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DIPARTIMENTO DII AGRONOMIA AMBIENTALE E PRODUZIONI VEGETALI

DOTTORATO DI RICERCA IN: VITICOLTURA, ENOLOGIA E MARKETING DELLE IMPRESE VITIVINICOLE
CICLO XXIII

RETI TROFICHE NELLE COMUNITÀ DI ACARI DELLA VITE: IMPLICAZIONI PER IL CONTROLLO BIOLOGICO

FOOD WEBS IN MITE COMMUNITIES OF GRAPE: IMPLICATIONS FOR BIOLOGICAL CONTROL

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Riassunto

Sono state condotte indagini di laboratorio sugli acari predatori *Amblyseius andersoni, Typhlodromus pyri* e *Kampimodromus aberrans.* Una prima indagine ha riguardato l'influenza di diverse fonti alimentari sui parametri demografici delle tre specie. Queste sono state allevate e monitorate, dalla deposizione dell'uovo alla morte, fornendo diversi tipi di alimenti: gli acari Tetranichidi *Panonychus ulmi* e *Eotetranychus carpini*, l'eriofide *Colomerus vitis*, una possibile preda alternativa (*Tydeus caudatus*), il polline di *Typha latifolia* e il micelio del fungo patogeno *Plasmopara viticola*. Sono stati calcolati i seguenti parametri: durata degli stadi di sviluppo, sopravvivenza, sex-ratio e fecondità. *T. caudatus* non rappresenta un alimento per le tre specie. *K. aberrans* ha esibito una sopravvivenza molto bassa su *P. viticola* e anche per *T. pyri* il numero dei sopravissuti sul patogeno è significativamente inferiore a quello dei sopravissuti sugli altri alimenti. Per *A. andersoni* non sono stati osservati differenze nel confronto tra il polline e le prede per la maggior parte dei parametri, mentre l'alimentazione su *P. viticola* ha conseguito un tasso di ovideposizione più contenuto. *T. pyri* ha evidenziato un tasso intrinseco di accrescimento della popolazione più elevato su polline e *C. vitis* che su *P. ulmi; E. carpini* è stato associato a valori intermedi, *P. viticola* ai valori più bassi. Per *K. aberrans* il polline risulta un alimento ottimale, *E. capini* è preferito a *P. ulmi*, mentre l'eriofide induce prestazioni simili a quelle viste su *E. carpini*. Il micelio di *P.viticola* non consente la riproduzione di *K. aberrans*. Indipendentemente dall'alimento fornito, *A. andersoni* mostra tendenzialmente tempi di sviluppo più brevi e fecondità più elevate rispetto a *T. pyri* e *K. aberrans.*

Una seconda indagine ha riguardato il comportamento alimentare delle femmine svernanti degli acari predatori. I predatori sono stati raccolti a fine inverno e allevati individualmente su polline di *Carpinus betulus* o su femmine svernanti di *E. carpini*. La preda è stata offerta a densità crescenti (1, 2, 4 e 8 *E. carpini* al giorno). In assenza di alimenti le femmine svernanti sono sopravvissute per pochi giorni e non hanno ovideposto. L'interruzione della diapausa è risultata più precoce nelle femmine che hanno avuto a disposizione un maggior numero di prede. Le femmine alimentate con polline hanno iniziato a ovideporre contemporaneamente a quelle allevate con ampie densità di preda. La longevità di *A. andersoni* si è ridotta al diminuire della disponibilità di preda. La fecondità è influenzata dall'apporto di preda. Il consumo di preda cresce con il numero di prede fornite. L'efficienza di conversione delle prede in uova è stata più elevata a basse densità di preda in *K. aberrans* e *T. pyri* e a moderate densità per *A. andersoni*.

