

Tobacco as Banker Plant for *Macrolophus Pygmaeus* to Control *Trialeurodes Vaporariorum* in Tomato Crops

Cécile Bresch, Lydia Ottenwalder, Christine Poncet, Pia Parolin *

French National Institute for Agricultural Research (INRA), Theoretical and Applied Ecology in Protected Environments and Agrosystems (TEAPEA); BP 167, 06903 Sophia Antipolis, France

*Corresponding Author: Pia.Parolin@sophia.inra.fr

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Abstract The predatory mirid bug *Macrolophus pygmaeus* Rambur (Heteroptera: Miridae) is commonly employed to control whiteflies *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae), a common pest on tomatoes. In greenhouses of the Mediterranean, tomatoes are important crops and the need to find alternatives to pesticide use is increasing. With the aim to find biocontrol plants which are suited to maintain and multiply the populations of predators and reduce pest presence, a greenhouse experiment was performed. Different combinations were used: crop plant and pest were always present, banker plant (abbreviated as BP hereafter) and predators were present or absent. Numbers of individuals of *M. pygmaeus*, *T. vaporariorum*, and plant health were assessed. *M. pygmaeus* reproduced efficiently on tobacco, with the highest reproduction when only BP were present. The number of pests was significantly reduced on the plants where predators had the highest densities. However, without the presence of predators tobacco acted as an attractive plant for *T. vaporariorum*. Plant growth in terms of height and leaf number was not significantly different between the treatments with different species combinations. Leaf damage was higher when the BP were in a cage with tomato plants. Tobacco acted as incubator for the pests when it was in a cage with tomatoes without predators present. This points to a complementarity of these two plant species to provide good reproductive conditions for the pest *T. vaporariorum*, an undesired synergy of plants to increase the presence of pests. Therefore, tobacco was an efficient banker plant to support the population of the predatory mirid bug *M. pygmaeus*, but under absence of predators it enhanced the proliferation of the pests. Its employment as BP in this combination of species is only efficient as long as predators are present.

Keywords Banker Plant, Biocontrol Plant, Biological Pest Control, Mediterranean, Greenhouse, *Nicotiana tabacum*, *Macrolophus pygmaeus*, *Trialeurodes vaporariorum*, *Solanum lycopersicum*

Banker plants are the plant components of the banker plant system, which is a “rearing and release system purposefully added to or established in a crop for control of pests in greenhouses or open field” (Huang et al. 2011, see also Frank 2010, Parolin et al. 2012a, Parker et al. 2013). Banker plants (in the following abbreviated as BP) are biocontrol plants (*sensu* Parolin et al. 2014) which are efficient in increasing the populations of predators to control pests such as whiteflies *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) (Barbera 2010). Whiteflies colonise greenhouses easily and build up high populations before the predators, which were released by inundative methods with the purpose of biological control, can establish in the crop (Gabarra et al. 2004). *T. vaporariorum* may be controlled by the mirid bug *Macrolophus pygmaeus* Rambur (Heteroptera: Miridae) (Perdikis & Lykouressis 2000, Bonato et al. 2006, Lykouressis et al. 2009). *M. pygmaeus* is a zoophytophagous predator which feeds on pests and plant sap (Castané et al. 2004, Ingegno et al. 2011). It is able to complete its nymphal development even in the absence of prey (Favas et al. 2003, Lykouressis et al. 2008). This way, a BP inoculated with mirid bugs and installed in a crop system can sustain the presence of a population of predators before the outbreak of pests. Therefore, BPs represent serious alternatives to potentially dangerous pesticides (McCaffery 1998, Geiger et al. 2010, Parker et al. 2013). However, there is little evidence of the efficiency of specific species of BP in certain species combinations in greenhouses in Mediterranean climate. The aim of the present study was to test if tobacco is suitable as BP to enhance the presence of *M. pygmaeus* in order to control the common whitefly *T. vaporariorum* on tomatoes which are important crops in greenhouses in Southern France. Tobacco (*Nicotiana tabacum* L.) is a plant species that has been employed as BP for the control of *T. vaporariorum* (Albajes & Gabarra 2003, Fischer & Terretaz 2003, Schoen 2003, Frank 2010, Huang et al. 2011). In order to understand the different roles within tritrophic interactions, an experiment using different species combinations (tobacco, tomato, pest and predatory insects) was performed. The main question was if the presence of tobacco plants as BP increases the control of whiteflies on

