New approaches in pest and disease control in Sicilian greenhouses*

Tropea Garzia G., Siscaro G., Polizzi G., Vitale A., Zappalà L.
University of Catania, Dipartimento di Scienze e Tecnologie Fitosanitarie, Via Santa Sofia 100, 95123 Catania, Italy. E-mail: gtgarzia@unict.it; gsiscaro@unict.it; gpolizzi@unict.it; alevital@unict.it; lzappala@unict.it

Abstract

Results of trials on mechanical methods to release beneficials and on new techniques to apply sulphur are reported. The former were carried out on two predators, a phytoseiid mite and an anthocorid bug. Laboratory and greenhouse tests were conducted with the aim of evaluating the vitality and the damage eventually caused and setting the machine. The latter dealt with new techniques of sulphur application on tomato crops using dispensers transforming granular sulphur into vapour. In order to evaluate the effects of sublimated sulphur both on fungal diseases and arthropod pests, different trials have been carried out. The incidence and the severity index for airborne pathogens were bi-weekly evaluated and the infestations caused by some of the most relevant pests have been regularly monitored. Moreover, the incidence of the viral disease TYLCD has been evaluated.

During the trials on mechanical methods, no physical damage to the beneficials was observed. The distribution in laboratory seems to be suitable for applications in biological control programs, and the results on protected sweet pepper crops showed a better spatial distribution and a more effective interaction beneficials/preys in the "mechanically released" plots than in the "manually released" ones.

The trials on sublimated sulphur confirmed the good effectiveness of the technique in controlling natural infection of tomato powdery mildew as well as a moderate efficacy on tomato late blight. A good protection has been registered in the case of arthropod pests: the tomato russet mite was totally controlled; appreciable results were also obtained on phytophagous insects. A significant reduction in viral infections was also observed.

Keywords: integrated pest management, mechanical device, sublimated sulphur.

Introduction

Integrated Production is "a farming system that produces high quality food and other products by using natural resources and regulating mechanisms to replace polluting inputs and to secure sustainable farming" (Boller *et al.*, 2004). In this context plant protection is a key factor and it is based on the use of biological, technical and chemical control methods carefully balanced.

In Sicily, among Mediterranean crops, a leading role is played by protected vegetables which cover an area of about 8,800 hectares with a production of around 430,000 tons per year (Bucca & Chinnici, 2008). The intensive exploitation of land, together with the altered environmental conditions and the very short cultivation cycles make this agro-ecosystem

---

* The trials were partially developed in the framework of the project "Progetto per lo sviluppo dell'agricoltura biologica in Sicilia", funded by the "Assessorato Agricoltura e Foreste della Regione Sicilia".

extremely unstable and therefore highly susceptible to attacks by biotic agents, both pathogens as well as insect and mite pests. The control of these noxious organisms is often performed by applying phytosanitary measures which are unacceptable from an ecological and toxicological point of view. The research on alternative ecologically sustainable control techniques is therefore fundamental to ensure high quality and safe products.

In Sicily, new experiences focused on one side on methods to improve biological control through mechanical releases of beneficials and on the other side on new techniques to apply sulphur as vapour phase against fungal diseases and pests.

Material and methods

Mechanical distribution of beneficials

Studies on potential mechanization of biological control are rather limited, then a first version prototype (Fig.1), based on the centrifugal principle (Blandini *et al.*, 2006) was designed by the "Dipartimento di Ingegneria agraria – Sezione Meccanica" at the University of Catania. The device was then evaluated for the mechanical release of *Phytoseiulus persimilis* Athias-Henriot and *Orius laevigatus* (Fieber), that control respectively *Tetranychus urticae* Koch and *Frankliniella occidentalis* (Pergande). These beneficials are normally commercialized mixed with vermiculite or buckwheat husk in plastic bottles and they are manually distributed on the infested plants. However, the manual-sprinkling method is time consuming and doesn't allow uniform spreading. The machine was used to release the beneficials which were placed, together with the carrier material into a hopper equipped with a distributor.