Un'ulteriore indagine di laboratorio ha riguardato la risposta degli acari Fitoseidi agli stimoli olfattivi provenienti da foglie infestate da acari Tetranichidi (*E. carpini* e *P. ulmi*) o infette dal patogeno *Plasmopara viticola*. La risposta è stata studiata utilizzando un olfattometro a Y. *K. aberrans* ha risposto agli stimoli emessi dalle foglie infestate da *E. carpini*. *A. andersoni* e *T. pyri* hanno risposto agli stimoli provenienti dalle foglie infestate da *P. ulmi* e da *E. carpini* nonché a quelli provenienti dalle foglie infette da *P. viticola*. Nella scelta tra i diversi stimoli, *A. andersoni* e *T. pyri* hanno preferito *P. ulmi* a *P. viticola*.

Sono state condotte indagini di campo finalizzate a valutare l'effetto dell'immissione di specie diverse di acari Fitoseidi sul controllo degli acari fitofagi. A tale scopo sono stati selezionati due siti. Nel primo erano presenti quattro vigneti della medesima cultivar (Glera) allevati a Sylvoz, cordone libero, doppia cortina e pergola. I vigneti erano caratterizzati da pullulazioni ricorrenti di acari Tetranichidi e dalla presenza di *A. andersoni*. Nel secondo sito è stato individuato un vigneto infestato da *E. carpini* nonostante la presenza di *Phytoseius finitimus* e *A. andersoni*. In entrambi i vigneti sono stati immessi *T. pyri* e *K. aberrans*, sia singolarmente che congiuntamente e a diverse densità di immissione. Entrambe le specie hanno contribuito al controllo biologico degli acari Tetranichidi. Tuttavia nel primo sito gli effetti dell'immissione sono apparsi significativi solo nell'anno successivo alla stessa. I predatori naturalmente presenti sono apparsi inefficaci. In numerose parcelle *A. andersoni* ha raggiunto densità inferiori nelle tesi in cui erano presenti *T. pyri* e *K. aberrans* che nel testimone. *Ph. finitimus* ha sofferto meno di *A. andersoni* la presenza dei competitori. *T. pyri* ha subito più di *K. aberrans* l'effetto del competitore.

Abstract

Laboratory experiments have been carried out on the predatory mites *Amblyseius andersoni, Typhlodromus pyri* and *Kampimodromus aberrans*. The first experiment concerned the effects of different food sources on biological and demographic parameters of predatory mites. The three predatory mites were reared from egg to mite death supplying different foods: *Panonychus ulmi, Eotetranychus carpini, Colomerus vitis*, a potential alternative prey (*Tydeus caudatus*) and two non-prey foods (*Typha latifolia* pollen and mycelium of the grape pathogen *Plasmopara viticola*). The following parameters were calculated: developmental times, survival, sex-ratio and fecundity. *T. caudatus* was not an alternative prey for the predatory mites. A low percentage of *K. aberrans* survived on *P. viticola*, and *T. pyri* survival was significantly lower on the grape pathogen than on other foods. *A. andersoni* did not show different parameters when fed with pollen or prey while *P. viticola* was associated to a lower oviposition rate. *T. pyri* had a significantly higher intrinsic rates population increase on pollen and *C. vitis* than on *P. ulmi*; *E. carpini* was associated to intermediate rates and *P. viticola* to the lowest. Pollen was an optimal food for *K. aberrans*. *E. capini* was preferred among tetranychids, while *C. vitis* induced similar performance to *E. carpini*. *P. viticola* mycelium did not allow for *K. aberrans* reproduction. *A. andersoni* exhibited a faster development and a higher fecundity than *T. pyri* and *K. aberrans*.

A second experiment deals with the behaviour of overwintered females of predatory mites. Mites were collected in late winter and reared on *Carpinus betulus* pollen or on *E. carpini* overwintering females. The prey was offered at increasing densities, i.e. 1, 2, 4 and 8 *E. carpini* females per day. Females without foods did not oviposit and died early. The diapause termination time was shorter in females fed with high prey densities or with on pollen. Females fed with one prey showed a low longevity. *T. pyri* and *A. andersoni* survived longer on pollen or as long as on the prey. The fecundity was positively affected by prey abundance, while on pollen the females showed intermediate values. Prey consumption increased with prey abundance, while proportion among consumed prey and available prey decreased. The efficiency of conversion of prey in egg biomass was higher with low prey densities for *K. aberrans* and *T. pyri*, but it was higher with 4 prey per day in case of *A. andersoni*.

