The use of *Harmonia axyridis* larvae (Coleoptera: Coccinellidae) against *Macrosiphum rosae* (Hemiptera: Sternorrhyncha: Aphididae) on rose bushes

André FERRAN[1], Hava NIKNAM[1], Firouze KABIRI[2], Jean-Luc PICART[3], Clotilde DE HERCE[3],
Jacques BRUN[1], Gabriel IPERTI[1] and Laurent LAPCHIN[1]

[1]INRA, Laboratory of Invertebrate Biology, 37, Boulevard du Cap, 06602 Antibes, France

[2]BIOTOP, 1382, route de Biot, 06500 Valbonne, France

[3]Direction des Parcs et Espaces Verts, 3, avenue de la porte d'Auteuil, 75016 Paris, France

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Abstract. Third and fourth instar larvae of *Harmonia axyridis* were released in spring into rose beds infested by the aphid *Macrosiphum rosae*. These biological treatments induced stabilization or a decrease of the aphid populations. Their efficiency is comparable with that of chemical treatments performed in neighbouring rose beds and the subsequent development of aphid populations was the same after these two types of treatments. The rearing condition of *H. axyridis*, particularly its feeding on a substitute prey (lepidopteran eggs), the climate, particularly the rainfall and low temperatures, sometimes near the development threshold of the coccinellid, and possibly the rose bush variety did not seem to affect its potential predatory efficiency. An aphid density of more than thirty aphids per rose bush appears to be necessary for the larvae to remain on the plants, when fifty larvae were released per four bushes.

INTRODUCTION

The efficiency of ladybirds has often been demonstrated in crops (Kring et al., 1985; Zou et al., 1986; Ohnesorge & Schier, 1989; Ghanin & El-Adl, 1991; Helenius, 1991). Their use in biological control against introduced prey is an established practice. The coccidophagous ladybirds *Novius cardinalis* Mulsant, *Cryptolaemus montrouzieri* Mulsant, *Cryptognatha nodiceps* Marshall (all Coleoptera: Coccinellidae) were introduced in different countries and controlled the scale insects *Icerya purchasi* Maskell (Margarodidae) (Marchal et al., 1913; Balachowsky & Molinari, 1930), *Planococcus citri* Risso (Pseudococcidae) (Marchal & Poutier, 1922) and *Aspidiotus destructor* Signoret (Diaspididae) (Taylor, 1935).

Many biological control experiments were also performed with aphidophagous ladybirds on sugar cane (Deng et al., 1987), cotton (Yang, 1985; Zhang, 1985; Dong, 1988) apple trees (Savoiskaya, 1970) and in greenhouses (Quilici et al., 1985). Today, in spite of promising results, aphidophagous coccinellids are used rarely in crop protection. Primarily, this situation is due to the complexity and cost of their production, which requires the simultaneous rearing of the predator and an aphid species on a given plant in artificial conditions. A simplification of this rearing technique is necessary to industrialize ladybird production and to promote their use in biological control, specifically, by inundative releases. This simplification involving substitute preys or artificial diets results, generally, in slower larval development, higher larval mortality and no reproduction (Okada &

Matsuka, 1973; Matsuka & Okada, 1975; Tanimoto, 1975; Deng, 1982; Kim & Choi, 1985).

The Asiatic polyphagous ladybird *Harmonia axyridis* Pallas was introduced to France by I.N.R.A. in 1982. Its continuous rearing (more than 100 generations) with an industrially produced prey, the eggs of *Ephestia kuehniella* Zeller (Schanderl et al., 1985, 1988) allowed a French private firm (BIOTOP) to perform a mass production and to develop biological control programmes against aphids.

The first biological control experiment was undertaken against the aphid *Macrosiphum rosae* L. on rose bushes located in town squares. Considering the risk of environmental pollution and the possible toxicity of insecticides for visitors, biological control could offer a suitable alternative to control with pesticides. This work presents the results obtained in 1993 with *H. axyridis* larvae in five squares located in Paris.