Trials were conducted under laboratory and greenhouse conditions to evaluate the vitality and the damage eventually caused to the predators, to determine the range of action of the machine at different working rates and to verify the distribution uniformity and effectiveness. Before being used in the trials the natural enemies were subjected to standard quality control tests (van Lenteren *et al.*, 2003).

The effects of the mechanical distribution were evaluated using 150 cm^3 of product (carrier material plus natural enemies) per each trial. Mortality of the antagonists was assessed both as they came out from the hopper and after the release. In the trials concerning the effects of the hopper, the material was collected into a container, while the release tests were conducted spreading the product on a horizontal surface (140 cm wide and 180 cm long), placed 40 cm below the level of the release device. This kind of distribution was selected as it simulates a field release on crops at the right stage to receive beneficials. The material was observed under a dissecting microscope to record the number of live and dead individuals and to detect the presence and degree of eventual physical damages caused by the mechanical release.

Subsequent tests were carried out to evaluate the quantity of beneficials collected after the release and their distribution on the same surface employed in the previous trials. This was subdivided into 15 sectors (5 contiguous rows made of 3 sectors each) using rectangular plastic trays (36×47 cm) for collecting the released specimens and the carrier material. In *P. persimilis* release tests, the trays contained a thin layer of water in order to make the collection more effective. In this case, the content of each tray was filtered using a siever (80 mesh) and the mites present in the separated material were then counted under a dissecting microscope.

The uniformity of distribution was evaluated collecting the material at the hopper every 10 seconds for *P. persimilis* and 30 seconds for *O. laevigatus*, due to the different size of dispersal material fragments and to the different amount per bottle. Instead, the manual distribution was performed releasing the beneficials and the dispersal material in "sub-samples" of about 2 g each, gently rotating the bottle to mix the content between 2 releases. The number of beneficials released was scored and the dispersal material weighted for both kinds of distribution.

Trials aiming at evaluating the distribution with the device in motion were also conducted analysing the distribution of the material (substrate and beneficials) in relation to different speeds of the operator in covering a fixed distance, simulating real field spaces. A row of plastic trays of the same width of a plant row (around 50 cm), placed at a distance of about 30 cm from the machine pathway was employed. For the trials with *P. persimilis* a thin layer of water was poured in the trays for the already mentioned reasons.

The field tests were conducted on protected sweet pepper crops comparing, for both beneficials, mechanical and manual release plots. The doses applied were 10 specimens/m^2 for *P. persimilis* and 1 specimen/m^2 for *O. laevigatus*. The manual distribution was performed spreading the product on 3 to 6 plants in a row, randomly chosen at regular intervals walking along all or every other inter-row corridor; the mechanical release was carried out distributing the product proceeding along all or every other inter-row corridor.

For *P. persimilis* only one release was performed in both manual and mechanical treatments, while for *O. laevigatus* one and two releases (at 6 days interval) were compared. The trial with *P. persimilis* was conducted in a 1,000 m^2 greenhouse, infested by *T. urticae* and divided in 2 sectors (manual and mechanical release) separated by an untreated area. The observations were carried out on a sample of 150 leaves/sector, uniformly collected from all the rows. *O. laevigatus* trials were conducted in 2 greenhouses (A and B) extended 1,000 m^2 each, where 1 and 2 releases were performed respectively. Both greenhouses were divided in 2 sectors: "manual" and "mechanical". The observations were carried out on a sample of 160 flowers/sector, uniformly collected from all the rows, scoring live thrips and predators.

<u>*Sulphur application*</u>

Studies on new techniques of sulphur application are based on the increased importance of agricultural and phytosanitary uses of this element in IPM, due to the recent knowledge on its physiological role and also on its reduced ecological impact. Curative activity is known for fungal diseases but only since last century this capacity was exploited in protection strategies also against arthropod pests.

Sulphur, even though solid at ambient temperature, is able to sublimate when heated at more than 95°C and, in the vapour phase, it uniformly covers the plant surface. After heating it cools down and returns to the solid phase with particles between 5 and 14 microns, scattering on the whole crop surface, even on the inner areas of the plant not easily reachable with other spraying methods. Inside greenhouses sublimation is performed using dispensers constituted by a cylinder (ø 8 cm – h 20 cm) and a thermic device able to reach 190-200°C, transforming granular sulphur into vapour, without generation of SO_2. The device, made out of aluminium, stainless steel, porcelain and a resistor (70-100 W), is entirely composed by non-corrosive materials and it is alimented by an electric single-phase energy system (Fig. 2).

