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Mechanical release of *Phytoseiulus persimilis* and *Amblyseius swirskii* on protected crops

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Abstract

The distribution of chemicals on protected crops can be critical for the operators who are forced to make frequent treatments in an enclosed environment and in the presence of high pesticide concentrations. The introduction of organic farming techniques limits these aspects but generally requires a substantial commitment of manpower for the release of beneficial organisms resulting in high costs. To evaluate the feasibility of improving the mechanical application of beneficial organisms a mechanical blower was used. The device was previously tested in the laboratory in terms of mites survival, reproduction and distribution patterns. The application of *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius swirskii* Athias-Henriot against *Tetranychus urticae* Koch and *Frankliniella occidentalis* (Pergande) on protected crops was investigated. Different application methods of the antagonists were considered: i) hand-sprinkling, ii) separate mechanical release, iii) combined mechanical release, iv) paper sachets (Swirskii-Breeding-System). Compared to hand application the mechanical release allowed a significant reduction in time needed for the distribution, while showing equal pest control effectiveness. *P. persimilis* was able to control *T. urticae* adequately in all treatments, while in the case of *A. swirskii* release a final spinosad application became necessary to provide effective *F. occidentalis* control.

Key words: application technology, biological control, *Frankliniella occidentalis*, mechanical distribution, natural enemy release, predatory mite, *Tetranychus urticae*.

Introduction

Chemical control of greenhouse pests has critical aspects for the operators who are forced to make frequent chemicals applications in an enclosed environment and in the presence of high pesticide concentrations. Added to this is the progressive increase of resistance to conventional pesticides, particularly in greenhouses where intensive management and repeated chemical applications produce a strong selection pressure on pests (Bielza, 2008; Pilkington *et al*., 2010). The introduction of organic farming techniques reduces these disadvantages but generally requires a substantial commitment of manpower for the release of beneficial organisms resulting in high costs.

About 100 species of beneficial organisms are currently available on the market for the control of insect and mite pests and are used by growers worldwide (van Lenteren, 2000). However, the application of natural enemies in a greenhouse is still almost always done manually even if the introduction of mechanical release systems would provide undoubted advantages in reducing distribution times and protecting operator health (Opit *et al*., 2005; Pezzi *et al*., 2015).

The main limitation to mechanical release is that the beneficial organisms may be damaged during their handling and distribution due to possible contact with mechanical elements and abrasion against carrier materials (Pezzi *et al*., 2015).

Mechanical release of the anthocorid *Orius laevigatus* (Fieber) (Hemiptera Anthocoridae) and *Phytoseiulus persimilis* Athias-Henriot would be particularly advantageous against *Frankliniella occidentalis* (Pergande) (Thysanoptera Thripidae) and *Tetranychus urticae* Koch (Acari Tetranychidae), respectively, which are economically important pests of many ornamentals and vegetables grown in greenhouses and fields all over the world (Helle and Sabelis, 1985; Silveira *et al*., 2004).

Examples of mechanical distribution are reported by Opit *et al*. (2005) who evaluated the distribution uniformity and survival of *P. persimilis* and *Amblyseius cucumeris* (Oudemans) released with two different mechanical blowers. *P. persimilis* distribution with a mechanical dispenser based on a sliding plate was also evaluated by Casey and Parrella (2005) for the control of *T. urticae* on greenhouse cut roses. Blandini *et al*. (2008) instead developed a centrifugal distribution system for the application of *P. persimilis* and *O. laevigatus* in the greenhouse. The system was used on pepper and also on flower protected crop (Zappalà *et al*., 2012). It was demonstrated to be suitable for organic plant protection treatment and the mechanical distribution working times were more than half compared to manual times. Pezzi *et al*. (2015) tested in the laboratory a mechanical blower for releasing the predatory mites *P. persimilis* and *Amblyseius swirskii* Athias-Henriot, intended for use in the greenhouse. They found that both the distribution pattern and effects of the mechanical application on beneficial arthropods were adequate for use on protected crops.