MATERIAL AND METHODS

Treatments of *M. rosae* with *H. axyridis* larvae were performed in five different public squares, on different rose bush varieties. In each square, two rose beds with the same geometrical shape and the same number of rose bushes were chosen. One of these two rose beds was treated with *H. axyridis* larvae, the other with an insecticide.

The aphid populations were estimated visually on 40 rose bushes, regularly distributed in each rose bed once a week from 24th April to 30th June. The observer quantified the aphids on all the leaves and stems of each rose bush (1 minute per rose bush). Each rose bush was then put into one of the following abundance classes, according to its total infestation: class 0 (0 aphid per rose bush), class 1 (1 to 3), class 2 (4 to 10), class 3 (11 to 30), class 4 (31 to 100), class 5 (101 to 300), class 6 (301 to 1,000) and so on.

The aphid population in each rose bed was expressed by the frequency of the 40 rose bushes in the different abundance classes and mean class was used to show frequency variations. In each biological rose bed, the predatory efficiency was estimated by rose bush frequency change in abundance classes before and after each treatment. As it was impossible to have proper rose bed checks (without chemical treatment) in these public squares, these changes were compared with those observed in the corresponding chemical rose beds. Comparison of the frequencies on two successive sampling dates was performed by a non-parametric statistical method. On these two dates, the rose bushes were classified according to their abundance class and their mean range was compared with the test of Kruskall and Wallis. This test calculates a variable (Hc) which follows the khi2 rule.

The larvae were reared in the BIOTOP biofactory and transported in transparent plastic boxes (height: 2.5 cm; length: 10.5 cm; width: 8 cm). Eggs of *E. kuehniella* were stuck to the bottom with arabic glue. These boxes were opened above the rose bushes to release the larvae. Fifty larvae were released on every four rose bushes which represent an approximate surface area of one square metre.

The biological treatments were performed at different dates in order to modify the variation of *H. axyridis* efficiency according to aphid density. The biological rose bed in square 1 was treated three times (on 25th April with last instar larvae, on 7th May and 22nd June with third instar larvae), that one in square 4, twice (on 25th April and 7th May with fourth and third larvae respectively) and those of squares 2, 3 and 5, once with fourth instar larvae (on 25th or 27th April).

Larval populations were quantified during aphid sampling. The ladybird densities were expressed at each date in each rose bed by the percentage of rose bushes on which at least one larva had been observed and by the mean number per rose bush.

Aphids mummified by parasitoids and larvae or adults of native aphidophagous predators (i.e., Syrphidae, Coccinellidae, Chrysopidae) were also noted. Their density was expressed by the percentage of rose bushes on which at least one of them had been observed.

The chemical rose beds were treated when the aesthetic quality of the rose bushes was threatened by *M. rosae* (7th May 1993). An organo-phosphorous insecticide (Phosalone) was pulverized on rose bushes according to the recommended concentration for ornamental plants (60 g/hl).

Data on the general climatic conditions in Paris (temperature and rainfall) were provided by the national meteorological service.

RESULTS

Square 1 (Figs 1A and 2A)

The rose bed was treated biologically three times. When the first biological treatment (25th April) was performed on very strongly infested rose bushes (median class: 5), the aphid population increased in the corresponding chemical rose bed (median class: 5 to 7, Hc: 27.0 p < 0.0001). Although this treatment presented a globally low efficiency (the median class remained constant, Hc: 0.1), it modified greatly the rose bush distribution in the different abundance classes (29th April). In comparison with the distribution observed before the treatment, the aphid populations on the different rose bushes either decreased from 1 to 3 classes (40% of the rose bushes), or increased from 1 to 2 classes (40%) or remained constant (20%).

Larvae of *H. axyridis* were present on every rose bush and their mean number was 8.2 ± 4.4 larvae per rose bush (Fig. 2A). The low efficiency of this treatment may have resulted from the release of last instar larvae (pupae were already present at the first observation date) and, possibly, from a particular sensitivity of the rose bush variety to *M. rosae*.

