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Mechanical application of *Orius laevigatus* nymphs for the control of *Frankliniella occidentalis* in greenhouse crops

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Abstract

The development of improved technology for use of beneficial organisms in greenhouse crops includes mechanical application methods to that can reduce labour costs compared to manual application while preserving the quality of the applied natural enemies. A pneumatic distribution system, verified as effective in previous study with mites (*Phytoseiulus persimilis* Athias-Henriot and *Amblyseius swirskii* Athias-Henriot), was tested for application of the predatory bug *Orius laevigatus* (Fieber). Laboratory and field tests were used to assess the efficiency of mechanical distribution of nymphs of *O. laevigatus*. The efficiency of mechanical distribution was evaluated comparing the biological parameters of the predator (survival, fecundity, fecundity rate and longevity) dispensed mechanically with to those following manual distribution. Nymphal survival immediately after and 10 days after the dispensing was calculated as proportion of nymphs reaching adulthood. We found that mechanical applications caused a reduction of predator survival in comparison to manual sprinkling of 21% and 36% immediately after and 10 days after the dispensing, respectively. The causes of the reduced survival from mechanical application were attributed to the combined effect of the extraction system and the velocity of airflow that transports the predator. Moreover, the biological parameters of adult females that survived mechanical distribution as nymphs, showed no significant differences compared to the same following manual distribution. In the greenhouse test, the mechanically released nymphs of *O. laevigatus* were as effective as those manually released in controlling *Frankliniella occidentalis* (Pergande) in a cucumber crop. Consistently, the yield and quality of cucumber fruits did not show differences in the mechanical *vs*. manual application. Mechanical application time was significantly lower compared to manual application, determining a 5.5 higher effective work capacity compared to traditional manual distribution.

Key words: application technology, *Orius laevigatus*, protected crop, cucumber, *Frankliniella occidentalis*, mechanical application, biological control, natural enemy release, survival, reproduction.

Introduction

The strong growth in the rate of adoption of biological control products and strategies for pest control is expected to continue because of the increasing demand for organic foods by the consumers and expanding government policies support pesticide reduction (Papa *et al*., 2018). Among factors promoting increase use of biological control and Integrated Pest Management (IPM), one of the most important has been increased resistance by pests to conventional pesticides in greenhouses (van Lenteren, 2000; Bielza, 2008; Pilkington *et al*., 2010). Other trends likely to increase in the use of biological control in greenhouses include the shortage of new pesticides due to the high cost of development and registration of new products in view of the relatively small market of greenhouse producers (van Lenteren, 2000). In addition, the perceived health risk for greenhouse workers applying pesticides in enclosed places encourages the adoption of biological control techniques (Pilkington *et al*., 2010; Pezzi *et al*., 2015).

Currently, about 100 species of beneficial organisms are commercially available for the control of insects and mite pests and a number of these are extensively used by growers worldwide (van Lenteren, 2000). The application of natural enemies in greenhouse crops is almost entirely carried out by manual application even though mechanical distribution systems would likely reduce application times and the incidence of allergies for applicators (Blandini *et al*., 2008; Zappalà *et al*., 2012; Lanzoni *et al*., 2014; Pezzi *et al*., 2015; Papa *et al*., 2018).

Research on application systems for dispersal of beneficial organisms is limited despite frequent requests by farmers, for more information on timely and cost effective application methods (Sargent and Hoddle, 2002), which would allow wider implementation of biological control (Sargent and Hoddle, 2000).

One limitation of mechanical application systems is that the beneficial organisms may be damaged by the machine parts during handling and distribution due to contact with mechanical elements and abrasion against carrier materials. In a greenhouse context, Pezzi *et al*. (2002) developed and tested an air-assisted system for *Phytoseiulus persimilis* Athias-Henriot that was characterized by high versatility and was able to effectively treat both crop plants growing near the ground and crops suspended vertically on supports. Opit *et al*. (2005) studied the distribution uniformity and survival of *P. persimilis* and *Amblyseius cucumeris* (Oudemans) applied with two different mechanical blowers, which resulted in average survival rates of 31% and 49% for *P. persimilis* and 61% and 79% for *A. cucumeris*. The resulting distribution of *P. persimilis* was also evaluated in relation to the control of *Tetranychus urticae* Koch on greenhouse cut roses (Casey and Parrella, 2005) using a mechanical dispenser with a sliding plate that did not appear to cause predator mortality and reduced application time by 67% compared to hand release. A centrifugal distribution system for the application of *P. persimilis* and *Orius laevigatus* (Fieber) was developed by Blandini *et al*. (2008) for biological control in greenhouses. In greenhouse pepper crops, the device proved to be suitable for the release of the two predators and cut application time in half. In the laboratory, Pezzi *et al*. (2015) tested a mechanical blower for application of the predatory mites *P. persimilis* and *A. swirskii* in greenhouses. The authors found that both the distribution pattern and the effects of mechanical application on predator quality were adequate for use in protected crops (Pezzi *et al*., 2015; Lanzoni *et al*., 2017).

A system for mechanical application of the anthocorid bug *O. laevigatus* was be advantageous as this species is an important predator in the Mediterranean area for control of the western flower thrips, *Frankliniella occidentalis* (Pergande), as well as aphids, mites and whiteflies (Alvarado *et al*., 1997; Montserrat *et al*., 2000; Tommasini *et al*., 2004). In particular, *F. occidentalis* is one of the most damaging pests of greenhouse crops worldwide (Tommasini and Maini, 1995). Release of *O. laevigatus* for *F. occidentalis* control in greenhouses is generally carried out by manually releasing adults, but it would be of very helpful to also release the preimaginal stages as they can also prey on thrips (Tommasini *et al*., 2004; Helyer *et al*., 2014). This practice would also allow us to reduce the rearing period in the commercial insectaries, reducing production costs and make it possible to employ predator at an earlier wingless stage incapable of dispersal by flight as can occur in the adult stage. Also the use of juveniles would avoid the damage of the air blast on the wings, which are delicate (Ade *et al*., 2010).

The goal of this study was to evaluate the use of the mechanical blower developed by Pezzi *et al*. (2002) for application of *O. laevigatus* nymphs in greenhouses. Our specific objectives were (*i*) to evaluate the feasibility of mechanically release of *O. laevigatus* at the nymphal stage; (*ii*) to find the most suitable settings of the blower for an effective distribution; (*iii*) to compare the survival and reproduction of *O. laevigatus* after mechanical vs manual application; (*iv*) to evaluate the practical functionality of the blower and its effectiveness for *F. occidentalis* control; and (*v*) to compare the application time using the blower respect to the traditionally hand release method.

