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Colonization of vineyards by the predatory mite
Typhlodromus pyri (Acari: Phytoseiidae)
using felt belts

Master Thesis

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1 Introduction

1.1 Historical aspects of vine-growing, plant protection and problems with mites

The role of beneficial mites as natural antagonists of pest organisms in ecosystems, including agro-ecosystems such as vineyards, was first discovered in the nineteenth century (Gerson et al., 2003). The first records date back to 1868 in the USA, when Shimer observed the mite *Hemisarcoptes malus* feeding on the oystershell scale. Charles Valentine Riley took notice of these observations and tried in 1873 to control the grape phylloxera, which had been a major pest at that time by establishing *Hemisarcoptes* sp. in vineyards but his experiments failed (Gerson et al., 2003). Later he found out that grafting with resistant rootstocks is the solution for the phylloxera problem and today he is known as a pioneer in the field of entomology and biological control of pests (Charles Valentine Riley Memorial Foundation, <http://rileymemorial.org/>). First records of the mites of the family Phytoseiidae as natural enemies of spider mites, which is nowadays the most studied and best known family of predacious mites, because of its relevance in natural and biological pest management, were made in 1906 by Parrott (McMurty et al., 1970, Gerson et al., 2003). For long time the importance of beneficial mites in agro-ecosystems was unnoticed or neglected, because problems with harmful mites were scarce until the end of World War II (van de Vrie et al., 1972, Duso et al., 2012).

The history of cultivation of *Vitis vinifera* L. goes back to the Neolithic period. Egyptians and Romans distributed this plant in their empires in Europe and Africa (Vincent et al., 2012). Around 1850, fungal diseases like powdery mildew and downy mildew and the herbivorous pest phylloxera invaded from North America into Europe and the grapevines were protected mostly with chemical and cultural measurements. Phylloxera-resistant rootstocks were used and copper and sulfur were applied as fungicides. After the Second World War chemical substances like DDT or organophosphates were widely used in vineyards (Huffaker et al., 1970). To that time, for obtaining quantitatively high yields, the grapevines were protected from a large range of fungal diseases and arthropod pests (Vincent et al., 2012).

First noticeable infestations of vineyards by the phytophagous mites *Tetranychus urticae*, *Panonychus ulmi* and some other species were reported in the second half of the nineteenth century, but the problems were negligible (Duso et al., 2012). Only after World War II, spider mites started causing serious damages to vineyards and, to that time, they were combated mainly with acaricides. Van de Vrie et al. (1972) reported about so called 'after World War II acaricides', which included organophosphates and a number of specific substances, such as Aramite (butylphenoxy isopropyl chloroethyl sulfite), chlorobenzilate (ethyl dichlorobenzilate) and others. However, these authors already mentioned that phytophagous mites developed

resistances against these substances. Also other fungicides and insecticides used at that time such as chlorinated hydrocarbon insecticides and ethylene-bis-dithiocarbamate fungicides, had serious impacts on the vineyard agroecosystem (Duso et al., 2012). At first, it was only a theory that with the use of modern pesticides the natural balance between herbivorous organisms and their natural enemies was impaired. However, then it was proven that many of the used chemical substances did not have a sufficient effect against phytophagous mites. The mites developed resistances very quickly, whereas most of their antagonists were killed and/or built up resistances much more slowly. Populations of harmful mites caused more and more damages to cultivated plants, because the chemical plant protection measurements were to their benefit but to the disadvantage of their predators. These explanations were widely and repeatedly confirmed (e.g. Narayanan et al., 1960; McMurty et al., 1970; Walters, 1976; Oberhofer, 1983).

Since the knowledge about the beneficial role of predatory mites gained more and more attention, pertinent measurements were taken and the frequency of spider mite outbreaks in vineyards consequently declined since the late 1980s. The measurements included reduced use of broad-spectrum pesticides, preferably only pesticides that are harmless for predatory mites, the adoption of action thresholds (Duso et al., 2012) and the promotion of predatory mites, mainly by conservation and release, with other words protection and augmentation of natural enemies.

1.2 Pest mites in Austrian vineyards

The following mite species described below occur as pests in Austrian vineyards. Further harmful mites on grapevines on a worldwide scale are, for example, the yellow grape-vine mite *Eotetranychus carpini*, which is currently a problem in Greece, Spain, southern Switzerland and Italy, the McDaniel mite *Tetranychus mcdanieli* in North America, the strawberry spider mite *Tetranychus turkestani* in France, Spain and Portugal and the citrus flat mite *Brevipalpus lewisi* in Spain (Duso et al., 2012).

1.2.1 True spider mites (Tetranychidae)

In springtime the European red mite *Panonychus ulmi* (Figure 1, left) is more frequently found in vineyards while in late summer the common or two-spotted spider mite *Tetranychus urticae* (Figure 1, right) can be found more often (Kadisch, 1986). Spider mites tend to live on the lower side of the grapevine leaves, have a size of about 0.5 mm when adult and have the ability to produce webs (Vogt et al., 1977). When hatching out of the egg, the larval mites have only six legs but after molting, as nymphs and as adult mites, they have eight legs (van de Vrie et al., 1972). Spider mites mostly occur in aggregations, so called hot spots, and are not evenly distributed across the whole vineyard. Spider mites suck out the content of the

parenchyma cells, which causes a loss of assimilation surface. As a consequence, infested leaves turn into yellow or brown, berries ripen slower, growth is retarded, fewer florescences are produced and plants are more sensitive to cold temperatures (Hassan et al., 1993). All this can lead to reductions in quality and yield. Besides predatory mites, antagonists of spider mites are also ladybird beetles, green lacewings and kissing bugs (Hassan et al., 1993).



Figure 1: Spider mites. Left: *Panonychus ulmi*, (<http://gardenerstips.co.uk/blog/2011/03/page/2/>), right: *Tetranychus urticae*, (<http://naturalenemiesbiocontrol.com/mite-control/>)

1.2.1.1 European red mite *Panonychus ulmi*

For overwintering, the females of *Panonychus ulmi* lay winter eggs on the bark (Figure 2, left) from August until October (Hillebrand et al., 1995). In springtime, small white larvae hatch out of these eggs. The weather conditions at the time when the larvae hatch have a big influence on the severity of the infestation (Hillebrand et al., 1995). Depending on temperature, the development of the eggs takes 3 to 15 days (Hillebrand et al., 1995). After 14 to 21 days, the nymphs molt to sexually mature adults that are first brown and later on red in color (Hillebrand et al., 1995). In Central Europe, the population size usually remains small until June and has its peak in summer at the end of July or beginning of August (Wermelinger et al., 1992). One female mite can lay 20 to 80 summer eggs on the lower side of the leaf within three weeks (Hillebrand et al., 1995). Four to six generations can occur in Central Europe; in Southern Europe seven are possible (Duso et al., 2012).

Upon infestation by *P. ulmi* at the time of shooting, leaves stay small with a brownish edge from dead cells. Later on, leaves get a yellow or bronze color and may get curled. Without any measurements, leaves and inflorescences can die and are shed at strong infestation (Hillebrand et al., 1995).

The population size of *P. ulmi* is often positively correlated to nitrogen fertilization (Oberhofer, 1983) and high temperature and negatively correlated to rainfall, high relative humidity (Wermelinger et al., 1992) and very dry air in combination with heat (Hillebrand et al., 1995).

1.2.1.2 Two-spotted spider mite *Tetranychus urticae*

For overwintering, the fertilized females of *Tetranychus urticae* hide in the bark but also in the groundcover on dead leaves and weeds (Vogt et al., 1977). At time of shooting in spring, they live on weeds in the vineyard and its surrounding area. Two-spotted spider mites can live on a large range of host plants (van de Vrie et al., 1972). During the season, four to five generations live mainly on weeds; the last two to three generations can migrate to the vine plants when the weeds do not provide enough food. The eggs are yellow and, depending on the temperature, egg development takes between six and ten days (Hillebrand et al., 1995). *Tetranychus urticae* is an economically extremely important pest because of its high reproduction rate and quick development. One female can deposit 80 to 120 eggs per week (Hofmann et al., 1995). In Central Europe, seven to eight generations can occur in one vegetation period. The juveniles, larvae and nymphs molt 3 times until becoming adult (Hillebrand et al., 1995). The adult mites are yellow-green or orange-reddish and have two large dark spots on their dorsal side. Larvae and nymphs only have faint spots.

The establishment of *T. urticae* on vine plants takes mainly place in July when weeds dry out and *T. urticae* needs another food source (Hillebrand et al., 1995). An infestation by *T. urticae* may cause the vine leaves changing their color into grey or brown, but also small malformed leaves that later on look torn or get curled (Vogt et al., 1977). The damage caused by *T. urticae* is visible in form of irregular necrotic areas, which means that parts of the leaf turn light green with dark spots from excrements and cells that have dried out. Spider mite infestations can be recognized based on the produced webs (Figure 2, right). At heavy infestations the side-twigs may grow into broom-like branches (Hofmann et al, 1995). In contrast to *P. ulmi*, *T. urticae* prefers high temperatures and low relative humidity (Hillebrand et al., 1995).



Figure 2: Left: red winter eggs of *Panonychus ulmi* (<http://www.ages.at/en/topics/harmful-organisms/obstbaumspinnmilben/>), middle: grapevine leaf damaged by *Tetranychus urticae* damaged leaf of grapevine (<http://de.wiki-videos.com/video/Gemeine+Spinnmilbe>) right: heavy infestation and damage caused by *Tetranychus urticae* on grapevine (<http://de.wiki-videos.com/video/Gemeine+Spinnmilbe>)

1.2.2 Eriophyoid mites

The grape rust mite *Calepitrimerus vitis* (Figure 3, left) and the grape erineum mite *Colomerus vitis* (Figure 3, right) have only a size of 0.15 mm when adult. Their body is wormlike, whitish, slightly curled and they have only four legs.

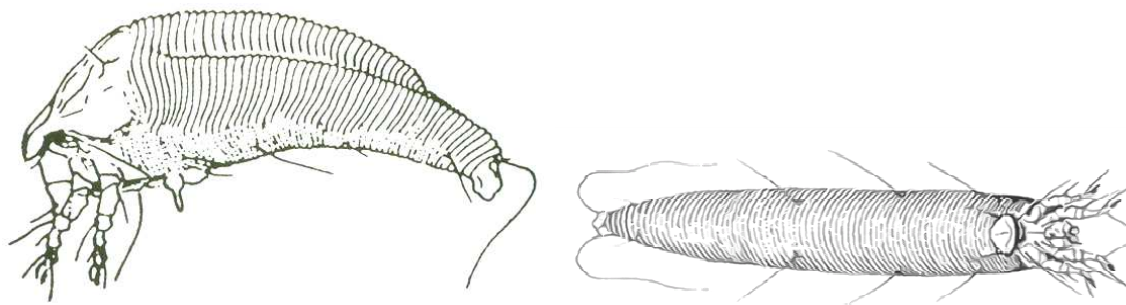


Figure 3: Eriophyoid mites: Left: *Calepitrimerus vitis* (<http://www7.inra.fr/hyppz/RAVAGEUR/6calvit.htm>) and right: *Colomerus vitis* (<http://ephytia.inra.fr/fr/C/16316/hypp-Biologie-du-ravageur>)

1.2.2.1 Grape rust mite *Calepitrimerus vitis*

Grape rust mites overwinter as adult mites in buds or in the bark of the trunk (Hofmann et al., 1995). The overwintering female mites are called deutogynes and they have no male equivalent (Duso et al., 2012). The protogynes, i.e. the summer females, are the females that reproduce in the same year as they were born and are similar in appearance to the males (Gerson et al., 2003). Grape rust mites only appear on young buds or on the lower side of young leaves. As leaves grow older and get thicker they move on again to younger leaves (Vogt et al., 1977). Rust mites have high reproductive rates and quick development. Eight to ten days after egg deposition, larvae hatch out of the tiny (0.039 mm diameter) eggs (Hillebrand et al., 1995). Two weeks after hatching, grape rust mites are sexually mature and start producing many eggs (Kadisich, 1986). A study showed that there is almost no egg production at less than 17°C or more than 34°C but at the optimum temperature of 25°C one female can lay up to 26 eggs per day (Walton et al., 2010). Due to their fast population growth, rust mites can cause big economic damages to vine growers. Especially young vineyards are concerned and the elevated summer temperatures in Europe in the last years favored outbreaks of grape rust mites (Duso et al., 2012).

In springtime, sprouts infested by rust mites grow more slowly and irregularly, and inflorescences stay underdeveloped, dry out and are shed (Vogt et al., 1977; Hillebrand et al., 1995). The edges of young leaves are getting curled and leaves can look as if torn. The apical growth of the shoots is suppressed by the saliva of the rust mites. As a consequence, side shoots or suckers grow out of the side buds making the branches look like brooms (Hillebrand et al., 1995). The mites are commonly spread by wind or use of equipment and

machinery. Rust mites can also become a problem in grapevine nurseries if infested wood is used for grafting (Vogt et al., 1977). The damage caused by rust mites (Figure 4, left) can easily be confused with symptoms of the diseases *Phomopsis* or *Eutypa* dieback (Hillebrand et al., 1995).

1.2.2.2 Grape erineum mite *Colomerus vitis*

Three strains of this species have been distinguished: the erineum-, the bud- and the leaf curl strain, with the erineum strain being highly common in Europe (Duso et al., 2012). The appearance and the mode of life of grape erineum mites (also called gall mites) and of grape rust mites are similar, just the damage they cause is very different. Grape erineum mites live on the lower side of leaves and overwinter as adults inside the buds (Vogt et al., 1977). In spring they start to suck inside the buds so that the plant sprouts later and less likely. The damage caused by these mites is characteristic and can be recognized easily (Figure 4, right). They inject their saliva into the leaf on the lower side and cause gall formation in the plant. The plant produces a white hairy felt on the underside of infested leaf spots, which is called *Erineum* (Hofmann et al., 1995, Hillebrand et al., 1995) and on the upper surface of the leaf, blisters (galls) are visible.

