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Release of *Aphidius rhopalosiphi* (Hymenoptera: Aphidiinae) for cereal aphid control: field cage experiments

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Abstract. The potential of the parasitoid *Aphidius rhopalosiphi* for controlling cereal aphids was tested in 16 m^2 field cage experiments in 1998 and 1999. In the first year, aphids and parasitoids were released in cages containing naturally occurring populations of aphids and their natural enemies. In the second year, aphids and parasitoids were released in cages which had been cleared of insects by applying insecticide. The growths of the aphid populations in the different cages were analysed and compared. In 1998, the release of 50 pairs of parasitoids per cage had no significant effect on aphid population growth relative to that in the control cages. Even though the aphid population growth rates were less than 60% of that in the control cages, in the cages in which 100 pairs and 200 pairs of parasitoids were released, it was not possible to show they statistically differed. The aphid populations in these three cages were held below 10 aphids per tiller. In 1999, the aphid density was higher and the population grew faster than in 1998. The release of 100 and 200 parasitoids per cage significantly reduced aphid population growth. *A.rhopalosiphi* seemed to be a good control agent in field cages, provided they were released at the beginning of aphid population growth.

INTRODUCTION

Parasitoids are promising biocontrol agents as they constitute 80% of the successful examples of biological control in the world (Van Lenteren, 1986). In all cases of successful biological control against aphids in glasshouses, the control agents have been Hymenopteran parasitoids and, with one exception, members of the Aphidiidae (Carver, 1989). However, according to Hughes (1989), no predator or parasitoid has proved to be an efficient biocontrol agent of aphids in field crops. This difference in efficacy between glasshouses and field crops could be due to differences in abiotic conditions and/or to the fact that fields are an "open system" allowing movement of pests, natural enemies and released biocontrol agents.

An intermediate step between laboratory and field tests is a field-cage experiment. It should help to determine if larger scale open field studies are warranted (Simmons & Minkenberg, 1994), and give an indication of the timing and the number of parasitoids to release into crops. Of course, the results must be interpreted with caution as although field cages allow the systems studied to experience approximately natural abiotic conditions the cage may influence these abiotic conditions and the behaviour of the insects. They are thus semi-natural conditions, which prevent the dispersal of the released parasitoids and the immigration of pests. The capacity of parasitoids to control aphids can differ in field cages and the laboratory. Höller & Haardt (1993) compared the parasitism by *Aphelinus abdominalis* Dalman (Hymenoptera, Aphelinidae) of *Sitobion avenae* Fabricius, and found that the parasitism levels in small field cages (enclosing two wheat tillers) reached only 25% of the laboratory levels. They concluded that *A. abdominalis* females were unable or unwilling to parasitize high numbers of aphids in the field, but could not explain this phenomenon.

The three pest aphid species of cereals in Belgium are *Sitobion avenae*, *Metopolophium dirhodum* Walker and *Rhopalosiphum padi* Linné (Latteur, 1985). Latteur (1985) showed that a density of 10 aphids per tiller induces a mean yield loss of 180 kg of wheat/ha and a density of 5 aphids per tiller induces a loss of 70 kg of wheat/ha.

Microhymenopteran parasitoids are naturally present in cereal fields in Belgium (Langer et al., 1997). One of the species, *Aphidius rhopalosiphi* De Stefani-Peres, was selected for mass release on the basis of many laboratory efficacy criteria. *A. rhopalosiphi* has a type III functional response (Hance, personal communication) and its numerical response maximizes the exploitation of dense patches (Stilmant, 1997). High fecundity is the most important factor determining the impact of *A. rhopalosiphi* on its host: it parasitizes a mean of 160 aphids during the first five days of its adult life (Stilmant, 1994).

To investigate the potential of *A. rhopalosiphi* for controlling cereal aphid populations, we conducted field cage studies in wheat at Louvain-la-Neuve, during the summers 1998 and 1999. Our aim was to test the capacity of *A. rhopalosiphi* to control cereal aphid populations, under (a) naturally occurring aphid and natural enemy densities, with added laboratory-reared aphids and parasitoids and (b) laboratory-reared aphids and parasitoids only.