Materials and methods

Dispensing device

The mechanical blower of Pezzi *et al*. (2002; 2015) consisted of a system of controlled extraction of the material carrying the *O. laevigatus* nymphs, which was then released by gravity into a diffuser where it is transported by an air flow (figure 1).

The mechanical extraction system consisted of a push rod with a metal spike with reciprocating movement generated by an electromagnet powered by a handheld battery. The push rod of the blower operating as originally designed by Pezzi *et al*. (2015) was modified by replacing it with a tapering and cone-shaped rod (diameter 15 mm, length 40 mm) better suited for the extraction of the buckwheat husk carrier material we used (figure 2).

Regulation of the movement rod (through frequency and stopping position) controlled the amount of carrier material released that fell into the pneumatic diffuser. The extraction system was fitted to the diffuser of a blower (Super Jolly model, Vibi Sprayers, Cremona, Italy) equipped with a 2.2 kW two-stroke engine that drove a radial fan. The tubular diffuser (60 mm diameter) had a flexible section, connected to the fan and a rigid end section which was adjustable by the operator and had a hand throttle to regulate engine speed. The air speed was measured using a Höntzsch fan anemometer (μP-ASDI, Höntzsch GmbH, Waiblingen, Germany) positioned at the blower outlet.

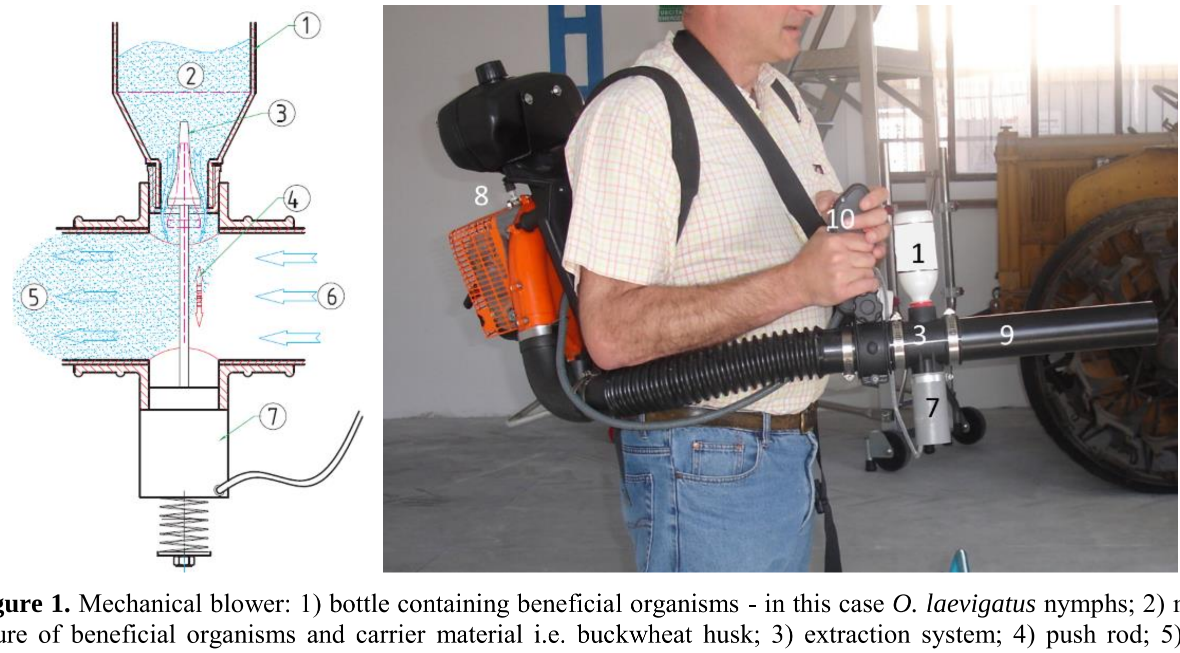

Figure 1. Mechanical blower: 1) bottle containing beneficial organisms - in this case *O. laevigatus* nymphs; 2) mixture of beneficial organisms and carrier material i.e. buckwheat husk; 3) extraction system; 4) push rod; 5) air stream blowing beneficial arthropods and carrier material; 6) air flow generated by the fan; 7) electromagnet for rod frequency regulation; 8) backpack blower; 9) air diffuser; 10) command lever.

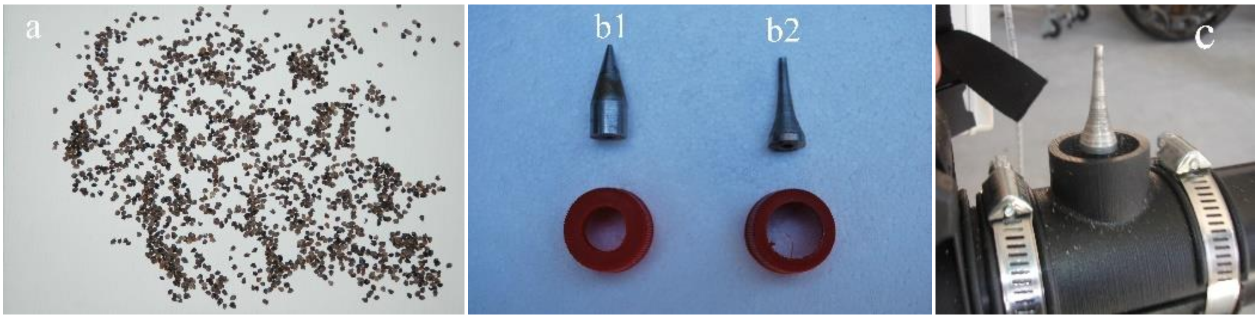

Figure 2. Buckwheat husk carrier material (a); push rod and bottle outlet section (mm 17) used for *P. persimilis* distribution (Pezzi *et al*., 2015) (b1); push rod and outlet section (mm 22) used for *O. laevigatus* application (b2); push rod emerging from the air diffuser (c).

