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Distribution of acarodomatia and predatory mites on *Viburnum tinus*

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Abstract

Domatia are small invaginations and hair tufts usually found at vein junctions on the undersides of leaves in many woody dicotyledonous plants. They are frequently occupied by predatory mites, sometimes at very high densities. They offer protection against adverse weather conditions and protect against other predators and intraguild predation. Plants may benefit from the presence of the mites through reduced densities of herbivores or plant-pathogenic fungi. It has therefore been suggested that domatia mediate a mutualistic interaction between plants and mites. Plants bearing domatia can be employed as efficient banker plants. In the context of a series of experiments designed to analyse the function and applicability of banker plants, in the present contribution we analyze the role of acarodomatia on *Viburnum tinus*, a Mediterranean plant which can be employed as banker plant to improve biological pest management in local crop production. Predatory mites, e.g. *Amblyseius californicus*, install efficiently on *V. tinus* and reduce the number of the common pest mite *Tetranychus urticae*. We analyzed the availability and distribution of domatia on greenhouse grown plants of *Viburnum tinus* and the presence of predators. The results indicate that there is a close positive relationship between the distribution of domatia and the presence of the predatory mite *Amblyseius californicus* on *Viburnum tinus*. Domatia were found more frequently on old and mature leaves, which is an important factor to take into account in the practical use of *V. tinus* as banker plant, when considering cutting cycles and the frequency of replacement of the banker plants in a greenhouse system. This study documents the suitability of *V. tinus* as banker plant to be employed especially in the Mediterranean region where it is native and adapted to the local climatic conditions.