Another experiment deals with the olfactory response of predatory mites to volatiles induced by leaves infested by spider mites (*P. ulmi* and *E. carpini*), or infected by the grape pathogen downy mildew (*Plasmopara viticola*). Response was tested by using a Y shaped tube olfactometer. *K. aberrans* responded positively to *E. carpini* infested leaves only. *A. andersoni* and *T. pyri*

responded positively to both prey *P. ulmi* and *E. carpini* infested leaves and did not show any preference between them. Leaves infected by downy mildew attracted only *A. andersoni* and *T. pyri*. When leaves with downy mildew and spider mite are compared, *A. andersoni* and *T. pyri* preferred *P. ulmi* to plant pathogen, while did not choose between *E. carpini* and the plant pathogen.

In field experiments the effect of predatory mite releases on biological control of spider mites was evaluated. The releases were performed in two farms. In the farm A, four vineyards of Glera cultivar were selected for the experiment. The vineyards were with four different training systems: Pergola, Sylvoz, G.D.C. and Free Cordon. Spider mite infestations were frequently observed in the seasons preceding the experiments. *A. andersoni* was the native predatory mite species. In the farm B, experiments were carried out in a vineyard of cultivar Verduzzo trevigiano and trained with Sylvoz pruning system. High population densities of *E. carpini* were recorded in the year preceeding the experiment. *Phytoseius finitimus* and *A. andersoni* were the native predatory mite species. *T. pyri* and *K. aberrans* were released singly or mixed at different densities. Both species contributed to the biological control of tetranychid mites. The effects of releases were higher in the second year in the farm A. The native species *A. andersoni* and *Ph. finitimus* were not effective in biological control. In most cases *A. andersoni* was reduced by *T. pyri* and *K. aberrans* releases. Trials with *Ph. finitimus* suggest that this species suffered less than *A. andersoni* interspecific competition. *T. pyri* suffered more than *K. aberrans* the presence of a competitor.

Chapter I

Effects of potential food sources on biological and demographic parameters of predatory mites *Kampimodromus aberrans*, *Typhlodromus pyri* and *Amblyseius andersoni*

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Introduction

Kampimodromus aberrans (Oudemans), *Typhlodromus pyri* Scheuten and *Amblyseius andersoni* (Chant) are dominant predatory mites in European vineyards (Duso and Liguori, 1984; Schruft, 1985; Maixner, 1990; Tixier et al., 1998; Pereira et al., 2003). They can keep the populations of tetranychid mites *Panonychus ulmi* (Koch) and *Eotetranychus carpini* (Oudemans) at non-damaging levels (Duso, 1989; Camporese and Duso, 1996).

Generalist phytoseiids can persist on plants when their main prey is absent or scarce by feeding on other foods (McMurtry, 1992; Eichhorn and Hoos, 1990; Bakker and Klein, 1992; Karban et al., 1995) and reaching high performances (McMurtry and Rodriguez, 1987). Because of these features, predatory mites can prevent herbivorous mite outbreaks on perennial plants, such as grapevines. The ability to exploit a wide range of food sources can be an advantage for generalist predators in competing with more specialized species (Symondson et al., 2002).