Laboratory tests

The predatory bugs were purchased from a commercial insectary (Bioplanet Srl, Cesena, Italy) in 0.5-l bottles each containing 500 5th instar nymphs of *O. laevigatus*. The product was specifically prepared for our study since *O. laevigatus* is normally sold only as adults. The carrier material used was the same buckwheat husk used in the commercial product. The predatory bugs were used in our experiments on the same day the shipment was received, with storage at 8 °C until actual use. Each bottle was divided into two parts, the first for mechanical release with the blower and the second for manual sprinkling. Before the release, the bottles containing predators were gently shaken and rotated to facilitate the mixing of nymphs with the carrier material.

Based on preliminary tests of achieved distribution, the blower was set to optimal distribution for greenhouse conditions (air speed 20 m s^{-1}; push rod moving frequency 3 Hz). To test the release capacity, the device was positioned horizontally at a height of 1 m and the material was applied on a test bench on which there were ten containers lined up in front of the dispenser, starting from the distal end of the air diffuser. Each container consisted of a box with a square-shaped top surface (0.5 × 0.5 m) with a funnel-shaped structure that facilitated the flow of the intercepted material into a 0.125-l test tube placed below the box to catch the applied sample (figure 3).

The manual distribution, representing the control treatment, was carried out by distributing the predatory bugs on the same test bench using the same amount of product as that used in the mechanical distribution and simulating the distribution movements usually followed during field application.

In order to define the distribution pattern of the *O. laevigatus* plus carrier material, each test tube' catch, following mechanical release, was weighed with a Sartorius BP 310 S balance (Sartorius AG, Göttingen, Germany) with a maximum weight: 310 g and an accuracy of 0.001 g.

Biological measurements in laboratory experiment

To evaluate the survival of individuals immediately after manual and mechanical application the numbers of live and dead nymphs of *O. laevigatus* collected from each box on the test bench at six interval distances from the distal end of the diffuser (0.5-1.0, 1.0- 1.5, 1.5-2.0, 2.0-2.5, 2.5-3.0, 3.0-3.5 m) were counted in both treatments with the aid of a microscope. The content of each 0.125-l test tubes was sprinkled onto a Petri dish (⌀ 110 mm) with a 10 × 10 mm grid on the base to facilitate counting of the nymphs. Afterwards, for each of the six interval distances, 15 nymphs were reared for 10 days in Plexiglas cylinders (⌀ 50 mm, h 70 mm; 3 nymphs per cylinder) in a climatic chamber at 25 ± 1 °C, RH 60-80%,

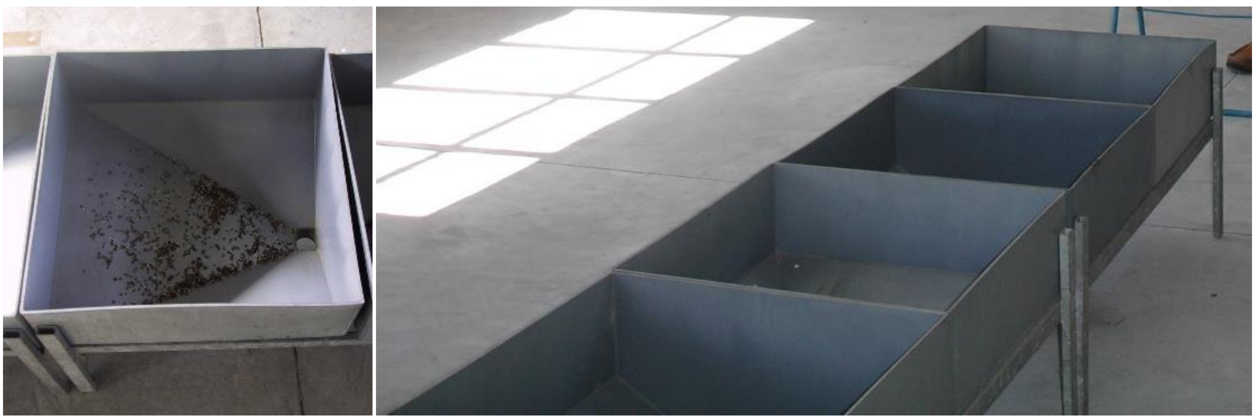

Figure 3. Test bench for laboratory application trials.

Table 1. Mechanical blower setting and treatment layout.

Test	ID	Push rod frequency (Hz)	Air speed (m s^{-1})	Air flow rate (m^3 h^{-1})
Effect of push rod alternative movement	MA1	0	0	0
	MA2	3.0	0	0
	MA3	6.6	0	0
Effect of air speed	MB1	-	20	203
	MB2	-	30	305
	HS	-	-	-
Combined effect of push rod movement and air speed	MC1	3.0	10	102
	MC2	3.0	15	153
	HS	-	-	-

L:D 16:8, to check for any deaths until adulthood. During this period, nymphs were fed frozen eggs of *Ephestia kuehniella* Zeller (Lepidoptera Pyralidae) obtained from a commercial supplier (Bioplanet Srl, Cesena, Italy). To prevent cannibalism, crushed pieces of tissue paper were added to the containers. The percentage survival of the *O. laevigatus* nymphs was calculated as proportion of original nymphs reaching adulthood.

In a second trial, the survival and reproductive performances of adult females of *O. laevigatus*, previously dispersed as 5th instar nymphs, were assessed in both treatments (mechanical and hand dispersal). In each treatment, newly emerged (<24 h old) adult females from individuals of the previous experiment, were collected randomly, without regard for test bench interval distances. Females were paired singly with one male of the same treatment and caged in individual plastic containers (⌀ 50 mm, h 70 mm). Cages were sealed with a fine net (⌀ 40 mm) on the top to favor air circulation and to avoid mold growth and were kept in a climatic chamber at 25 ± 1 °C with a R.H. of 60-80%, L:D 16:8. The adults were fed as during their nymphal life, and green bean pods were added as oviposition substrate. Every other day green bean pods were replaced with fresh ones and checked for oviposition. Survival of females was also recorded. After 12 days the experiment was stopped. Each experiment had three independent replicates.

Mechanical blower settings

Since the mechanical distribution of *O. laevigatus* resulted in a significant reduction of nymphal survival, an additional test was run to investigate the reasons of the observed results. In particular, the effect of i) the extraction system through the alternating movement of the push rod, ii) the air speed inside the air diffuser (the air flow generated by the blower), and iii) the combination of the extraction system and the air speed (air flow) on nymphal survival were analysed.