These mites normally do not cause big difficulties in vine production because they commonly only occur on single plants or groups of adjacent plants (hot spots). A reduction of yield caused by grape erineum mites is only possible if the inflorescences of the grapevine are infested (Kadisch, 1986) but the erineum strain is not considered an economically important pest as the reduction of photosynthetic active leaf surface area is negligible (Duso et al., 2012). However, the bud strain of the grape erineum mite may cause yield reductions of about 50% in California (Duso et al., 2012).

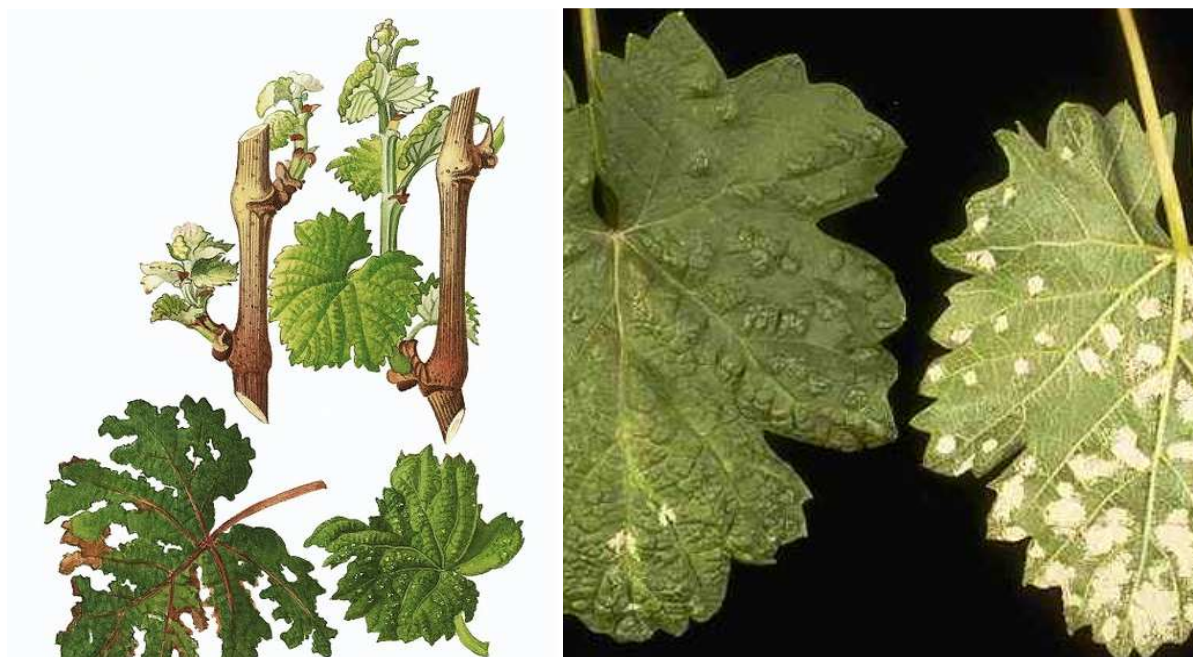


Figure 4: Left: damage of *Caleptrimerus vitis* (<http://www.ages.at/themen/schaderreger/kraeuselmilbe/>), right: damage of *Colomerus vitis* (https://www.uni-hohenheim.de/lehre370/weinbau/bild_html/phytopat/pockmilb.htm)

1.3 Pest mite management options in vineyards

Until the mid 1980s, the use of acaricides was the most common and most wide spread measurement to control spider mites in vineyards. However, due to the application of the same chemical agents for several times, resistances occurred and plant protection products that preserve beneficial mites were developed (Hillebrand et al., 1995). As biological control with beneficial mites worked well, the use of acaricides is nowadays only an emergency measurement and the use of pesticides that are harmless to predatory mites is one of the key principles of integrated pest management (IPM) in vineyards (Duso et al., 2012). To allow the combination of chemical and biological control options, predatory mites with resistance genes against certain pesticides can be released (Hluchy et al., 1996).

1.3.1 Chemical control

In some cases, when beneficial arthropods are absent or insufficient and their establishment is difficult, chemical control of herbivorous mites might still be necessary. If so, the following aspects should be considered: the action threshold, the side effects on beneficial arthropods, the mode of action of pesticides and their correct application and resistance management (Duso et al., 2012). Especially in hot regions of vine production pest mites can rapidly develop resistances against acaricides because of their high reproduction rate in combination with short life cycles (Duso et al., 2012).

1.3.2 Biological control

The damage caused by harmful mites is usually small in vineyards with little man-made interference, because of the natural presence and action of several macropredators but mainly because of predatory mites (Hofmann et al., 1995). The most important macropredators, belonging to the Thysanoptera (Aelothripidae), Heteroptera (Anthocoridae and Miridae), Coleoptera (Coccinellidae and Staphilinidae), and Neuroptera (Chrysopidae), have a high reproductive potential, a relatively long developmental time and they can significantly reduce large populations of phytophagous mites. However, when prey is scarce, the efficacy of the macropredators is relatively low (Duso et al., 2012). In contrast, predatory mites are able to build up stable populations and are therefore the most important beneficial organisms in biological control of pest mites in vineyards (Duso et al., 2012). In the long term, the use of acaricides can usually be avoided at a density of 1 to 2 predatory mites per leaf (Fortmann, 1993).

1.4 Natural and biological control with the help of predatory mites

Natural control means the reduction of living organisms by their natural enemies such as predators, parasites, antagonists and diseases; when control of pests by natural enemies is influenced by man, this process is called biological control (Hajek, 2004). Biological control has the aim to avoid an excessive increase of pest populations with the help of natural enemies in order to keep or re-establish the natural balance within agro-ecosystems (Börner, 2009). With biological control, the application of chemical substances can be reduced or totally avoided, the biocenosis is preserved and no problems with resistances or residues have to be managed (Börner, 2009). In the context of biological control, predators are defined as organisms that hunt, attack, kill and consume prey (Wegensteiner, 2013). While obligatory predators need to hunt for prey in at least one developmental stage, facultative predators can also live on alternative food sources (Wegensteiner, 2013). Regarding the use of predatory mites, different types of biocontrol options – classical, augmentation, conservation – can be distinguished (Hajek, 2004).

1.4.1 Biological control strategies using predatory mites

Classical biological control means the introduction of exotic species in order to use them against exotic pests, which often have a shared co-evolutionary history (Wegensteiner, 2013). In most cases, the introduced beneficial species come from the same regions as the exotic pest (Walten et al., 2012). Before releasing exotic species into a new environment, they have to be checked for their effects on non-target organisms. For this reason, the quarantine processes can be very complex and strict and predators with general feeding habits are rarely accepted (Walten et al., 2012). According to Hajek et al. (2007), until 2007

three out of 131 programs in classical biological control were dealing with pest mites. One example is the use of predatory mites in Africa against the extremely harmful coconut mite *Aceria guerreronis* that invaded from Latin or South America (Negloh, 2012).

Augmentative biological control is the release of natural enemies with and without the goal of permanent establishment (Hajek, 2004). Two different ways can be distinguished.

When many natural enemies are released, possibly several times per season, it is called inundative release (Walton et al., 2012). This method became popular because here, natural enemies can be referred to as bio-pesticides because their application works very similar to the use of chemical pesticides. Immediate or rapid control is wanted but no reproduction and establishment is expected. Accordingly, this method is often used in short term crops and crops with low pest thresholds. An example is the inundative release of the predatory mite *Neoseiulus cucumeris* against thrips in seasonal greenhouse crops (Hajek, 2004).

On the other hand, when relatively small numbers of natural enemies are released, preferably at a time with favorable environmental conditions, it is called inoculative release. It is expected that the natural enemies successfully reproduce and the progeny of the released individuals control the pests. The positive impact in pest management is not as quickly visible as with inundative release but this method aims at a long-term and self-sustained control (Hajek, 2004). The study presented in my thesis can be referred to as an example for inoculative release.

Conservation biological control means the enhancement of existing populations of natural enemies without release. The knowledge and understanding of the biology, ecology and behavior of pests and their natural enemies are required. Interruptions of the pest-natural enemy interactions, caused by pesticides, have to be set to a minimum; food and shelter can be provided, abiotic conditions can be optimized and many other cultural measurements can be taken for the promotion of natural enemies (Hajek, 2004). The choice of plant cultivar is, for example, a key factor that can enhance the habitat for beneficial antagonists of pests. A morphological plant characteristic that is particularly important for predatory mites are leaf trichomes. For example, on crops lacking leaf trichomes, biological control with *T. pyri* was not as successful as on crops with plenty of leaf trichomes (Loughner et al., 2008). These trichomes serve as protective structures but also pollen, spores of fungi and other substances that can be used as alternative food are captured so that the mites generally tend to stay on these plants (Roda et al., 2003). Not only phytoseiid mites benefit from leaf trichomes, also tydeid mites, which can reduce the spores of powdery mildew and suppress this disease, prefer varieties with plenty of leaf trichomes (Hajek, 2004).

1.4.2 Predatory mites as natural enemies of pest mites

Predatory mites belong to the class Arachnida and the order Acari. Almost all predatory mites that are important for viticultural pest management belong to the family of the Phytoseiidae (Hofmann et al., 1995). More than 2000 species of Phytoseiidae have been described in the past 50 years and many of them play an important role as natural or biological control agents (Kostianinen and Hoy, 1996). Also some species of the family Stigmaeidae have biocontrol potential, especially for the control of eriophyoid mites, but they might be more important in warmer regions and are usually less effective than phytoseiid mites (Gerson et al., 2003).

Hundreds of scientific studies from different countries and crops showed that populations of harmful mites can be successfully kept under economic thresholds by predatory mites, provided that they occur at high enough population densities (Karg, 1994). Phytoseiid mites can also function as bioindicators as they reliably indicate negative developments of ecosystems, caused for example by adverse anthropogenic impacts such as pollution (Karg, 1994).

McMurty and Croft (1997) described four different life style types of Phytoseiidae that have importance in biological control, based on their feeding habits and traits. To **type 1** belong *Phytoseiulus* species, which are specialized on spider mites that belong to the genus *Tetranychus*. They are frequently used as beneficial organisms in greenhouse crops, for example for fruit-vegetable and ornamental production. **Type 2** phytoseiid mites are selective predators of *Tetranychus* species, have a broader range of prey and even feed on pollen or plant exudates. Representatives of this type are for example *Galendromus* species, some *Neoseiulus* species and a few *Typhlodromus* species. *G. occidentalis* is used, for example, in the USA in many different crops, like apple, prune, almond, grape, strawberry, cotton, mainly against *Tetranychus* species. Sometimes this species is used complementary to *T. pyri* and, as *Tetranychus* species become rare, it may disappear. However, *G. occidentalis* is usually less susceptible to chemical pesticides than is *T. pyri*. Mites of **type 3**, to which *T. pyri* belongs to, are generalist predators. Most of them can reproduce on pollen, which is for some as valuable a food source as its prey. Besides mites and thrips, type 3 predators feed on a wide range of substances, like plant exudates, pollen, spores and mycelia of different fungi, hemipteran honeydew or, on rare occasions, plant sap. They have no preference for tetranychid mites but are still capable of controlling spider mite pests (Gerson et al., 2003). Compared to mites of type 1 and 2 they have a lower reproductive rate, intraguild predation is more common and they show more often cannibalistic behavior (Gerson et al., 2003). They have rather evolved in response to the features of their habitat than to any specific species of prey (McMurty and Croft, 1997). Several studies revealed that some generalist phytoseiid species that belong to type 3 are dominant in European vineyards, like

Typhlodromus pyri, *Kampimodromus aberrans*, *Amblyseius andersoni* and *Phytoseius finitimus* (Duso et al., 2012). Finally, mites that belong to **type 4** are only members of the genus *Euseius*. They are specialized pollen feeders and also generalist predators but they develop and reproduce better on pollen than on prey (McMurty and Croft, 1997).

1.4.3 Specialists vs. generalists

The most common and simpler classification of predatory mites is to distinguish between specialists (equivalent to type 1 and 2 after McMurty and Croft, 1997) and generalists (equivalent to type 3 and 4). Specialists are very well studied because of their efficacy in biological control. They reproduce rapidly when abundant prey is available and diminish populations of phytophagous mites more quickly than generalists. In contrary, their populations are unstable when prey is absent. They can be considered as 'r-selected' strategists, whereas generalists are more likely 'k-selected' (McMurty, 1992). Generalists feed on a wide range of substances, have a lower reproductive potential, are sometimes characterized by a close association with the host plant, their distribution is not related to spider mite populations and they are dominant in stable agro-ecosystems with minimal use of pesticides (McMurty, 1992). Their role in vineyards is crucial (Duso et al., 2012). The most abundant predatory mite occurring in Austrian vineyards is *Typhlodromus pyri* (El-Borolossy and Fischer-Colbrie, 1989).

1.5 *Typhlodromus pyri*

The predatory mite *Typhlodromus pyri* (Figure 5) belongs within the order Acari to the family of the Phytoseiidae.

1.5.1 Biology

Typhlodromus pyri has a size of 0.4 to 0.6 mm when adult, its body is pear-shaped and its color is variable from light reddish to yellow or light brown, and sometimes even colorless (Fortmann, 1993), depending also on the ingested food. Adult females are twice as large as adult males. Only the fertilized females are able to enter diapause and overwinter. For overwintering, they hide in small crevices of wood bark or in dead wood but, depending on the harshness of the winter, 60 to 90% of them can die even though they can survive frosty temperatures as low as -30°C (Fortmann 1993). In spring, at temperatures of about 10°C and increasing length of daylight, the diapause is broken and the mites become active again (Hoffmann et al., 1995, Fortmann, 1993). The mites have a dorsal shield with small hairs; the number of hairs is, amongst other traits, an important characteristic for species identification. For breathing they have stigmata at both sides in the middle of their body (Karg, 1994). Mature females can only lay eggs if they were fertilized by a male mite and each female can

lay between 30 and 50 eggs (Hillebrand and Eichhorn, 1988). The first mating of *T. pyri* mites may take between 7 and 8 hours and mating has to be repeated several times to reach maximum egg production, whereas other phytoseiid mite species copulate for shorter periods and mate only once in their life time (Overmeer et al., 1982). The larvae hatching from the eggs have only six legs. The two following nymphal stages (proto- and deutonymph) have already eight legs, as is common for most species that belong to the order Acari. During molting the mites do not move. The duration of development from egg to adult mite varies, depending on temperature and relative humidity (Karg, 1994). At 15°C it takes about 23 days, at 25°C only about seven days (Dosse, 1956). A temperature increase of 10°C doubles or triples the developmental speed (Karg, 1994), but the study by Imtiaz (2003) showed that the longevity of both sexes is reduced when temperature increases from 25°C to 30°C. Also prey consumption was lower at 30°C, compared to prey consumption at 25°C (Imtiaz, 2003). Six to eight generations can occur per year under the climatic conditions of Central Europe (Karg, 1994).