The range of food sources for generalist phytoseiids can include arthropods in particular: mites like tetranychids, eriophyids, tarsonemids, tenuipalpids (e.g., Helle and Sabelis, 1985; Overmeer, 1985; McMurtry et al., 1984; Ozman-Sullivan, 2006), and other phytoseiids (Schausberger, 2003); insects like thrips, coccids and whiteflies (e.g., Engel and Ohnesorge, 1994; Schausberger, 1998; Nomikou et al., 2001). Eriophyids can be considered the most important pests after tetranychids. *Calepitrimerus vitis* (Nalepa) is responsible of stunted shoot growth, bud death

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and leaf necrosis and distortions. *Colomerus vitis* Pagestencher causes leaf erinea, but is only occasionally economically damaging. A strain of *Col. vitis* damaging buds is considered more dangerous (Carew et al, 2004; Bernard et al. 2005; Walton et al 2007; Duso et al. 2010). Demographic parameters of *A. andersoni* and *T. pyri* feeding on *Col. vitis* have been studied in laboratory and showed a high suitability of this prey (Duso and Camporese, 1991). In consideration of its low harmfulness, the interest on *Col. vitis* is associated to its possible function as alternative prey for predatory mites in vineyards. Tydeids were also suggested as potential prey for phytoseiids. *Homeopronematus anconai* (Baker) was a food source for *Metaseiulus occidentalis* (Nesbitt) in California (Flaherty and Hoy, 1971). He can favour predator persistence in vineyards when the main prey *Tetranychus pacificus* McGregor is scarce (Knop and Hoy, 1983). *Tydeus caudatus* Dugès was considered a suitable prey for *T. pyri* in the laboratory (Calis et al., 1988). This tydeid is very common in European vineyards where it can be exploited by the phytoseiid mite *Paraseiulus talbii* (Athias-Henriot) (Camporese and Duso, 1995). However, relationships between generalist phytoseiids and *T. caudatus* are little known.

Several studies suggest that among alternative foods, pollen is suitable for many phytoseiid species (e.g., Dosse, 1961; Overmeer, 1985; McMurtry and Rodriguez, 1987; McMurtry et al., 1991; Addison et al., 2000; Duso et al., 1997, 2004; Kasap, 2005; Roda et al., 2003). Pollen often favours faster developmental times and greater oviposition rates than tetranychids (Abdallah et al., 2002; Duso and Camporese, 1991; Kasap, 2005). However, the abundance of pollen in crop perennial ecosystems is inconstant throughout the season (Eichhorn and Hoos, 1990; Duso et al., 1997; Addison et al., 2000).

An additional possible alternative food source for predatory mites is represented by plant pathogens (Daftari, 1979; Zemek and Prenerovà, 1997; Pozzebon and Duso, 2008; Pozzebon et al., 2009). The Grape downy mildew (GDM), *Plasmopara viticola* (Berk and Curtis), is a major disease in temperate humid areas. It often spreads in late summer when the use of fungicides has ceased. A correlation between the spread of GDM foliar symptoms and the abundance of *T. pyri* and *A. andersoni* has been found in north-Italian vineyards (Duso et al., 2003, Pozzebon et al., 2010).

Generalist predatory mites species can be involved in competitive interactions (e.g., Duso et al., 1991, 1996 , 2009; Duso, 1992; Schausberger, 1997, 1998). It is known that food-mediated effects can influence intraguild interactions among predators (e.g., Rosenheim et al., 1995; Holt and Polis 1997; Okuyama, 2009). Predatory mites can show a different fitness on the same resource (Dicke et al., 1990; Duso and Camporese, 1991), therefore competition among predatory mites may be affected by the availability of different resources with implications for biological control.

Knowledge of the influence of food sources on phytoseiid life history parameters is not exhaustive for many species and a comprehensive comparison among *K. aberrans*, *T. pyri* and *A. andersoni* fitness has not been performed yet. We investigated the effects of six food sources, the herbivorous mites *P. ulmi*, *E. carpini* and *Col. vitis*, the non-specialized feeder mite *T. caudatus*, the pollen of *Typha latifolia* and the mycelium of *P. viticola*, on the development and reproduction of *K. aberrans, T. pyri* and *A. andersoni*. We also estimated fecundity table parameters as fitness estimation of each predatory mite species on different food sources. The intrinsic rate of increase was used to compare the responses of the three predatory mites to each of the food sources considered.