This variation of the rose bush distribution induced by the first treatment was still observed when the second treatment with third instar larvae was performed on 7th May. Its efficiency increased from 13th May (median class: 5, Hc: 0.6) to 18th May (median class: 5 to 1, Hc: 29.3, p < 0.0001). This biological treatment was as efficient as the chemical treatment (7th May, median class: 7 to 2, Hc: 60.7, p < 0.0001). The belated effect of this biological treatment may depend on the high aphid density or the increase in feeding capacities after the moult to
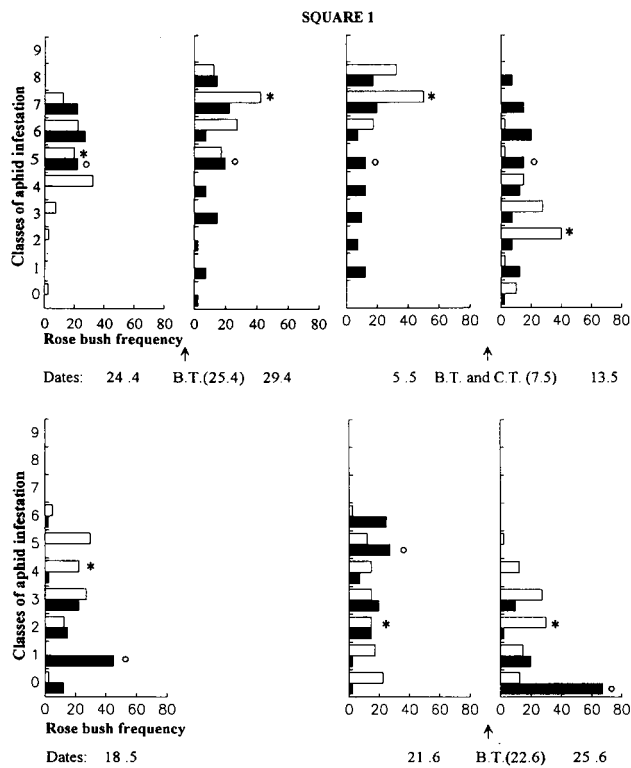

Fig. 1A. Square 1 – variation of rose bush frequency in the aphid infestation classes according to biological (B.T., solid columns) and chemical (C.T., open columns) treatments (○ and *, median class of the rose bush distribution in the biological and chemical rose beds).

the last instar larvae. The adults observed in this rose bed did not prevent an increase of the aphid populations till 21st June (median class: 5).

The third biological treatment with third instar larvae (22nd June) was effective immediately. The median class decreased from 5 to 0 (Hc: 50.9, p < 0.0001), whereas for the same period of time, it was approximately constant in the corresponding chemical rose bed (median class 2, Hc: 0.01). On 25th June, *H. axyridis* larvae were present on all rose bushes (mean number: 6.8 ± 3.6 larvae per rose bush).

There were few parasitoids and predators of *M. rosae* in the different biological rose beds, especially at the start of the experiment (24th April). Nevertheless, in square 1, mummified aphids, syrphid larvae, ladybird larvae and adults (*Coccinella septempunctata* L. and *Adalia bipunctata* L.) were present on 45%, 30% and 7.5% of the rose bushes respectively, due to an early and heavy aphid infestation.

Square 2 (Figs 1B and 2A)

Only one biological treatment with last instar larvae was performed at a time (27th April) when the density of the aphid populations was low (median class: 3). On the first observation (5th May), the median class was much lower; it decreased to 0 (Hc: 33.3, p < 0.0001) whereas in the corresponding chemical rose bed it increased from 3 to 4 (Hc: 8.8, p < 0.0003). The development of the aphid populations were identical in the two rose beds of this square during the following week (13th to 18th May). The *H. axyridis* larvae quickly disappeared from the rose bushes.