In this study, the blower device developed and tested in the laboratory by Pezzi *et al*. (2015) was used for controlling eggplant pests in a protected crop. The application of *P. persimilis* and *A. swirskii* to *T. urticae* and *F. occidentalis* control in the greenhouse was evaluated to compare mechanical and manual distribution using different application strategies and predatory mite formulations: hand-sprinkling, separate mechanical release, combined mechanical release and paper sachets.

Materials and methods

Greenhouse experiments

The experiment was conducted at Igea Marina, Rimini Province, Northern Italy, one of the main areas for protected eggplant production in the Po Valley. A typical local (44°07'09"N 12°29'31"E) horticultural farm, producing vegetables in greenhouses covered by polyethylene film, was used. Four identical commercial greenhouses (30 × 8 m) with an arched roof (height 3.3 m) were chosen, cultivated with eggplant (*Solanum melongena* L.) cv. Nilo from May to August 2010.

In each greenhouse there were 7 rows of plants spaced 1.2 m apart; the distance of plants on the row was 0.7 m, for a total of 300 plants. Treatments were: i) separate mechanical release (SMR) for consecutively application of the two mites, ii) combined mechanical release (CMR) for simultaneously application of both mites, iii) paper sachets (PS) for *A. swirskii* release along with mechanical *P. persimilis* release, iv) hand sprinkling of both mites (HS). Each treatment was randomly assigned to a greenhouse (table 1).

A. swirskii for manual and mechanical release was purchased in 0.1 litres cardboard tubes each containing 10,000 mites (Swirskii-system, Biobest Biological Systems, Belgium) dispersed on vermiculite and bran for the first release, and in 0.1 litres cardboard tubes each containing 25,000 mites (AMBLYPAK25000SW, Bioplanet, Italy) dispersed on vermiculite and bran for the other release dates. Since these containers did not fit on the extraction system of the blower, the mixture carrying the mites was carefully poured into a 2 litres bottle fastened on the blower that acted as a reservoir. Paper sachets each containing 250 predatory mites were purchased from Koppert Italia (SWIRSKI-MITE PLUS). *P. persimilis* was purchased from Bioplanet (Italy) in 0.5 litres bottles each containing 1,000 mites (FITOPAK1000) dispersed on vermiculite. These containers were fitted directly on the extraction system of the blower without the need for mites handling (Pezzi *et al.*, 2015). In the CMR, just before being released, 2 bottles each containing 1,000 *P. persimilis* plus carrier material and a cardboard tube containing 25,000 *A. swirskii* plus carrier material were contemporarily poured into the 2 litres reservoir. For all treatments and application systems, the beneficial dose distributed was chosen according to the manufacturer's recommended release rate. Mites release rate, application dates and patterns are reported in table 1. The combined mechanical release of both mites was adopted only on the last release date (7 July). Indeed, the simultaneous release of both beneficials only resulted as being necessary on this date.

With the aim of evaluating the efficacy of the different application methods, on each of the eight sampling dates (from 27 May to 02 August 2010) the mean number of *T. urticae*, *F. occidentalis*, *P. persimilis* and *A. swirskii* per leaf was determined by counting immature life stages (with exception of eggs) and adults using a 5X hand-held magnifying lens. On each sampling date, one randomly chosen leaf per plant on 50 plants per greenhouse was sampled.

The evaluation of efficacy of the different application systems was performed under the assumption of "observational survey" (Schwarz, 1998). With this approach, four greenhouses were selected, each one, as previously stated, with a different application system. Sample plants were located in each greenhouse, and the number of pests and predatory mites was counted on one leaf of each sample plant; the response variables were the number of pests and predatory mites on a leaf of each plant. The results are only applicable to the four sampled greenhouses and cannot be extrapolated to other greenhouses (Schwarz, 1998).

Application methods

The beneficials mechanical distribution (SMR and CMR) was performed with a blower device (figure 1) previously tested in the laboratory (Pezzi *et al.*, 2015). The device was set at maximum air speed (30 m s^{-1}) that allowed a release distance of ≈8 m with a width of 1.2 m. At this regulation most of the dispersed material (beneficials + carrier material) was concentrated between 3 and 6 m, which resulted as suitable for distribution in the confined spaces of the greenhouses ensuring

Table 1. Beneficials applications and treatment layout. SMR, separate mechanical release; HS, hand sprinkling; CMR, combined mechanical release; PS, paper sachets for *A. swirskii* and mechanical release for *P. persimilis*.