Materials and Methods

Stock cultures

Kampimodromus aberrans, T. pyri, *A. andersoni* and *T. caudatus* were collected from vineyards in North-East of Italy and reared in laboratory conditions (24°C, 70 ± 10% R. H., 16 L: 8 D regime of photoperiod) for at least three generations prior to the experiments. Colonies were maintained in the laboratory at the Department of Environmental Agronomy and Crop Science, University of Padua, Legnaro (PD), on grape leaves cv. Isabella placed on a wet sponge in a plastic tray containing water. Pollen of different taxa was provided as food. Strips of wet tissue paper were placed on the plastic arena, along its periphery, for drinking water supply and to prevent mite escape. A piece of transparent plastic sheet (1–2 cm^2) folded in the shape of a tent, was placed over each arena as shelter and oviposition site for the mites.

GDM infected leaves used in the experiments were obtained from a GDM laboratory colony. Samples of leaves infected by GDM were collected from fungicide untreated vineyards. Lesions were induced to sporulate by incubating leaves in a humid chamber in darkness for 12 hours. Using a water suspensions of freshly-produced sporangia, GDM was inoculated to detached leaf sections (5 x 5 cm) obtained from grape plants grown in greenhouse. Afterwards infections were induced by incubating inoculated leaf portions overnight in darkness. Leaf portions were maintained in Petri dishes over moistened filter paper, at room temperature (25°C) with a 16 L: 8 D regime for 7 days.

Laboratory experiments

The effect of different food sources on developmental times of predatory mites were evaluated using coeval eggs obtained by transferring predatory mite females from laboratory colonies onto new leaves and allowed to lay eggs for 12 hours. The eggs were then singly transferred to experimental units consisting of a section of grape leaf (2 x 2 cm.) placed botton-side down on a wet layer of cotton within a plastic box. The experimental units were maintained in climatic chambers at controlled conditions (24°C, 70 ± 10% R.H.., 16 L: 8 D regime of photoperiod). We compared six food sources: the mites *P. ulmi*, *E. carpini*, *Col. vitis* and *T. caudatus*, *Typha latifolia* pollen, and *P. viticola* mycelium. Cattail pollen had been previously collected, dried and stored in a freezer (- 18° C) and was replenished every day (about 0.5 mg of pollen per day) to phytoseiids with a fine paint brush. *Panonychus ulmi*, *E. carpini* and *Col. vitis* were collected from infested vineyards. *Tydeus caudatus* was obtained from laboratory colonies. Twenty tetranychid or tydeid mite individuals and about 60 eriophyid mite individuals of various developmental stages were supplied to each predatory mite every day. GDM mycelium was provided to phytoseiids using leaf discs obtained from infected leaves with fresh downy felt of GDM sporulations. Every two days leaf discs were replenished by placing them close to the older discs to allow mite transfer.

To evaluate developmental times, and immature mortality, experimental units were monitored every 12 hours. The presence of an exuvium was the criterion used to prove successful molting to the next developmental stage. When adults were obtained, one male and one female reared on the same food source were placed together in the same experimental units to allow mating. Thereafter, oviposition and longevity of mites were monitored every 24 hours. When necessary, an additional male was added to ensure mating. Laid eggs were removed from experimental units and offspring mortality and sex ratio were determined.

Data analysis

The effects of different food sources on phytoseiid development and oviposition were analyzed with one-way ANOVA using the GLM procedure of SAS (SAS Institute Inc., 1999). We evaluated the effect of food source on the duration (h) of developmental stages, fecundity (number of eggs per female), and female and male longevity (days) applying the Tukey–Kramer's test (a = 0.05). Only individuals that reached the adult stage were included in the analysis. Juvenile survival (number of females + males/number of initial eggs) and sex-ratio (number of females/number of females + males) were analyzed by applying a logit model using the GENMOD procedure of SAS (SAS Institute Inc., 1999). Treatment effects on the survival curves were estimated using the Kaplan–Meier method and were compared by Wilcoxon χ^2 test using the LIFETEST procedure of

SAS (Allison, 1995).