Square 3 (Figs 1B and 2A)

This biological treatment with last instar larvae was also performed on rose bushes with low infestation (median class: 2). As in the previous square, it was highly efficient, the median class decreased from 2 to 0 (Hc: 28.4, p < 0.0001), whereas it increased in the corresponding chemical rose

Fig. 1B. Square 2 and 3 – variation of rose bush frequency in the aphid infestation classes according to biological (B.T., solid columns) and chemical (C.T., open columns) treatments (O and *, median class of the rose bush distribution in the biological and chemical rose beds).
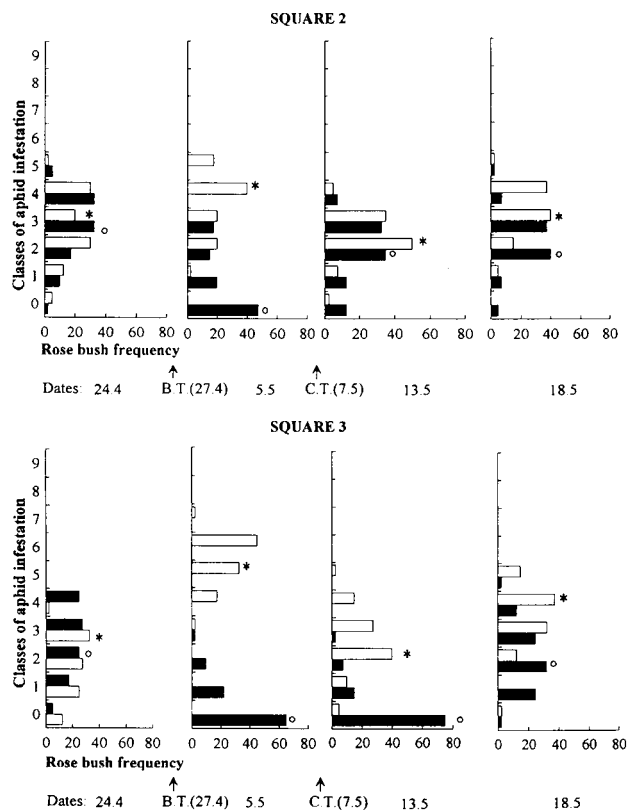

SQUARE 2

SQUARE 3

Fig. 1C. Square 4 and 5 – variation of rose bush frequency in the aphid infestation classes according to biological (B.T., solid columns) and chemical (C.T., open columns) treatments (○ and *, median class of the rose bush distribution in the biological and chemical rose beds).

SQUARE 4

SQUARE 5

bed from 3 to 5 (Hc: 53.7, p < 0.00001). *H. axyridis* larvae quickly left the rose bushes and the aphid populations were identical in these two rose beds on 18th May.
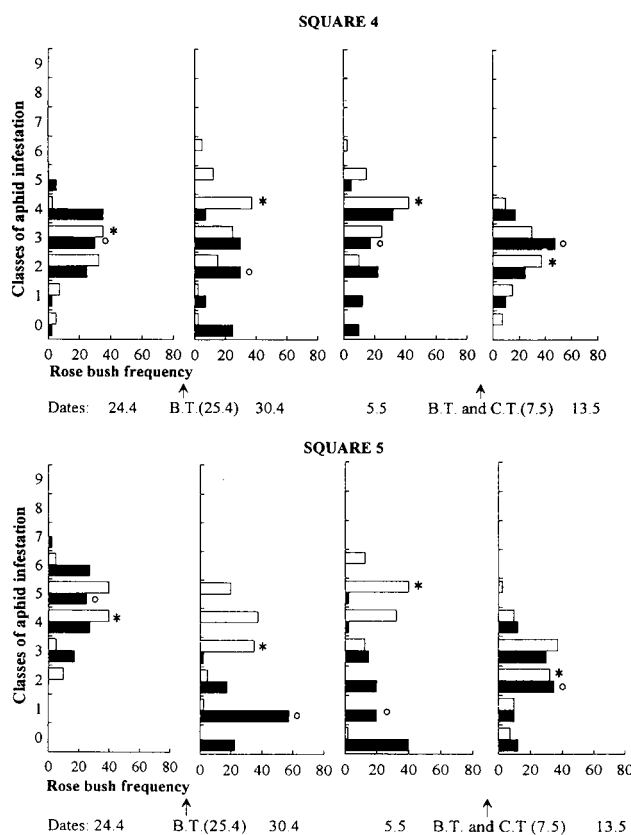
Square 4 (Figs 1C and 2B)