Treatment	4 June		17 June		22 June		7 July	
	Beneficial	Dose	Beneficial	Dose	Beneficial	Dose	Beneficial	Dose
SMR	*A. swirskii*	42 mites m^{-2}	*P. persimilis*	8 mites m^{-2}	*A. swirskii*	100 mites m^{-2}	*A. swirskii* and *P. persimilis* [a]	100 mites m^{-2} 8 mites m^{-2}
HS	*A. swirskii*	42 mites m^{-2}	*P. persimilis*	8 mites m^{-2}	*A. swirskii*	100 mites m^{-2}	*A. swirskii* and *P. persimilis*	100 mites m^{-2} 8 mites m^{-2}
CMR	*A. swirskii*	42 mites m^{-2}	*P. persimilis*	8 mites m^{-2}	*A. swirskii*	100 mites m^{-2}	*A. swirskii* comb. with *P. persimilis* [a]	100 mites m^{-2} 8 mites m^{-2}
PS	*A. swirskii*	0.4 sachets m^{-2}	*P. persimilis*	8 mites m^{-2}	*A. swirskii*	0.4 sachets m^{-2}	*A. swirskii* and *P. persimilis*	0.4 sachets m^{-2} 8 mites m^{-2}

[a] In SMR the two phytoseiids were consecutively released with the blower instead in CMR they were mixed and simultaneously released (see materials and methods).

Figure 1. Blower device (Pezzi *et al*., 2015), for predatory mites mechanical release. 1) Bottle containing beneficial organisms, 2) air diffuser, 3) extraction system operated by electromagnet for rod frequency regulation, 4) command lever for air speed regulation.

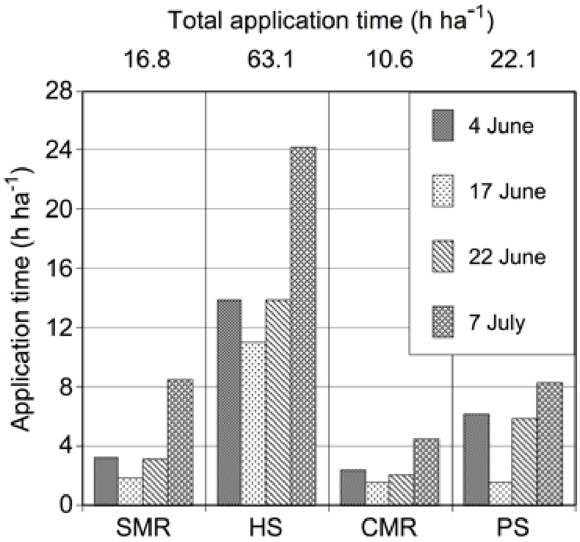

Figure 2. Application times (distribution plus additional times) for each treatment. SMR, separate mechanical release; HS, hand sprinkling; CMR, combined mechanical release; PS, paper sachets.

an uniform distribution pattern (Pezzi *et al*., 2015) thus favouring an evenly predatory mite coverage.

During the application the operator oscillated the blower with an angle of about 90°. In this way, moving along the second and fifth interrow of the greenhouse it was possible to distribute the product on all the plants. The advancement speed was adapted by the operator according to the bottle emptying time. On each date, all beneficial releases were performed by the same operator.

In the manual application (HS) the operator dispensed the material directly on eggplant leaves spilling it from the bottle and intervening on a row at a time. In the paper sachets application (PS) the operator moved along the interrow applying 100 sachets per greenhouse to the supporting wires of the plants.

For all treatments, the distribution times and additional times were measured to calculate the total application time. The additional times were constituted by the time for procuring the sachets for PS treatment and times of mixing and substitution of the bottles for the other treatments.

Results

Field capacity

Table 2 summarizes, for the four treatments, the operator advancement speed and additional times for the distribution in each greenhouse.

In the mechanical distributions the speed of the operator was very variable (0.2-0.7 m s^{-1}) depending on the emptying times of the container according to the type and moisture of carrier material in which mites were dispersed. The substitution times varied from 25 to 60 s mainly depending on the number of bottles used (1 or 2) in each greenhouse.