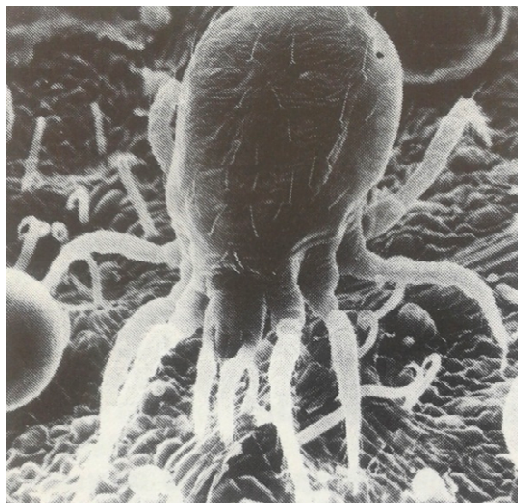


Figure 5: Adult female of *Typhlodromus pyri* (Karg, 1994).

1.5.2 Food and behavior

Typhlodromus pyri is a type 3 generalist, according to McMurty and Croft (1997), which can feed on a large range of animal, plant and fungal substances.

It can feed up to 20 spider mites per day, but also on grape rust mites, gall mites, or other mite species, thrips, pollen, the mycelia or spores of different fungi. In times where no food is available it can also survive without food for a couple of days (Fortmann, 1993). *T. pyri* has a higher reproductive potential on rust mites than on *Tetranychus* species; among spider mites it showed a preference for the European red mite *Panonychus ulmi* (McMurty and Croft, 1997). If the predators feed only on pollen, their development takes longer and the reproductive rate decreases, as compared to feeding on prey. It was observed that, if there are only a few spider mites available, they suck out the whole contents of the mites but if

there are plenty, they tend to hunt and kill many of them but suck only a bit on each (Fortmann, 1993). In the field, often the plant-provided nutrients such as pollen and sugar exudates determine the number of generalist phytoseiids more strongly than the presence of prey (McMurty, 1992).

The mites are eyeless but have well-developed tactile and chemical senses, using their first pair of legs to find the prey (Hofmann et al., 1995). At the distal end of the leg, called tarsus, small hairs serve as organs of perception of volatile chemicals like kairomones (Karg, 1994). For proper orientation and perception of environmental features, they move the tarsi of the first pair of legs constantly up and down; if there is prey they grasp it very quickly with their mouthparts called chelicerae (Figure 6). They use their chelicerae for holding and opening the prey. The two chelicerae are located between the paired sensory organs, called pedipalpalae; the chelicerae are serrated and each has an immobile part (*Digitus fixus*) and a mobile part (*Digitus mobilis*) so that they can be used as pincers (Karg, 1994). As the esophagus is very tight, the mites inject digestive liquids into the prey because they can only take up liquid food. They suck out the prey with the help of their pharynx (situated underneath the chelicerae) that is provided with muscles for sucking (Karg, 1994).

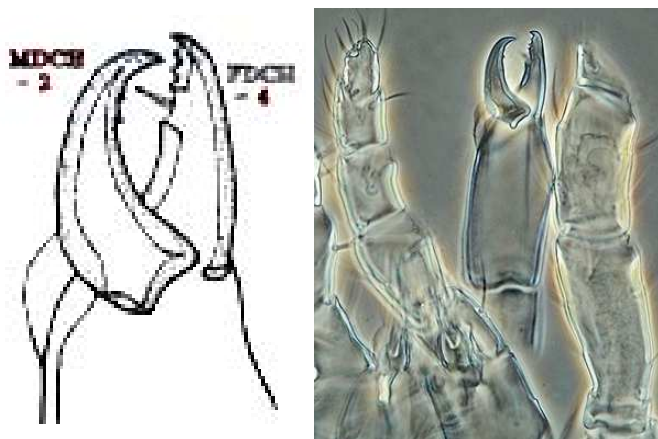


Figure 6: chelicerae of *Typhlodromus pyri* (Papadoulis, Emmanouel and Kapaxide, 2009, left); chelicerae under the microscope (key.lucidcentral.org, right)

1.5.3 Habitat

Typhlodromus pyri is not necessarily specialized to vineyards or fruit orchards, and can be found on a large range of plant species (Fortmann, 1993). However, in Austria and other Central European regions, they are the dominant phytoseiid species in vineyards (El-Borolossy and Fischer-Colbrie, 1989). Phytoseiids, including *T. pyri*, prefer to live on the lower side of leaves and commonly inhabit leaf domatia (Walter, 1996). These domatia are small invaginations in the surface of leaves or small tufts of hairs in the angles of the veins, which are commonly inhabited by several taxa of carnivorous mites and they are therefore also called acarodomatia (Norton et al., 2000). Plants with domatia provide a more favorable

habitat to beneficial mites than plants without domatia (Walter and O'Dowd, 1992). This plant-mite interaction is a type of a functional relationship: beneficial carnivorous mites protect the plant from herbivorous mites and it was also shown that plant domatia protect the predatory mites from other larger predatory arthropods (Norton et al., 2000). Experiments by Karban et al. (1995) suggest that populations of phytoseiid mites are rather limited by the availability of sheltered habitats than the availability of food. It was found that pubescent leaves can harbor more predatory mites because the numerous glandular and non-glandular trichomes hold back many pollen grains and spores of fungi, which can serve as alternative food sources for generalist predators (Roda et al., 2003, Karban et al., 1995). Accordingly, *T. pyri* populations increase more rapidly on grape varieties with pubescent leaf undersurfaces, whereas some other predatory mite species prefer varieties with glabrous leaf undersurfaces (Duso, 1992).

1.5.4 The role of *Typhlodromus pyri* as biological control agent

Typhlodromus pyri is an important biological control agent not only on grape but also on apple, cherry, hop, gooseberry and other crops. It is a key predator of pest mites due to its wide distribution and its abilities to feed on a large range of food and to survive relatively long in periods of prey scarcity (Duso and Camporese, 1996, Prischmann et al., 2002, Gadino et al., 2011). The efficacy of *T. pyri* in controlling pest mites has been shown in many studies (e.g. Boller and Frey, 1990, Duso and Camporese, 1996, Duso and Vettorazzo, 1999, Prischmann et al., 2002,). Although generalist predators are not as effective in controlling pest mites as are specialists, some of the most important species in vineyards like *Amblyseius andersoni*, *Kampimodromus aberrans*, *Phytoseius finitimus*, *Typhlodromus exhilaratus* and *T. pyri* are generalists. Thus, their value for natural and biological control in vineyards is undoubted. *T. pyri* is the dominant species in vineyards of Central Europe and the best studied species in the genus *Typhlodromus* (McMurty and Croft, 1997, Duso et al., 2012). Not necessarily the ability to reduce high populations of pest mites within a short time, but its persistence and ability to keep phytophagous mite populations on a constantly low level, are the reasons for the success of *T. pyri* and other generalist predators. An additional reason for the wide distribution and occurrence of *T. pyri* is probably its well-developed ability to evolve resistances against different pesticides (Hluchy et al., 1996).

1.5.5 Different ways of releasing *Typhlodromus pyri* in vineyards

Boller and Remund (1986) described three different possibilities to colonize vineyards with *T. pyri*. General advantageous conditions that should be fulfilled are the use of plant protection products that conserve beneficial mites, soil between the rows covered with plants that can provide the mites with pollen, and a small resident population of phytophagous mites.

The first possibility is to transfer young saplings that were thinned out from a donor vineyard harboring a population of predatory mites (commonly from an old vineyard) in spring to the receiver vineyard. The population is concentrated on a small leaf area because there are not yet so many fully grown leaves on the saplings. As the leaves of the saplings wilt quickly, the mites move on to the fresh leaves of the receiver vine plants. Diseases like *Pseudopeziza tracheiphila* may also be transferred, so for using this method a healthy donor vineyard with an existing population of predatory mites is required.

The second possibility is to use the cut twigs or timber of an aged vineyard with predatory mites. Bunches of wooden twigs or timber have to be prepared in winter and also transferred to the receiver vineyard in the cold season, so that the mites do not move out and disperse. This also requires a healthy donor vineyard with predatory mites, having no pests or diseases on or inside the wood like the pest *Lygus spinolai*. It is an elaborate method that should be done only by experts so that no harmful organisms are transferred from the donor to the receiver vineyard.

The third possibility is to colonize the vineyards with beneficial mites via textile carriers such as strips or belts. The material can be any textile that is roughly woven or a felt, so that the overwintering mites can hide inside. The textile strips/belts should be fixed to the twigs or trunks in the donor vineyard in September or at least two weeks before harvesting grapes. In order to avoid removing all predatory mites, the textile carrier should only be fixed on half of the vine plant. The transfer of the textile strips/belts from the donor to the receiver vineyard should be done as quickly as possible, in an ideal case on the same day. If this is not possible, the textile strips/belts containing overwintering mites should be stored in a cool and not too dry place, so that the mites stay inside the material. The receiving vineyard should have been already cut when the strips/belts are applied. This method has several advantages to the ones mentioned before: it is less elaborate and less time-consuming, less material has to be transported and the risk of transferring pests or diseases is relatively low (Boller and Remund, 1986).

This third method was used for the experiments described in my thesis.

1.5.6 OP-resistant population “Mikulov”

First noted in an inadvertently introduced population in New Zealand, resistance of *T. pyri* against organophosphate-based pesticides (OP-resistance) became an issue of interest in the 1970s (Gerson et al., 2003). This New Zealand strain was imported to England where another OP-resistant strain was found, coincidentally selected after the widespread use of OPs (Fitzgerald and Solomon, 2000). Among other pesticides, the fungicide mancozeb eliminated indigenous phytoseiid populations in Europe (Gerson et al., 2003). After the discovery of the OP-resistant strain “Mikulov” in a vine-growing area near the town of Mikulov in the Czech

Republic, this strain was released in various European region and replaced previously established predatory mite populations. It is resistant against a broad spectrum of pesticides, especially the OP-based ones and also against mancozeb (Hluchý et al., 1996, Gerson et al., 2003). Biological characteristics like longevity or fecundity can be, but are not necessarily, linked to the pesticide resistance status of the predators (Fitzgerald and Solomon, 2000). The mites that were released in the course of my thesis were tested for their resistance against OPs about 15 years ago. As the use of OP-based pesticides generally diminished, their resistance level was not tested any longer but the mites still most likely possess resistance gene alleles (company Biocont Laboratory, personal communication).

1.6 Study objectives

Several possibilities for colonizing vineyards with predatory mites were described (Boller and Remund, 1986). The most practical and simple way for vine growers is to introduce overwintering mites with textile carriers made of felt because the risk of transmitting diseases or pests is the lowest (Boller and Remund, 1986). However, although widely used, this method and the success in mite transfer, respectively, have been poorly scientifically investigated.

Why is the size of predatory mite populations relevant? In absence of the predators or if their populations are too small, phytophagous mites can take over and cause damage to the plants. If there are enough “leaf-guards” on the plants, this happens very unlikely. The used textile carriers were felt belts, which serve as artificial overwintering sites for the mites. Under natural conditions, overwintering predatory mites hide in the bark of wooden plants. In very young vineyards, the twigs and trunks often do not provide enough shelter as they are slim and have a smooth surface. Accordingly, in the receiving vineyard, the textile carriers do not only serve as means to introduce the mites but the mites can also take refuge in winter.

In my thesis, I describe a comprehensive study, consisting of field and laboratory experiments, conducted in a vine-growing region of Burgenland, Austria, with the ultimate goal of evaluating the textile carrier method to introduce and augment beneficial predatory mites of the species *T. pyri* in the vineyards. It is known that in many temperate vine-growing regions especially *T. pyri* plays an important role as natural antagonist of pest mites (Huffaker et al., 1970, Boller, 1978). Similarly, also in the vineyards of my study, a preliminary species determination showed that *T. pyri* was the only resident predatory mite species. This species is present in many different countries all over the world; El-Borolossy (1988) reported that it is numerously distributed in both eastern and western parts of Austria, especially on grapevines.

The core field study ran for two years to monitor the predatory mite populations in six different vineyards before and after application of the felt belts. Additionally, the textile

carriers, which were provided by a commercial company dealing with beneficial mites and insects, were analyzed in the laboratory for the contained mites.

2 Materials and methods

In order to assess the use of felt belts containing hibernating predatory mites for colonization of vineyards, a two-year field experiment was started in 2013. For evaluating the felt belts they were fixed in different vineyards and the number of predatory mites was determined before and after the release. Additionally, the felt belts were analyzed in the laboratory to determine how many predatory mites can be found inside.

2.1 Experimental design

The field experiment, running over two years, was conducted in six different vineyards. Within each vineyard, 12 plots were chosen randomly, half of which were assigned to mite release and the other half served as control. Each plot consisted of 15 plants (vinestocks). During the vegetative season of 2013, two samplings were carried out, one in June and one in July, in order to determine the existing predatory mite population in these vineyards before application of the felt belts. For predatory mite species identification, from each vineyard 4 plots were chosen randomly and at least one adult female mite per plot that was found on the leaves was embedded in a drop of Hoyer's medium on a microscope slide. In January 2014, felt belts containing predatory mites were applied in six randomly picked plots, out of 12, in each vineyard. In June and July 2014, all plots were sampled again to assess the effect of the felt belt applications on the colonization of the vineyards with predatory mites. In total, 1080 leaves were collected and analyzed during four time periods. Additionally, the numbers of leaves from 10 different plants in each vineyard were counted.