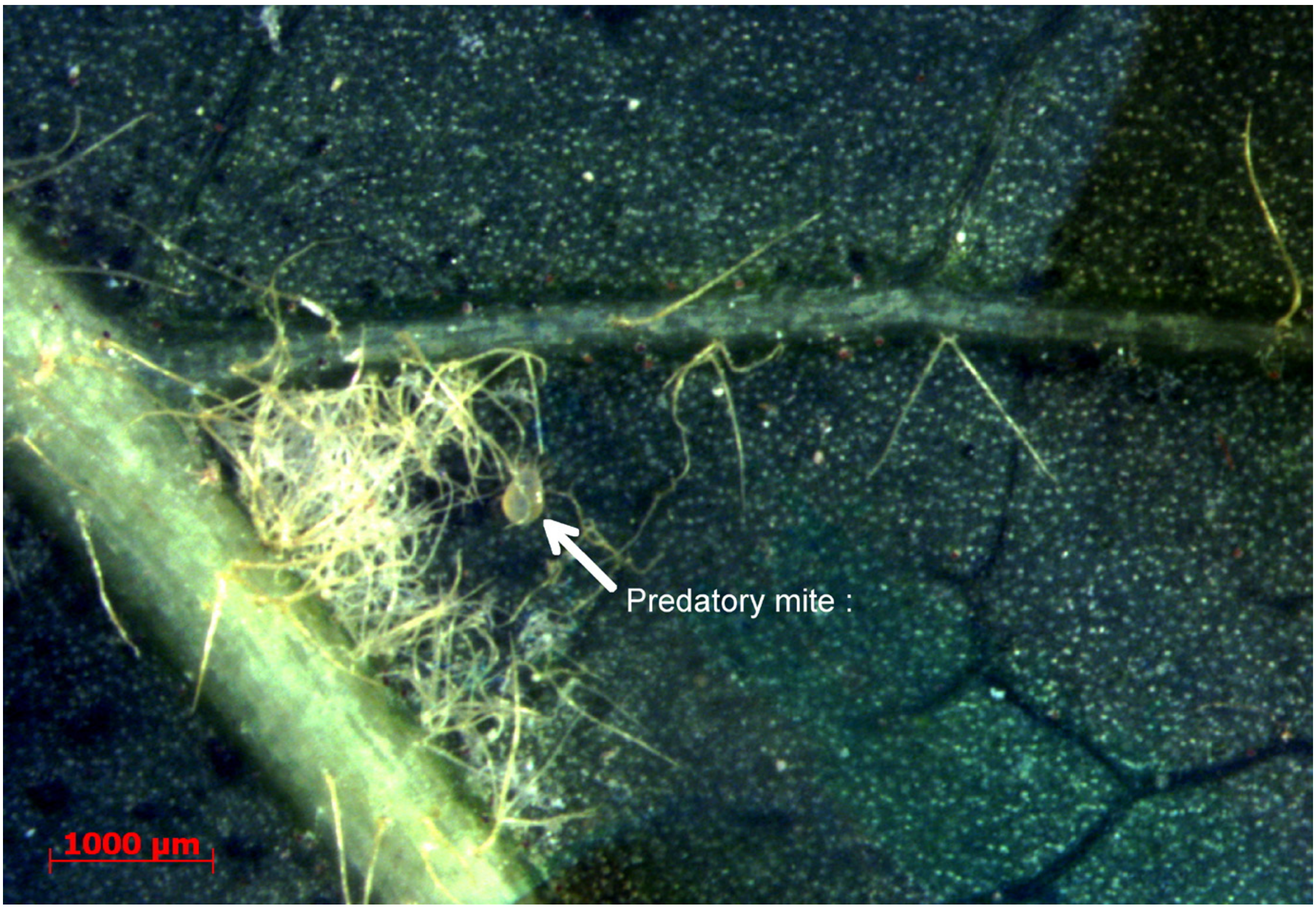
Introduction

Domatia (from the Latin « domus » meaning home) are structures on plants used in symbiotic relationships with small animals (e.g. ants, mites). Acarodomatia (Figure 1, Figure 2) are domatia specifically for mites (Acari). They are small invaginations and hair tufts usually found at vein junctions on the undersides of leaves in many woody dicotyledonous plants (Pemberton and Turner 1989). Domatia are frequently occupied by predatory and mycophagous mites. They are found in more than 2000 plant species of several families (Agrawal et al., 2000) and offer protection against adverse weather conditions and protect against other predators and intraguild predation. Plants that possess acarodomatia have more

Acarodomatium on the lower leaf side of *Viburnum tinus* formed by hairs in the junction of leaf veins.

abundant and persistent populations of predaceous and microbivorous mites, resulting in greater consumption of damaging phytophagous mite species and fungal epiphytes and pathogens (Walter and O'Dowd, 1992; Grostal and O'Dowd, 1994; Karban et al., 1995). It has therefore been suggested that domatia mediate a mutualistic interaction between plants and mites (Lundström, 1887). Acarodomatia play an important role for shelter and reproduction, and thus can influence predator-prey-relationships and tritrophic interactions in the context of biological pest control and Integrated Pest Management (IPM). Therefore, plants with acarodomatia are suited as "banker plants" employed in biological control (Huang et al., 2011; Parolin et al., in press 1).

Banker plants are the plant component of the banker plant system in a rearing and release system purposefully established in a crop for control of pests in greenhouses or open field (Huang et al., 2011). A banker plant is specifically associated with biological control whose aim is to increase the probability of establishment of natural enemies (Murphy, 2004; Osborne et al., 2005; Sanderson and Nyrop, 2008; Huang et al., 2011; Parolin et al., in press 2). The banker plants maintain a population of natural enemies and thus increase the likelihood of natural enemies for crop pests to be present in the crop plant system (Frank, 2010). Predators and parasitoids create reproducing populations on the banker plants which guarantees ongoing release of natural enemies with a stabilizing effect on population



Acarodomatium on the lower leaf side of *Viburnum tinus*, with predatory mite.

Plant of *Viburnum tinus* grown in a greenhouse, as it can be employed as banker plant in Integrated Pest Management.

fluctuation within the crop system and thus provides long-term pest suppression (Frank, 2010; Huang et al., 2011; Parolin et al., in press 2).

Viburnum tinus L. (Caprifoliaceae; Figure 3) is an evergreen shrub that grows naturally in Mediterranean ecosystems. It is frequently used as ornamental plant. A few studies focused on the potential of *Viburnum tinus*, which possesses acarodomatia, in IPM context (Walter and O'Dowd, 1992; Grostal and O'Dowd, 1994).

To investigate the banker plant mechanisms, we have conducted a series of experiments in Southeastern France where we study the ecological, physiological and phenological characteristics of secondary plant species added to greenhouse systems with the purpose to enhance biological control. In one experiment (Parolin et al. unpublished) we found that the predatory mites *Amblyseius californicus* and *Phytoseiulus persimilis* installed themselves efficiently on *V. tinus* and reduced the number of pest mites *Tetranychus urticae* on the rose crops placed nearby. As a side effect of these experiments which are focussed on the question of how secondary plants can influence a system of biological control (Parolin et al. in press 1), we analyzed the distribution of acarodomatia on *Viburnum tinus* and their influence on the presence of the predatory mite *Amblyseius californicus*. The present paper deals with the question how domatia are distributed on *Viburnum tinus* and how the distribution of predators is related to the presence of domatia on the plant.