Life table parameters [Reproductive rate (Ro); mean generation time (T); intrinsic rate of increase (r_m); doubling time (Dt); finite rate of increase (λ)] were estimated and compared using a SAS-based procedure developed by Maia et al. (2000) which applies a Jackknife method to the variance estimate and performs specific pairwise comparisons between treatments using a two-tailed t test ($\alpha = 0.05$). Assuming a stable age distribution and under the same climatic and food conditions, the intrinsic rate of natural increase is a useful comparative statistic of population growth potential (Southwood, 1978). The intrinsic rate of population increase (r_m) can be used for natural enemies comparisons (Messenger 1964). Using the above mentioned procedure we compared r_m values of predatory mites species on the same food source using a two-tailed t test ($\alpha = 0.05$).

Results

Kampimodromus aberrans

Food sources affected immature survival significantly ($\chi^2 = 321.48$; df = 6; $P < 0.001$). In the control, protonymphs died before to become deutonymphs. Similar results were obtained when *T. caudatus* was offered as a prey. Development was completed on the other food sources, but immature mortality rate was particularly high when predators were reared on GDM (Table 1).

No effects of food sources were observed on the duration of egg and larval stages (female eggs: $F_{4, 81} = 0.31$; $P = 0.869$; female larvae: $F_{4, 81} = 1.22$; $P = 0.311$; male eggs: $F_{4, 79} = 1.04$; $P = 0.390$; male larvae: $F_{3, 79} = 0.77$; $P = 0.517$; Tables 2 and 3). In contrast, developmental times of female protonymphs were affected significantly by food sources ($F_{4, 81}=19.45$; $P < 0.001$). Protonymphs reared on GDM or *P. ulmi* required longer times to reach the deutonymphal stage compared to those reared on pollen, *Col. vitis* or *E. carpini* (Table 2). Female deutonymphs development was affected by food sources ($F_{4, 81} = 62.13$; $P < 0.001$), since they needed a longer time to reach the adult stage when reared on GDM (Table 2). Developmental times were longer on *P. ulmi* than on pollen, while *E. carpini* and *Col. vitis* were associated to intermediate values (Table 2). Development of *K. aberrans* females was faster on pollen, *Col. vitis*, and *E. carpini* than on *P. ulmi*, while predators reared on GDM developed in the longest times ($F_{4, 81} = 85.40$; $P < 0.001$; Table 2). Food source influenced *K. aberrans* male protonymphs development ($F_{4, 79} = 18.45$; $P < 0.001$), which was shorter on pollen and *Col. vitis* than on *P. ulmi* and GDM (Table 3). Feeding on *E. carpini* rather

than on *P. ulmi* resulted in shorter developmental times. No differences on protonymphs development were observed offering GDM or tetranychids (Table 3). Male deutonymphs required longer times to reach the adult stage when fed with GDM ($F_{4, 79} = 17.20$; $P < 0.001$; Table 3). The complete development of *K. aberrans* males was shorter on pollen than on other foods, on *Col. vitis* was shorter than on *P. ulmi* and GDM, on *E. carpini* development showed intermediate values among pollen and *Col. vitis* ($F_{4, 79} = 51.54$; $P < 0.001$; Table 3). Food source did not affect offspring sex ratio ($\chi^2 = 0.30$; df = 4; $P < 0.990$, Table 1).