In order to evaluate the effects of this technique of supplying sulphur both on fungal diseases and arthropod pests, different trials have been carried out on Sicilian protected vegetable crops in comparison with control plots.

a) Fungal diseases

Two experimental trials were conducted in October 2006 through February 2007 and October 2007 through February 2008 in a typical Mediterranean greenhouse (900 m^2) located at Marina di Ragusa (RG). The greenhouse was divided into five sectors, three of which with activated dispensers of sublimated sulphur placed between plant bines at 2.0÷2.5 m from the ground. Each dispenser covered a surface of 100 m^2 and was turned on 11 hours/night (from 19.00 to 06.00). In both trials transplant of tomato seedlings was performed within the first decade of October (2006 and 2007), the plant density was about 2.5 plants/m^2 and cultivars "Shannon" and "Ikram" were respectively used for the first and second trial.

Observations were periodically performed to evaluate the incidence and severity of infections caused by important airborne pathogens of tomato, as *Leveillula taurica* (Lév.) G. Arnaud, *Phytophthora infestans* (Mont.) de Bary, *Cladosporium fulvum* (Cooke), *Botritys cinerea* Pers.:Fr.. The experimental design for both trials consisted in greenhouse subdivision into four randomized blocks (sectors), two with dispensers of sublimated sulphur and two without. Each sector was divided in two parts.

The disease incidence was evaluated as percentage of infected plants. An empirical 6-scoring points system was used to evaluate the severity of *L. taurica* natural infections on tomato: class 0 = healthy leaf; class 1 = 1-10% of infected leaf area; class 2 = 11-25% of infected leaf area; class 3 = 26-50% of infected leaf area; class 4 = 51-75% of infected leaf area; class 5 > 76% of infected leaf area. The severity of *P. infestans* natural infections on tomato was evaluated according to Barrat/Horsfall scale, consisting of 12 classes (index values from 0 to 11) (Horsfall & Barratt, 1945).

Analysis of variance was performed on the diseases parameters data using STATISTICS 7 (Statsoft Inc.). The data concerning disease incidence were previously transformed using the arcsine transformation ($\sin^{-1}$ square root x).

b) Pests

Observations on protected tomato crops were carried out in 2004-2007 in greenhouses located respectively at Vittoria, Ispica and Marina di Ragusa (RG) in order to monitor arthropod pests. In all cases the cultivar was "Shannon" and the density was 2.5 plants/m^2. In the first two trials one greenhouse was considered as a single thesis and the device was turned on for 6 hours (August-November 2004) and 12 hours/night (October 2004-March 2005). The last trial (October 2006-January 2007) was performed in the same greenhouse used for the trials on pathogens where sublimated sulphur was supplied for 11 hours/night.

The infestations were regularly monitored: whitefly adults [*Bemisia tabaci* (Gennadius)] on the growing apex of 5% of the plants and leafminer adults (*Liriomyza huidobrensis* Blanchard) on yellow sticky traps placed inside the greenhouse were bi-weekly scored. Moreover, in the last trial, the evaluation of the damage caused by the tomato russet mite, *Aculops lycopersici* (Massee), was conducted by marking the number of plants with symptoms every 15 days, while TYLCD infection was monitored monthly counting the plants that showed clear viral evidence.

Results

Mechanical distribution of beneficials

During the trials, no physical damage to the beneficials was observed. The data obtained suggested to use the machine at a rotational speed of 600 rpm, that turned out to be optimal for the distribution of both beneficials tested, jointly avoiding waste of product. Also the distribution, evaluated first in the laboratory on a horizontal surface, seems to be suitable for applications in biological control programs.