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MATERIALS AND METHODS

Rearing aphids and parasitoids

The aphid, *Metopolophium dirhodum*, used in this experiment was reared on winter wheat (*Triticum aestivum* L. cv. "Torfrida"), at 20±1.5°C, 60% relative humidity and a 16L : 8D regime. Each year, the rearing started with individuals collected in summer.

The parasitoid, *Aphidius rhopalosiphi*, has been in laboratory culture in 0.3 m^3 cages, on *M. dirhodum* at the above conditions. Individuals were collected each summer, and reared during the year.

1998-cage experiment

This experiment was carried out in cages, 4 × 4 m and 1.6 m high. They were placed in a wheat field in May 1998 on the experimental farm of the Université Catholique de Louvain (UCL), Louvain-la-Neuve, Belgium. The wheat was at a density of 420 tillers/m^2.

Because the natural aphid population (*M. dirhodum* and *S. avenae*) in the field was very low in 1998, 1500 laboratory-reared *M. dirhodum* (25% adults, no alates) were added to each cage. The day after the aphids were released, pairs of laboratory-reared *A. rhopalosiphi* parasitoids were also released in the centre of the cages.

We started two experiments. On 28 May, 200 pairs of parasitoids we released in cages 200a and 200b, 100 pairs in cages 100a and 100b and none in two control cages, control a and control b. On 5 June, we released 50 pairs of parasitoids in cages 50a and 50b and none in control c and control d.

Each week, starting on 2 June for the first six cages and on 9 June for the four remaining cages, the aphids were counted on 80 randomly chosen tillers until the wheat wilted (14 July).

1999-cage experiment

This experiment was carried out in the same cages as in 1998, placed in a wheat field in April at the experimental farm, UCL, Louvain-la-Neuve.

As the results of the 1998 experiment were very difficult to analyse due to variation between cages within a treatment, in 1999 the naturally occurring aphids and natural enemies were eradicated by spraying a synthetic insecticide with a short residual activity (Duoflor®, 100 ml in 10 l of water, 3.3 l sprayed in each cage, which is 0.2 l per m^2) twice in each cage, at one week interval. We released 1000 laboratory-reared *M. dirhodum* (25% adults, no alates) in each cage on 10 May 1999. One month later, on 10 June, we released the parasitoids: 200 pairs in two cages, 100 pairs in three cages and none in the four control cages.

The aphids were counted twice a week, from 10 May to 28 June, on 15 randomly selected tillers in each quarter of the cage. We increased the sampling frequency in 1999, in comparison to 1998. By sampling the 4 quarters of each cage in 1999, we obtained four data points (instead of one in 1998) for each date and each cage. The wheat wilted earlier in 1999 than in 1998. In order to understand this, we compared the abiotic conditions in 1998 and 1999 (IRM, 1999 and IRM, 2000). The same quantity of rain fell in the 2 years and the temperature was similar in May and June 1998 and 1999. However, the duration of insolation per month was different: 1998 – 205 h in May, 165 h in June,140 h in July; 1999 – 210 h in May, 205 h in June and 260 h in July.

Statistical analysis: comparison of aphid population growth

For the first six cages in 1998, we fitted a linear regression line to the ascending part of the aphid population growth curves, from 23 June to 14 July. For the four last cages in 1998 and the cages in 1999, we fitted an exponential curve to aphid population growth. The data was then logarithmically transformed (ln). To compare the population growth rates, a slope heterogeneity test was performed on each of the three groups (proc GLM, SAS version 6.12 SAS. Institute Inc.1989). To determine where the differences lie, we fitted a linear regression line to the relationship between the slope coefficients and the parasitoid density. The Dagnélies (1970) tables gave the critical correlation coefficients.

RESULTS

1998-cage experiment

The data for the two series of cages were analysed separately, because of the difference in starting dates.