Two biological treatments were performed in this square. The first (25th April) resulted in a non significant decrease of the aphid populations (median class: from 3 to 2; Hc: 0.01), but aphid infestation increased in the corresponding chemical rose bed during the same period of time (from 3 to 4; Hc: 3.9). The second treatment (7th May) did not modify the median class (median class: 3, Hc: 0.01) and was less efficient than the chemical treatment performed at the same time (median class from 4 to 2; Hc: 46.0, p < 0.0001). The aphid infestation was similar on 13th May in both rose beds, the median class was 3 for the biological rose bed and 2 for the chemical rose bed.

H. axyridis larvae were present on many rose bushes (65%) soon after the first treatment but their number remained low (1.5 ± 0.7 larvae per rose bush) and did not increase after the second release (51.0%; 0.6 ± 0.8).

Square 5 (Figs 1C and 2B)

The first treatment (25 April) with last instar larvae was performed on highly infested rose bushes (median class: 5) and was efficient: median class decreased from 5 to 1 (Hc: 60.8, p < 0.0001). In the meantime, the aphid populations decreased in the chemical rose bed only slightly (median class: from 4 to 3, Hc: 25.2, p < 0.001). On the contrary, the aphid infestation was low (median class: 1) when the second treatment (7th May) was performed. It did not prevent a slight increase of aphid populations (median class: from 1 to

SQUARE 1

% of rose bushes with *H. axyridis*

100
80
60
40
20
0

Larvae
Adults

B.T. (25.4)
B.T.(7.5)
B.T.(22.6)

24.4   5.5   18   3.6   21   30.6

Dates

N: 2.5   11.1   2.4   1.0 6.8   2.1
±sd: ±0.6   ±6.4   ±2.3   ±1.3 ±3.6 ±2.1

SQUARE 2

100
80
60
40
20
0

B.T.(25.4)

24.4   5.5   18   3.6

N: 0.6   0.1   **Dates**
±sd: ±0.7   ±0.3

SQUARE 3

100
80
60
40
20
0

B.T.(25.4)

24.4   5.5   18   3.6

N: 0.4   0.4   0.2   0.1   **Dates**
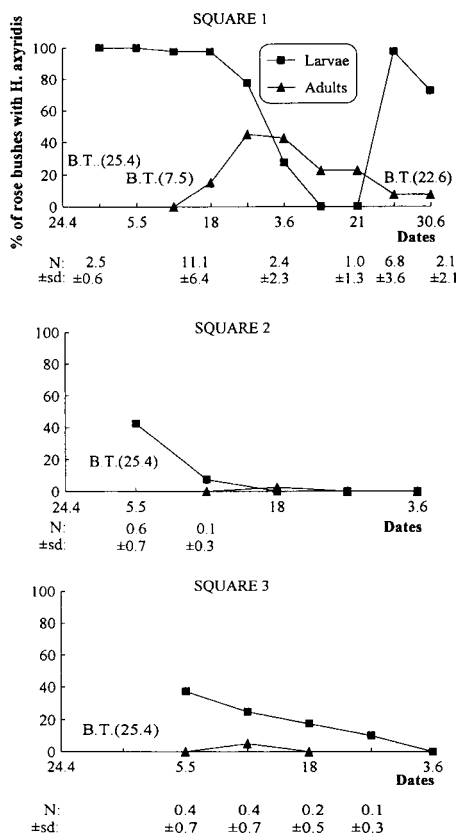±sd: ±0.7   ±0.7   ±0.5   ±0.3

Fig. 2A. Square 1 to 3 – variation of *H. axyridis* larvae and adults. The results are expressed in two ways: I. The percentage of rose bushes with at least one larva or adult with respect to the total number of observed rose bushes (Figure); II. The average number of larvae per rose bush (numbers below).