Kampimodromus aberrans females reared on GDM did not oviposit (Table 2). Pre-oviposition period was shorter on *P. ulmi* than on pollen or *E. carpini* ($F_{4, 81} = 3834.80$; $P < 0.001$; Table 2). However, egg to egg time period was longer on *P. ulmi* than on other food sources ($F_{4, 81} = 68255.20$; $P < 0.001$; Table 2). Total fecundity was higher on pollen than on prey ($F_{4, 65} = 791.22$; $P < 0.001$; Table 2). Female longevity depended on food source ($F_{4, 65} = 87.16$; $P < 0.001$), resulting higher on pollen than on *E. carpini* or GDM. Longevity of females reared on *P. ulmi* and *Col. vitis* was not significantly different from that reported for pollen or *E. carpini* (Table 2). Longevity of males was lower on GDM than on other food sources ($F_{4, 55} = 62.01$; $P < 0.001$; Table 3). *Kampimodromus aberrans* showed higher r_m, Ro and λ, and lower Dt when feeding on pollen than on *Col. vitis*, *E. carpini* or *P. ulmi*. A higher r_m was recorded for predatory mite females fed with *Col. vitis* or *E. carpini* compared to those fed with *P. ulmi*. The parameter T was not affected by food types (Table 4).

Typhlodromus pyri

Juvenile survival was different among treatments ($\chi^2 = 170.73$; df = 6; $P < 0.001$). *Typhlodromus pyri* completed its development on pollen, *E. carpini*, *P. ulmi*, and *P. viticola* but not in the control and *T. caudatus* treatments where protonymphs did not molt to deutonymphs (Table 1). Juvenile survival resulted lower on GDM than on pollen and *E. carpini*, while *P. ulmi* was associated to intermediate values (Table 1).

No effects of food type were observed on the development of female egg, larval and protonymphal stages (eggs: $F_{4, 70} = 0.07$; $P = 0.992$; larvae: $F_{4, 70} = 0.52$; $P = 0.720$; protonymphs: $F_{4, 70} = 0.70$, $P = 0.593$; Table 2). Female deutonymphs required longer times to reach the adult stage when fed with GDM compared to other foods ($F_{4, 70} = 7.59$; $P < 0.001$, Table 2). Food source affected total developmental times of females ($F_{4, 70} = 8.27$; $P < 0.001$), which resulted higher on GDM than on remaining foods (Table 2). Food source did not affect male complete development ($F_{4, 50} = 0.01$, $P = 0.999$) as well as developmental times of different male stages (eggs: $F_{4, 50} = 0.27$;

$P = 0.895$; larvae: $F_{4, 50} = 0.38$; $P = 0.823$; protonymphs: $F_{4, 50} = 0.54$; $P = 0.707$; deutonymphs: $F_{4,50} = 0.29$; $P = 0.8861$, Table 3), and sex ratio ($\chi^2 = 0.46$; df = 4; $P = 0.978$; Table 1).

Pre-oviposition was shorter on GDM than on *Col. vitis*, *P. ulmi* or *E. carpini*, while pollen was associated to intermediate values ($F_{4, 70} = 3.18$; $P = 0.019$) but egg-egg time periods were similar ($F_{4, 70} = 0.29$; $P = 0.886$). *Typhlodromus pyri* fecundity ($F_{4, 62} = 164.59$; $P < 0.001$) was higher on pollen than on *P. ulmi* and GDM. The fecundity of females fed with *E. carpini* or *Col. vitis* was not significantly different from that of predators reared on pollen or *P. ulmi* (Table 2). Female longevity was lower on GDM than on other food sources ($F_{4, 62} = 46.90$; $P < 0.0001$; Table 3), but male longevity was not affected by food type ($F_{4, 49} = 0.18$; $P = 0.948$; Table 3).

For *T. pyri*, feeding on GDM resulted in the lowest r_m, *Ro*, λ and *T*, and the highest *Dt*. Predatory mites fed with pollen or *Col. vitis* had higher r_m, *Ro* and λ and lower *Dt* than those reared on *P. ulmi*, while demographic parameters of predatory mites fed with *E. carpini* were similar to those associated to pollen *and Col. vitis* or intermediate between the first two food sources and *P. ulmi* (Table 4).

Amblyseius andersoni

Food source affected juvenile survival ($\chi^2 = 167.25$; df = 6; $P < 0.001$).

