False yellowhead (*Dittrichia viscosa*) causes over infestation with the whitefly pest (*Trialeurodes vaporariorum*) in tomato crops

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**Key words:** banker plant, biocontrol plant, tomato, whitefly, biological pest control.

**INTRODUCTION**

*Dittrichia viscosa* L. (W. Greuther) (False Yellowhead, Asteraceae; Figure 1) is a common ruderal plant of the Mediterranean region (Parolin et al., 2014a). It is highly entomophilous (Lambion, 2011), and enhances the presence of predatory arthropods (Fimiani and Digilio, 1993; Alomar et al., 2002; Broumas et al., 2002; Favas et al., 2003; Perdikis et al., 2007, 2008; Franco-Micán et al., 2010; Ingegno et al., 2011). *D. viscosa* is the most important natural host plant conserving and augmenting the predators of the genus *Macrolophus*, which is native in the Mediterranean area (Pasini et al., 1998; Favas et al., 2003; Fischer and Terrettaz, 2003; Perdikis et al., 2007). It is efficiently employed in agro-ecosystems and in greenhouses of the Mediterranean region, contributing to the efficiency of the biological control agent to protect crops against several important insect pests such as whiteflies, aphids and thrips (Albajes and Alomar, 1999; Favas et al., 2003; Castané et al., 2004; Gabarra et al., 2004; Perdikis et al., 2007). The advantage of the interactions between *D. viscosa* and mirid bugs of the genus *Macrolophus* is, is that the predator is zoophytophagous and feeds on both pests and plant sap (Castané et al., 2004; Ingegno et al., 2011). This way, the predators can complete their nymphal development even in the absence of prey; although nymphal development is faster if prey is available (Favas et al., 2003; Lykouressis et al., 2008). A biocontrol plant *sensu* Parolin et al., (2014b) inoculated with mirid bugs and installed in a crop system can thus sustain the presence of a population of predators before the pest outbreak. This way, pest outbreak may be avoided, representing serious alternatives to potentially dangerous pesticides (McCaffery 1998; Geiger et al., 2010).
Furthermore, *D. viscosa* may act as site for the winter survival of *Macrlophus*, thus contributing to the conservation and increase of their populations in agro-ecosystems (Favas et al., 2003).

The study tested *D. viscosa* in its function as banker plant (Frank 2010; Huang et al., 2011; Parolin et al., 2012a). This is defined as the plant component of the banker plant system, which together with alternative food and beneficial organisms, 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). The study wanted to assess if *D. viscosa* is suitable as banker plant to control the common tomato pest - the greenhouse whitefly *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) - on tomato crops in a greenhouse.

In an experiment in Southern France, the study tested its function to act as a multiplier for the beneficial arthropod *Macrolophus pygmaeus* (Heteroptera: Miridae); a mirid bug which predates on *T. vaporariorum* (Perdikis and Lykouressis 2000; Lykouressis et al., 2009). The study measured the population development of *M. pygmaeus* and *T. vaporariorum* on *D. viscosa* and crops. In a study performed in the open field in Greece, *D. viscosa* did not prove to be particularly efficient as banker plant for *M. pygmaeus* (Lykouressis et al., 2008). However, our projects involve *D. viscosa*, *M. pygmaeus* and tomato crops, bearing in mind those interactions may vary with the local conditions (Lykouressis et al., 2008; Parolin et al., 2012a). Detailed investigations on the role of secondary plants as a source of alternative food are essential. The study therefore, tested this species combination in greenhouse.

Gabarra et al. (2004) states that whiteflies colonise greenhouses easily and build up high populations before predators establish in the crop. Whether *D. viscosa* can influence these interactions, and the underlying mechanisms that lead to its role in tritrophic interactions under given circumstances should give interesting insights into the general understanding of the function of secondary plants and their functional traits (Cortesero et al., 2000; Parolin et al., 2012a, b). The main question addressed by this study was whether potted non-flowering individuals of *D. viscosa* fulfil the functions of a biocontrol plant, and more precisely of a banker plant, which should be able to maintain and multiply populations of predatory arthropods and ultimately cause a reduction of the presence of pest insects in the greenhouse crop system. For this purpose, the study conducted an experiment in a greenhouse in Southern France for eight weeks and counted the number of individuals of the predator *M. pygmaeus* and of the pest *T. vaporariorum* on the potential banker plant *D. viscosa*, in the presence of tomato *Lycopersicon esculentum* Mill. (Solanaceae) (cv Marmande) as crop plant.

