Distribution of pest and predatory mites on plants with differing availability of acarodomatia

Plants which are added to a crop system may enhance the stable presence of predators. Acarodomatia could be a valuable functional characteristic for biocontrol plants employed in Integrated Pest Management (IPM). We tested the hypotheses that presence and availability of domatia on potential banker plants increases the stability of predatory mites’ populations by improving their reproduction, and that the number of domatia is positively correlated with the number of mites and eggs. We chose three plant species with differing presence of domatia in a greenhouse experiment lasting five weeks, and determined the presence of pest mites Tetranychus urticae and predatory mites Neoseiulus californicus and Phytoseiulus persimilis. Adults were present on the plants independently from the presence of domatia, but eggs were laid preferentially inside domatia by both predators, but not by the pest. The predators preferred banker plants with domatia, although correlations with the number of available domatia were not relevant for the presence of both predators. In summary, the two Vitis species and Viburnum tinus are good candidates to implement biological control of T. urticae by means of a banker plant system, with a positive role of acarodomatia for the reproduction of P. persimilis and N. californicus.

Key words: Banker plants; biocontrol plants; domatia; plant functional types; rose ornamental crops.

INTRODUCTION

Crop plants are strongly affected by numerous pest species which have deleterious effects on yield and quality. Many of these pests are resistant to chemical pesticides and it has become ever more difficult to control them in case of infestation (Van Lenteren 2000). The two-spotted spider mite Tetranychus urticae Koch 1836 (Acari, Tetranychidae) can resist to at least 80 commercial acaricides (Badawy et al. 2010). This pest is known to damage more than a thousand plant species worldwide, such as grapes, fruit trees, green beans, cucumber, ivy, geranium and roses (Van Lenteren 2000; Badawy et al. 2010). The urgent need to find alternatives to pesticides has been recognized over the past decades, increasing the interest for biological control of pests in crops. Crop growers buy and release predatory mites on their crops, but the predators tend to disperse away from or not reproduce on the crops, making frequent releases necessary, which are expensive. Methods to enhance sustainability and maintain a reproducing population of natural predators to provide long-term pest suppression are needed. One efficient method is the addition of biocontrol plants (Parolin et al. 2014) such as banker plants (Stacey 1977; Frank 2010; Huang et al. 2011; Xiao et al. 2012; Avery et al. 2014) to the crop system. Biological control agents like predatory mites are released onto the banker plants where they reproduce and increase in numbers. Less numerous pests were found in crops where banker plants were added in comparison with control crops (Stacey 1977; Pickett et al. 2004; Wong and Frank 2012; Xiao et al. 2012). This preventive control system avoids repeated and costly releases of natural enemies (Frank 2010; Huang 2011).
Although banker plant systems have been investigated for about thirty years, it is still difficult to implement such systems in crops. This is mainly due to the complexity of the interactions which also depend on the functional characteristics of the plants (Cortesero et al. 2000). The presence of domatia on the leaves, for example, has positive effects on the abundance, distribution and reproduction of predatory mites (Walter and O'Dowd 1992; Grostal and O'Dowd 1994; Agrawal et al. 2000; Norton et al. 2001; Ferreira et al. 2010). In this context, we wanted to answer the question if the presence and availability of domatia on banker plants plays a role for the presence of predatory mites and for their reproduction on plants. The hypothesis was that the more domatia present, the more the mites find shelter for reproduction and can be found in large numbers on the respective plants or in the system. For this purpose, we performed a greenhouse experiment over a period of five weeks in the Mediterranean climate of Southeastern France with two predatory mites (Neoseiulus californicus McGregor 1954 and Phytoseiulus persimilis Athias-Henriot 1957, Phytoseiidae) and one pest mite species (T. urticae). We offered the mites a combination of four plants, consisting of roses as ornamental crop (no domatia) and three potential banker plants, local species of the Mediterranean area, bearing domatia in different quantities. Our work is based on the assumption that the presence of banker plants with domatia aids the installation of the predatory mites. In the greenhouse experiment, we measured how the different availability of domatia influences the presence of adult and egg individuals of predatory and pest mites. This way, we want to contribute to the understanding of biological pest control via biocontrol plants with the final scope to increase the efficiency of biological control of pests.