Methods

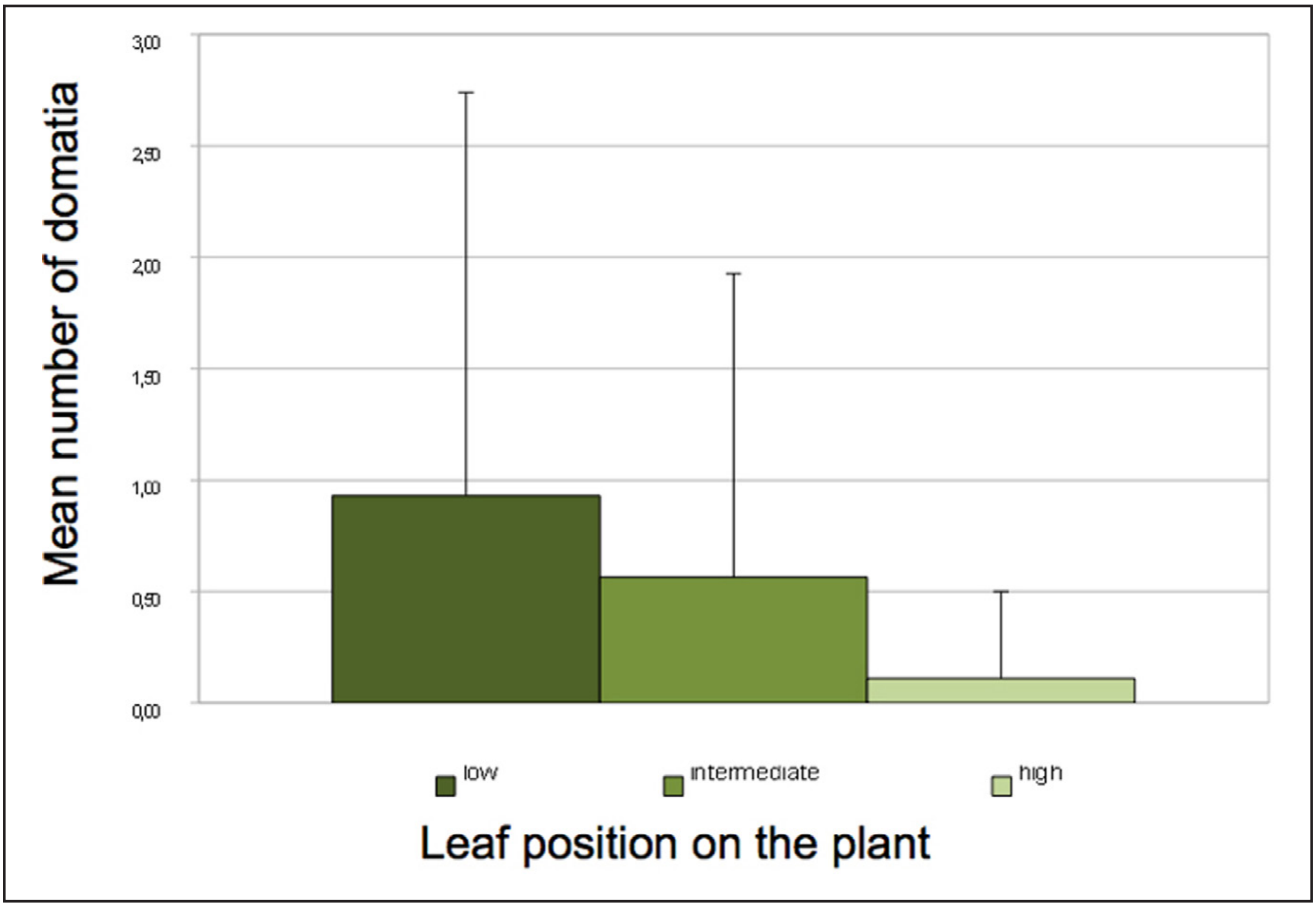
Three plants of *Viburnum tinus* were grown in an IPM greenhouse and inoculated with *Amblyseius* (*Neoseiulus*) *californicus* (McGregor) (Arachnidae, Acari, Phytoseiidae). This is a generalist predatory mite that feeds on various arthropods and pollen. The commercial strain Spical® was ordered at Koppert's and installed within 2 days upon arrival. Pollen from *Pinus halepensis* P. Mill. collected on trees outside the greenhouse was added to the plants at regular intervals in order that food was not limiting for the predatory mites on the plants.