**MATERIALS AND METHODS**

**Production of the plants**

Seeds of *D. viscosa* were collected in the open fields surrounding 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). Seeds of *D. viscosa* and of tomato were sown in a mixture of 1:3 per litre and 2:3 loam in 20 cm pots, kept in a greenhouse (25°C ± 2°C, RH 70 ± 10%) and watered daily. The plants did not flower during the period of experiments as their natural flowering occurs in October (Parolin et al., 2014a). All plants (*D. viscosa* and tomato) were about 20 cm high when the experiment started.

**Production of insects**

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**

In spring 2012, the study tested six combinations of plants, pests and predators in six separated cages of 2 x 1, 5 x 0, 8 m made of muslin. This was placed in a 40 m² greenhouse compartment covered with single glass (Figure 2). A randomized block design was used for each treatment with *D. viscossa* as banker plant and tomato as crop plant.

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

**Inoculation of the plants**

To prevent insect transfer between treatments in the greenhouse, the different treatments were isolated from one another using cages made of mesh material. Pesticide applications were strictly avoided. In every cage, the plants were pre-incubated with 6 *M. pygmaeus* predators (three males and three females). Seven to eight days of inoculation were considered as a necessary introduction period in the nursery (Ridray et al., 2001). After one week, the plants were inoculated with 30 individuals of whiteflies *T. vaporariorum* per cage. The pests were placed in an open Petri dish on the floor of the cage.

**Environmental conditions**

A fog and cooling system (Aria) was used to maintain stable climatic conditions (25°C temperature and 70% humidity) in the greenhouse. The plants were watered and fertilized with a drip irrigation system (set point values for the parameters of electrical conductivity EC was 1.5 µS and pH was 6).

**Monitored parameters**

Number of individual pests and beneficial species were counted in each treatment after eight weeks. The sampling started two weeks after predator release. Using a lens with a magnification of 10 x, all adults and larvae on all the leaves of *M. pygmaeus* and *T. vaporariorum* were counted on each plant.

At the end of the experiment, a destructive sampling enabled to assess plant damage by counting the percentage of damaged leaves. In each cage a yellow stick trap was introduced to evaluate the population of flying insects (adult *T. vaporariorum*, adult and larvae *M. pygmaeus*). After eight weeks, all plants were separated, leaves detached, transferred into zip plastic bags and taken to the lab for detailed counting of eggs, larvae and adults of pests and predators using lenses and a stereo microscope.

The percentage of damaged leaves of the total number of leaves present was counted at the end, before the destructive sampling. Leaves were defined as damaged when they were heavily affected by the herbivores and coloured differently from healthy leaves.

**RESULTS**

**Plants**

The percentage of damaged leaves was significantly higher when only tomatoes were present in the cage in comparison to the treatments where only *D. viscossa*, or *D. viscossa* and tomato were present - with or without the presence of pests (Figure 3). No significant differences were found in the percentage of damaged leaves with or without the presence of pests when *D. viscossa* was present in the
Figure 3: Percentage of damaged leaves of total leaves present in the three combinations of plants (D. viscosa + D. viscosa, D. viscosa + tomato, tomato + tomato) with only predators M. pygmaeus, and with predators and pests T. vaporariorum present (all numbers in %).

Figure 4: Number of individual predator M. pygmaeus present in the treatments with only predators (no pests) inoculated on the plants in distinctive combinations.

cage. There were some differences with or without the presence of pests when only tomato was present in the cage, with less leaves damaged if pests were available. The tomato plants were more attacked by the predators than the leaves of D. viscosa when no pest prey was available.

Presence of predators

The survey found only very few predators on D. viscosa after eight weeks (Figure 4). No predators were found on tomato, and no larvae were found in any treatment when no pests were present.

When pests were present (Figure 5), the presence of predators were high on both D. viscosa and tomato. Larvae of the predator were found on both, D. viscosa and tomato. The highest reproduction of the predator was measured during the treatment with only tomato plants.