To test the possible damage attributable to extraction system, three treatments were identified (table 1): i) push rod kept motionless, the bottle content was allowed to flow into the air diffuser by gravity simply shaking the blower (MA1); ii) push rod moving at low frequency (3.0 Hz) (MA2); and iii) push rod moving at high frequency (6.6 Hz) (MA3). The mixture carrying the nymphs was expelled by tilting the blower over the test bench as described above. With the aim to test possible effects due to air speed, the mixture carrying the natural enemies was dispersed over the test bench setting the blower at two different air speed, i.e. 20 m s^{-1} (MB1) or 30 m s^{-1} (MB2) (table 1). In both treatments, the bottle content was made to flow into the air diffuser by shaking the blower, with the push rod removed. The effect of hand-sprinkling (HS) over the test bench was used as control. Finally, the combination of extraction system and air speed was tested running the blower with two different setting, i.e. low air speed and push rod moving at low frequency (MC1) and high air speed and push rod moving at low frequency (MC2) (table 1). Again, the effect of hand-sprinkling was used as control. In all trials the survival of *O. laevigatus* nymphs immediately after being released and 10 days after the releasing, were evaluated as previously described.

Field tests

The field experiment was conducted in a greenhouse at ASTRA (Agency for Technological Research and Innovation in Agriculture and Environment, Imola, Bologna, Italy).

The greenhouse (33 × 7 m) was planted with cucumber (*Cucumis sativus* L.) var. Dinero. There were 6 rows of plants, spaced 1 m between rows; the distance along rows was 0.45 m for a total of 420 plants. Treatments were hand sprinkling or mechanical release. The greenhouse was divided into 6 plots, each measuring 11 × 3 m. Plots were delimited using transparent plastic sheets, which were hung on the structure of the greenhouse (figure 4). To guarantee air circulation, the greenhouse was opened laterally during daytime, and the plastic sheets did not reach the ceiling of the greenhouse. In every plot, there were 3 rows of plants for a total of 70 plants/plot. Treatments were randomly assigned to the plots, three to mechanical release and three to hand sprinkling. In both treatments, each plot was considered as a replicate.

As for the laboratory tests, the predatory bug was purchased in 0.5-l bottles each containing 500 5th instar nymphs of *O. laevigatus*. The predator was released on July, 12. Based on the predator application rates recommended by the producer (5 nymphs of *O. laevigatus* m^{-2}) and to cope with the higher mortality ascertained in the laboratory tests, the application rate in the mechanical release was increased to 6.5 nymphs m^{-2}. To allow a

Figure 4. Test plots on the cucumber greenhouse. Each plot, 11 × 3 m, was delimited by transparent plastic sheets.

better operability of the blower, the nominal product concentration used in the mechanical release, was reduced to 250 nymphs per 0.5-l bottle by dividing each of the purchased bottles into two parts and pouring each part together with additional carrier material, into a new 0.5-l bottle. The resulting mixture was then, gently rotated to homogeneously mix the nymphs and the carrier material. Before *O. laevigatus* release, three samples were taken from the obtained 0.5-l bottle; each sample was checked to confirm uniform distribution of the nymphs inside bottles using a 5× hand-held magnifying lens. Throughout the experiment, the blower was run at the lowest setting, i.e. air speed 20 m s^{-1} and push rod frequency 3.0 Hz. During the application, the operator oscillated the blower with an angle of about 90°. In this way, moving along the first interrow in each plot, it was possible to distribute the product on all the plants.

For both release methods (hand and mechanical), the additional times for bottle preparation and blower setting before distribution, as well as the application time, were measured to determine the field efficiency (E_f) and effective field area capacity (C_a) of the two application systems (Eq. 1) (ASAE, 2007).

$$C_a = 0.1 \cdot w \cdot f_s \cdot E_f \quad \text{(Eq. 1)}$$

where, C_a represents effective field capacity (ha h^{-1}), w the working width (m), f_s the field speed (km h^{-1}) and E_f the field efficiency (ASAE, 2007).

On each of the eight sampling dates (7, 14, 21, 28 July and 4, 11, 18, 25 August), the mean number of *F. occidentalis* and *O. laevigatus* specimens was determined by counting immature life stages and adults on one randomly chosen leaf and flower per plant using a 5× hand-held magnifying lens. On each sampling date, 15 plants per plot were sampled.

Different parameters related to the production of cucumber plants were also measured. For each plot, 15 plants coming from three different collection areas, each consisting of 5 plants, were considered. The traits measured were weight of marketable production (fruits that have the proper traits to be sold); weight of discarded fruits (deformed cucumbers); fruit number; fruit average weigh; earliness index, expressed as the quantity of product obtained in the first week of harvest.

Statistical analysis

The data relating to post-release nymphs survival were analysed using a factorial ANOVA, considering treatment (mechanical and manual release) and distance from the blower output, as main factors. Data on fecundity, longevity, fecundity rate, and survival of females 12 days after release were analysed by one-way ANOVA. Percent data were arcsine transformed before analysis. Data on nymphs survival related to different device settings were analysed with a 2 × 2 χ^2 test. Data on productive parameters of cucumber plants in the green house were analysed by one-way ANOVA. All statistical analyses were performed using STATISTICA 13 (StatSoft, 2017).

Results

Carrier material characteristics and distribution pattern

The average diameter of particles of the carrier material (buckwheat husks) and their relative distribution by size class were measured after sieving the particles using laboratory metal sifters. The moisture content, bulk density, texture, and friction coefficient of the carrier materials are reported in table 2.

The movement of the push rod was set at a frequency of 3 Hz, which emptied the bottle in 23 s, and an air exit speed of 20 m s^{-1}, corresponding to flows of 200 m^3 h^{-1}; the carrier material and nymphs' distribution pattern related to this blower setting is shown in figure 5.

The material flow was detectable until the distance of 5 m with most of the product (84%) falling between 1.0 and 3.0 m. This distribution pattern was adequate for application in the greenhouse plots (11 × 3 m), so the machine setup adopted for laboratory tests was used in the greenhouse trial.

Table 2. Physical and texture characteristics of carrier material (buckwheat husks).