The trials on the distribution uniformity showed that in the mechanical release *P. persimilis* was more abundant in the final release intervals; comparing this result with data on the amount of carrier material, which follow the same trend, a positive and significant correlation emerged between the 2 parameters ($y= -19.26+56.41\ x$; $r^2= 0.80$; $r= 0.90$; $p= 0.000$). In the same kind of distribution, the amount of *O. laevigatus* was instead higher at the beginning of the release. Also these data were positively and significantly correlated with the quantity of dispersal material ($y= -15.22 + 9.88\ x$; $r^2= 0.44$; $r= 0.66$; $p= 0.000$).

In the manual distribution the amount of *P. persimilis* was uniform among the sub-samples, with a positive and significant relation between the number of individuals released and the quantity of dispersal material ($y= -49.25 + 58.629\ x$; $r^2= 0.44$; $r= 0.66$; $p= 0.000$). The quantity of manually released *O. laevigatus* slightly increased in the last sub-samples and also in this case a positive and significant correlation with the weight of the dispersal material was recorded ($y= -1.94 + 16.56\ x$; $r^2= 0.57$; $r= 0.76$; $p= 0.000$).

The laboratory tests carried out with the device in motion aimed at making the necessary adjustments to the speed of the prototype in order to release the right amount of beneficials per area unit. The trials conducted in greenhouses on sweet pepper showed a better spatial distribution and a more effective interaction of the beneficials with the respective preys in the "mechanically released" plots than in the "manually released" ones.

The results of *P. persimilis* greenhouse trials were difficult to interpret because of the presence on the crop of an indigenous population of the predatory mite which somehow influenced *T. urticae* dynamics. But given the similar initial densities of both prey and predator in the 2 plots, the larger reduction in *T. urticae* infestation in the mechanically released area (Fig. 3) suggests a more efficient control of the pest after the release with the device.

The data collected in the *O. laevigatus* trials showed that in the greenhouse A (1 release) (Fig. 4a) the amount of predators was higher in the mechanically released plot, thus demonstrating that the more uniform the distribution is, the more rapidly the population density increases, possibly due to higher probability of sexes encounter and mating. In the greenhouse B (2 releases), calculating the percentage of rows where the presence of *O. laevigatus* was recorded, the predator was more widely spread in the mechanically released plot than in the manual one both after 6 days (35.29% vs. 11.76%) and at the end of the trial (70.59% vs. 41.18%) (Fig. 4b).

Sulphur application

a) Fungal diseases

In the 2006-2007 trial, the occurrence of powdery mildew infections (*L. taurica*) was detected on cv "Shannon" tomato since the first week of November. The final survey (30.11.2006) demonstrated a significant reduction of disease incidence and severity in the sectors where sulphur dispensers were activated (table 1). Tomato late blight (*P. infestans*) infections on leaves and stems were instead ascertained since the first decade of December. Two monthly surveys were performed on the treatments effectiveness. The results of the 1st survey

(19.12.2006) showed lower values of disease incidence and severity in sectors where sulphur was supplied as vapour phase. However, no significant differences were highlighted among treatments (table 1). Only following disease progression on the final survey (19.01.2007), it was possible to observe in the sectors treated with sublimated sulphur that tomato late blight incidence values were significantly lower than in the untreated sectors (table 1).

Table 1. Effectiveness of sublimated sulphur against *L. taurica* and *P. infestans* infections on protected tomato crops (cv "Shannon") in 2006-07 experimental trial

Treatments	*Tomato powdery mildew* (survey, 30.11.2006)			
	Incidence (%)		**Severity**	
Control	98.2 b		0.986 b	
Sublimated S	17.5 a		0.175 a	
Treatments	*Tomato late blight*			
	1st survey (19.12.2006)		2nd survey (19.01.2007)	
	Incidence (%)	**Severity**	**Incidence (%)**	**Severity**
Control	47.86	0.671	90.71 b	4.27
Sublimated S	3.21	0.036	40.71 a	0.563

Each value represents the average of 4 replicates each constituted by 70 plants. Values followed by different letters on the same column are significantly different at P=0.05. The analysis of variance on disease incidence was performed on percent values previously transformed in angular values. Severity was referred to Barrat/Horsfall scale.

In regard to the 2007-2008 trial, first infections of tomato powdery mildew were observed since late November but, because a very low disease level was detected, it was not possible to compare the treatments' effectiveness (*data not shown*).