1. Introduction

tomato crops. This is an indirect process as the optimum would be that the BP increases the continuous presence of the predators which then feed on the pests which should not reproduce on the BP and decrease in number. This implies that in separate treatments, the plant combinations BP+crop or BP+BP have a higher number of predators and a lower number of pests than crop+crop under the same environmental conditions. Furthermore, leaf damage should be reduced in presence of the BP. Whether the BP influences these interactions and which underlying mechanisms lead to its role in the tritrophic interactions under the given circumstances should give interesting insights into the general understanding of the function of biocontrol plants and their functional traits (Cortesero et al. 2000, Parolin et al. 2012a, b, 2014).

2. Materials and Methods

An experiment lasting eight weeks was performed in summer 2012 in a glass greenhouse at the INRA Sophia-Antipolis experimental centre in the South of France (43°36'44.9" N latitude, 07°04'40.4" E longitude and 125m altitude). The development of the populations of the predator *M. pygmaeus* and the pest *T. vaporariorum* were analyzed on tobacco as BP, in presence and absence of tomato *Solanum lycopersicum* Mill. (Solanaceae) (cv Marmande) as crop plant.

Origin of plants and insects. Seeds of tomato and tobacco were sown in a mixture of 1:3 perlite and 2:3 loam in 20 cm pots, kept in a greenhouse with climatic parameters set-points 25°C ± 2 °C, RH 70 ± 10 %, and watered daily. The plants were put in 1.3 litre pots with 2/3 soil and 1/3 substrate. When the experiment started, the plants were about 30 cm tall.

Adult *T. vaporariorum* were collected from greenhouse colonies on roses and bred on tomato plants. Adult *M. pygmaeus* were ordered at Biotop Company, Valbonne, France.

Experimental design. Six combinations of plants, pests and predators were tested in separate cages (size: 2x1x1m) made of muslin (Figure 1) placed in a 40 m² greenhouse compartment covered with single glass. A randomized block design was used for each treatment with the six combinations which had five repetitions each, adding up to 30 cages in total:

- Treatment 1: Banker plant + crop plant + 30 pests
- Treatment 2: Banker plant + crop plant + 30 pests + 6 predators
- Treatment 3: Crop plant + 30 pests
- Treatment 4: Crop plant + 30 pests + 6 predators
- Treatment 5: Banker plant + 30 pests
- Treatment 6: Banker plant + 30 pests + 6 predators



Figure 1. Cages in the greenhouse compartment at INRA Sophia Antipolis with the different combinations of plants, pests and predators, and plants used for our experiments (left tomato crop, right tobacco BP).

Inoculation of the plants. To prevent insect transfer between treatments inside the greenhouse, the different treatments were isolated from one another using cages made of fine net material. Pesticide applications were strictly avoided. In half the cages the plants were pre-inoculated with 6 *M. pygmaeus* (3 males and 3 females). Since one week is considered as a necessary introduction period for the predator (Ridray et al. 2001), after eight days the plants were inoculated with 30 individuals of whiteflies *T. vaporariorum* per cage, in all cages and treatments. The pests were placed in an open petri dish on the floor of the cage and freely colonized the plants.

Environmental conditions. A pad-and-fan cooling systems (Aria) was used to keep the greenhouse climate parameters around 25°C temperature and 70 % humidity. Additional shading screens were not used as the cages filtered much light. The plants were watered and fertilized with a drip irrigation system (set point values for the parameters of EC were 1.5 mS.cm⁻¹ at 25°C and pH=6).

Monitored parameters. The following parameters of the plants and insects were monitored in the different combinations after eight weeks:

Presence of predators and pests. All adults and larvae of *M. pygmaeus* and *T. vaporariorum* were counted on each plant at the end of the experiment with destructive sampling. All plants were separated, leaves detached, transferred into zip plastic bags and taken to the lab for detailed countings of larvae and adults of pests and predators using lenses with a magnification of 10x and a stereo microscope. When the

number of larvae of *T. vaporariorum* was so high, photos were taken and the numbers were counted later on the computer screen.