MATERIALS AND METHODS

Plant material

*Rosa sonia* Meilland, var. 'Sweet Promise' (Rosaceae) was the focal ornamental crop species. Roses were multiplied in vitro and were genetically uniform. They were grown for 3 months in climate chambers at 18.8°C and 94.8% relative humidity (RH), 4500 lux, 16h day-8h night. The plants were then planted into 1.3l pots filled with perlite (proportion: 1/3) and with horticultural and professional compost « Humomot » (2/3) with pH 6.5, conductivity 1.08 mS/cm. Before the beginning of the experiment, they were acclimated in the greenhouse for one month at a temperature of 23°C-26°C and RH: 64%-67%.

*Vitis riparia* var. Gloire de Montpellier and *Vitis rupestris* Scheele (Vitaceae, grape), and *Viburnum tinus* L. (Caprifoliaceae, laurel tinus) were ordered as one year-old plants of about 20cm height at “Les 3 chênes”, a nursery in Orléans, France. They were planted into 1.3l pots filled at 1/3 with perlite and 2/3 horticultural and professional compost « Humomot », with pH 6.5, conductivity 1.08 mS/cm, and field capacity 153%. The plants were free from mites and were installed for a few weeks in the greenhouse before the experiments started. *V. tinus* were cut so that there were about 30 leaves per plant. Nutrients in the form of a slow-release fertilizer were added to all plants which were watered daily.

Pest and predators

The two-spotted red spider mites *T. urticae* were bred in the laboratory for 5 months at 23.7°C and RH 26% on 2 week old bean plants *Phaseolus vulgaris* L. The generalist predatory mite *Neoseiulus californicus* (Arachnidae, Acari, Phytoseiidae) was ordered as commercial strain Spical® at Koppert’s. To ensure food availability, pollen was sprayed on the plants before the experiment. *P. persimilis* is a specialist predator of *Tetanychus* species feeding on any developmental stage of the pest mite (Mc Murtry and Croft, 1997; Malais and Ravensberg 2006). The commercial strain Spidex® was ordered at Koppert’s.

Experimental design

Quartets of the four plant species (one of each species; Figure 1) were formed in seven replicates. The plants were close enough to allow mites to move from one plant...
to another, with touching leaves and additional woody sticks which acted as bridges in order to guarantee mobility of the mites (Casey and Parrella 2005). As predatory mites are likely to move on the ground, each pot was placed on Petri dishes (10 cm diameter) in a tray filled with water up to 5mm below the base of the pots (10 cm diameter).

The experiment began when ten mites of each of the three species were randomly put on each plant quartet. Climatic conditions in the greenhouse were settled so as to make a compromise between the requirements for each mite species in order not to promote one’s growth rather than another’s (Malais and Ravensberg, 2006). Temperature ranged from 18°C-31°C with an average daily temperature of 23°C±5°C, relative humidity ranged from 45% to 92%, with an average daily humidity of 63%±14%. To ensure food availability and not to stimulate migration towards other places where *T. urticae* was more abundant, pollen was sprayed on all plants, control plants included, before the inoculation with mites, and again 2 weeks after the beginning of the measurements.

After five weeks, at the end of the experiment, 20 leaves of every plant were harvested to count the number of domatia per leaf under the stereomicroscope (magnifying power ×20). Eggs were counted and their location on the leaves was recorded (adaxial, abaxial or inside domatia). A fine hair brush was used to softly separate trichomes and investigate the presence of the eggs inside the domatia. Age (young-mature-old) and position on the plant (top-middle-bottom) were assessed for the leaves analysed.

**RESULTS**

**Distribution of domatia and eggs on the plants**

The mean number of domatia per leaf varied from around 19 in *V. riparia* over 8 in *V. rupestris* to 2.5 in *V. tinus* (Figure 2A). The roses had no domatia.

The percentage of domatia bearing mite eggs was inversely related to the number of available domatia: *V. riparia* had the lowest percentage of domatia with predator eggs, *V. tinus* the highest (Figure 2B).

