *E. quadripustulatus* and the parasitoid *C. semicircularis* have to be considered as the most promising beneficials. Additionally, it is possible to optimize such control measures by the combination of the aforementiond together with other beneficial species like *C. lycimnia* or *M. flavus.* But, accurate time schedule, combined releases and optimized beneficials/pest ratio would lead to greater success of the control strategy. Furthermore, additional supporting measures in the field would help implement permanent control of the horse chestnut scale insect *P. regalis* on ornamental trees in urban areas.

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