Presence of pests

In the treatment where tomato plants and D. viscosa were mixed, relatively few pest larvae were found on the tomato plants (44), as compared to the highly infested plants of D. viscosa (with 1775 larvae; Figure 6). The number of pest larvae found on D. viscosa in combination with tomato plants was significantly higher than in the other treatments; indicating that this treatment is best suited for the propagation of the pest T. pygmaeus. On the same plants, 427 adult pest individuals were counted, compared to 2 adult predators and no larvae. The mean number of pest
larvae summed up for each plant species was less elevated when only tomatoes (238 larvae) or only *D. viscosa* (7 larvae) were present.

In the cages containing plants that had been inoculated with both pests and predators, when the only plant available was *D. viscosa*, the number of pests on the plants was rather low (cumulative 34 adults + larvae; Figure 6). The tomato plants in the cage without *D. viscosa* had 241 pest individuals. When the plant species were mixed – *D. viscosa* and tomato – the number of pests rose to an over infestation with 2246 adults and larvae of *T. vaporariorum* in the cage. The tomato plant had 471 pest individuals and *D. viscosa* harboured 1775 adult and larvae individuals of *T. vaporariorum*.

### DISCUSSION

*D. viscosa* clearly does not fulfil the desired functions as banker plant in the given species combination. On the contrary, it multiplied the population of the pest, enhancing the presence of adults and larvae of *T. vaporariorum* to an incomparable extent. This indicates the complexity between tropic relationships of predators, pests and secondary plants which have to be analyzed in detail for every species combination in order to avoid undesired results. The identification and evaluation of features of the agro-ecosystem that are involved in the conservation of predators employing secondary plants requires detailed case studies (Lykouressis et al., 2008). The attempt of this study to contribute more to this knowledge has resulted in undesired results. However, these unexpected outcomes can still be useful for the general understanding of the employment of banker plants. Despite the negative results for this species combination, the use of banker plants in general and of *D. viscosa* in particular, is promising as alternatives to pesticides (Geiger et al., 2010; Isenring, 2010). The combinations of *D. viscosa* and *M. caliginosus* worked well as other studies have shown (Favas et al., 2003).

Since it is important to improve the robustness of greenhouse crop rising against pests without the employment of pesticides, the producers should allow immigration and establishment of predator populations without disturbing them with toxic and non-selective insecticides (Castané et al., 2004). Further research should be carried out to test the extent to which predators migrate into the greenhouses, and if *D. viscosa* guarantees the survival of predator populations in winter; perhaps pollen or *Ephestia kuehniella* should be employed as factitious food (Vandekerkhove and De Clercq, 2010).

**D. viscosa** as banker plant for the predator *M. pygmaeus*

The predators of the genus *Macrolophus* which in other studies were observed to reproduce well on *D. viscosa* (Pasini et al., 1998; Favas et al., 2003; Fischer and Terrettaz, 2003; Perdikis et al., 2007) almost did not reproduce under the given experimental conditions. *M. pygmaeus* performed poorly on *D. viscosa*, although some larvae were found. This indicates that it completed its development while feeding on *D. viscosa*, but only in the presence of the prey *T. vaporariorum*.

The finding that *D. viscosa* is not suitable as secondary plant to enhance *M. pygmaeus* is already stated by Lykouressis et al. (2008) in Greece, and confirmed under the environmental conditions of this study's greenhouse in Southern France. Other plant species may act as banker plants for *M. pygmaeus*, e.g. *Solanum nigrum* can support and enhance the maintenance of these predators in the field (Lykouressis et al., 2008).

Lykouressis et al. (2008) establish that the non-performance of *M. pygmaeus* on *D. viscosa* was related to
the high mortality of the young nymphs caused by the entrapment on the dense sticky trichomes of *D. viscosa*. The legs of the nymphs stuck to their body, and tarsi or antennal segments were detached (Lykouressis et al., 2008). Other species of the genus *Macrolophus* seem to cope with the sticky hairs by means of their particular walking behaviour (Pasini et al., 1998) but not so with *M. pygmaeus* (Perdikis and Lykouressis 2000).