The mechanical release treatments required less working time (distribution plus additional times), namely 16.8 h ha^{-1} and 10.6 h ha^{-1} for the SMR and CMR treatment respectively (figure 2). The lower time of CMR treatment was determined by the mixture of both beneficials in the last release on 7 July, which allowed both predatory mites to be applied in one pass. Good performances were also achieved in the PS treatment

Table 2. Operator advancement speed and additional times for mites application in each greenhouse. SMR, separate mechanical release; HS, hand sprinkling; CMR, combined mechanical release; PS, paper sachets for *A. swirskii* and mechanical release for *P. persimilis*.

Treatment	4 June		17 June		22 June		7 July		7 July		7 July	
	A. swirskii		*P. persimilis*		*A. swirskii*		*A. swirskii*		*P. persimilis*		*A. swirskii* combined with *P. persimilis*	
	Speed (m s^{-1})	Time (s)	Speed (m s^{-1})	Time [a] (s)	Speed (m s^{-1})	Time (s)	Speed (m s^{-1})	Time (s)	Speed (m s^{-1})	Time [a] (s)	Speed (m s^{-1})	Time [a] (s)
SMR	0.24	32	0.57	55	0.25	30	0.11	36	0.50	50		
HS	0.18	28	0.23	60	0.18	32	0.18	30	0.25	54		
CMR	0.33	25	0.71	55	0.39	25					0.18 [b]	60 [b]
PS	0.25	60	0.70	54	0.26	55	0.25	60	0.47	50		

[a] 2 bottles distributed; [b] speed and time relative to combined release of *A. swirskii* plus *P. persimilis*.

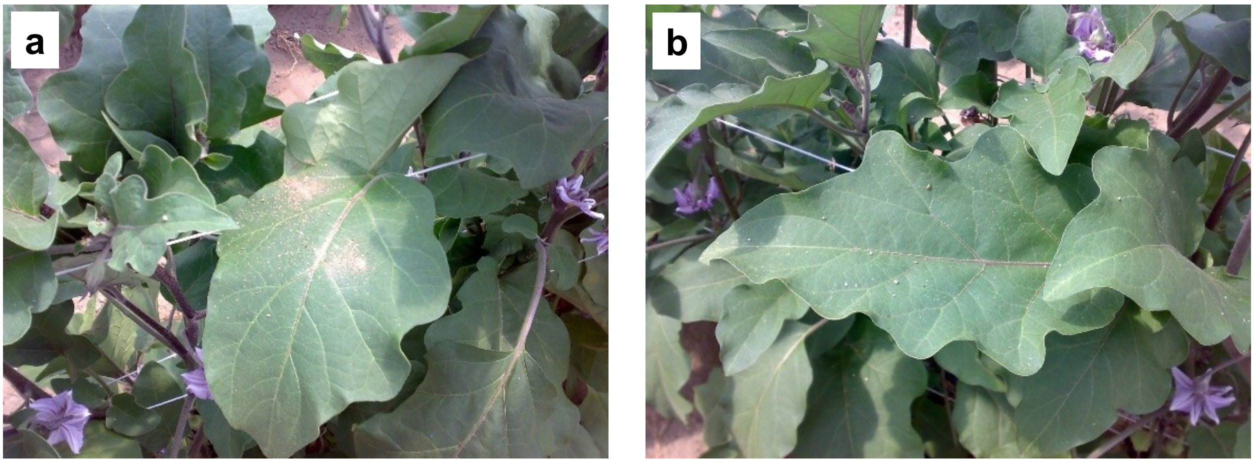

Figure 3. Eggplant leaves after beneficials application: (a) manual release; (b) mechanical application.

(22.1 h ha^{-1}) where *A. swirskii* were distributed from paper sachets and *P. persimilis* with the blower device. The longest times were those of the HS treatment that required 63.1 h ha^{-1}.

The outcome of the beneficials application on the plants is shown in figure 3 where a more even distribution of the product (beneficials + carrier materials) can be observed in the mechanical application, while the product appears clumped in the manual release. The distribution pattern generated by the blower used in this study guarantees a uniform horizontal distribution of predatory mites, as previously reported by Pezzi *et al*. (2015).