2.1.1 The vineyards

All six vineyards were located in Gols, Burgenland, Austria. The vineyards are managed by three different farmers and are all organic or bio-dynamic. Basic information about the vineyards was obtained from the farmers. Before my study, none of these six vineyards ever received predatory mites using felt belts. Vineyard 6 had later to be omitted from statistical analyses because the grower himself applied additional felt belts in the control plots.

Vineyard 1 was planted in 1999 and is a bio-dynamic vineyard since 2007. The variety is Pinot Noir. **Vineyard 2** consists of the variety Neuburger, was planted in 2009 and is also bio-dynamic. Both vineyards are surrounded by other vineyards. As not all the surrounding vineyards are organic or bio-dynamic, pesticide influence by wind drift cannot be excluded. The area between the vine rows is planted with grass and flowers. In spring time there are phacelia, spring vetch, vetchling and white clover seeded, in autumn hairy vetch, wheat and white clover. Normally no irrigation is made but in 2013 there was an extreme drought and plants were irrigated, using a tank, with 20 liter per vinestock. The vineyards are fertilized

with compost made of cow dung. As plant protection measurements, herbal extracts are applied on the soil and on the leaves, for example from common horsetail *Equisetum arvense* for the soil, chamomile *Matricaria chamomilla* for the leaves but also prophylactic applications of sulfur and copper against pathogens (personal communication Mathias Beck, owner of vineyards 1 and 2).

Vineyard 3 was planted in 2004. The variety is Blaufränkisch. Since 2006 it is a bio-dynamic vineyard. **Vineyard 4** was planted in 2011 and the variety is Zweigelt. These vineyards are surrounded by other vineyards and roads. For fertilization, self-made compost made of cow dung, reed, woodchips and pomace is used. Vineyard 3 is on a slope and measurements against erosion of soil are carried out. The area between the rows is covered with planted vegetation. The ground is also covered with straw and reed in order to support the soil fauna and to support the accumulation of humus. In spring, the plants between the rows are not cut down but are steamrolled so that sufficient food can be offered to beneficial insects for a longer time. For plant protection also herbal extracts are used as well as sulfur and copper against pathogens like powdery mildew (pathogenic agent: *Oidium tucker*) or downy mildew *Peronospora* (pathogenic agent: *Plasmopara viticola*) (personal communication Gerhard Dödl, manager of vineyards 3 and 4).

Both **Vineyard 5** and **vineyard 6** consist of the variety Merlot and they are located next to each other. Vineyard 5 was planted in 1998 and is organic since 2006 whereas vineyard 6 was planted in 2011. Next to vineyard 5 are some trees and bushes on one side. On two sides there are farm lanes and one side is bordered by vineyard 6. Both vineyards are located on a gentle slope. The ground between the vine plants is covered with grass and flowers but partly there are sites with bare soil. For plant protection, herbal extracts and several prophylactic products, such as sulfur and copper are applied (personal communication Hans Nittnaus, owner of vineyards 5 and 6).

The differences between varieties, ages, management measurements, surrounding and soil cover have to be considered for the interpretation of results as all these factors can influence, promote, or interfere with the colonization by predatory mites.

2.1.2 Sampling

In June and July 2013, leaves were collected from 12 randomly chosen plots from each of the six vineyards. To avoid edge-effects, no leaves were collected from the borders. Each plot consisted of 15 adjacent plants and from each plant one fully developed leaf was sampled from the middle section of the plant. The leaves were put into plastic bags that were closed right after collecting. To keep the leaves fresh the bags were put into a cooling box

right after sampling and stored in the fridge of the laboratory until analysis. Within each vineyard, the plots were spaced apart by at least five vinestocks.

Each plot was marked (Figure 7, left) and a map of each vineyard was drawn to have an overview of the relative location of the plots within the vineyard. The maps contained information about the numbers of the rows, in which rows the plots were located, and how many plants were between the plots (Figure 7, right).

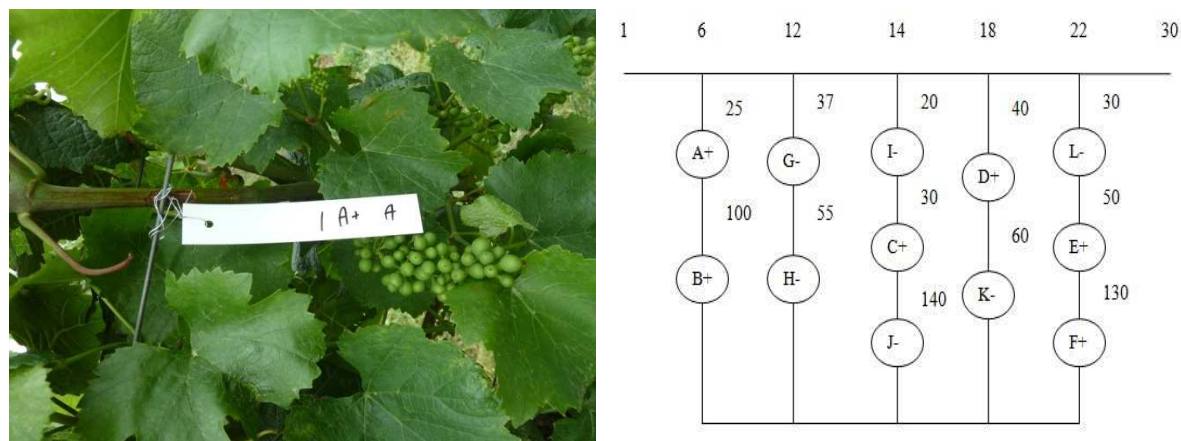


Figure 7: Mark in vineyard (left) and example for a map of a vineyard (right).

In June and July 2014 the same plants were sampled again in exactly the same way as in 2013. In addition, the presence of the felt belts was verified and any deviation from the original concept was recorded.

2.1.3 Application of the felt belts and release of the predatory mites

In January 2014 the felt belts were brought to the vineyards. In each vineyard, six plots were assigned as control plots and no mites were released. In the other six plots, every second plant received a felt belt attached to the top section of the main trunk. The felt belts were fixed to the trunk, using a stapler, as tight as necessary to prevent that they would fall down or get lost by other means (Figure 8). Before release, the belts were constantly kept in a cooling box so that the inactive female mites would stay inside the felt belts.



Figure 8: Fixation of the felt belts (left) and young vinestock with felt belt (right).

2.1.4 Counting of predatory mites on leaves

For counting of the predatory mites, every sampled leaf was scrutinized in the laboratory. Especially the main veins on the abaxial side of the leaves were observed carefully because the mites tend to hide near the veins (Figure 9). First, the leaves were visually inspected with the naked eye to get an overview. Then, both sides of the leaves were visually inspected under the binocular. Additionally, a soft brush was used to sweep over the leaf in order to activate mites that were hiding near the leaf-veins. As predatory mites can run quite quickly it was necessary to count them immediately upon sight, so that none was counted twice. The last step was to fold the leaf along the main vein. This vein provides a shelter for predatory mites and they are hiding often close to this vein.



Figure 9: Leaf of grapevine and preferred places of predatory mites for hiding.

2.1.5 Species determination of predatory mites

For species determination of the sampled predatory mites, from each vineyard four adult female predatory mites from four different plots were randomly selected and embedded in a drop of Hoyer's medium on a microscope slide (Figure 10, left). Then the mites were identified under a microscope according to the key provided by El-Borolossy (1988). Only adult female mites can be used for the identification of the species.

El-Borolossy described in 1988 the mite species that occur in Austrian vineyards and fruit orchards. Considering the differences between possibly occurring species, the mite species were determined using mainly one trait, the number of hairs at the dorsal shield (Figure 10, right).

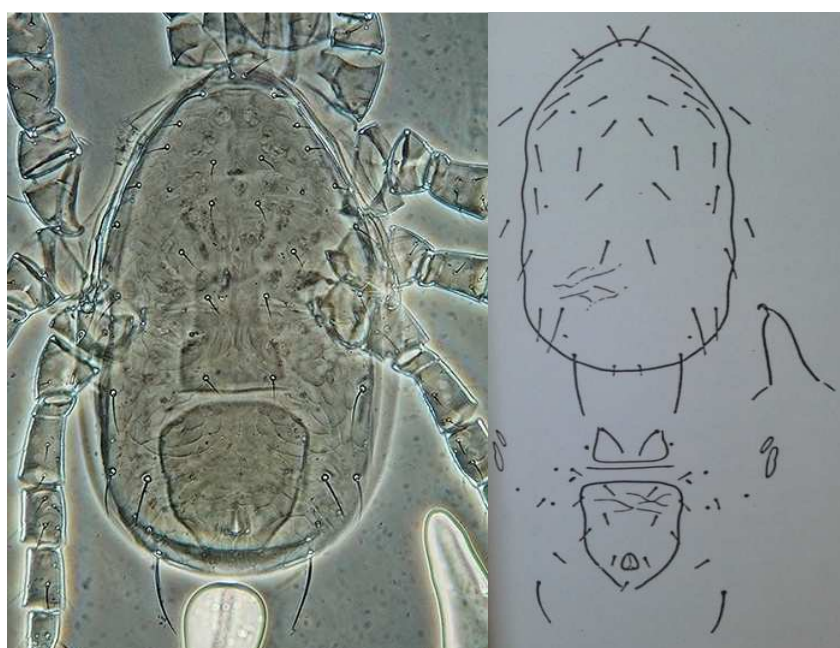


Figure 10: Dorsal view of *Typhlodromus pyri* Scheuten, 1857, under the microscope (key.lucidcentral.org) and graphic identification plate (El-Borolossy, 1988)

2.2 Felt belts

The felt belts used in this study (product name TYRON) were obtained from Biohelp, Vienna, but producer of the felt belts is the Czech company BiocontLaboratory (<http://www.biocont.cz/cz/biologicka-ochrana-rostlin.htm>). Each felt belt has a size of 7x14 cm. According to the provider, the felt belts can be stored in the fridge, at temperatures of 1 to 5°C, for a maximum of 10 days but the most favorable way of application is to apply them in the vineyard or orchard as quickly as possible after receipt. Application has to be done during winter time, in January or February, or even earlier depending on weather conditions. The recommended application rate is 1000 to 1500 felt belts per ha and there should be 30 to 50 predatory mites, *Typhlodromus pyri*, inside each textile carrier. Resistance against

organophosphates was tested about 15 years ago but since the use of these substances decreased; the current status of OP-resistance of the mites is unknown. The felt is an imitation of the overwintering sites of the predatory mites (Biohelp, Merkblatt zu Tyron, s.a.). Normally, predatory mites overwinter in the bark of trees and shrubs including vinestocks. Felt belts fixed to the stem of the vinestocks in fall are used by the predatory mites as overwintering sites.

2.2.1 Analysis of felt belts in the laboratory

When the felt belts were brought to the vineyards in January 2014, 20 additional felt belts, taken from different charges, were kept for analysis. They were stored in a cooling box and brought to the laboratory. Artificial arenas (Figure 11) were used to find out how many predatory mites are hibernating inside the felt belts. Each felt was cut into small pieces and placed on top of an acrylic plate delimited by strips of moist tissue paper. The plate rested on top of a water-soaked foam cube inside a plastic box half filled with water. Water-saturated tissue strips were used as a barrier in order to prevent the mites escaping from the plate. Pollen from cattail *Typha angustifolia* (product name: "Nutrimite" from the Belgian company Biobest) was dusted onto the plate to be used as food by the mites. Cotton tufts under cover slips were provided as shelters. These arenas were placed inside climate chambers set at 25°C to activate the predatory mites and make them come out of the felt. Each visible mite was counted and picked up from the arena using a brush so that none was counted twice. The arenas were checked every day. After one week no more mites could be found. Then the felt pieces were put into a glass jar containing 75% alcohol, rigorously shaken and the alcohol rinsed over a fine sieve so that all mites that were still in the felt were washed out from the felt into the sieve. According to the provider, 30 to 50 predatory mites *Typhlodromus pyri* should hibernate in each felt belt (Biohelp, Merkblatt zu Tyron, s.a.).

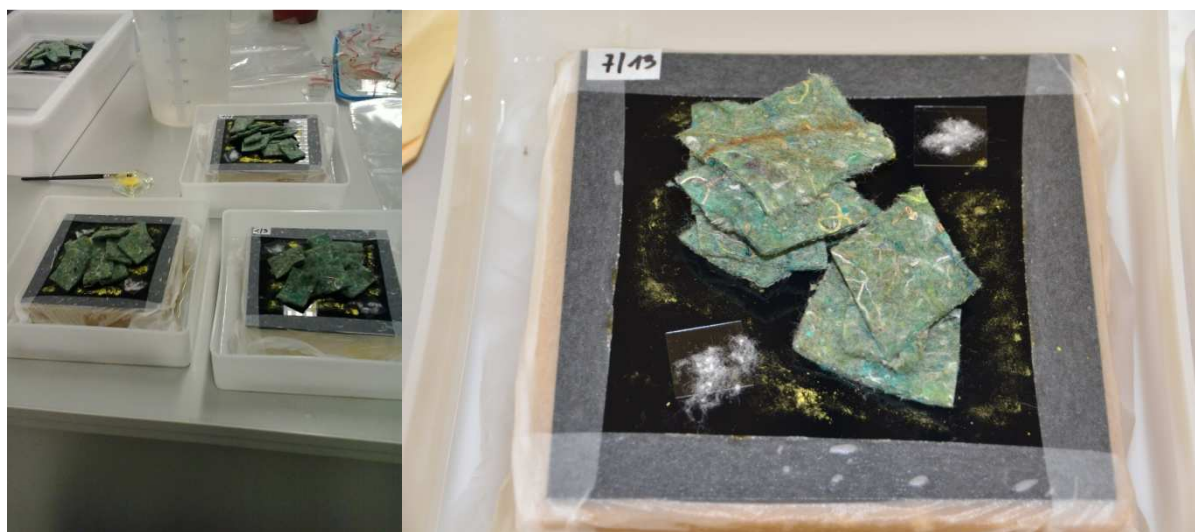


Figure 11: Arenas with felt pieces, pollen and cotton tufts under cover slips.