Since the last decade of December 2007 it was also possible to observe the occurrence of tomato late blight, but only in control sectors. However four surveys were needed to evaluate the treatments' efficacy (on 24, 29 December, 5 and 19 January). For each survey the disease incidence and severity index were determined in all different treatment plots. The data analysis regarding the examined disease parameters has allowed to ascertain a good effectiveness of sublimated sulphur (significant data) already since the second survey and through all the remaining ones (table 2). In both trials no relevant *B. cinerea* or *C. fulvum* infections were reported on tomato crops.

Table 2. Effectiveness of sublimated sulphur against *P. infestans* infections on protected tomato crops (cv "Ikram") in 2007-08 experimental trial

Treatments	*Tomato late blight*					
	2nd survey (29.12.2007)		3rd survey (5.01.2008)		4th survey (19.01.2008)	
	Incidence (%)	**Severity**	**Incidence (%)**	**Severity**	**Incidence (%)**	**Severity**
Control	7.5 b	0.16	31.25 b	0.56 b	83.96 b	2.25 b
Sublimated S	0.21 a	0.002	2.91 a	0.060 a	24.37 a	0.29 a

Each value represents the average of 4 replicates each constituted by 60 plants. Values followed by different letters on the same column are significantly different at P=0.05. The analysis of variance on disease incidence was performed on percent values previously transformed in angular values. Severity was referred to Barrat/Horsfall scale.

b) Pests

A good crop protection has been also registered in the case of arthropod pests. Appreciable results were obtained on leafminers and partially on whiteflies (Figg. 5a-e). The best results were obtained with low numbers of pests and 12 hours of sulphur application especially in the case of whiteflies (Fig. 5c). In addition, a significant reduction in viral infections on the plants was also observed (Fig. 5g), probably due to a disturbing effect of sulphur on the behaviour of adult whiteflies. Further studies are therefore needed to investigate the mechanism of this interference. Preliminary observations didn't demonstrate also any harmful effect on pollination activity. The tomato russet mite has been totally controlled (Fig. 5f), obtaining a considerable saving of expenses, thanks to the absence of manual sulphur application.

Conclusions

The development of the device to mechanically release beneficials could be of great help to the farmers, ensuring better economic investment in terms of work, especially considering that no physical damage to the beneficials was observed and that the field trials showed a better spatial distribution and a more effective interaction beneficials/preys.

Sulphur in vapour phase has a good effectiveness in controlling tomato powdery mildew, although its activity in solid phase is already well known in literature. For the first time a collateral activity of sublimated sulphur against some airborne pathogens of tomato was observed. In detail this work showed an effectiveness, although moderate, of vapour applied in gaseous phase against tomato late blight infections on which a possible delaying effect has been observed. These encouraging results could suggest the employment of sublimated sulphur in mixture or alternation with other molecules or active ingredients authorized in biological production systems. The plots provided with the sulphur sublimation devices were protected against the arthropod pests considered, with a relevant reduction in expenses and levels of pollution. Moreover fruits didn't show any dusty sulphur residues in comparison with the traditional powdered application. Further investigations are in progress to verify the potential secondary effects on beneficials, the residues on fruits, in the environment, on the covering plastic films and the metallic structure of the greenhouses.

References

Blandini G., Failla S., Manetto G., Tropea Garzia G., Siscaro G., Zappalà L. 2006. Prove preliminari di distribuzione meccanica di antagonisti naturali. Atti Giornate Fitopatologiche, 1, 557-562.

Boller E.F., Avilla J., Joerg E., Malavolta C., Wijnands F.G., Esbjerg P. (eds). 2004. Integrated Production: Principles and Technical Guidelines, 3rd Edition. IOBC wprs Bulletin, 27 (2), 1-12. http://www.iobc.ch/iobc_bas.pdf

Bucca M., Chinnici G. 2008. Sicilia leader italiano nell'orticoltura protetta. L'Informatore Agrario, 23 (suppl.), 35-38.

Horsfall J.G., Barratt R.W. 1945. An improved grading system for measuring plant disease**.** Phytopathology 35, 655.