Three weeks after inoculation of the plants with the predatory mites, the number of predatory mites and their position on the plants was noted. The mites were observed on the leaves with a lupe while taking care not to touch the plants too much because handling of the leaves during the experiment induces stress and movements of the predators. Later, all leaves (n=143) present on the three plants of *V. tinus* were removed and observed under the binocular in the laboratory. On every leaf, presence of predatory mites and the position and number of domatia were counted. We differentiated three categories of leaf age (young, mature, old) and three categories of the leaf's position on the plant (leaf on bottom, middle, top part of the plant), as well as upper / lower leaf side. The presence of predators was then related to leaf age and to the position on the plant.

We used Chi^2 test to analyze the distribution of domatia as related to leaf side (upper, lower), leaf position on the plant (top, middle, bottom), leaf exposition (sun, shade) and leaf age (old, mature, young) and to the presence of predatory mites.

Results

Position and number of domatia. The correlations between presence of domatia and leaf side was highly significant, there were no domatia on the upper leaf sides, only on the low sides. The presence of domatia was not correlated with leaf position on the plant (Figure 4; $\chi 2$ =4.3433, P =0.114). Despite some differences and a higher total number of domatia on the lower leaves, differences were not significant for the presence of domatia. Related to this, also the exposition of the leaf to sun or shade was not significant. Exposure to sunlight (light / shade) was not significantly related to the number of domatia present (Figure 5A). The relation between the presence of domatia and leaf age (young, mature, old) was highly significant (Figure



Number of domatia on leaves of *Viburnum tinus*, in relation to leaf position on the stem (low, intermediate, high).

6A; χ^2=19,25, P =1,329. 10^{-7}). The older the leaf, the more domatia were present. Also mature leaves bear many domatia.

Position and number of predatory mites. The distribution of predatory mites on the plant was closely related to the distribution of domatia (χ^2=18,121, p value=2,073. 10^{-5}). There were significantly more predators where there were many domatia on the leaves (Figure 6B). The exposition to sunlight enhanced the presence of predatory mites on the leaves but the differences as compared to shaded leaves were not significant (Figure 5B).