3 Results

3.1 Field experiment

The data obtained from the field sampling, representing the mean number of predatory mites per leaf and the number of leaves per vinestock were statistically analyzed using Generalized Estimating Equations (GEE) with year used as auto-correlated inner subject variable. GEE for the number of predatory mites was used at two levels: first, across vineyards (with vineyard as additional independent variable) and, second, within each vineyard.

In both analyses, we compared the mean number of predatory mites on leaves assigned to the control and release plots before (2013) and after (2014) predator release by application of the felt belts.

3.1.1 Result of all five vineyards together

Across the five vineyards, the mean number of predatory mites per leaf did not differ between the two years but differed among vineyards and was overall higher in predator release than control plots (Figure 12, Table 1). The significant pairwise interactions indicate that the relative comparisons between the two years and between the control and predator release plots varied significantly among vineyards. Additionally, the difference between control and predator release plots varied between the two years and was much higher after (2014) than before (2013) predator release (Figure 12, Table1). While in the control plots the number of predators decreased from 2013 to 2014, it increased in the predator release plots.

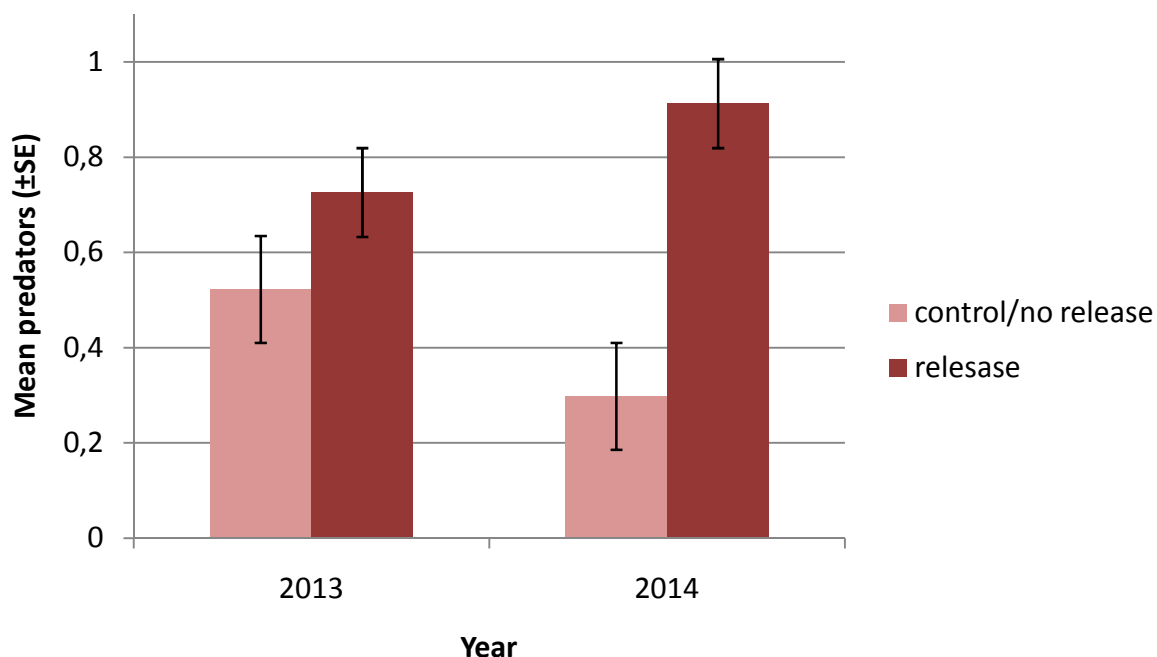


Figure 12: Mean number of predatory mites per leaf in plots assigned to control and predator release in all five vineyards together before (2013) and after (2014) release of the predators by the application of felt belts.

Table 1: Results of generalized estimating equations (GEE) for the effects of year, vineyard and plot assignment (predatory mite release or control) on the mean number of predatory mites per leaf.

Source	Type III		
	Wald Chi-Square	df	Significance (P)
(Intercept)	744.101	1	<0.001
Year	0.498	1	0.480
Vineyard	363.832	4	<0.001
Plot assignment	144.443	1	<0.001
Year * Vineyard	171.368	4	<0.001
Year * Plot assignment	69.898	1	<0.001
Vineyard * Plot assignment	46.984	4	<0.001

3.1.2 Vineyard 1

In vineyard 1, year had a significant main effect on the number of predatory mites per leaf (Wald $\chi^2=34.313$, $P<0.001$): more predators were found in 2014 than 2013 (Figure 13). Plot assignment (control or predator release) did not have a significant main effect (Wald $\chi^2=0.764$, $P=0.382$) but this effect varied between the two years, as indicated by the interaction between year and plot assignment (Wald $\chi^2=18.489$, $P<0.001$). The latter indicates that in 2013 more predators were found in plots assigned to the control than to predator release, whereas the reverse was true after the predator release in 2014 (Figure 13).

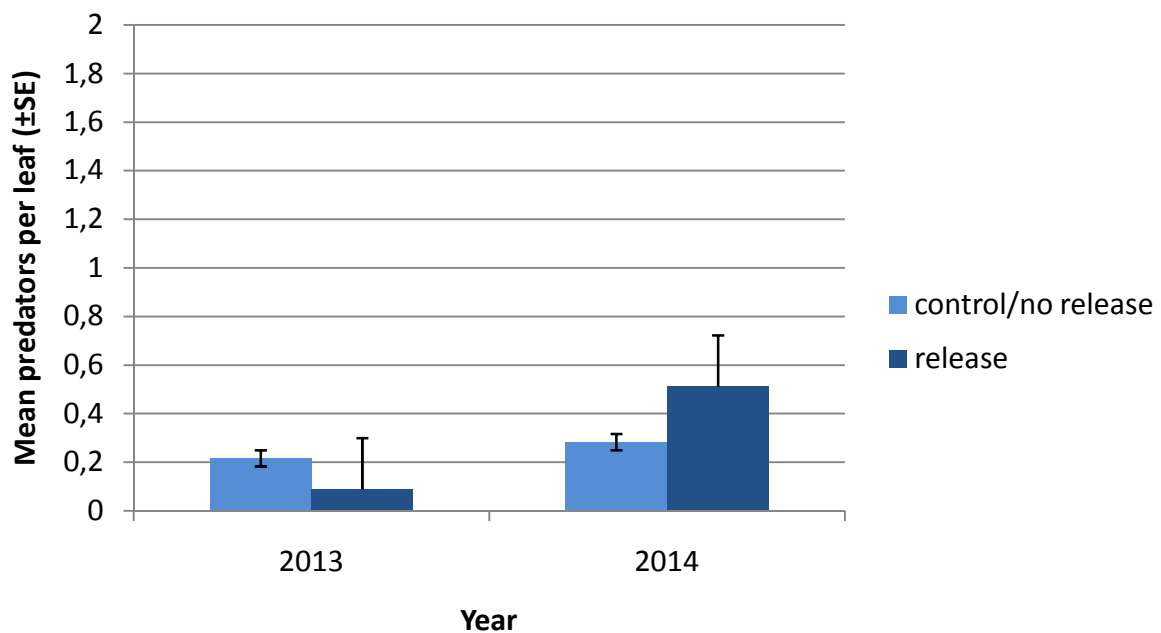


Figure 13: Mean number of predatory mites per leaf in vineyard 1 in plots assigned to control and predator release before (2013) and after (2014) predator release by the application of felt belts.

3.1.3 Vineyard 2

In vineyard 2, year had the significant main effect that more predatory mites were found before the predator release, i.e. in 2013, than after the release in 2014 (Wald $\chi^2=39.239$, $P<0.001$; Figure 14). Plot assignment also had a significant effect (Wald $\chi^2=20.264$, $P<0.001$): more mites were found in plots assigned to mite release than in control plots. In 2013, similar numbers of mites were found in plots assigned to control and predator release, whereas in 2014, a larger difference between the plots was observed. The decrease of predatory mites in plots where mites were released was much smaller than the decrease of predatory mites in the control plots, as indicated by the significant interaction between year and plot assignment (Wald $\chi^2=11.808$, $P=0.001$).

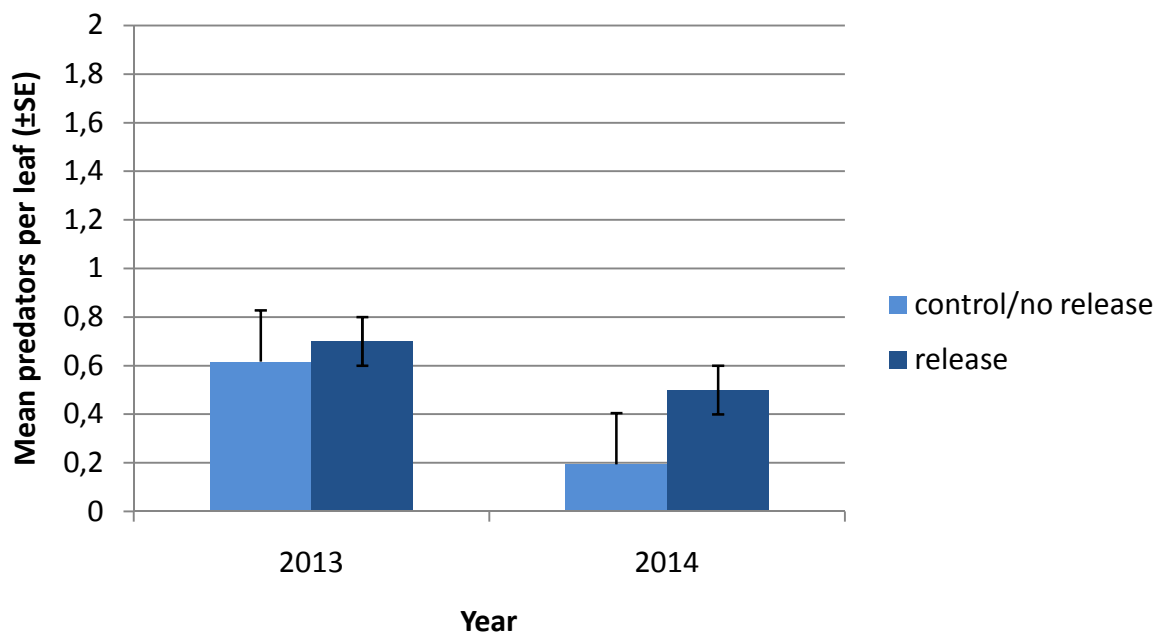


Figure 14: Mean number of predatory mites per leaf in vineyard 2 in plots assigned to control and predator release before (2013) and after (2014) predator release.

3.1.4 Vineyard 3

In vineyard 3, year did not have a significant effect (Wald $\chi^2=1.611$, $P=0.204$). The effect of plot assignment was highly significant (Wald $\chi^2=135.772$, $P<0.001$): more predatory mites were found in the plots assigned to mite release than in control plots (Figure 15). Before predator release in 2013, the number of mites in these plots was already higher than in control plots but after the release of mites, in 2014, the number increased significantly, as indicated by the interaction between year and plot assignment (Wald $\chi^2= 28.654$, $P<0.001$), whereas in control plots the number of predators slightly decreased (Figure 15).

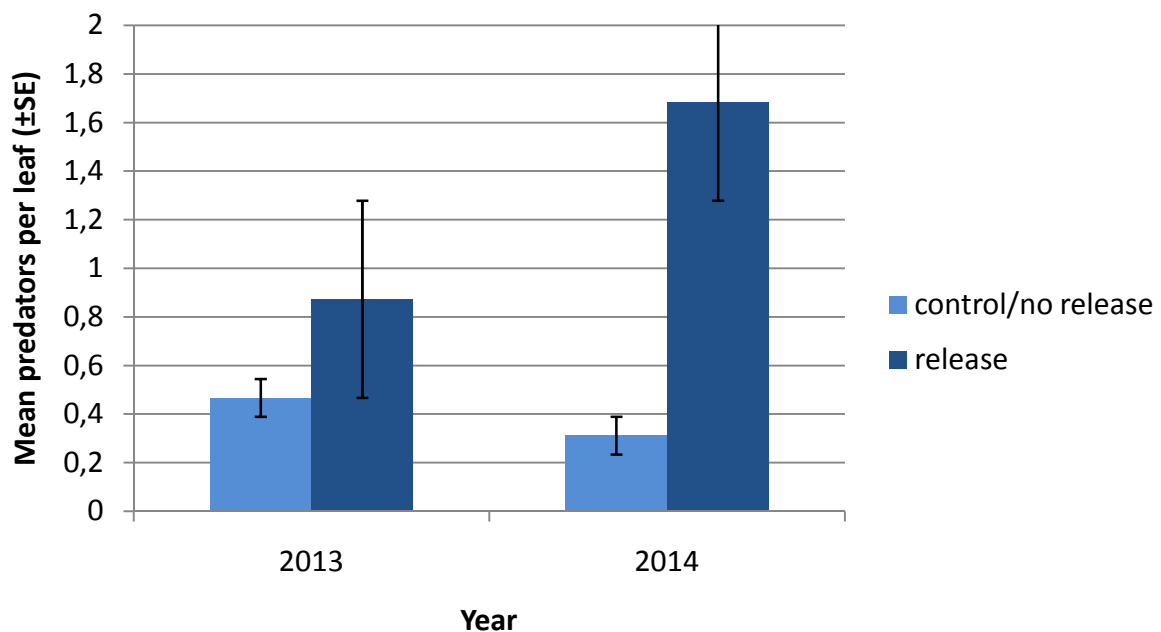


Figure 15: Mean number of predatory mites per leaf in vineyard 3 in plots assigned to control and predator release before (2013) and after (2014) predator release.

3.1.5 Vineyard 4

In vineyard 4, plot assignment had a significant effect (Wald $\chi^2=19.163$, $P<0.001$): more mites were found in the plots assigned to mite release than in control plots (Figure 16). The effect of year was also significant (Wald $\chi^2=11.320$, $P=0.001$). In 2014, more mites per leaf were counted than in 2013. The significant effect of the interaction between year and plot assignment (Wald $\chi^2=23.391$, $P<0.001$) shows that in 2014 only in plots assigned to predator release a higher number of predatory mites was found, whereas in control plots the number of predatory mites slightly decreased (Figure 16).