Lenteren J.C. van, Hale A., Klapwijk J.N., Schelt J. van, Steinberg S. 2003. Guidelines for quality control of commercially produced natural enemies. *In*: Quality Control and Production of

Biological Control Agents. Theory and Testing Procedures (J.C. van Lenteren ed.). Cap. 19, CABI PUBLISHING, WALLINGFORD, OXON, UK. 265-303.

Figure 1. Mechanical prototype

Figure 2. Sulphur dispenser

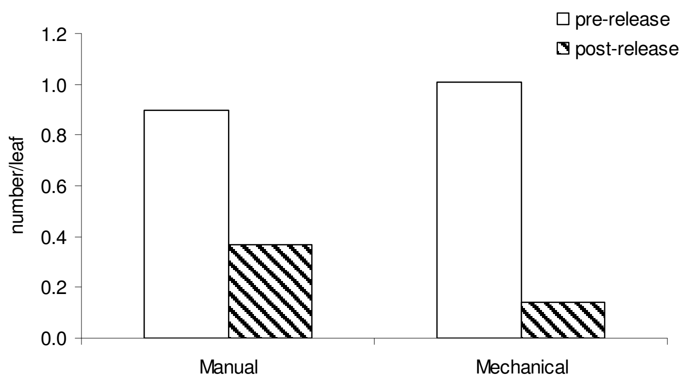


Figure 3. Mean number of *T. urticae*/leaf before and after the manual and mechanical release