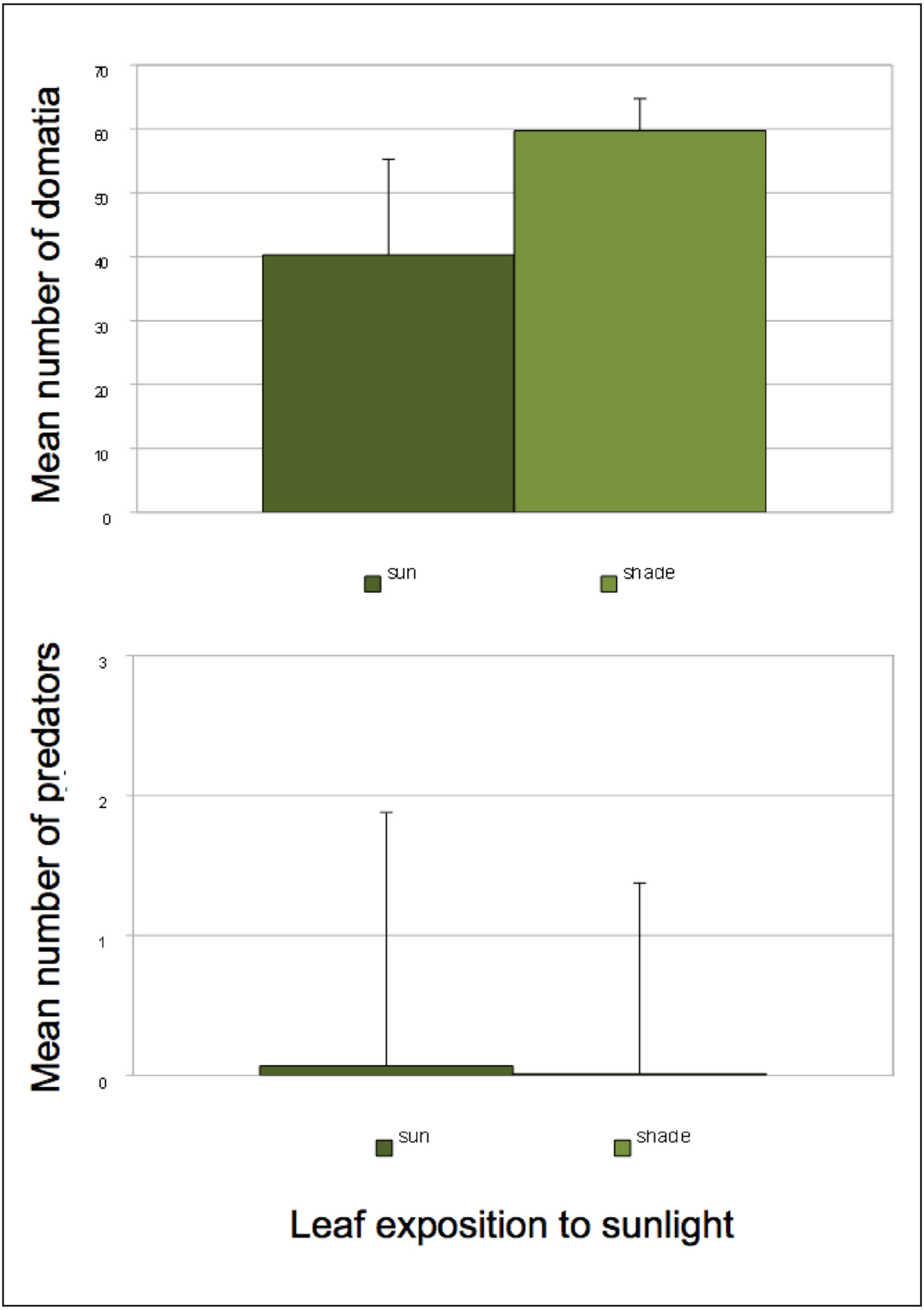
Discussion

There was a close relationship between the distribution of domatia and the presence of the predatory mites *Amblyseius californicus* on greenhouse grown plants of *Viburnum tinus*. This was documented earlier (Walter and O'Dowd, 1992; Grostal and O'Dowd, 1994; Karban et al., 1995) but the importance of the distribution of domatia for the distribution of A. californicus on *V. tinus* was not analyzed to date. The present data indicate the importance of domatia for the distribution of predatory mite populations on *V. tinus* which has a high potential as banker plant, especially in the Mediterranean region where it is native and adapted to the local climatic conditions, e.g. high temperatures and low water availability in summer (Salleo et al., 1997).