Plant growth. Plant height was measured from stem basis to apex and leaf number was counted on each plant using a common measuring tape, as well as the number of healthy and damaged leaves. Measurements were performed before and at the end of the experiment.

3. Results

Presence of pests and predators. After 8 weeks of experiment, few predator larvae and some adults were found when only tomato crop plants were present in the treatments (Figure 2). When only tobacco plants were present, the number of predators was highest, especially the proportion of larvae (Figure 2 middle bar). In the combination tomato crop + BP there were few larvae and many adults, the highest number of adults of the three combinations. The predator *M. pygmaeus* reproduced efficiently on tobacco plants, and the highest reproduction was found when only BP were present in the cage (Figure 2).

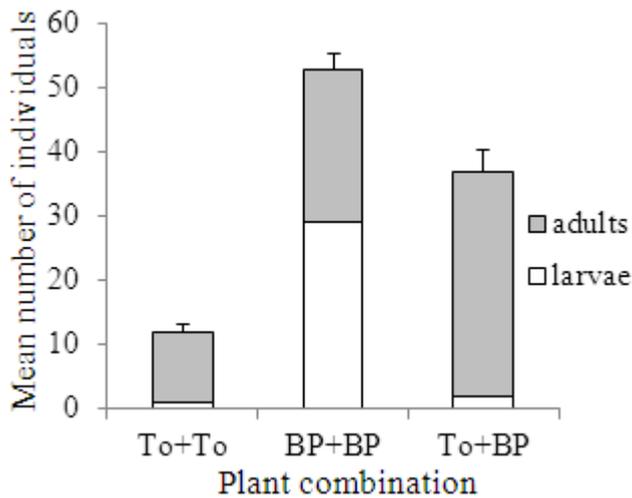


Figure 2. Mean number of predators *M. pygmaeus* (adults grey, larvae white) in the different plant combinations (to= tomato crop plants, BP = tobacco banker plants; To+To = two tomato plants present; BP+BP = two banker plants present; To+BP = one tomato and one banker plant present) nine weeks after inoculation with predators and eight weeks after inoculation with pests.

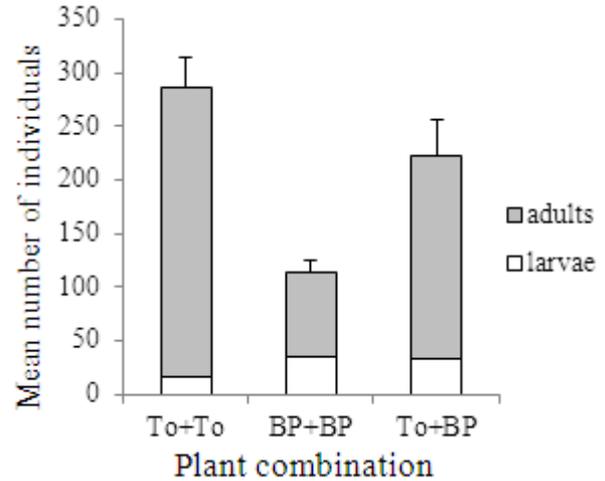


Figure 3. Mean number of pests *T. vaporariorum* (adults grey, larvae white) in the different plant combinations (to= tomato crop plants, BP = tobacco banker plants; To+To = two tomato plants present; BP+BP = two banker plants present; To+BP = one tomato and one banker plant present) nine weeks after inoculation with predators and eight weeks after inoculation with pests.

When both pests and predators were present, the mean number of pests was highest when only tomatoes, or when tomatoes and BP were present (Figure 3). When only BP were present, the pests were significantly lower than when tomato plants were in the cage, almost one third than when only tomatoes were present. The number of adults accounted for the differences whereas the number of larvae was not significantly different between the plant combinations.

Comparing Figure 2 and Figure 3 it becomes evident that the number of pests was reduced on the plants where predators had the highest densities (BP). However, when only pests and no predators were present (Figure 4) the highest number of pests was found in the combination of crop and BP, and the number of pests was not as high when only tomatoes or only BP were in the cage. This indicates that the presence of the predators cannot be responsible alone for the reduced number of pests found in some plant combinations.