Pests and beneficials behaviour

No differences were observed among the four greenhouses in the number of both *F. occidentalis* and *T. urticae* on the first sampling date (figures 4a and 5a) hence the initial infestations were considered similar among treatments.

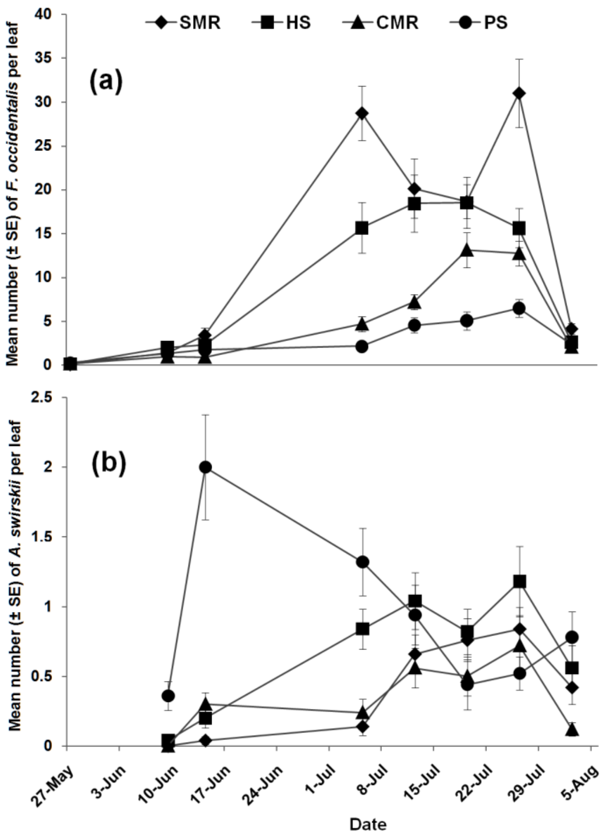

Figure 4. Mean number (± SE) of *F. occidentalis* (a) and *A. swirskii* (b) on each sampling date in the four greenhouses.

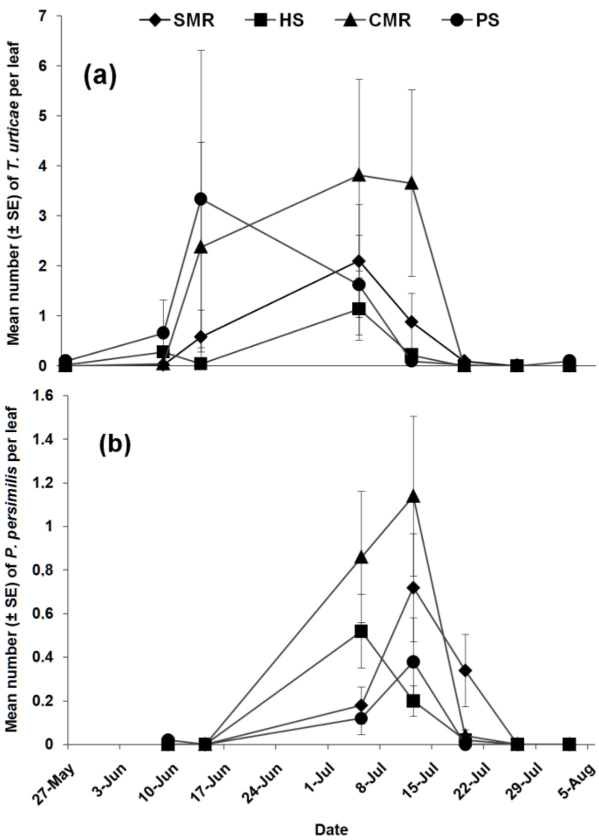

Figure 5. Mean number (± SE) of *T. urticae* (a) and *P. persimilis* (b) on each sampling date in the four greenhouses.