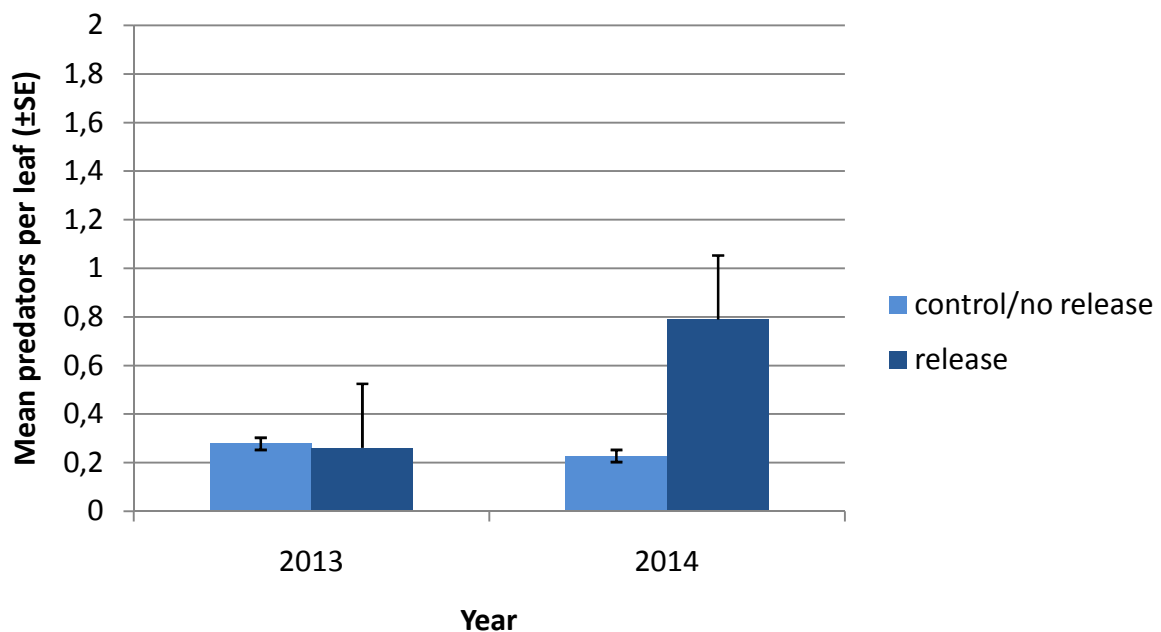


Figure 16: Mean number of predatory mites per leaf in vineyard 4 in plots assigned to control and predator release before (2013) and after (2014) predator release.

3.1.6 Vineyard 5

Year had a significant effect in vineyard 5 (Wald $\chi^2=60.375$, $P<0.001$). More mites were found before the release in 2013 than after the release of predatory mites in 2014 (Figure 17). Also the effect of plot assignment was significant (Wald $\chi^2=68.841$, $P<0.001$). More predatory mites were found in the plots assigned to predatory mite release but this was true for both years, i.e. before and after release. Also the interaction between year and plot assignment was significant (Wald $\chi^2=4.111$, $P=0.043$). In control plots, the number of mites per leaf was reduced approximately by half, whereas in plots assigned to the predator mite release the number was reduced only by 35%. The release of predatory mites via the application of felt belts in the latter plots did not lead to a higher population of predatory mites in this vineyard but somewhat buffered the decrease from 2013 to 2014 (Figure 17).

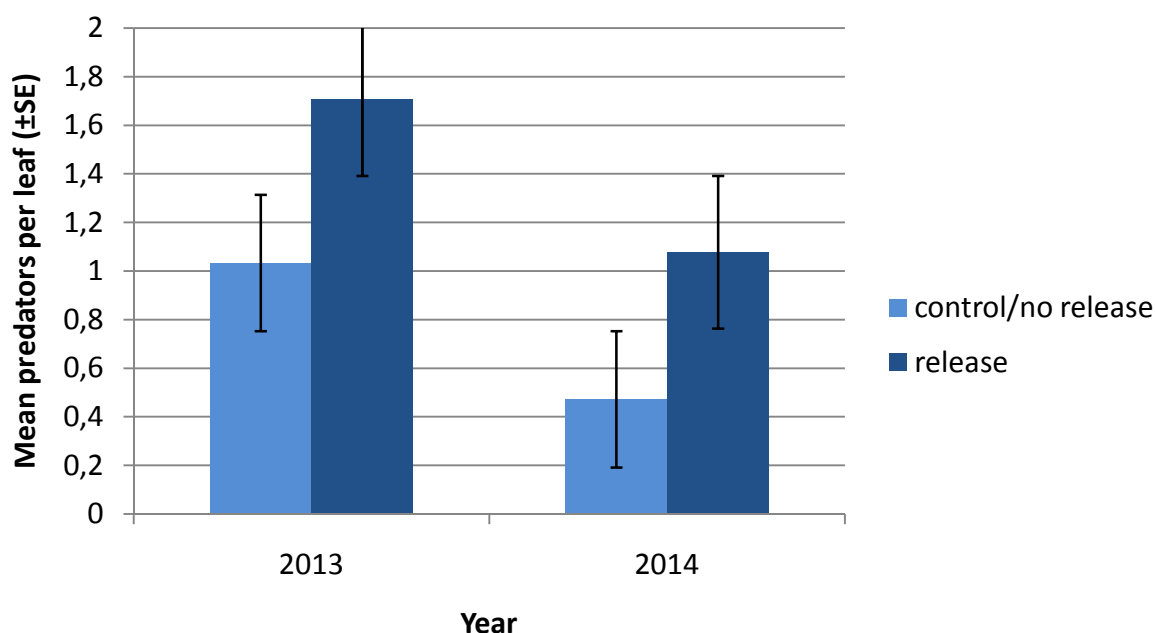


Figure 17: Mean number of predatory mites per leaf in vineyard 5 in plots assigned to control and predator release before (2013) and after (2014) predator release.

3.1.7 Species determination

From each vineyard, at each sampling date in 2013 four adult predatory mite females were sampled and their species was determined. The plots, from which the mites were collected, were randomly chosen but each time two adult females from plots assigned to mite release and two adult females from control plots were collected. Only the species *Typhlodromus pyri* was found.

3.1.8 Leaves per vinestock

The number of leaves per vinestock differed between 2013 and 2014 (GEE: Wald $\chi^2=9.064$, $P=0.003$), among vineyards (Wald $\chi^2=39.084$, $P<0.001$) and also the interaction between year and vineyard was significant (Wald $\chi^2=25.117$, $P<0.001$).

In vineyard 1, an average of approximately 150 leaves per plant was determined and this number did not differ strongly between the two years. In vineyard 2, the vinestocks carried almost a third fewer leaves in 2013 than in 2014. Similar to vineyard 1, the average of both years was approximately 150 leaves per plant. The highest leaf number was counted in vineyard 3: around 170 leaves per plant averaged over both years. Vineyard 4 had the lowest number of leaves with a mean of about 120 leaves per plant which is 30% less than in vineyard 3. Vineyard 5 had, similar to vineyard 1, around 150 leaves per plant in both years (Figure 19).

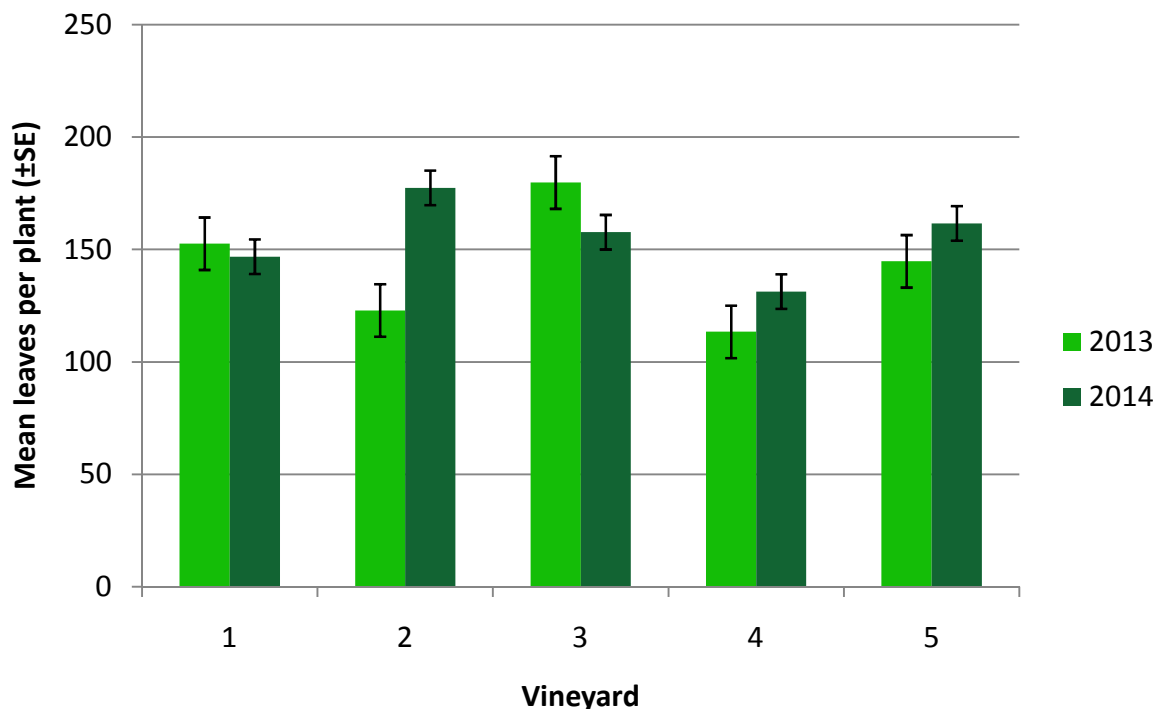


Figure 18: Mean number of leaves per vinestock in the five experimental vineyards.

3.2 Analysis of felt belts in the laboratory

An average number of 16.65 ± 2.41 (SE) predatory mites were found in each felt belt. The range went from 1 mite to 40 mites per felt belt. Approximately 90% of the mites were found on the artificial arenas, i.e. were definitely alive because they had actively left the felt. The remaining 10% of the mites were found after soaking and washing out the felt pieces and these mites could have been dead or alive.

4 Discussion

4.1 Field experiment

The results across the five vineyards showed that the application of felt belts containing predatory mites increased the population densities of predatory mites. In contrast, in control plots, a decrease in the population densities of predatory mites was observed from 2013 to 2014. However, in two out of five vineyards (vineyards 2 and 5) the application of the felt belts did not lead to a higher number of predatory mites but the decrease from 2013 to 2014 was lower in plots assigned to mite release than in control plots.

For interpretation of the differences in results among vineyards, the differences in experimental circumstances must be considered. In the five vineyards, five different grape varieties were grown. The vinestocks were planted in five different years, and were thus differently aged, and are managed by three different vine growers. These are probably the main categories of reasons why the application of the felt belts had vineyard-specific impacts on the population development of predatory mites from 2013 to 2014.

In general, food sources (the occurrence of prey and alternative food sources such as pollen or fungal mycelia) and the areas surrounding the vineyards are factors that markedly influence the population dynamics of predatory mites (Pozzebon et al., 2005; Duso et al., 2012). From surrounding vegetation like woody margins, natural colonization of vineyards by phytoseiid mites can occur because of dispersal by wind (Tixier, 1998). The local availability of alternative food can be influenced by several factors, such as leaf pubescence, canopy and cover crops in between the vine rows, but also the surrounding vegetation. Chemical plant protection measurements can markedly influence the arthropod communities in vineyards. Application of plant protection substances that are harmful for predatory mites can have a very negative impact on predatory mite abundance but in none of the vineyards this was the case in my study. However, wind drift of pesticides from neighboring vineyards could have had a possible negative impact in all vineyards, except for vineyard 5. The age of the vineyard is another factor that is important to be considered. The differences in predatory mite abundance between old and young vineyards were visible already before the felt belts were applied. All five experimental vineyards are located in Gols, Burgenland so the weather conditions were similar and do not necessarily have to be considered in detail. In a study by Duso and Pasqualetto (1993) also the interspecific competition, the phytoseiid strain and the presence of macropredators were factors that influenced the occurrence and abundance of predatory mites. For example, the occurrence of anthocorids in high density caused a strong decline in the abundance of phytoseiid mites (Duso and Pasqualetto, 1993). However, in my study no high densities of macropredators were observed and interspecific competition was negligible as *T. pyri* was the only species that was found and released.

4.1.1 Factors possibly influencing the population dynamics of predatory mites

Occurrence of prey

The occurrence of prey is decisive and certainly advantageous for the growth of predatory mite populations but *T. pyri* does not necessarily need prey for survival and reproduction because it can also live on alternative plant-derived food sources. In a study by Lorenzon et al. (2012) the developmental times of *T. pyri* feeding on prey and on pollen were similar but the fecundity was even higher on pollen than on the European red mite *P. ulmi*. Some damage caused by grape rust mite *Calepitrimerus vitis* was noticed in all five experimental vineyards but the occurrence of prey was not recorded in detail. Tydeid mites, which were also present in the vineyards, are generally an unsuitable prey for *T. pyri* (Lorenzon et al., 2012).