Only relatively few larvae were present when only tomatoes were in the cage, compared to many larvae when banker plants were present. This might lead to the assumption that tobacco enhances the development of the pests, which obviously is not the aim of biological pest control via BP.

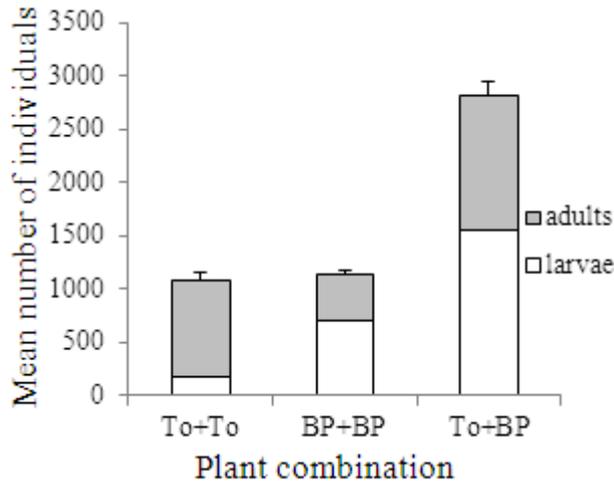


Figure 4. Mean number of pests *T. vaporariorum* (adults grey, larvae white) when no predators were present in the different plant combinations (to= tomato crop plants, BP = tobacco banker plants; To+To = two tomato plants present; BP+BP = two banker plants present; To+BP = one tomato and one banker plant present) eight weeks after inoculation.

The number of adult whiteflies with and without presence of predators was significantly different between the plant combinations, with many more pests present in all treatments (Figure 5).

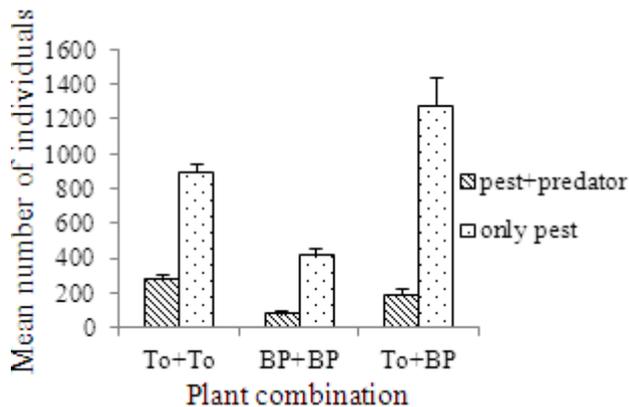


Figure 5. Mean number of adult pests *T. vaporariorum* with and without presence of predators in the different plant combinations (to= tomato crop plants, BP = tobacco banker plants; To+To = two tomato plants present; BP+BP = two banker plants present; To+BP = one tomato and one banker plant present) eight weeks after inoculation.



Figure 6. Infestation with adult whiteflies *T. vaporariorum* on the lower leaf side of a tobacco plant eight weeks after inoculation.

However, if the number of pests present in the to+to treatment are set as 100% (Figure 7), the number of adult pests present on the banker plants alone was significantly lower both in the presence and in absence of the predator, whereas in the combination of BP+to in the presence of the predator the number of pests was reduced. If no predator was present, the number of pests was significantly higher. This confirms the finding that without the presence of predators tobacco may act as incubator for the pest *T. vaporariorum*.

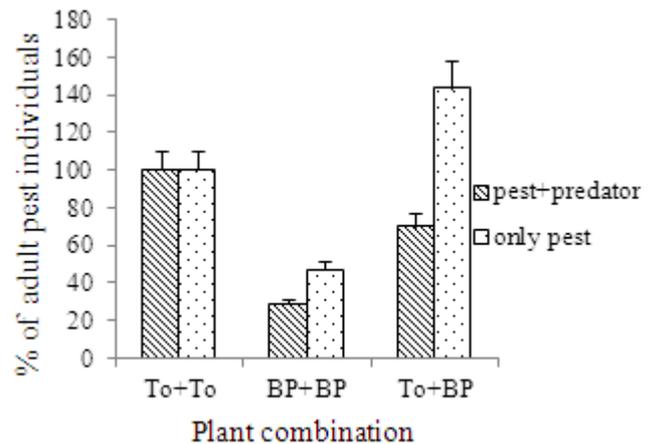


Figure 7. Percentage of adult pests *T. vaporariorum* if the number of pests present in the to+to treatment is set as 100%, with and without presence of predators in the different plant combinations (to= tomato crop plants, BP = tobacco banker plants; To+To = two tomato plants present; BP+BP = two banker plants present; To+BP = one tomato and one banker plant present) eight weeks after inoculation.