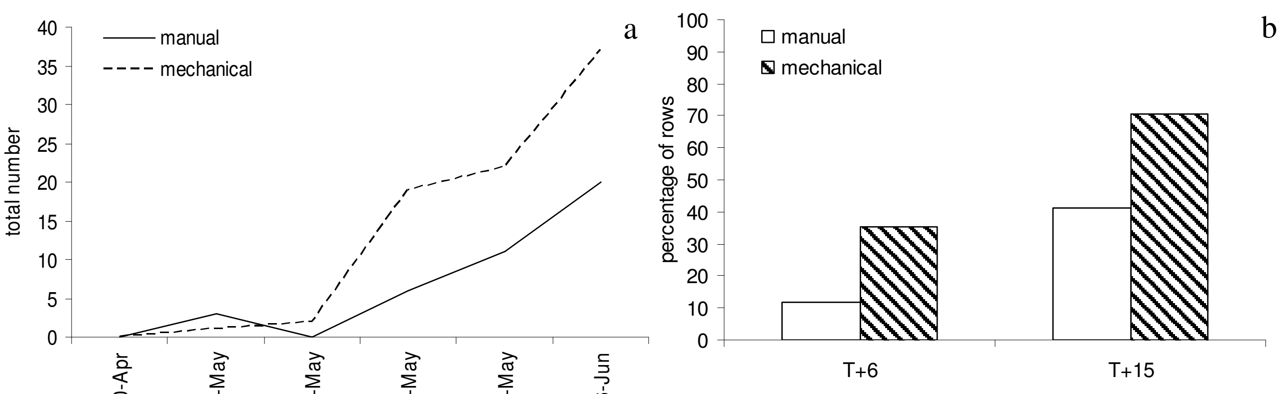
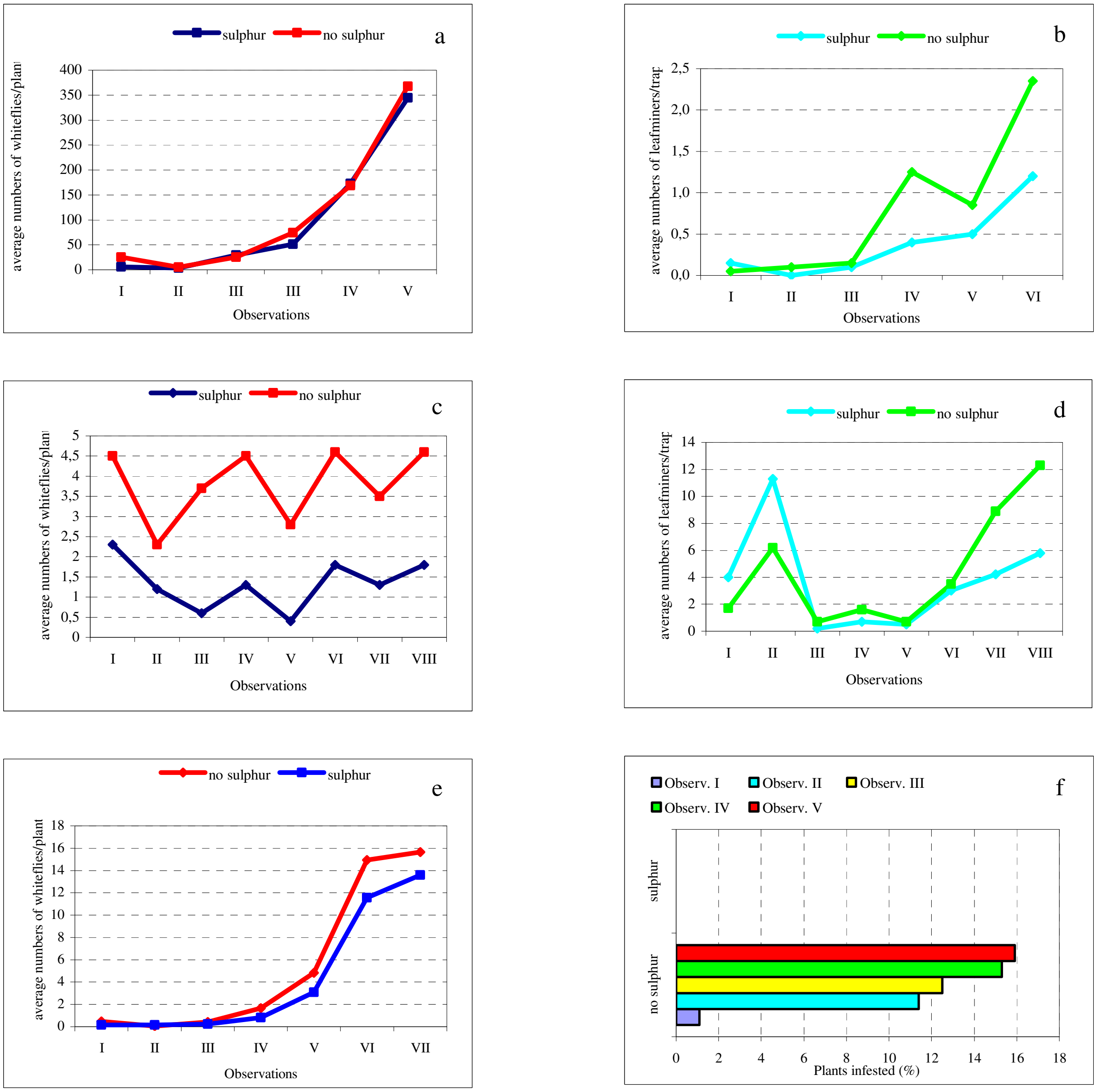


Figure 4. *O. laevigatus*: total number of specimens on the flowers in the manually and mechanically released plots (a); percentage of rows with presence of the beneficial in the manually and mechanically released plots, 6 and 15 days after the release (b)



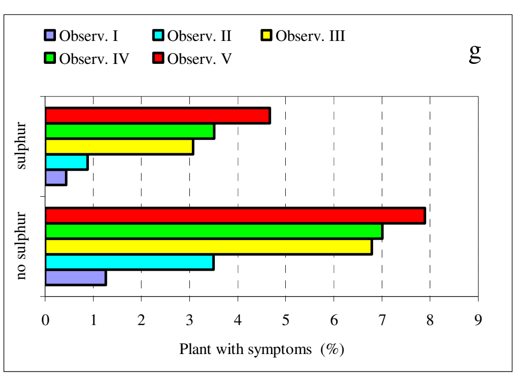


Figure 5. Infestation trend in greenhouse and presence of viral infections; a-b: whiteflies and leafminers (I trial); c-d: whiteflies and leafminers (II trial); e: whiteflies (III trial); f: tomato russet mite (III trial); g: infections of TYLCD