2, Hc: 10.3, p = 0.013). On 13th May, the mean class (2) was the same in both rose beds in this square.

As in the previous square, the *H. axyridis* larvae were dispersed on many rose bushes (95% on 25th April then 80% on 7th May) and their number per plant remained low (1.3 ± 0.9 then 1.8 ± 1.8).

DISCUSSION

In biological experiments, the prey sampling method should be accurate enough without being time consuming for its achievement, repetitive to reduce rough counting errors and rapid enough to be applied to a great number of plants in order to analyse results. Before this experiment, the observers became accustomed to the aphid distribution on the plants and verified their estimation of the total number of aphids per plant by accurate countings. During the experiment, each observer always controlled the same plants.

Although this method, used frequently for estimation of aphid populations, is probably not adequate for entomophagous insects, it gave a satisfactory evidence that parasitoids and predators were rare when the aphid population outbreaks took place.

Nine biological treatments with third or fourth instar larvae of *H. axyridis* (50 larvae per m²) and five chemical treatments were performed in spring in rose beds of five public squares.

The chemical treatments resulted in a strong decrease but not a complete elimination of the aphid population. The mean class generally decreased to class 2 in the different rose beds. Consequently, the efficiency of biological and chemical treatments was often similar and the subsequent development of the aphid populations was the same in the different rose beds. Some aphids apparently survived in shelters, such as rose buds which had opened. The chemical treatment could be inefficient due to rainfall after the spraying (Fig. 3) and/or the resistance of the aphid to the pesticide used.

Nine biological treatments resulted either in stabilization of aphid population in four cases or distinct decrease in five cases. The larvae of *H. axyridis* are able to limit and reduce the number of *M. rosae* on outdoor rose bushes. This efficiency was often confirmed by the increase of aphid populations in the chemically treated rose beds, while the released
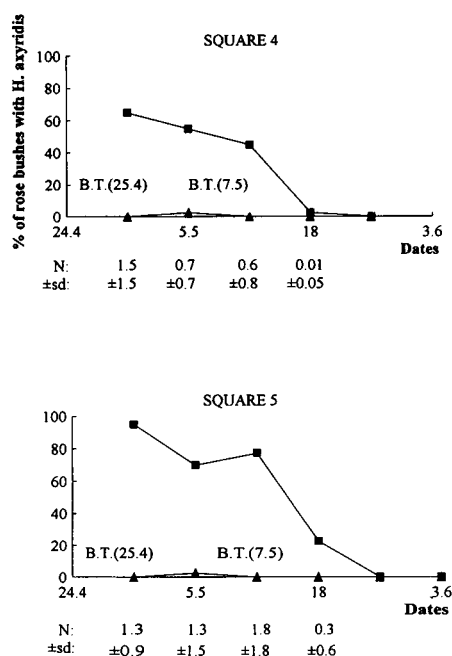
Fig. 2B. Square 4 and 5 – variation of *H. axyridis* larvae and adults. The results are expressed in two ways: I. The percentage of rose bushes with at least one larva with respect to the total number of observed rose bushes (Figure); II. The average number of larvae per rose bush (numbers below).

larvae were active in the corresponding biological rose beds. These results also showed that this predator quickly adapts itself to this aphid species, in spite of continuous rearing on a substitute prey of lepidopteran eggs.