There were no differences in the mean number of *F. occidentalis* sampled among treatments on the first three sampling dates (figure 4a). Afterwards the SMR greenhouse showed the highest thrips counts on the 4th and 7th sampling dates. However, the numbers on the other dates were the same as in the HS greenhouse. The CMR greenhouse always had lower thrips counts with respect to HS. Instead, the PS treatment showed the lowest ones.

There was little difference among mechanical treatments (both SMR and CMR) and HS on the mean number of *A. swirskii* sampled, even if the HS greenhouse had the highest numbers on four sampling dates (figure 4b). Nevertheless, in these three greenhouses, a spinosad application on 30 July resulted as being necessary to provide effective control of *F. occidentalis*. Conversely, the *A. swirskii* formulated in sachets consistently had the highest number of mites on the first three sampling dates, providing the best thrips control even without pesticide applications.

All the greenhouses with mechanical application (SMR, CMR and PS; the latter had mechanical distribution of *P. persimilis* since the sachets were used only for *A. swirskii* release) suffered relatively higher *T. urticae* infestation than the HS greenhouse (figure 5a). However the very low mite infestation, the clumping of individuals and large number of zero observations makes it difficult, in this case, to separate the contribution of the different treatments.

There were some differences among treatments in the mean number of *P. persimilis*, with the CMR and HS greenhouses having higher counts from the 3rd sampling date, the former peaking on the subsequent sampling date (figure 5b). A rapid decrease of *P. persimilis* followed, mainly due to a fast decline of *T. urticae*. The other two greenhouses in which *P. persimilis* was applied alone with the blower, instead, showed a slower population onset. Nonetheless, *T. urticae* was successfully controlled in all greenhouses on the 6th sampling date.

Discussion

This study demonstrated that the mechanically released *A. swirskii* and *P. persimilis*, both in separate or combined mode, were as effective as those manually released, in controlling *F. occidentalis* and *T. urticae*, respectively. However, the best control of *F. occidentalis* was achieved with the slow-release sachet formulation of *A. swirskii*. Previous laboratory tests demonstrated that mechanical application of *P. persimilis* and *A. swirskii*, in separate or combined release, does not reduce their viability or reproductive capacity respect to manual sprinkling (Pezzi *et al*., 2015).

Dispersal capability of released mites appears to be a key factor for biological control success, influencing the application strategy and the release pattern throughout the greenhouse (Skirvin *et al*., 2002; Skirvin and Fenlon, 2003). Different studies have dealt with the dispersal capability of *A. swirskii* (Buitenhuis *et al*., 2010) and *P. persimilis* (Casey and Parrella, 2005; Alatawi *et al*., 2011), concluding that predators released only in areas of mites infestation can increase the risks of pest outbreaks and that an even and good coverage should be preferred for both predatory mites.

Obtaining a good coverage of the crop with manual release at each predatory mite application requires a lot of time and thus costs that cannot favour biological control practices. The use of air-assisted systems, such as the one utilized in this study, other than ensuring an even distribution of predators, dramatically reduces the beneficials application time to 3.7 times shorter than the manual distribution for the separate mechanical release (SMR) and reaching 5.9 in the case of the combined mechanical distribution of both predatory mites (CMR). A 47% reduction in application time by using a custom-made mechanical dispenser for *A. cucumeris* release was observed by Van Driesche *et al*. (2002) for the control of *F. occidentalis* in commercial greenhouses.

Likewise, the application of the sachet formulation required half the time than manual distribution of *A. swirskii*. However, it took 1.9 time longer compared to mechanical application (see 4 and 22 June releasing) even if a better performance of the sachet formulation was found in this study. To overcome this and to obtain a satisfactory control of *F. occidentalis*, also with the mechanical blower, the release strategy should be optimized especially with regard to the number of *A. swirskii* released and/or the release frequency.

The results obtained led us to conclude that the control of *F. occidentalis* and *T. urticae*, adopting a strategy with mechanical application of predatory mites, is consistent with that obtained with manual application. The manual distribution of biocontrol agents is usually a time-consuming and expensive process that in protected crops, also forces the operator to remain in an unhealthy environment while performing a strenuous task. The use of mechanical blower could therefore considerably decrease pest control costs in terms of working time thus making biological control more affordable and providing a substantial support to its development and adoption in protected crops.

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