The number of larvae of the pests *T. vaporariorum* with and without presence of predators was highly significantly different between the plant combinations. When predators were present, the number of pest larvae was very low in all plant combinations (below 36 individuals) whereas it was elevated without predators, and especially when the BP was present (Figure 8). When both tomato and tobacco were present in the cage, the number of juvenile *T. vaporariorum* reached a mean of 1550 per plant.

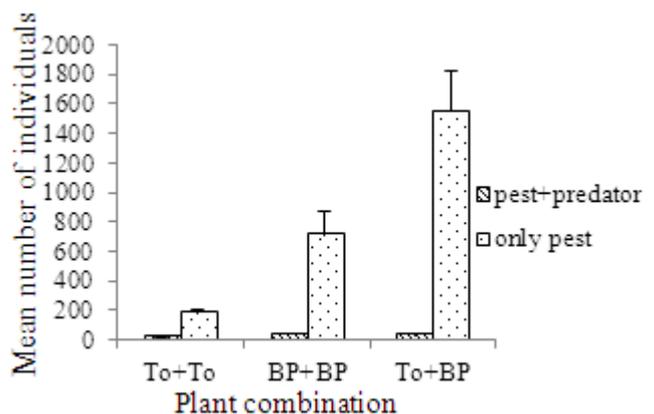


Figure 8. Mean number of larvae of the pest *T. vaporariorum* with and without presence of predators in the different plant combinations (to= tomato crop plants, BP = tobacco banker plants; To+To = two tomato plants present; BP+BP = two banker plants present; To+BP = one tomato and one banker plant present) eight weeks after inoculation.

Plant height. After eight weeks, there were no significant differences between the treatments (Figure 9). Initially the plants had the same height. After eight weeks, the influence of the presence of predators, and of sharing the cage with the same or the other species did not have an influence on plant height

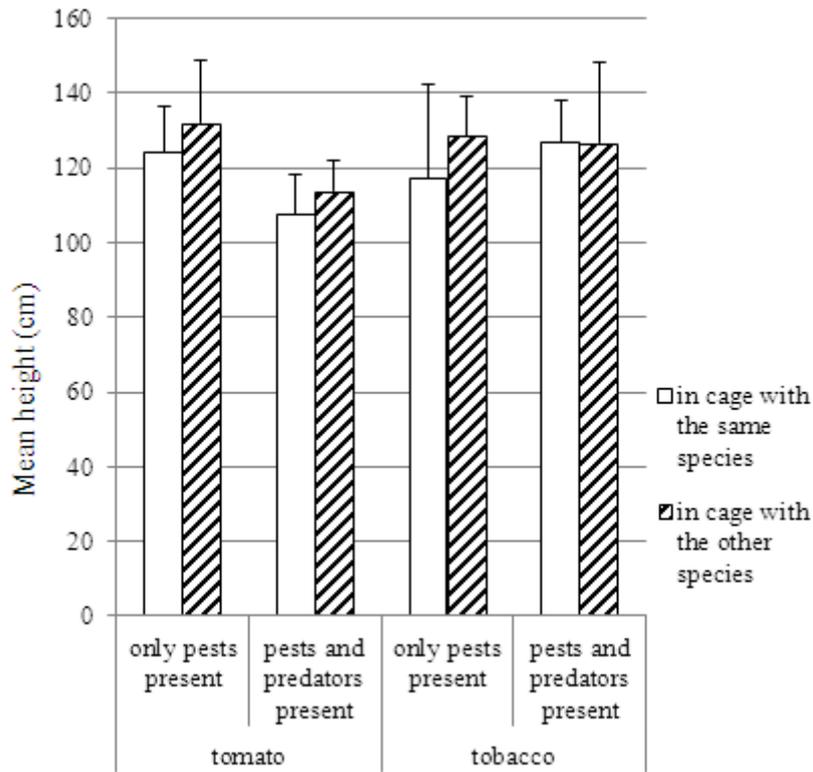


Figure 9. Mean plant height (in cm) after eight weeks in the different species combinations and treatments.