100 and 200 pairs of parasitoids released vs. control

Fig. 1 represents the growth of aphid populations on 80 tillers. Aphid population growth was lower in the two cages with 100 pairs of parasitoids and in one of the cages with 200 pairs, than in the control cages. Indeed, the slopes of the increase phase of the aphid populations (Table 1) were less than 60% of that of the controls. The cage 100a was the only cage for which the correlation coefficient (Table 1) was too low to be significant. The two cages with 100 pairs of parasitoids had the same slope. The aphid population trend in cage 100a, unlike that in cage 100b, took the form of a flattened curve indicating a better level of control, but it is more difficult to derive a linear approximation for such a curve. In order to see if these differences were significant, a slope heterogeneity test was performed. Cage 100a could not be tested because the linear approximation was not significant. The slope heterogeneity test indicated a significant difference between the slopes of the six cages ($F(5,4) = 16,91$ $p = 0.0001$). However, the number of parasitoids released in the cages did not account for the heterogeneity in population growth slopes. Indeed, the correlation between the two criteria was not significant (Fig. 2): r=0,365 and $r_{critical}(n=6, \alpha=0.05)=0,7067$.

TABLE 1. Slopes and correlation coefficients of the regression lines fitted to the increase phase of aphid population growth in 1998 for cages in which 100 and 200 pairs of parasitoids were released.

Cage	200 a	200 b	100 a	100 b	control a	control b
b	64.10**	12.46**	18.67	18.56**	101.51**	32.04**
R^2	0.988	0.923	0.663	0.962	0.990	0.984

Regression lines are: non significant or ** highly significant, with r critical = 0.8114 for $\alpha = 0.05$ and r critical = 0.9172 for $\alpha = 0.01$, and number of data points k = 4

TABLE 2. Slopes and correlation coefficients of the regression lines fitted to the aphid population growth in 1998, for the cages in which 50 pairs of parasitoids were released.

Cage	50 a	50 b	control c	control d
b (ln of data)	0.161**	0.073**	0.102**	0.082**
R^2	0.773	0.976	0.876	0.962

Regression lines are ** highly significant, with r critical = 0.7067 for $\alpha = 0.05$ and r critical = 0.8343 for $\alpha = 0.01$, and number of data points k = 6

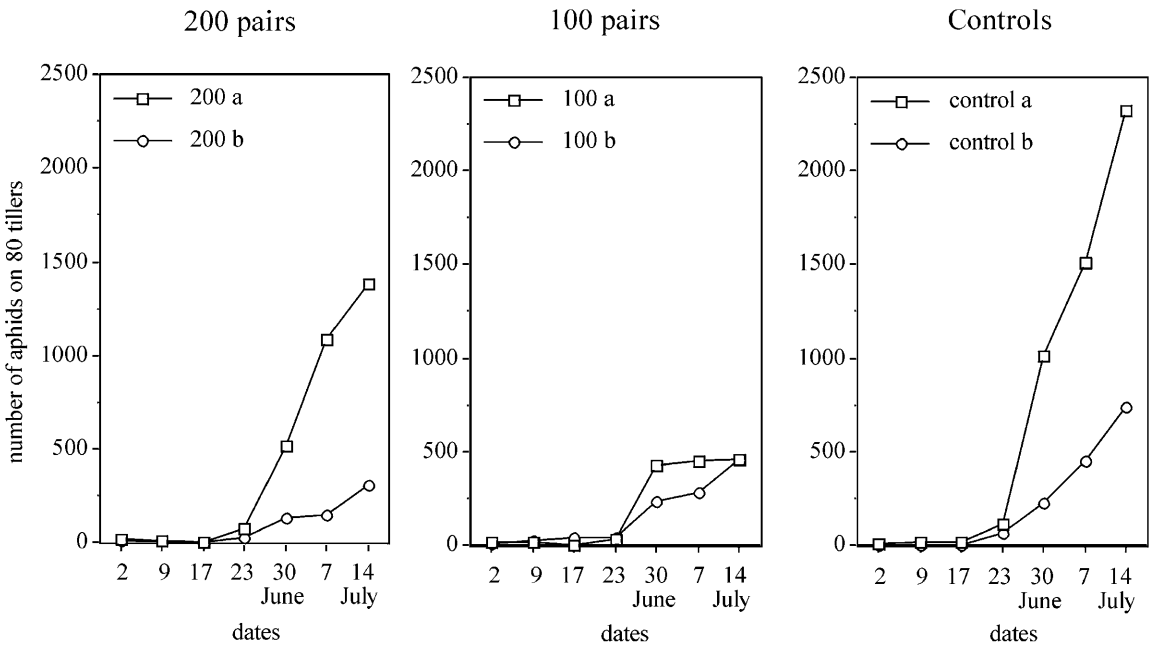

Fig. 1. Growth of the aphid population in the cages in which parasitoids were released in 1998. 100 and 200 pairs were released on 28 May.