No plant had domatia on the upper leaf sides, all were...
on the lower leaf sides. The distribution of domatia on the plant differed among the studied plants (Figure 3A): *V. riparia* had most domatia in the top and middle height of the plant, in *V. rupestris* the distribution was rather uniform, and in *V. tinus* most domatia were in the bottom part of the plant. Differences of the number of domatia depending on the position on the plant were statistically significant. Leaf age did not play a role for the distribution of domatia on *V. tinus*, while in *V. riparia* and *V. rupestris* most domatia were present on young leaves, and only few on the oldest leaves (Figure 3B).

**Distribution of adults on the plant species**

The presence of adult predatory mites was not linked to domatia presence in the chosen plant species (Figure 4). *N. californicus* had high numbers of individuals on *V. riparia* (many domatia) and *V. tinus* (few domatia) and very few individuals only on *V. rupestris* with intermediate availability of domatia, or rose with no domatia. Rose plants were particularly infested by adult pest mites. *P. persimilis* occurred in low numbers on all plants, and had the highest presence on the plants with little or no domatia (*V. tinus* and rose).

**Presence of eggs**

*N. californicus* reproduced well on the plants with most domatia, less eggs were present with decreasing number of domatia on the banker plants (Figure 5). Roses, which had no domatia, had a number of eggs which was as high as *V. riparia*. Eggs of *P. persimilis* occurred in very high numbers on *V. riparia* and decreased with decreasing availability of domatia on the plant species. On roses, eggs
Figure 4: Mean number of mite adults (pest *T. urticae*, predators *N. californicus* and *P. persimilis*) on the four plant species, sampled after five weeks in the experiment.

Figure 5: Mean number of mite eggs (pest *T. urticae*, predators *N. californicus* and *P. persimilis*) on the four plant species, sampled after five weeks in the experiment.
of *P. persimilis* were present in similar numbers as in *V. tinus* which had few domatia.

*T. urticae* reproduced only on roses, no eggs were found on the three species of banker plants.

**Presence of mites depending on leaf position on the plant**

The number of adult mites did not show significant differences between top, middle or bottom positions (Figure 6A-D). Slight differences were found for the predator *P. persimilis* which tended to prefer middle and bottom positions.

Mite eggs were also equally distributed over top, middle and bottom plant parts in *T. urticae* and *N. californicus*, with a tendency towards more eggs in the lower plant parts than in the higher ones in *T. urticae*, whereas *P. persimilis* had 100% of the eggs on the bottom part of the plants (Figure 7).

**Presence of mite adults and eggs depending on position on the leaf**

Adult *T. urticae* and *P. persimilis* preferred the lower leaf sides over upper leaf sides or domatia wherever they occurred (*V. rupestris, V. tinus, Rosa*) (Figure 8). Adult *N. californicus* preferred domatia (Figure 8). Some individuals were found also on the lower leaf sides. No mites were found on the upper leaf side, in all mite and plant species.

The eggs of *T. urticae* were found predominantly on the lower leaf side. Only single eggs were found on the upper side of rose leaves and domatia of *V. riparia* (Figure 9). The two predators never laid their eggs on the upper side of leaves whatever the plant species was. The eggs of *N. californicus* were found predominantly on the lower leaf side when domatia were not present. On the plants with domatia, eggs were laid preferentially inside the domatia (Figure 9). *P. persimilis* laid most eggs inside the domatia, only very few eggs were laid on the lower leaf sides.

**Role of leaf age for presence of mite adults**

Most adult mites and their eggs were found on mature leaves, as compared to young or old leaves (Figure 10, Figure 11). *T. urticae* was restricted to mature leaves with few eggs also present on old leaves. Adult *N. californicus* occurred on mature leaves only in the two *Vitis* species, but on all age classes in *V. tinus*. Their eggs were found only on mature leaves. *P. persimilis* occurred on mature leaves only except for *V. tinus*, there it was found also on old leaves, just as were the eggs.

**DISCUSSION**

The results of our study show that, despite good mobility of the adult predatory mites and their presence on all plant species, the eggs were laid inside domatia to a high percentage whenever these were available. Acarodomatia show to have a particularly important role for the reproduction of the chosen predatory mites, particularly for *N. californicus*. The pest mites were not found inside the acarodomatia, nor did they reproduce there. This is the desired behaviour, as the objective of introducing banker plants is to enhance the reproduction of the predators alone, and not to act as multipliers for the pests.