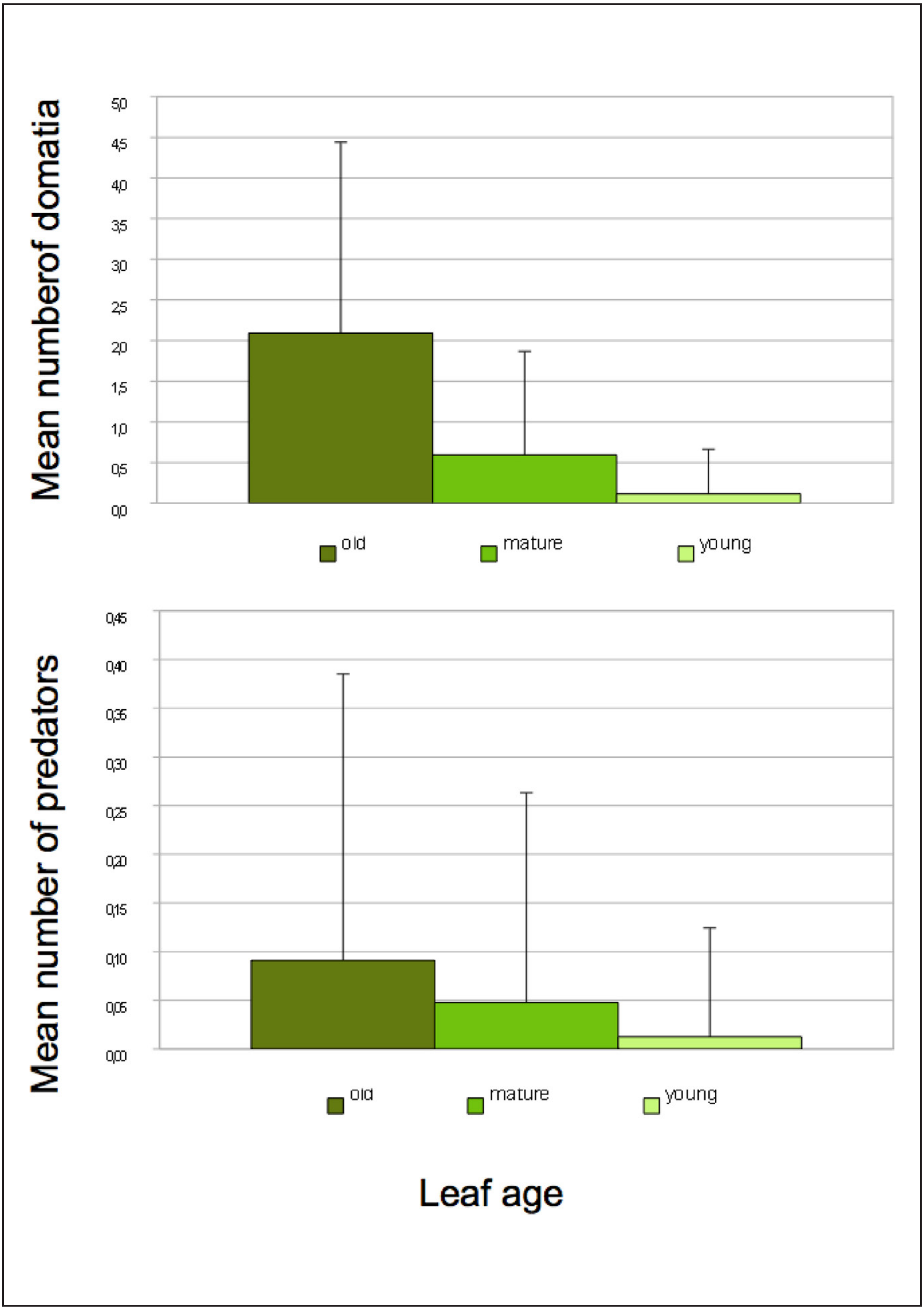
Domatia were found more frequently on old and mature leaves, which is an important factor to take into account in the practical use of *V. tinus* as banker plant, when considering cutting and pruning cycles and the frequency of replacement of the banker plants in a greenhouse system.

Other experiments have to be conducted to understand the presence, distribution and size of domatia over the annual cycle. The plants employed in this experiment were grown in greenhouses. In the field, presence, size and position of domatia may be different. The present data however can be compared to the conditions employed by local producers who grow their crops in greenhouses and can use *V. tinus* as banker plant, e.g. to protect roses, an important crop in the region.

The next step on the way to our understanding of the installation of predatory mites on *V. tinus* must be to analyze how the presence of domatia is triggered. Experimental results demonstrated that predatory mites benefit from domatia presence (Walter and O'Dowd,



Number of domatia on leaves of *Viburnum tinus* (A) in relation to leaf exposure to sunlight (high / low) and number of predatory mites (B) depending on the exposition to sunlight of *Viburnum tinus*.



Number of domatia on leaves of *Viburnum tinus* (A) in relation leaf age (young, mature, old) and number of predatory mites (B) on different leaf age classes of *Viburnum tinus*.

1992; Grostal and O'Dowd, 1994; Karban et al., 1995). However the induction of acarodomatia is less documented. Norton et al. (2000) demonstrated a strong positive relationship between leaf domatia size and the abundance of beneficial mites. In a rain forest tree, ants were also found to induce domatia (Blüthgen and Wesenberg, 2001). The presence of mites is known to induce the formation of acarodomatia but in our experiment we cannot answer the question whether the mites were responsible for their presence, or the domatia were responsible for the presence of reproducing mites, or both.

Furthermore, the presence of domatia probably varies over the annual cycle, and thus influences the reproductive success of the predatory mites which are installed on the plants in different ways along the year. This is important in the context of IPM and biological pest control because a stable supply of predatory mites is necessary if they are to be employed as pest control in IPM.

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