Alternative non-animal food sources

The factors leaf pubescence, canopy, cover crop and adjacent vegetation influence the availability of alternative non-animal food sources, which was proven to affect the population size of predatory mites in vineyards (Pozzebon et al., 2005). Alternative food is mainly pollen but *T. pyri* can also develop and reproduce on spores and mycelia of powdery mildew although the oviposition rate is rather low (Zemek and Prenerova, 1997). Studies on pollen drift and deposition showed that especially wind-pollinated plants are favorable for predatory mites because the pollen is trans-located by the wind onto the leaves of the vinestocks, while the pollen of plants that are pollinated by insects is not (Wiedmer and Boller, 1990). Therefore, until the middle of June, pollen from trees can be an important alternative food source for the predators. Later in the season, pollen from cover crops like grasses and other wind-pollinated plants and herbs provide the predominant pollen that can be found on vine-leaves (Wiedmer and Boller, 1990). However, predatory mites can only use pollen of particular plant species as food source. A study from Chile assessing the suitability of pollen from 11 different plant species showed that *T. pyri* did not suffer any mortality during the experiment only on pollen of *Hirschfeldia incana* from the family Brassicaceae (Bermúdez et al., 2010). Pollen of *Vitis vinifera* var. Chardonnay and Merlot has no nutritional value for *T. pyri* neither has *Poa annua* (Poaceae) and other pollen tested in this Chilean study (Bermúdez et al., 2010). In a study by Remund and Boller (1992) in Switzerland, pollen of seven different grasses were tested, for example *Poa pratensis* or *Lolium multiflorum*. The mites fed with grass-pollen had a similar life span as the mites fed with spider mites but their oviposition rate was much lower (Remund and Boller, 1992).

The pollen occurrence in the experimental vineyards of my study was not examined so the differences of pollen quality and quantity are unknown but the vegetation in and around the vineyards could give a hint on pollen occurrence.

Leaf pubescence and vine variety

Because of their small size, predatory mites are strongly affected by the morphological features of the surface of the leaves. For example, pubescence, i.e. the occurrence of hairs, is a variety-specific trait that can have a strong influence on predatory mites. An advantage of pubescent leaves is the enhanced capture and retention of pollen and fungal spores serving as alternative food sources for the predatory mites (Roda et al., 2002). *Typhlodromus pyri* has a preference for pubescent leaves and it will likely be less abundant on cultivars lacking leaf trichomes (Duso and Vettorello, 1999, Loughner et al., 2008). None of the five varieties in the experiment had completely glabrous or strongly pubescent leaves. The varieties are all described as slightly or moderately pubescent (Duso and Vettorello, 1999, BMLFWU, 2010, own observation). Thus, leaf pubescence alone cannot be the explanation for differing predatory mite densities among vineyards.

The leaf canopy

The leaf canopy is important for the climatic microhabitat conditions experienced by the mites inside the vineyards. As such, the leaf canopy may have an influence on the whole community in the vineyard. However, Prischmann et al. (2006) tested the effect of canopy structure on generalist phytoseiid mites but did not detect a significant influence on the densities of pest mites or predatory mites. In my study, the number of leaves per vineyard could have influenced the number of predatory mite per leaf: the more leaves a vineyard carries, the less predatory mites per leaf might be found. There were differences between the vineyards in the numbers of leaves per vineyard and they have to be considered when comparing different vineyards. In my study, a direct correlation was not observed. Vineyards 1 and 5 had about the same numbers of leaves but vineyard 5 had much higher numbers of predatory mites per leaf than vineyard 1. On the other hand, when comparing the same vineyard in different years, a correlation can be observed in vineyard 2. In this vineyard the number of predatory mites per leaf was higher in 2013 when the number of leaves was relatively low and it was lower in 2014 when the vineyards carried more leaves.

Cover crops, adjacent vegetation and mite dispersal by wind

Two key aspects of the natural occurrence, abundance and diversity of phytoseiid mites in vineyards are (1) the plant composition in neighboring areas because of the tight relationship

between phytoseiid population development and the plant species and (2) the main phytoseiid species occurring on the neighboring natural vegetation is often also the prevalent species in the vineyard (Duso et al., 2012). A study by Tixier et al. (1998) showed that the most stable source for natural colonization of vineyards by phytoseiid mites is a deep, dense and tall woody area containing suitable host plants for phytoseiid mites close to the vineyard. This was the case in vineyard 5 where relatively high numbers of *T. pyri* were found in 2013, probably promoted by dispersal from tall trees at the border of the vineyard. It was also found, that the main vector of dispersal was the wind. Narrow border hedges were not a good source of predatory mites neither were neighboring vineyards except if the density of phytoseiid mites was particularly high (Tixier et al., 1998). That means that management of bordering areas around the vineyards can also be an important tool for the promotion of beneficial arthropods. The reasons why phytoseiid mites start dispersing by passive aerial transport or active ambulatory locomotion can be a deficient habitat with poor food quality or lacking suitable food, plant senescence, an overcrowded habitat or interspecific competition. Regarding aerial dispersal, phytoseiid mites perform specific behaviors like adopting a standing posture with raised forelegs or walking near the edge of the leaf before takeoff (Tixier et al., 1998). Cover crops and the adjacent vegetation influence phytoseiid occurrence and abundance also because of the supply of alternative food such as pollen (Boller and Frey, 1990, Duso et al., 2012). However, not only predatory mites but also many other arthropods in the vineyard may benefit from cover crops, amongst them the pest mite *T. urticae* (Vogt et al., 1977).

The area where the experiment was conducted is a very windy area (see also *Pesticide drift by wind*), which makes it very likely that the wind influences the predatory mite occurrence and abundance. *T. pyri* commonly disperses by wind only over short distances of about 12 m, which is less compared to other species (Dunley and Croft, 1990, Tixier et al., 1998). The surrounding area has an influence on predatory mite abundance to a certain extent and this influence likely contributed to the difference among vineyards already in 2013 before the felt belts were applied. However, within vineyards border effects were avoided so the surrounding area cannot explain differing results between the vineyards regarding predatory mite density arising from the application of felt belts.

Cultural and pest management in the vineyards

Different types of management may affect predatory mite populations, especially because of differences in the application of plant protection substances. However, none of the vineyards in my study is under conventional or integrated pest management. Four vineyards (1 to 4) are bio-dynamic and one vineyard (5) is organic. There is no difference between organic and bio-dynamic management concerning soil-management, fertilization restrictions and allowed

plant protection measurements. The main difference is the basic definition because bio-dynamic management considers, amongst other aspects, the phases of the moon and includes additional plant extracts that are used in homeopathic concentrations (Bauer et al., 2013). For control of harmful mites in both organic and bio-dynamic vineyards, vegetable oil (for example colza oil), mineral oils such as liquid paraffin and sulfur compounds can be used. As a fungicide against *Peronospora*, for example copper can be applied (Bauer et al., 2013). Sulfur can be harmful to predatory mites at high concentrations of 320 g per 10 l water or 0.23% per 100 m² (Schmid and Henggeler, 2000). Fungicides containing copper have negligible effect on predatory mites (Duso et al., 2012). According to the declaration of the felt belts, paraffin oil and sulfur are harmless for the predators (Biohelp, Merkblatt zu Tyron, s.a.) but in a study by Gadino et al. (2011) 91.2% paraffin oil (JMS Stylet) caused more than 50% mortality of *T. pyri*. In all five vineyards of my study, sulfur and copper were applied in concentrations that should not affect the predatory mites. Paraffin oil was only used in vineyards 1 and 2. There are differences between dates and frequency of applications and concentrations but in general very similar plant protection substances were used by the three different vine growers (personal communication).

Time interval since conversion to organic or bio-dynamic production

The time elapsed since conversion from conventional to organic or bio-dynamic pest management is a possible factor responsible for differences in mite populations because it varies among all the experimental vineyards (Table 2). Regarding this factor, also the age of the vineyard should be considered. Vineyards 2 and 4 were managed bio-dynamic from the beginning on but these vineyards did not have the highest numbers of predatory mites, probably also because of their youth. Vineyards 1, 3 and 5 were probably treated with synthetic chemical pesticides before becoming organic vineyards. Vineyards 3 and 5 were either biodynamic or organic for eight years which is the longest time interval of all experimental vineyards and they had the highest numbers of predatory mites per leaf. On the other hand, vineyard 1 that was managed bio-dynamic for seven years had the lowest numbers of predatory mites of all experimental vineyards. With exception of vineyard 1, the general tendency in my study is: the older the vineyard and the longer the time interval since conversion to organic or bio-dynamic production the higher the number of predatory mites per leaf.

Pesticide drift by wind

Gols is located in a very windy area, so the experimental vineyards were partly exposed to strong wind. According to the information of the meteorological station of Neusiedl/See, which is close to Gols, between May and July 2014 there were only two days without wind

and the mean wind-speed was 2.67 m per s (ZAMG, www.zamg.ac.at). It is possible that pesticide drift by wind influenced the populations of predatory mites in vineyards 1 to 4 because of bordering conventional vineyards. Even under still wind conditions, spray from an air-assisted broadcast sprayer can drift 10 to 15 m (Otto et al., 2013). Hedgerows between vineyards can act as drift barriers and protect beneficial arthropods in neighboring fields. Tixier and Kreiter (2003) found out that predatory mite populations in neighboring areas, although more susceptible to pesticides than predatory mites in the vineyards, have relatively high levels of resistance to pesticides. Pesticide pressure is a major limitation for establishment of immigrant predatory mites from woody margins (Tixier et al., 2006). Vineyard 5 was the only vineyard where no conventional vineyard was bordering and it had the most abundant populations of predatory mites in 2013. As there were no hedgerows in vineyards 1 to 4, serving as buffer between the experimental vineyards and the neighboring conventional vineyards, it could be that pesticide drift partly influenced the general occurrence and abundance of predatory mites but could not be responsible for any differences between mite release and control plots.

Age of the vinestocks

Young newly planted vineyards normally do not have any or only small populations of predatory mites. In contrast, older vinestocks provide natural shelters and overwintering habitats for predatory mites in their barks and therefore harbor generally a larger number of predatory mites. Young vinestocks lack these overwintering habitats as the surface of the bark is smooth without any crevices and cracks.

Table 2: Overview of some characteristics of the experimental vineyards 1 through 5 (manager, variety and level of pubescence, age in 2014, management, surrounding area, planted cover crops between rows and average numbers of leaves in 2013 and 2014) that can promote or interfere with the occurrence of, and colonization by, predatory mites (Duso and Vettorazzo, 1999, BMLFWU, 2010, personal communication with vine growers and own observation).

Vineyard + Manager	Variety, level of pubescence	Age (years), Management	Surrounding area	Planted cover crops between rows	Average number of leaves in 2013 and 2014	
1 Beck	Pinot Noir, slightly pubescent	15 Bio-dynamic since 2007	Conventional vineyards on 4 borders	Phacelia, spring vetch, vetchling, white clover, hairy vetch, wheat	153	147
2 Beck	Neuburger, pubescent	5 Bio-dynamic since 2009	Conventional vineyards on 3 borders, meadow	Phacelia, spring vetch, vetchling, white clover, hairy vetch, wheat	123	177
3 Heinrich	Blau-fränkisch, pubescent	10 Bio-dynamic since 2006	N, S: Roads W: organic vineyard E: conv. vineyard	Soil covered with straw and planted vegetation	180	158
4 Heinrich	Zweigelt, pubescent	3 Bio-dynamic since 2011	N, S, E: asphaltic road W: conv. vineyard	Soil covered with straw and planted vegetation	113	131
5 Nittnaus	Merlot, slightly pubescent	16 Organic since 2006	Field paths, organic vineyard, Trees	Soil covered with vegetation, partly open sites	145	162

4.1.2 Discussion of vineyard specific results

Vineyard 1

Although vineyard 1 was 15 years old, the predatory mite abundance was very low before the release of predatory mites, in particular compared to vineyard 5. Vineyard 5 has about the same age as vineyard 1 but harbored a much larger population of predatory mites before application of the felt belts. A reason for this could be that vineyard 1 is only surrounded by conventional vineyards where as vineyard 5 is surrounded by trees, other natural vegetation and an organic vineyard. In plots where felt belts were applied, the average number of

predatory mites per leaf increased from 0.09 to 0.51, which corresponds to an increase of more than 500%, while in control plots the number of predatory mites per leaf stayed at approximately the same level. Although the application of felt belts successfully led to a higher number of predatory mites per leaf, this number is only half of the wanted density of at least 1 mite per leaf, which is presumably necessary for sustainable natural/biological control of pest mites (Fortmann, 1993). Unfavorable microclimatic conditions, lack of suitable habitats in the surrounding vegetation, lack of food sources or pesticide drift from neighboring vineyards by wind could be an explanation for the generally low density of predatory mites in this vineyard. However, it is very likely that the diverse cover crops, the slight pubescence of the variety Pinot Noir and the aged wood stocks in this vineyard will promote the occurrence of predatory mites in the future.

Vineyard 2

In this five-year-old vineyard the number of predatory mites per leaf was relatively high in 2013, compared to vineyard 1, which is 10 years older and under bio-dynamic management for seven years and also compared to the three-year-old vineyard 4. At a distance of about 10 m there is a tree and also a meadow is bordering the vineyard on one side. This neighboring vegetation as habitat can be a source of predatory mites for vineyards because of wind dispersal (Tixier et al., 1998). For some reasons, the number of predatory mites per leaf generally decreased from 2013 to 2014, even in plots with the application of felt belts. This could have been due to negative impacts like pesticide drift by wind as this vineyard is surrounded by three other conventional vineyards. Most likely, the decrease in abundance of predatory mites was also due to the number of leaves per vinestock. In 2014, the number of leaves per vinestock was much higher than that in 2013. It is evident that, within the same vinestock between two years, a higher number of leaves correlates with a lower number of predatory mites per leaf. While in control plots there was a decrease of predatory mites by two thirds from 2013 to 2014, it was less than one third in plots with mite release. In this vineyard, to provide beneficial mites with sheltered habitats in order to enhance overwintering, more felt belts could be applied because of the scarcity of overwintering sites on the relatively young vinestocks. Pesticide drift by wind from neighboring vineyards can hardly be controlled or avoided. Planting vegetation at the borders and between the rows of the vineyard that provide suitable pollen for predatory mites (e.g. *Hirschfeldia incana* for *T.pyri*) could promote the occurrence of predatory mites in this vineyard.