Characteristics	Unit	Values (mean ± SE)[a]
Apparent bulk density	kg m^{-3}	140.0 ± 4.0
Friction coefficient		0.40 ± 0.01
Moisture content	%	20.1 ± 0.6
Average diameter	mm	3.06 ± 0.1
Distribution per diameter class	%	
>4.0 mm		2.3 ± 0.1
3.15-4 mm		32.7 ± 1.0
2.5-3.15 mm		61.7 ± 1.8
2.0-2.5 mm		1.80 ± 0.05
1.6-2.0 mm		0.80 ± 0.02
1.25-1.6 mm		0.020 ± 0.017
0.5-1.25 mm		0.010 ± 0.006
<0.5 mm		0.70 ± 0.02

[a]Mean and standard error of four samples.

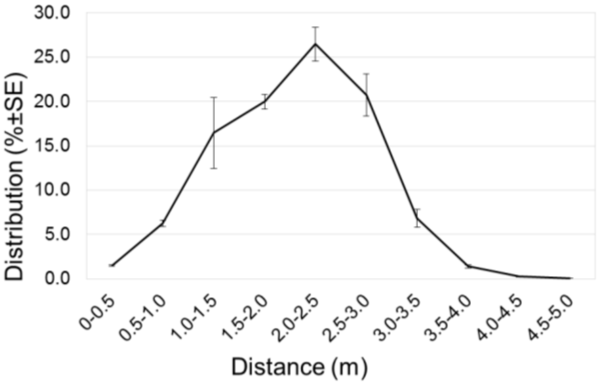

Figure 5. Distribution pattern of three replicates of *O. laevigatus* mixed with buckwheat husk carrier material.

Laboratory tests

The method (mechanical and manual) of releasing *O. laevigatus* had a significant effect on the percentage of nymphs found alive immediately after the release ($F_{1, 24}$ = 27.400, P < 0.0001) (figure 6A) and on the percentage of the dispensed nymphs that reached adulthood ($F_{1, 24}$ = 49.051, P < 0.0001) (figure 6B).

In contrast, no significant differences were found between launch distances both immediately after the release ($F_{5, 24}$ = 1.027, P = 0.424) and after 10 days after releasing ($F_{5, 24}$ = 0.652, P = 0.663). Likewise, no significant interaction between release method and distance achieved was found in the two sampling periods ($F_{5, 24}$ = 0.419, P = 0.831 and $F_{5, 24}$ = 0.807, P = 0.556 respectively).

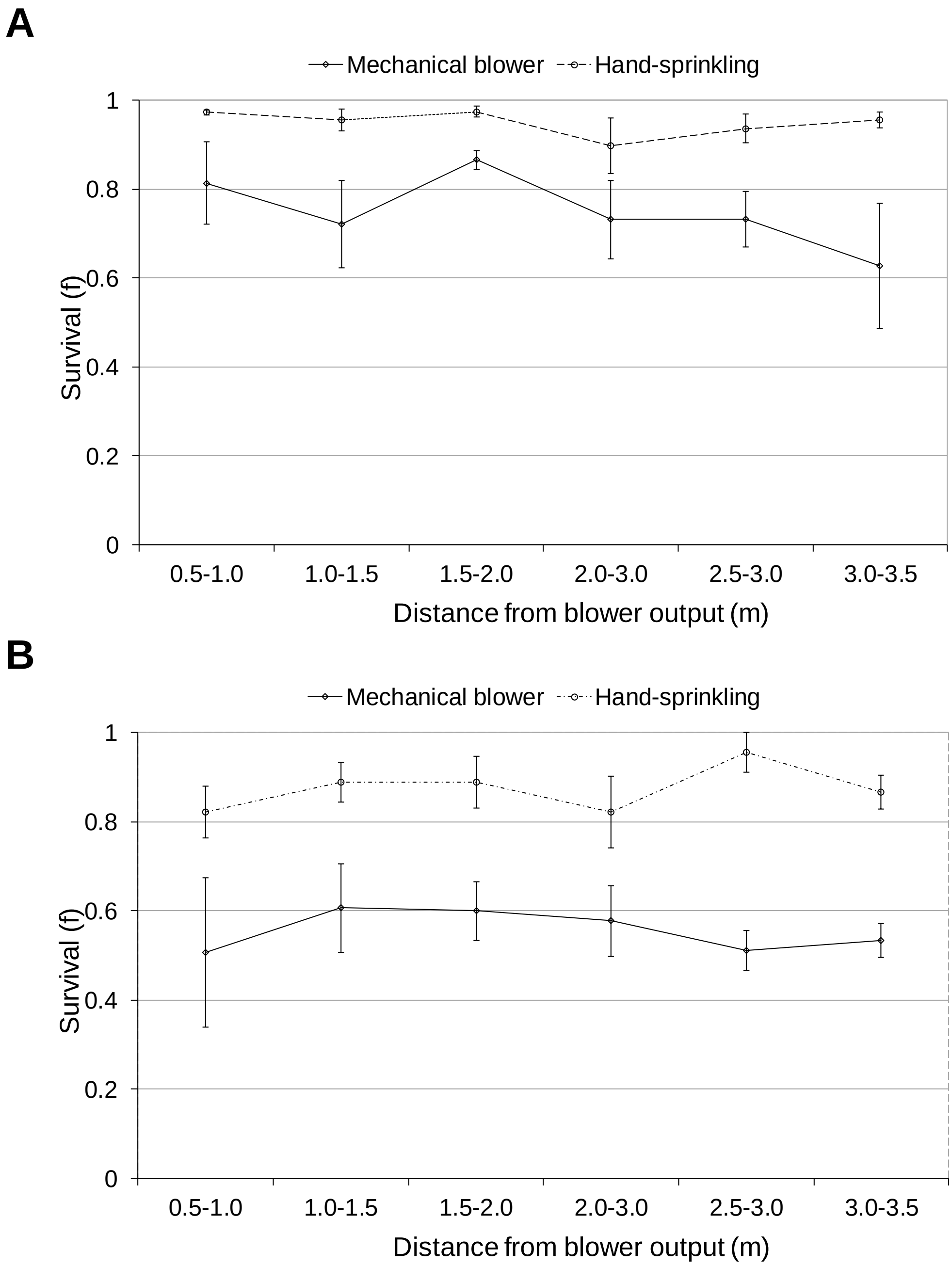

Figure 6. Survival frequencies (mean ± SE) of *O. laevigatus* nymphs after mechanical and manual application evaluated A) immediately after releasing and B) after 10 days from the release.