50 pairs of parasitoids released vs. control

Fig. 3 represents the growth of aphid populations on 80 tillers. There was no visible difference between the aphid population growth in the two cages with parasitoids and in the control cages. Table 2 records the slope of the linear approximation of the logarithm (ln) of the data and the correlation coefficient. All curves were highly significant. A slope heterogeneity test was performed. It indicated a significant difference between the four cages ($F(3,6) = 3{,}50$ $p = 0.0418$). However, (Fig. 4) the number of parasitoids introduced was not significantly correlated with the slopes ($r=0{,}365$ and $r_{critical}(n=4, \alpha=0.05)=0{,}8114$). The introduction of 50 pairs of parasitoids did not affect the growth of the aphid populations.

1999-cage experiment

Exponential curves gave a satisfactory approximation to the aphid population growth (correlation coefficients Table 3), which seemed to slow in the cages with parasitoids (Fig. 5). Table 3 records the slope of the linear approximation to the logarithm (ln) of the data and the correlation coefficients, which are highly significant. A slope heterogeneity test showed a significant difference between the slopes of the 9 cages ($F(8,20)=2{,}11$ $p=0.0376$). The number of parasitoids released was significantly correlated with the slopes of the aphid poplution growths (fig. 6): ($r=0{,}908$ and $r_{critical}(n=9, \alpha=0.05)= 0{,}6021$).

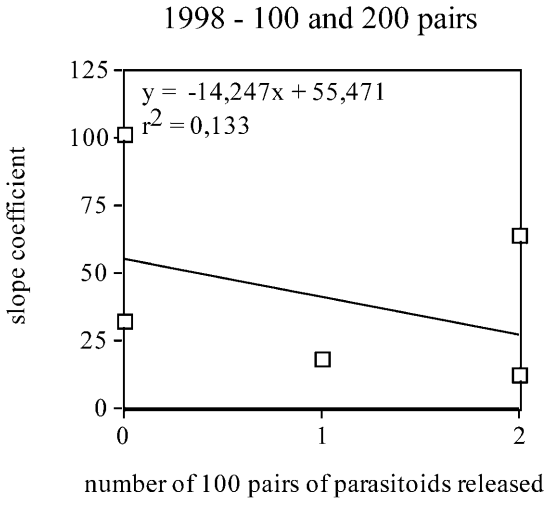

Fig. 2. Correlation between the number of parasitoids released and the slopes of the increase phase of the aphid population curves in 1998. 100 and 200 pairs were released.

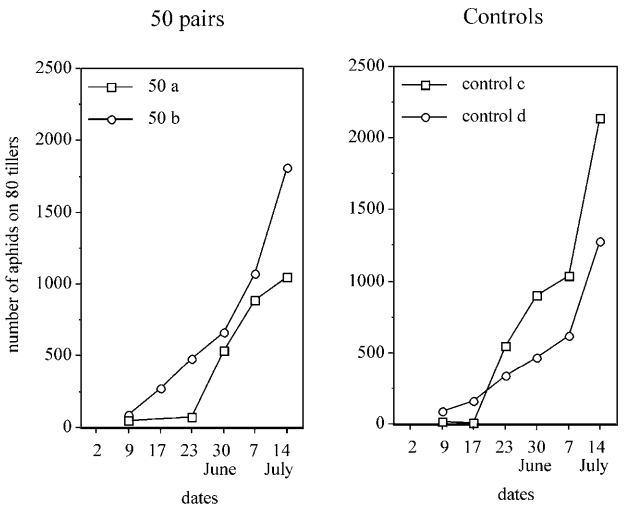

Fig. 3. Growth of the aphid population in the cages in which parasitoids were released in 1998. 50 pairs were released on 5 June.