Vineyard 3

In vineyard 3, the number of predatory mites per leaf strongly increased in the plots assigned to mite release whereas the number of mites decreased in control plots. This shows that the

colonization by *T. pyri* using felt belts was highly successful. Also the cover crop in between the rows and the age of the vinestocks (10 years old) were advantageous for the occurrence and establishment of beneficial mites. The number of leaves per vinestock was relatively high in this vineyard but there was a slight decrease from 2013 to 2014. In the plots where the felt belts were applied the number of 1.68 predatory mites per leaf would be high enough for natural/biological control as prevention against damage caused by pest mites. Although there is one conventional vineyard bordering, pesticide drift is probably negligible because the main direction of the wind is from west to east and the conventional vineyard is in the east of the study vineyard (personal communication with vine grower). For better colonization of the whole vineyard by *T. pyri*, more felt belts could be applied area-wide or twigs and leaves from plots with felt belts could be redistributed in the entire vineyard. For the latter measurement, the risk of transfer of diseases has to be taken into account. The strong increase in predatory mite abundance in plots where the felt belts were applied shows that the general conditions for establishment and occurrence of predatory mites were very good.

Vineyard 4

The three-year-old vineyard 4 was the youngest vineyard in this study, which also explains the lowest number of leaves per vinestock among the five experimental vineyards. Also in 2013, low numbers of predatory mites per leaf were counted, especially compared to the five-year-old vineyard 2. The application of felt belts led to numbers of predatory mites per leaf that were four times higher than in the year before and in control plots. No other factors could have caused this strong increase. On average, 0.79 predatory mites per leaf were found in plots with felt belt applications, which is almost the recommended density of one predatory mite per leaf for successful natural/biological control, to prevent damages caused by pest mite infestations. Compared to vineyard 2, the conditions for phytoseiid mites must have been better in plots where mites were released. The numbers of leaves slightly increased in 2014 and, as one conventional vineyard is bordering, pesticide drift cannot be excluded. These two factors could have generally lowered the mite abundance in this vineyard. For obtaining higher numbers of beneficial mites, more felt belts could be applied and suitable food sources and niches for *T. pyri* could be provided. The felt belts would serve as additional overwintering sites for the predatory mites. It might take some time to establish a stable population but a first step was done in plots where the felt belts were applied.

Vineyard 5

Vineyard 5 was the oldest vineyard, with 16-year-old vinestocks, and it had the highest numbers of mites per leaf of all vineyards in 2013. Woody richness close to this vineyard going hand in hand with wind dispersal by predatory mites and old vinestocks, which have a

thick cracked bark, favored a high density of predatory mites already before the application of felt belts. No conventional vineyard is bordering vineyard 5 so pesticide drift can be excluded. This vineyard is under organic management for eight years, which is the longest period with organic management of all experimental vineyards. For some reasons, the density of predatory mites generally decreased from 2013 to 2014. In this vineyard, partly open sites with bare ground between the rows were observed, especially in 2014 (Figure 19). Thus, the decrease in predatory mite abundance could have been caused by a lack of food sources and suitable spatial niches, in particular at sites located more distantly away from the woody margin. Maybe also unfavorable weather conditions occurred locally or the predatory mites were blown away by strong wind. In any case, more leaves per vinestock were counted in 2014 than 2013, partly explaining the decreasing numbers of predatory mites per leaf between years. Nonetheless, in plots where felt belts were applied, on average still more than one mite per leaf were found whereas it was only about 0.5 mites per leaf in control plots. It is possible that the density of 1.7 predatory mites per leaf in 2013 was already close to the carrying capacity because of limited sheltered habitats and food sources in the vineyard. Even without further application of felt belts, the mite populations could be further promoted by distributing twigs from vinestocks close to the woody margin in the whole vineyard. More cover crops between the rows should be planted in order to avoid erosion and to provide food and spatial niches for beneficial arthropods.



Figure 19: A lack of cover crops could have caused the general decrease of predatory mite density from 2013 to 2014 in vineyard 5.

4.2 Laboratory analysis of the felt belts

The average number of predatory mites detected per felt belt was only 16.65, which is contradictory to the declaration of the provider, which states that 30 to 50 predatory mites *T. pyri* should hibernate in each felt belt (Biohelp, Merkblatt zu Tyron, s.a.). Also, this number is only half of the number detected by Hluchy et al. (1996), who found on average 33

hibernating adult females of *T. pyri* in one textile carrier of the same size as in my study, i. e. 7x14 cm.

A possible explanation for the difference between the observed and the company-claimed number of predatory mites per felt belt could be a problem with quality management in the vineyards or production of the felt belts containing hibernating females of *T. pyri*. The number of predatory mites per felt belt can hardly be controlled by the producer or provider and in my study the range went from 1 mite to 40 mites per felt belt. Since the felt belt is a product containing living organisms, it is almost inevitable that differences occur but it is in the responsibility of the producer and provider to keep the fluctuations as low as possible, for example, by strict monitoring or standardized processes, monitor the numbers at regular intervals and adjust the product declarations accordingly. The results of my study suggest that the felt belts are rather heterogeneous and that the commercial companies overstate the contents of the belts.

4.3 Discussion of the use of felt belts and comparison with other studies

Comparison of the results of my study with those of other studies dealing with man-influenced colonization of vineyards by predatory mites is difficult because of different starting points, environmental conditions and treatments. Moreover, studies on this topic are generally rather scarce. The method of distributing branches of donor vineyards in receiver vineyards is a wide spread and common method but it requires comprehensive knowledge and a healthy donor vineyard with high densities of predatory mites. In the studies by Camporese and Duso (1996) and Duso and Vettorazzo (1999), two-year-old branches were used for colonization of vineyards by different phytoseiid species. Approximately 100 mites per plant were released, which is many more than those released in the present study, where one felt belt, containing about 17 predatory mites, was applied on every second plant. Nonetheless, the densities of *T. pyri* described in the study by Camporese and Duso (1996) are similar or a bit higher compared to the densities found in the present study, ranging from 0 to about 1.4 active forms of *T. pyri* per leaf. In the study by Duso and Vettorazzo (1999), higher densities of up to five *T. pyri* per leaf were found. Marshall and Lester (2001) used vine leaves to introduce *T. pyri* into a vineyard in Ontario, Canada. *T. pyri* was released at two different levels of about 8.5 and 25.5 predatory mites per plant. The treatment of 8.5 predatory mites per plant is at about the same level of release as in the present study. In the first year, *T. pyri* established and was found in significantly higher numbers, but the numbers of the pest mite *P. ulmi* remained at the same level. In the second year, *T. pyri* successfully controlled *P. ulmi* and was still found at the same density as in the first year. The highest recorded density of *T. pyri* was only 0.26 mites per leaf, but the mean densities were about 0.18 mites per leaf. Thus, *T. pyri* effectively suppressed *P. ulmi*, although occurring at very

low densities. In the study by Marshall and Lester (2001), *T. pyri* was introduced as a new species for the habitat, whereas in my study *T. pyri* already occurred in each vineyard at different densities. The mite increase in plots assigned to mite release was generally higher in my study than in the study by Marshall and Lester (2001) in three out of five vineyards, but decreased in two of five vineyards. Therefore, just regarding these comparisons it is not possible to state which method is more effective but it seems that similar levels of predatory mite abundance can be achieved using twigs and leaves of vinestocks. For direct comparison of the two different methods, a targeted study on this topic would be required.

Using branches for colonization of vineyards by predatory mites is cheaper but more laborious and time-consuming than applying felt belts. Predatory mites have to be taken away from a donor vineyard in which consequently the number of predatory mites may decrease and the risk of an infestation by pest mites may increase. The latter is no problem if the donor vineyard is an abandoned one.

Another question when comparing different methods of predatory mite release is the risk of concurrent transfer of pests. According to Boller and Remund (1986) it is not likely but can it be excluded for felt belts? In any case, the risk is rather low compared to the other described methods of predatory mite transfer in vineyards. However, there is no guarantee that there are no pests inside the felt belts and also a constant number of hibernating females of *T. pyri* cannot be guaranteed for 100%.

One phenomenon, albeit not relevant to my study but described in several other pertinent studies, is that locally established indigenous species were outcompeted by the artificially released *T. pyri* or other predatory mite species used in biological control like *Amblyseius andersoni* or *Kampimodromus aberrans* (Duso and Vettorazzo, 1999, Marshall and Lester, 2001). If non-indigenous species are released a change in the predatory mite diversity and associated organisms has to be taken into account. If several species of generalist phytoseiid mites co-occur, interspecific competition intensifies in conditions of food scarcity (Schausberger, 1997). These phenomena have to be considered for sustained effective biological control by generalist phytoseiid mites.

The provision with natural shelters and food sources is a good supplement to any artificial colonization method. Would it be sufficient for sustained biological control of pest mites to rely on natural control? Tixier et al. (2006) assessed the immigration of phytoseiid mites from uncultivated vegetation margins into a young newly planted vineyard and observed an increase from an average of 0.04 to 0.15 phytoseiid mites per leaf within three years. It is evident that natural colonization of vineyards by predatory mites takes place but it works rather slowly. Additionally, predatory mite species that occur naturally sometimes do not effectively control the pest mites (Marshall and Lester, 2001). For vineyards surrounded by areas with very low occurrence of phytoseiid mites, an artificial method for colonization is

advisable. The management of surrounding areas is one way of promoting natural colonization of vineyards by wind-borne dispersal of predatory mites. However, deep, tall and woody margins serving as habitat for predatory mites cannot be easily integrated into each landscape. The presence of trees can also cause problems with mass-occurrence of birds feeding on grapes like starlings, which are a big problem in Gols and the surrounding vine-growing areas.

4.4 Conclusion

My study shows that the application of felt belts containing hibernating females of *T. pyri* can effectively lead to higher numbers of predatory mites in vineyards and can therefore be recommended as a very useful measurement enhancing biological control of pest mites. However, it also showed that the number of predatory mites inside the felt belt can vary a lot and that the commercial producers and providers overstate the contents of the felt belts. Cover crops and woody margins can promote natural colonization of vineyards by beneficial mites and can be favorable for their long-term establishment. Natural colonization is slower than colonization using felt belts. Felt belts containing hibernating predatory mites *T. pyri* are a good option for inoculative release compared to other methods because this method is less laborious, has a lower risk of transferring pests and diseases, higher densities of predatory mites can be achieved and the felt is useful for several years because it can remain permanently on the vinestocks and serve as overwintering site for the mites. The most promising way of using the felt belts would be to apply them in newly planted or young vineyards, already before problems with pest mites occur, because development and establishment of a stable population of generalist predatory mites take some time. Therefore, the application of felt belts has the potential to prevent damage before it can even arise.

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Abstract

In a two-year-study, the application of felt belts for colonization of vineyards by the predatory mite *Typhlodromus pyri* was assessed in a field experiment and in the laboratory. The field experiment was conducted in five vineyards in Gols, Burgenland, Austria. Populations of predatory mites were determined in the years before and after the application of felt belts containing hibernating females of *T. pyri* and differences between control plots and plots assigned to mite release were analyzed. In the laboratory experiment, artificial arenas were used to assess the number of living predatory mites inside each of 20 felt belts taken from different charges of belts. With an average of 16.65 predatory mites per felt belt, the number was lower than expected because, according to the declaration of the commercial provider, 30 to 50 predatory mites should be in each belt. In three out of five vineyards the number of predatory mites increased significantly in plots assigned to mite release, as compared to control plots. Across the five vineyards, the numbers of predatory mites decreased in control plots and increased in plots where felt belts were applied. A density of 1 to 2 predatory mites per leaf is recommended to prevent damages by pest mites but in my study in plots with felt belt application on average only a number of 0.91 mites per leaf could be attained. The advantages of the application of felt belts as compared to other methods of man-influenced colonization of vineyards by predatory mites are discussed. Overall, my thesis suggests that the application of felt belts is an appropriate strategy for inoculative release of predatory mites, which can be especially useful in newly planted and young vineyards.

Key words: Predatory mites, colonization, Phytoseiidae, *Typhlodromus pyri*, inoculative release, vineyards, biocontrol.

Zusammenfassung

In einer zweijährigen Studie wurde die Anwendung von Filzbändern zur Ansiedlung der Raubmilbe *Typhlodromus pyri* in Weingärten in einem Feldexperiment und im Labor untersucht. Das Feldexperiment wurde in fünf verschiedenen Weingärten in Gols, Burgenland, Österreich durchgeführt. Die Raubmilbenpopulationen wurden in den Jahren vor und nach dem Ausbringen der Filzbänder, welche Weibchen von *T. pyri* in Winterruhe enthielten, bestimmt, und die Unterschiede zwischen Kontrollplots und Plots mit Filzbändern wurden analysiert. Zur Analyse der Filzbänder im Labor wurden künstliche Arenen verwendet um die Anzahl der lebenden Raubmilbenweibchen von 20 Filzbändern aus verschiedenen Chargen zu eruieren. Mit durchschnittlich 16.65 Raubmilben pro Filzband war die Anzahl niedriger als erwartet, denn laut Firmendeklaration sollte jedes Filzband 30 bis 50 Raubmilben beinhalten. In drei von fünf Weingärten konnte ein signifikanter Anstieg der Raubmilbenpopulationen in Plots, in denen Filzbänder montiert wurden, festgestellt werden. Fasst man die Ergebnisse aller fünf Weingärten zusammen, so nimmt die Anzahl der Raubmilben in den Kontrollplots ab, während sie in den Plots mit Filzbändern zunimmt. Eine Dichte von ein bis zwei Raubmilben pro Blatt wird empfohlen, um Schäden durch Schadmilben vorzubeugen. In meiner Studie konnte allerdings nur eine durchschnittliche Anzahl von 0.91 Raubmilben pro Blatt erzielt werden. Die Vor- und Nachteile der Verwendung von Filzbändern im Vergleich zu anderen Ausbringungsmethoden werden diskutiert. Die Ausbringung von Filzbändern ist eine geeignete Möglichkeit um Raubmilben inokulativ in Weingärten anzusiedeln und ist speziell in jungen Weingärten zielführend und empfehlenswert.

Schlüsselwörter: Raubmilben, Ansiedlung, Phytoseiidae, *Typhlodromus pyri*, Weingärten, inokulative Methode, biologische Schädlingskontrolle.

Curriculum vitae

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02-06/2014	Semester abroad as exchange student in Toulouse, France at ENSAT (École Nationale Supérieure Agronomique de Toulouse)
2008-2011	Bachelor study Agricultural Sciences, University of Natural Resources and Life Sciences, Vienna
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Professional experience

04/2016 - continuous	Internship at Agro Trial Center Gerhaus, trial assistant
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