Table 3. Effects of releasing method on the biological traits (mean ± SE) of *O. laevigatus* females in the 12 days following the release.

Parameter	Mechanical blower	Hand sprinkling	Statistics
Fecundity (total eggs/female)	26.4 ± 3.8	39.0 ± 11.2	$F_{1,4}$ = 1.136, P = 0.347
Fecundity rate (eggs/female/day)	2.4 ± 0.3	3.6 ± 1.0	$F_{1,4}$ = 1.360, P = 0.308
Longevity (days)	10.2 ± 0.5	9.5 ± 1.0	$F_{1,4}$ = 0.353, P = 0.584
Survival (%)	73.4 ± 8.3	71.8 ± 16.1	$F_{1,4}$ = 0.001, P = 0.973

Immediately after release, a very high mean value of nymphal survival was found for manual release (0.95) with little variance in the data (cv = 6%). With the mechanical release average survival was 0.75, with a higher variance (cv = 21%), and a minimum value of 0.63 survival at the greatest distance (3.0-3.5 m).

Ten days after application, the manual release treatment showed low variance (cv = 11%) of the data and an average survival of 0.87. With the mechanical release, mean survival was 0.56 with a higher variance (cv = 26%).

The effects of mechanical and manual release on the biological parameters of dispensed females are summarized in table 3. Fecundity and longevity are represented, respectively, by the mean number of eggs laid per female and the mean number of days of survival during the 12-day experiment. The fecundity rate is the average daily number of eggs per female, while survival was the percentage of surviving specimens during the 12-day experiment.

For all the biological parameters considered, no significant effect produced by mechanical release with respect to the manual one, was found (table 3).

Mechanical blower setting, and effects on *O. laevigatus*

The effects of mechanical distribution on *O. laevigatus* survival immediately after application and 10 days later in relation to the different settings of the machine (table 1) are summarized in figure 7 and table 4).

No significant effect was observed in survival of *O. laevigatus* immediately following application due to different frequencies of the alternative movement of the rod (0 Hz, 3.0 Hz, and 6.6 Hz; MA treatments) or due to a different levels of air speed set (20 m s^{-1} and 30 m s^{-1}; MB treatments).

Conversely if we consider the combined effect of the rod movement and of the air speed, in the case of MC2 treatment (flow air = 15 m s^{-1}) there is a significant reduction in the survival of the anthocorid compared to HS treatment (−31%) (χ^2 = 13.21, df = 1, P < 0.001) and compared to MC1 treatment (flow air = 10 m s^{-1}) (−24%) (χ^2 = 6.38, df = 1, P = 0.012) (figure 7A).

Even 10 days after application, there was no evidence of a change in predator survival in relation to the variation in the rod frequency movement (MA treatments) or to a different air speed (MB treatments). In the case of combined effect of the rod movement and of the air

Table 4. Results (P values) of chi-square analyses on *O. laevigatus* survival in relation to different setting of dispenser device.

Test	Treatment comparison	df	χ^2	P
Nymphal survival evaluated immediately after releasing				
Effect of push rod alternative movement	MA1 *vs* MA2	1	1.13	0.2879
	MA1 *vs* MA3	1	2.59	0.1075
	MA2 *vs* MA3	1	0.49	0.4841
Effect of air speed	MB1 *vs* HS	1	0.45	0.5029
	MB2 *vs* HS	1	0.25	0.6172
	MB1 *vs* MB2	1	1.15	0.2835
Combined effect of push rod movement and air speed	MC1 *vs* HS	1	3.05	0.0810
	MC2 *vs* HS	1	13.21	0.0003
	MC1 *vs* MC2	1	6.38	0.0115
Nymphal survival evaluated after ten days from the releasing				
Effect of push rod alternative movement	MA1 *vs* MA2	1	1.03	0.3091
	MA1 *vs* MA3	1	1.03	0.3091
	MA2 *vs* MA3	1	0	1
Effect of air speed	MB1 *vs* HS	1	1.03	0.3091
	MB2 *vs* HS	1	0	1
	MB1 *vs* MB2	1	1.03	0.3091
Combined effect of push rod movement and air speed	MC1 *vs* HS	1	6.00	0.0143
	MC2 *vs* HS	1	3.33	0.0679
	MC1 *vs* MC2	1	0.68	0.4090