Number of leaves. There were no significant differences of the number of leaves between the treatments after eight weeks (Figure 10). At the beginning of the experiment, the plants had the same number of leaves. After eight weeks, predator and plant species presence did not influence the number of leaves.

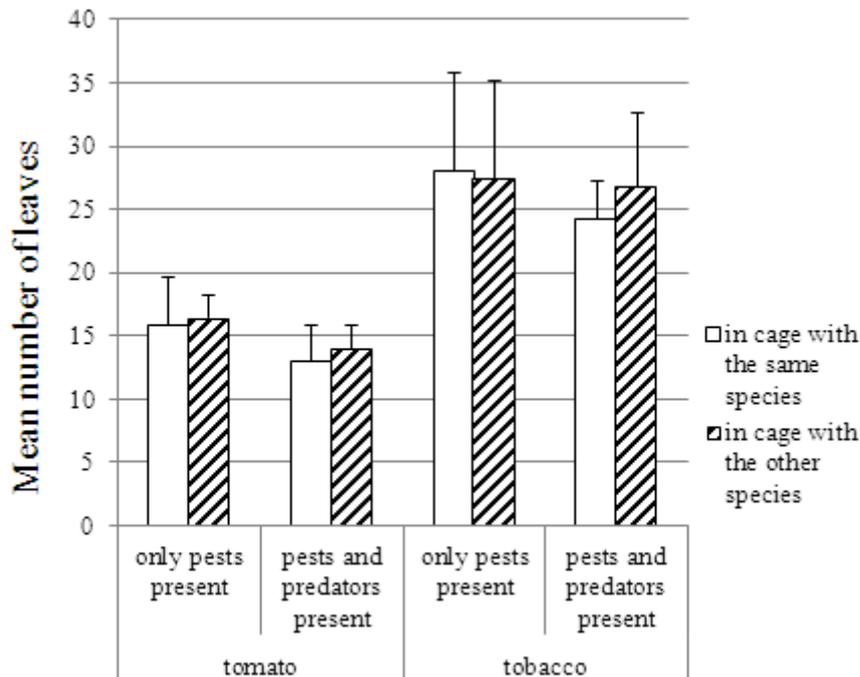


Figure 10. Mean number of leaves after eight weeks in the different species combinations and treatments.

Leaf damage. The percentage of damaged leaves was not significantly different between the treatments with the exception of the BP tobacco when only pests were present. Those plants had a significantly higher percentage of leaves damaged when the BP was in a cage with a tomato plant.