These biological treatments with two last instar larvae were performed in spring at different climatic conditions, rose bush varieties and initial densities of preys. The heavy rainfall observed in April did not appear to reduce the larval efficiency (squares 2, 3 and 5). Low temperatures near the lower developmental threshold of the larvae (10.7°C, Schanderl et al., 1985) could increase their effect on aphid populations by limiting the larval dispersion from treated rose bushes (Fig. 3). The biological treatment performed in square 1 in June was particularly effective.

It is impossible in this study to separate the peculiar effect of rose bush variety and microclimate in individual replicates. These environmental factors may explain the early appearence of aphid populations in square 1 and the inefficiency of biological treatments in square 4.

When rose bushes were infested densely with aphids (median class > 3), the larvae of *H. axyridis* were numerous and some adults settled. Conversely, when the aphid infestation was low (median class < 3), the larvae disappeared quickly; even so the biological treatment was efficient. As shown by Obata & Johki (1990) (for coccinellid adults), the larvae of *H. axyridis* are able to remain longer on the most infested plants (numerical response to aphid density). Median classes from 3 (30 aphids per rose bush) to 5 (100 to 300 aphids per rosebush) seem necessary for larvae to settle on rose bushes and probably also for the efficiency of the biological treatments.

H. axyridis larvae are very mobile insects, particularly the third and fourth instar larvae. They are able to change plants several times and even prospect again previously explored plants. Consequently, it appears that a time interval of more than five days between the first observation of the aphid populations and the biological treatment could prevent us from precisely quantifying the number of the larvae which are really efficient. This is especially true when the aphid density on rose bushes is low.

Although a few egg-clutches were observed on rose bushes in square 1, the length of time that the adults stay on herbaceous plants is probably short. In its native country, this coccinellid is regularly seen on wild or cultivated trees (Aksyutova & Gul'dyaeva, 1977). Thus, there is an innate tendency for adults, emerging from larvae released on rose bushes,
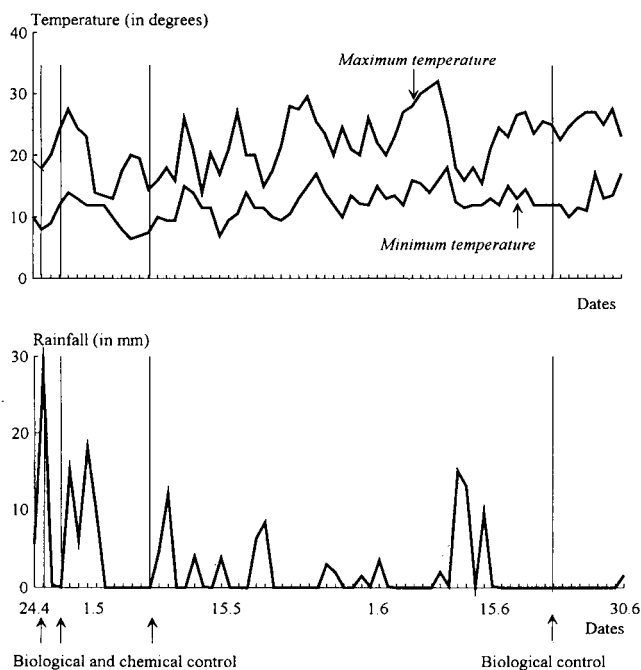
Fig. 3. Biological (*Harmonia axyridis* larvae) and chemical control (Phosalone) against *Macrosiphum rosae* on rose bushes: temperature and rainfall during the experiment.

to search for trees to lay their eggs.

Third instar larvae should be preferred for the treatment. In two or three days, they become voracious fourth instar and, consequently, their effect on aphid populations is quick and important. The food consumption of these two larval instars represents 13% and 81% respectively of the total needs for larval growth (Schanderl et al., 1985). The use of egg-clutches instead of third or fourth instar larvae in biological treatments would be less expensive and their transportation would be easier. However, it is generally assumed that first instar larvae are very fragile as they have low movement and defence capacities (Dixon, 1970). Moreover, a delayed efficiency (they reach the fourth stage in approximately ten days) requires careful forecasting with regard to the growth of aphid populations.

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