TABLE 3. Slopes and correlation coefficients of the regression lines fitted to the aphid population growth in 1999.

Cage	200 a	200 b	100 a	100 b	100 c	control a	control b	control c	control d
b	0.080**	0.048**	0.085**	0.082**	0.074**	0.127**	0.111**	0.116**	0.115**
R^2	0.621	0.338	0.738	0.529	0.551	0.755	0.743	0.947	0.766

Regression lines are: ** highly significant, with r critical = 0.5368 for $\alpha = 0.01$ and number of data points k = 20

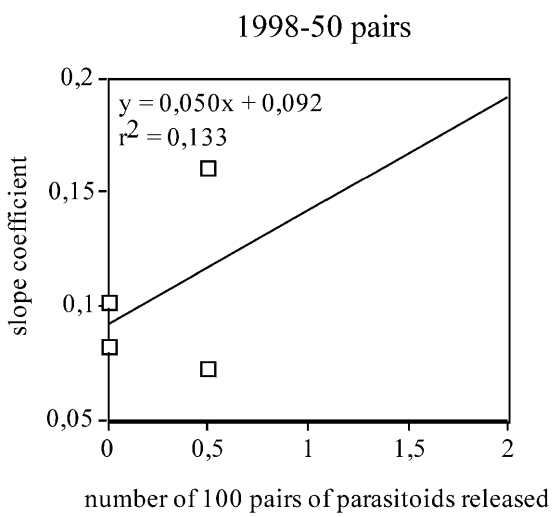

Fig. 4. Correlation between the number of parasitoids released and the slopes of the aphid population curves in 1998. 50 pairs were released.

DISCUSSION AND CONCLUSIONS

In 1998, aphid population growth was the result of naturally occurring aphids (two species: *S. avenae* and *M. dirhodum*) and introduced laboratory-raised aphids (*M. dirhodum*). Natural enemies were left in the cages: parasitoids of different species (*Aphidius* spp. and *Praon* spp.) were seen in the cages, but they were not counted. We assume that the differences in aphid population growths, particularly in the two control cages, were due to differences in numbers of parasitoids present in the cages before the release of *A. rhopalosiphi*. This probably obscured the effect of parasitoid introduction. The introduction of 50 parasitoid pairs did not alter aphid population growth. We were not able to prove statistically that 100 and 200 pairs of parasitoids altered the aphid population growth. However, when 100 pairs were released in the 16 m^2 cages, the aphid populations remained below that in the control cages. So it appears that 12 parasitoid individuals/m^2 might control low aphid populations (< 1 aphid/tiller), and keep them under the 10 aphid per tiller limit.

In 1999, the cages were sprayed with insecticide before aphid and parasitoid introduction, in order to diminish the heterogeneity between cages. The aphid population growth was much higher than in 1998. This was possibly due to the higher number of hours of insolation in 1999 (IRM 1999 and 2000), most probably raising the temperatures in the cages (not recorded), and thus enhancing population growth. Another explanation could be the absence of other natural enemies. When parasitoids were released, the aphid populations were already at 9 aphids per tiller. The introduction of parasitoids diminished sig-