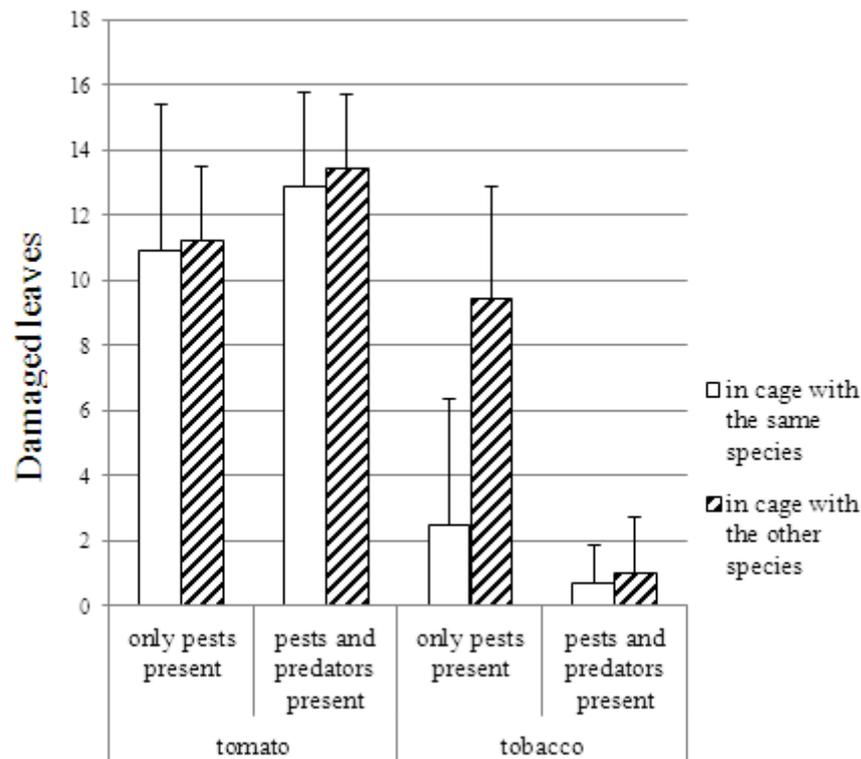


Figure 11. Number of damaged leaves in the combinations of plants with only predators *M. pygmaeus*, and with predators and pests *T. vaporariorum* present.

4. Discussion

Tobacco acted as efficient BP for the mirid bug *M. pygmaeus*, but it was not an efficient BP in this species combination as it increased the pest population when the predators were absent. The efficiency in increasing the predator population is congruent with other studies which showed that tobacco may host the predatory bugs, and that established populations of mirids maintain whiteflies at low densities and therefore drastically reduce pesticide use (Alomar et al. 2002; Bonato & Ridray 2007).

One important aspect for the use of BP by the local producers is to diminish costly releases of predators and thus save money. The cost of biological pest control using beneficials is high, and for this reason growers wish to conserve a part of the predator population from one crop season to another (Schoen 2003). In the case of *M. caliginosus* applied for tomato protection with tobacco as BP, the cost/efficacy ratio supports the BP method (Fischer & Terretaz 2003) which deserves further development. In a study over winter, tobacco could conserve a population of the predators in the tomato free periods in winter in non-heated greenhouses (Arno et al. 2000, Fischer & Terretaz 2003). Although population size was rather low in December, *M. caliginosus* recovered by March when the

next crop season started. This way, its reestablishment into the tomato crops was guaranteed without the necessity of repurchasing predators (Fischer & Terretaz 2003). The results of this study show that, however, in the combination with the pest *T. vaporariorum* to protect tomatoes, the employment of tobacco cannot be recommended. In the experiments the pests were able to increase their populations very strongly when predators were absent. As long as *M. pygmaeus* was well established in the banker plant system, the presence of tobacco was efficient in indirectly reducing pest attacks. However in practical applications the stable presence of a predatory population is not always guaranteed, and the danger that the BP causes an increase of the pest insects is significant.

To date, documentations in the literature are quite controversial regarding tobacco as an efficient BP for *Macrolophus* to protect tomato crops. The results of the present study support the finding that tobacco needs to be employed with caution. An additional danger is represented by the potato virus Y which can be transported into the system by the tobacco plants or by virus-infected *Bemisia tabaci* so that tobacco is not suited for use in greenhouse tomato productions (Schoen 2003).

The fact that tobacco acted as incubator for the pest in the species combinations when predators were absent and

tomatoes present, points to a complementarity of the two plant species to provide good reproductive conditions for *T. vaporariorum*, e.g. sites for reproduction and food. This unwanted synergy of plants to increase the presence of pests needs to be avoided.

Furthermore, the presence of the BP did not increase the productivity of the crop. This goal of IPM should be achieved via indirect tritrophic interactions of the present organisms, where the BP increases the number of predators which in turn reduce the presence of pests and have a lower impact on crop plants which hence can grow better and increase their biomass (Parolin et al. 2012a). This was clearly not the case in the present species combination.

5. Conclusions

The employment of tobacco as biocontrol plant was efficient in that it increased the population of predators. However, it bears a risk as tobacco may enhance the presence of the pests if the predator population is not stable. In the light of new legislations prohibiting pesticide use, the employment of biocontrol plants for biological pest control is a promising alternative (Parolin et al. 2014). Further studies are needed to find suited biocontrol plants which enhance the predator but not the pest populations. The better the biocontrol plant is suited for the species combinations of pests encountered and predators introduced, the more resilient and cost efficient the crop system will be.

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