Other plants with sticky trichomes such as the tomatoes used in our experiment affected *M. Pygmaeus* less; since it survived and reproduced on tomato as previously observed by Perdikis and Lykouressis (2000). Physiological constraints such as plant sap or prey nutritional balance may play a role in the different tolerance to trichome stickiness (Perdikis and Lykouressis 2000). However, this study did not find a high number of predators on tomato, and no larvae were found in any treatment when pests were absent.

*D. viscosa* causes over infestation with the pest *T. vaporariorum*

Contrary to its use as biological pest control, the presence of *D. viscosa* enhanced the presence of the whitefly pest *T. vaporariorum* in our experiment. This insect showed an explosive reproduction mainly on plants of *D. viscosa* despite its sticky hairiness which seemed not to affect the whiteflies.

The study observed that oviposition was hampered by not only the trichomes on leaves of *D. viscosa* but also on tomato and the whiteflies could not make their typical circular oviposition (Byrne and Bellows, 1991), it was rather dispersed on the whole leaf or in the stems (whereas in a parallel experiment with tobacco leaves the same population of whiteflies laid eggs in circular position; unpubl. pers. obs.). All in all, over infestation with these pests indicates a highly efficient reproduction despite the constraints for oviposition due to the trichomes. The presence of *D. viscosa* was responsible for the high reproduction of this insect, but only in presence of tomato plants. Since the development of the predators was only partly successful on the same plants, this study cannot estimate the importance of *M. pygmaeus* for the distribution of *T. vaporariorum* on the plants. More experimental evidence is required here.

**Plant damage**

Tomato leaves were more attacked by the present pests and probably also by the polyphagous predators when the plants were in monoculture (to+to, Dv+Dv) than in the combination of Dv and crop plant (Dv+to). As the percentage of healthy leaves in comparison to damaged leaves did not differ significantly in the treatments with or without the presence of predators, the present data cannot conclude that the predators played a big role for plant health or damage. This is mainly because the predators did not install significant populations in any of the plant combinations.

Interestingly, tomato leaves were less damaged when pests were present. This is probably due to the fact that the presence of pests meant less leaf attack by the predators that are polyphagous and feed on the plants if prey is unavailable. The tomato plants were attacked more by the predators than the leaves of *D. viscosa*. In other species combinations, trophic switching of *M. pygmaeus* between prey and plant feeding could have favourable implications on the biological control and the predator’s conservation in the agro-ecosystems (Lykouressis et al., 2008).

**D. viscosa** in the greenhouse

The plants of *D. viscosa* were not easy to rear in the greenhouse. First, the seeds collected in the open field...
germinated slowly and the young plants grew slowly as well. This is because *D. viscosa* is a high-light species (Parolin et al., 2013, 2014a) and light was often limiting in the cages installed in the greenhouse, particularly in the long and dark winter months. The growing conditions for this ruderal species were not optimal as only about 30% of the global radiation entering the greenhouse was received in the cage.

Also the adult plants did not tolerate the shade in the cages very well; their stems were weak and did not stand as upright as the prosperous individuals growing outside of the greenhouse. This species is not well suited for light conditions in the greenhouse which must be kept in mind especially if thinking of utilizing it for the maintenance of over wintering populations inside of greenhouses. In an absence of prey, polyphagous predators might seriously affect these plants if grown inside.

**Conclusion**

*D. viscosa* is probably suited as banker plants for other species combinations but not for the protection of tomatoes with the predator *M. pygmaeus* against the whitefly pest *T. vaporariorum*. The use of potted plants of *D. viscosa* has some potential in combinations of other species of predators, but their best employment for biological control should be expected in open sites next to semi-open greenhouses. From this study, it is advised that producers in the Mediterranean region should take advantage of the exploitation character of the plant which is closely linked to its flowering habit enhances its function in the open field (Parolin et al., 2014a). The entomophilous character of the plant which is closely linked to its flowering habit enhances its function in the open field (Parolin et al., 2013). The overall goal is to improve the effectiveness of predators in greenhouses by employing novel biotic and abiotic technologies and this must be accompanied by specific case studies.

**ACKNOWLEDGEMENTS**

This research was part of the project PURE: Integrated pest management systems of major importance for Europe supported by EC-FP7-KBBE-2010-4.

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