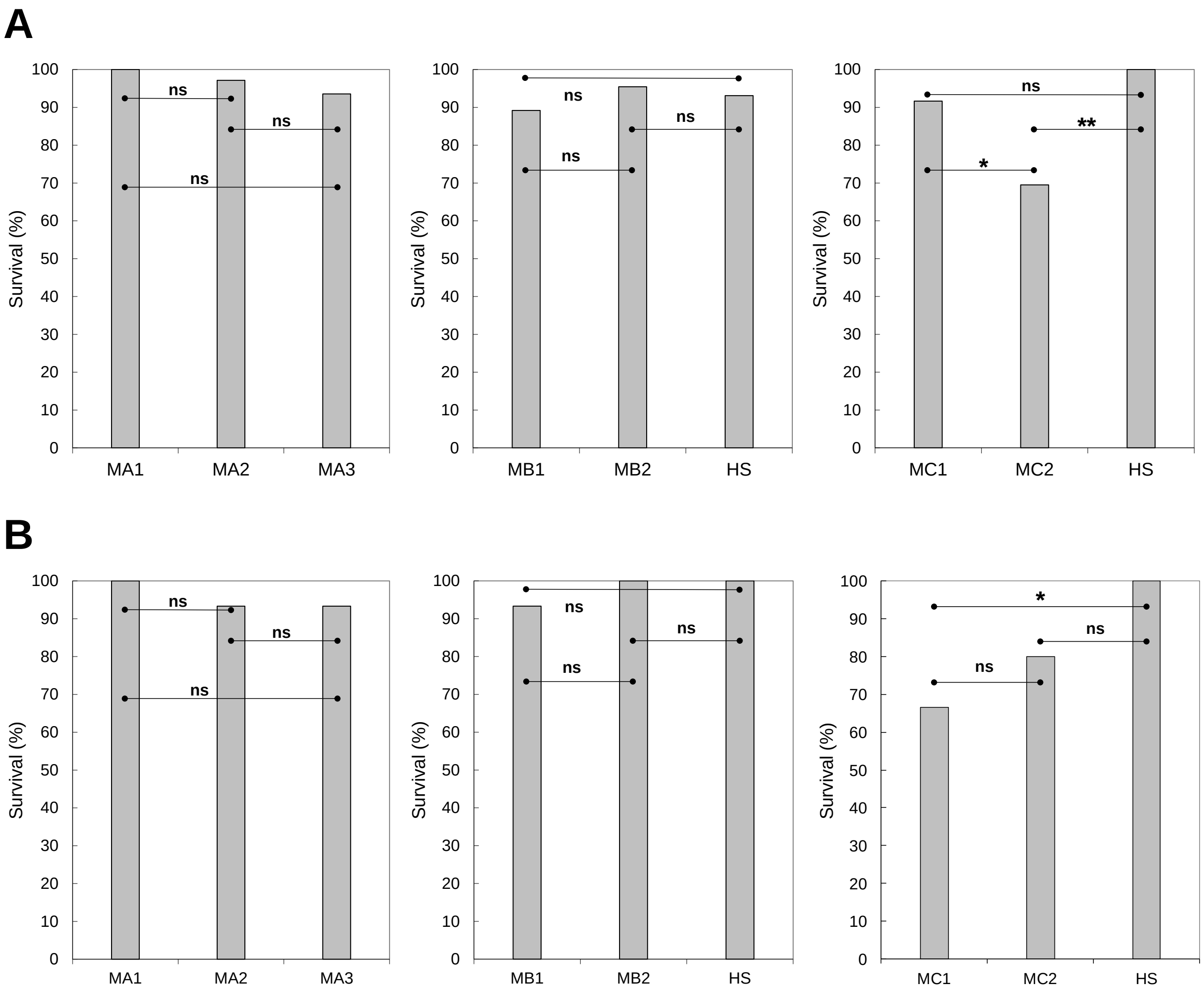

Figure 7. *O. laevigatus* survival evaluated immediately (A), and after ten days from the releasing (B), in relation to different setting of dispenser device. MA, mechanical application without air flow, push rod frequency: MA1 = 0; MA2 = 3.0; MA3 = 6.6. MB, mechanical application without push rod movement, air speed: MB1 = 20 m s^{-1}, MB2 = 30 m s^{-1}. MC, mechanical combined effect, air speed + push rod movement, MC1 = push rod frequency 3.0 Hz, air speed 10 m s^{-1}; MC2 = push rod frequency 3.0 Hz, air speed 15 m s^{-1}. HS, hand sprinkling. * = $P < 0.05$, ** = $P < 0.01$, ns = not significant, χ^2 test.

flow (MC treatments), there was a significant reduction in the survival of the predator (−33%) in the MC1 treatment (push rod frequency 3.0 Hz, air speed 10 m s^{-1}) compared to hand release ($\chi^2 = 6.00$, df = 1, $P = 0.014$) (figure 7B).

Field tests

Device operational parameters

Table 5 summarizes the main operational parameters, predator dose distributed, operator advancement speed (field speed), field efficiency E_f and effective field capacity C_a. The E_f takes into account the additional times for the *O. laevigatus* distribution, in particular it considers the time required for gently mixing the bottle to guarantee an uniform distribution of the nymphs and the carrier material inside the bottle. Moreover, the E_f takes into account the additional time needed for refuelling and setting the machine.

The effective distribution time per bottle was 23 s for the mechanical release treatment and 257 s for the hand-sprinkling, thus the theoretical working capacity of the blower was eleven times higher than that of hand sprinkling. If the additional times were also considered by introducing field efficiency, the effective field capacity of mechanical application was nearly six time higher than manual application.

F. occidentalis control

The population trends of *F. occidentalis* and *O. laevigatus* are shown in figure 8. Because the density of *F. occidentalis* was low, thrips were recovered only on flowers, not leaves.

The release of *O. laevigatus* nymphs has been shown to provide effective control of *F. occidentalis*, even with mechanical application. Immediately after the predator release, there was a rapid increase in the density of *O laevigatus*, reaching 0.16 and 0.19 anthocorids per flower, respectively, for manual and mechanical application. These values showed rapid population growth of the predator, with an increase of 54% and 40% in the

Table 5. Operating parameters, field efficiency and effective field capacity for *O. laevigatus* nymphal application in greenhouse.

Application system		Mechanical	Manual
Plants number per plot		70	70
Emptying bottle time	s	23	257
Predator dose	n. m^{-2}	6.5	5.0
Field speed	km h^{-1}	1.72	0.46
Working width	m	3.5	1
Field efficiency E_f		0.37	0.90
Effective field capacity C_a	ha h^{-1}	0.22	0.04