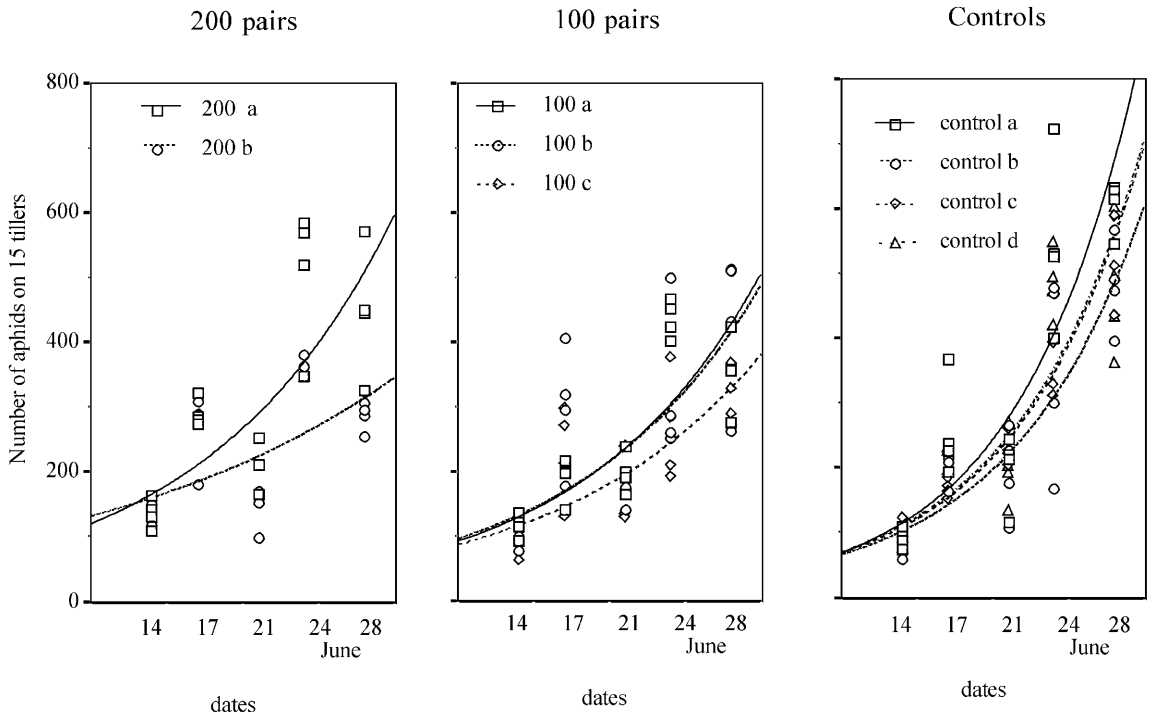

Fig. 5. Exponential model, fitted to the aphid population growth in the cages in which parasitoids were released in 1999. 100 and 200 pairs were released on 10 June.

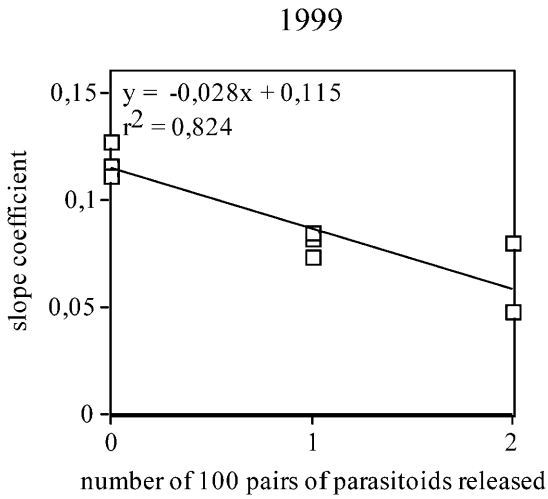

Fig. 6. Correlation between the number of parasitoids released and the slope of the aphid population curves in 1999. 100 and 200 pairs were released.

nificantly the aphid population growth. The latter was inversely related to the number of parasitoids released in a cage.

In contrast to 1999, there were two species of aphids in the cages in 1998. However, *A. rhopalosiphi* does not prefer *S. avenae* or *M. dirhodum* (Stilmant, 1997).

Other field cage experiments show that control can be obtained using parasitoids. Indeed, Simmons & Minkenberg (1994) evaluated the impact of the parasitoid *Eretmocerus* nr. *californicus* (Hymenoptera: Aphelinidae) on *Bemisia argentifolli* (Homoptera: Aleyrodidae), in 5.5 m^2 field cages. They found that low releases (14–59 parasitoids per m^2) resulted in whitefly populations that were not significantly different from the controls, whereas high releases (113–367 parasitoids per m^2) increased parasitism, suppressed whitefly levels and increased cotton yield. These results show that parasitoids can have a strong influence on the host population growth in field cages.