The hypothesis that with more domatia present, more mites would reproduce on the respective plants, can be stated as we found that the more domatia on the plant, the more eggs of the predators were counted. However, also other functional characteristics of the banker plants may be responsible for the efficient reproduction of the mites in our study. We employed whole plants with all their complexity on purpose, in order to simulate as much as possible real conditions in the greenhouse. However this way, we cannot exclude that other factors, such as trichomes or plant chemistry, played a role for the encountered results. Furthermore, despite provision with pollen for food on all plants, whatever the plant, the predators would always prefer the one with spider mites as food. The predators present on the roses probably followed their abundant preys on these plants, and thus give a distorted view of their plant preferences. Trichomes may play an important role for mites: *N. californicus* prefer plants with non-glandular trichomes (Gotth et al. 2006). Some Phytoseiid mites could not establish on trichome-free plants such as *Typhlodromus pyri* Scheuten (Loughner et al. 2010). Looking at our results, the absence of trichomes on the roses might affect the presence of *N. californicus*. However, not all Phytoseiid mites respond in the same way to the presence or absence of trichomes. *P. persimilis* was more present on roses and *V. rupestris*, the least ‘hairy’ banker plant in this study. The effects of trichomes on *P. persimilis* have been investigated in previous researches. Contrary to *N. californicus*, the searching efficiency and predation rate of *P. persimilis* females was negatively affected by the density of trichomes on *Gerbera jamesonii* (Krips et al. 1998). Besides, life functions of *Phytoseiulus* species are carried out almost exclusively in spider mite colonies (McMurtry and Croft 1997) thus, on roses. So the plant characteristics can explain why *P. persimilis* individuals are mainly found on roses and *N. californicus* individuals on the banker plants.

In fact in our study, the predators do not need domatia for reproduction. They can reproduce very well in their absence, if prey is present, as was the case on the roses. However, basing on our results we assume that the domatia enhance their reproduction significantly. Probably in our experiment there was not enough time to disclose all the eggs. Future generations in a longer-lasting experiment might have given a different picture of the importance of domatia for the mite populations. We found that the more domatia were on the plant, the more eggs of the predators were present on the plants. Thus, mite reproduction was related to the availability of
Figure 6: Mean number of mite adults (TU pest T. urticae, NC predator N. californicus and PP predator P. persimilis) on three positions on the plants: top, middle and bottom in the four species of plants, A V. rupestris, B V. riparia, C V. tinus, D Rosa
Figure 7: Distribution of mite eggs (TU pest *T. urticae*, NC predator *N. californicus* and PP predator *P. persimilis*) on top, middle and bottom part of the plant, in percentage of total number of eggs.
Figure 8: Mean number of mite adults (TU pest *T. urticae*, NC predator *N. californicus* and PP predator *P. persimilis*) on the four plants on the upper leafside, lower leafside, or inside domatia. *Rosa* has no domatia, *V. rupestris* only very few.

Figure 9: Mean number of mite eggs (TU pest *T. urticae*, NC predator *N. californicus* and PP predator *P. persimilis*) on the four plants on the upper leafside, lower leafside, or inside domatia. *Rosa* has no domatia, *V. rupestris* only very few.

domatia on the banker plants, emphasizing the role of plant functional characteristics (Cortesero et al. 2000; Avery et al. 2014). Further studies are needed to provide insights here, e.g. focusing on experiments with one plant species where the availability of domatia is manipulated so that the differing availability of acarodomatia is not linked to other factors than to the domatia alone. Also, in our study, we did not consider possible negative effects of
Figure 10: Mean number of mite adults (TU pest *T. urticae*, NC predator *N. californicus* and PP predator *P. persimilis*) on the four plants on young, mature and old leaves.
the presence of two predatory mite species which may influence each other via undesired interactions and competition.

**Conclusions**

Our hypothesis, the more domatia are available the more predatory mites are present, was tested in an experiment. The presence of banker plants with domatia aided the installation of the predatory mites in the present species combination. Basing on our results, banker plants were efficient in making the predatory mite populations in a crop system more stable. Thus, we suggest local farmers and producers who work with banker plants to use species with acarodomatia if they employ *N. californicus* and *P. persimilis*.

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**Conflicts of interest**

We have no conflicts of interest. Research involving Human Participants and/or Animals: we have no conflicts here.

**REFERENCES**