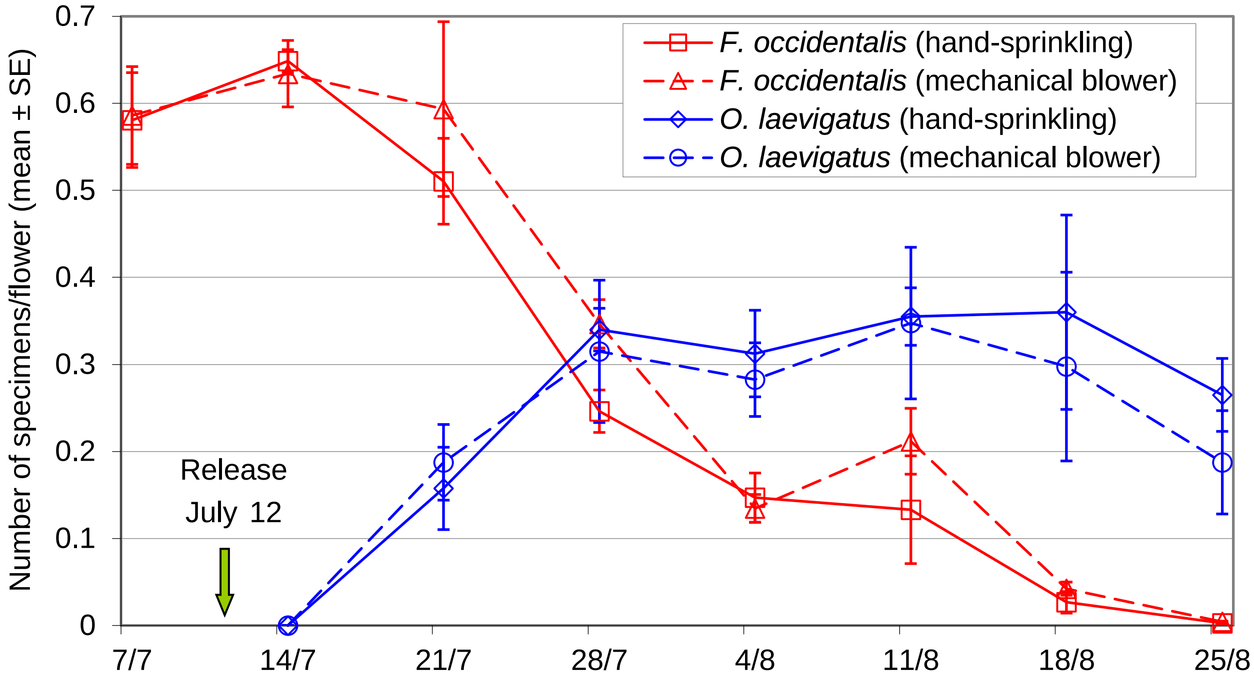

Figure 8. Population trend of *F. occidentalis* and *O. laevigatus* in the cucumber greenhouse after *O. laevigatus* release (The arrow points out the release date).

subsequent 6 days following manual and mechanical application, respectively. Afterwards the *O. laevigatus* population remained fairly stable. Concurrently we observed a steady decrease in *F. occidentalis* population density by 77% and 71%, for mechanical and manual application, respectively (from 21 July to 4 August), that dropped almost to zero by the last sampling date (25 August).

Crop performances

Table 6 summarizes the average production values in the different plots with manual or mechanical application of the predator.

None of the measured crop parameters showed any significant difference between the two application methods, although the number of fruits harvested was slightly higher (+15%) for mechanical distribution.

Table 6. Productive parameters of cucumber plants in the greenhouse.

Test	Marketable fruits (kg)	Discarded fruits (kg)	Earliness (kg)	Fruits per plot (number)	Fruit weight (g)
Manual	95.9 ± 3.7	1.8 ± 0.4	0.4 ± 0.0	532.3 ± 18.7	180.3 ± 2.6
Mechanical	108.5 ± 3.6	1.0 ± 0.1	0.6 ± 0.1	614.7 ± 21.5	176.7 ± 0.9
Statistics	$F_{(1, 4)} = 6.005$ $P = 0.070$	$F_{(1, 4)} = 3.064$ $P = 0.155$	$F_{(1, 4)} = 7.287$ $P = 0.054$	$F_{(1, 4)} = 8.329$ $P = 0.045$	$F_{(1, 4)} = 1.780$ $P = 0.253$

Values are mean ± SE of 15 plants for each plot. Marketable fruits: fruits meeting specification requirements for sale; Discarded fruits: fruits that do not meet the qualitative traits suitable for sale; Earliness: quantity of product obtained in the first week of harvest.

Discussion

The optimal operating characteristics and the blower settings for *O. laevigatus* application in greenhouses were determined. Using those values, most of the mixture (predator plus carrier material) was distributed in a band 1 to 3 m from the point of application, which in a typical tunnel greenhouse allows the applied material to reach all areas with two passages (there and back of the operator in the greenhouse).

The possibility of using a mechanical system such as the blower proposed for greenhouse application requires that the crops do not grow excessively in height, as they would create barriers to the mechanical distribution of the blower requiring more steps by the operator to guarantee the coverage of the entire surface. In any case, the target crops for *O. laevigatus*, mainly sweet pepper, strawberry, aubergine, some ornamental, does not develop excessively in height. Furthermore the control with *O. laevigatus* is essentially based on an inoculative biological control strategy that requires an early release of the predator (when the crops are therefore not yet fully developed) to guarantee an effective pest control.

Even if the application of *O. laevigatus* in greenhouse is generally carried out by manually releasing of adults that are then independently able to disperse, the success of releasing wingless preimaginal stages is linked to the possibility of an even distribution inside the greenhouse. We found that the mechanical blower was able to evenly dispense the anthocorid nymphs as showed by the uniform distribution pattern obtained, thus allowing a good dispersion of the predator. Moreover, release of *O. laevigatus* as nymphs reduces the necessary period of mass rearing in commercial insectaries, thus reducing production costs, potentially decreasing *O. laevigatus* cost for farmers.

Our laboratory tests showed that there is certain level of damage to the nymphs of *O. laevigatus* not seen in previous tests carried out with the same device for the distribution of *P. persimilis* and *A. swirski* (Pezzi *et al.*, 2015). The analysis of the different dispenser settings aimed to identify the causes of this mortality showed a concomitant effect of mechanical extraction and pneumatic transport that was particularly harmful at the higher air speed. However, even with the lower air speed setting a significant lower nymphal survival was observed after 10 days from the release. It is plausible to speculate that higher nymphal survival could be obtained by using a lower air speed (less than 10 m s^{-1}). However, such a setting could probably result in a different predator + carrier material distribution diagrams not necessarily adequate for a greenhouse distribution. The higher mortality of the individuals distributed mechanically was not considered particularly limiting since *O. laevigatus* is generally used in strategies that involve inoculative biological control, in which predator reproduction is expected in the crop (Nicoli and Tommasini, 2000). Indeed, we showed that the specimens that survived the mechanical distribution suffered no reduction of their fecundity or longevity. In the worst case, the higher mortality from release can be managed, as carried out in our field trial, increasing the numbers of predators released. Our greenhouse test showed that the mechanically released nymphs of *O. laevigatus* were as effective in controlling *F. occidentalis* as those released manually.

The possibility that biological control may be more costly than chemical control can lead to a reduced adoption of biological control. The costs of releasing natural enemies in greenhouse includes beneficial arthropods purchase and labour. The mechanical release of *O. laevigatus*, as shown in this study, is an effective alternative to manual distribution thanks to the faster working times that allow a greater timeliness of the treatment and limit the time an operator must work in uncomfortable conditions. Overall, the mechanical distribution of *O. laevigatus* nymphs, while not reducing intervention costs with respect to manual sprinkling due to the increase of predator dosage, can represent an interesting alternative to manual distribution. Indeed it allows a substantial reduction of distribution time making biological control more competitive with chemical control.

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