A. rhopalosiphi can control aphid density in the laboratory. Stilmant (1997) studied the control of *S. avenae* by *A. rhopalosiphi* in 0.9 m^2 laboratory cages. He released 70 aphids per m^2, and 6, 13 and 26 parasitoids per m^2: half of the parasitoids were released 5 days after the aphids and the other half 10 days later. The cages with 13 and 26 parasitoids per m^2 had significantly lower aphid populations than the cages with 6 individuals per m^2 or the controls. Laboratory aphid populations probably grow faster than those in field-cages. On the other hand, parasitoid mortality must be lower in the laboratory. However, in both cases, we observed that a minimum of 12 parasitoids (6 pairs) per m^2 had a significant impact on aphid population growth. It is not usual to observe similar levels of control in field and laboratory cages. For example, the level of parasitism of *Sitobion avenae* by *Aphelinus abdominalis* is 4 times higher in laboratory than field cages (Höller & Haardt, 1993).

Our results indicate that the parasitoids can influence aphid population growth. A minimum of 6 pairs per m^2 was necessary to obtain a significant reduction. However, the control of aphid populations below the economic threshold (placed at 10 aphids per tiller) can only be achieved under semi-natural field conditions if the parasitoids are released early in the aphid population increase. Large scale open field studies will now be attempted to study the effect of parasitoid mass release on aphid populations.

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REFERENCES

CARVER M. 1989: Biological control of aphids. In Minks A.K. & Harrewijn P. (eds): *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control, Vol. C.*, Elsevier, Amsterdam, pp. 141–165.

DAGNÉLIES P. 1970: Théorie et méthodes statistiques. 1st ed. Vol. 2. p 427.

HÖLLER C. & HAARDT H. 1993: Low field performance of an aphid parasitoid, Aphelinus abdominalis, efficient in the laboratory (Hym., Aphelinidae). *Entomophaga* **38**: 115–124.

HUGHES R.D. 1989. Biological control in the open field. In Minks A.K. & Harrewijn P. (eds): *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control, Vol. C.* Elsevier, Amsterdam, pp. 167–198.

IRM: Institut Royal Météorologique 1999: Résumé climatologique de l'année 1998. *http://www.meteo.oma.be/ IRM-KMI/ climate/na98_fr.htm*

IRM: Institut Royal Météorologique 2000: Bilan climatologique 1999 pour la Belgique *http://www.meteo.oma.be/IRM-KMI/ climate/ba99_fr.htm*

LANGER A., STILMANT D., VERBOIS D. & HANCE TH. 1997: Seasonal activity and distribution of cereal aphid parasitoids in Belgium. *Entomophaga* **42**: 185–191.

LATTEUR G. 1985: Appercu des travaux effectués en Belgique de 1971 à 1983 en vue de rationaliser la lutte contre les pucerons des céréales en froment d'hiver. *OILB-Bulletin SROP.* **8**: 40–44.

SAS Institute Inc. 1989: SAS/STAT®. User's guide, version 6.12, fourth edition, volume 2. Cary, N.C.:S.A.S. Institute Inc.

SIMMONS G.S. & MINKENBERG O.P.J.M. 1994: Field evaluation of augmentative biological control of Bemisia argentifolii (Homoptera: Aleyrodidae) in southern California cotton with the parasitoid Eretmocerus nr. californicus (Hymenoptera: Aphelinidae). *Envir. Entomol.* **23**: 1552–1557.

STILMANT D. 1994: Differential impact of three Sitobion avenae parasitoids. *Norw. J. Agric. Sci.* **16**: 89–99.

STILMANT D. 1997: *Adaptation des parasitoides à leurs hôtes: étude du modèle formé par les principales espèces de pucerons de céréales (Homoptera: Aphididae) et par leurs hôtes (Hymenoptera: Aphidiidae).* Ph.D.Thesis, Université Catholique de Louvain, Louvain-la-Neuve, 216 pp.

VAN LENTEREN J.C. 1986: Parasitoids in greenhouse: successes with seasonal inoculative release systems. In Waage J. & Greathead D. (eds): *Insect Parasitoids – 13th Symposium of the Royal Entomological Society of London.* Academic Press, London, pp. 341–374.

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