Development was not completed in the control and in *T. caudatus* treatment where protonymphs did not molt to deutonymphs (Table 1). No differences were found among other food sources.

The duration of female developmental stages was not influenced by food types (eggs: $F_{4, 72} = 0.39$, $P = 0.819$; larvae: $F_{4, 72} = 0.94$; $P = 0.446$; protonymphs: $F_{4, 72} = 1.02$, $P = 0.401$; deutonymphs: $F_{4, 72} = 1.75$; $P = 0.148$; Table 2). However, overall development was affected by food ($F_{4, 72} = 5.33$; $P = 0.001$; Table 2). In particular, predators fed with pollen or *Col. vitis* developed faster than those fed with *P. ulmi* or GDM (Table 1). Developmental times of predators fed with *E. carpini* were not different from those reported for *Col. vitis*, pollen or *P. ulmi*. Food source did not affect male development (eggs: $F_{4, 58} = 0.32$; $P = 0.866$; larvae: $F_{4, 58} = 0.30$; $P = 0.879$; protonymphs: $F_{4, 58} = 0.68$; $P = 0.610$; deutonymphs: $F_{4, 58} = 1.92$; $P = 0.119$; total development: $F_{4, 58} = 3.21$; $P = 0.070$; Table 3), and sex ratio ($\chi^2 = 0.52$; df = 4; $P = 0.971$, Table 1).

Pre-oviposition period was similar among treatments ($F_{4, 72} = 0.62$; $P = 0.648$; Table 2). *Amblyseius andersoni* females fed with GDM evidenced lower fecundity than those reared on other food sources ($F_{4, 49} = 53.08$; $P < 0.0001$; Table 2). Female longevity was shorter on GDM than on

other foods ($F_{4, 49} = 60.79$; $P < 0.0001$) (Table 2) while male longevity was not affected by food source ($F_{4, 51} = 0.01$; $P = 0.998$).

Amblyseius andersoni fed with GDM showed lower r_m, Ro, λ and T, and higher Dt than on other foods (Table 4). There were some differences between pollen and *P. ulmi* (Table 4).

Interspecific variation in capacity of population increase

Amblyseius andersoni exhibited higher r_m values than *T. pyri* and *K. aberrans* when fed with prey (Figure 1). On these food sources, *T. pyri* showed higher r_m values than *K. aberrans*. When fed with pollen, *A. andersoni* and *T. pyri* had higher r_m values than *K. aberrans* (Figure 1). In the comparison involving GDM, a higher r_m was associated to *A. andersoni* than on the remaining predatory mite species (Figure 1).

	Pollen	E. carpini	P. ulmi	Col.vitis	GDM	T.caudatus	Control
Kampimodromus aberrans	(n = 71)	(n=55)	(n = 60)	(n = 58)	(n = 102)	(n = 58)	(n = 60)
Survival to adult	0.97 ± 0.72 a	0.93 ± 0,52 a	0.90 ± 0.43 a	0.97 ± 0.72 a	0.26 ± 0.22 b	–	–
Sex ratio	0.51 ± 0.24	0.55 ± 0.28	0.50 ± 0.27	0.52 ± 0.27	0.52 ± 0.39	–	–
	(f = 35; m = 34)	(f = 28; m = 23)	(f = 27; m = 27)	(f = 29; m = 27)	(f = 14; m = 13)		
Typhlodromus pyri	(n = 52)	(n = 56)	(n = 56)	(n = 51)	(n = 69)	(n = 64)	(n = 60)
Survival to adult	0.94 ± 0.60 a	0.93 ± 0.52 a	0.88 ± 0.40 ab	0.92 ± 0.52 a	0.75 ± 0.28 b	–	–
Sex ratio	0.60 ± 0.30	0.61 ± 0.29	0.59 ± 0.29	0.62 ± 0.30	0.63 ± 0.29	–	–
	(f = 35; m = 34)	(f = 28; m = 23)	(f = 27; m = 27)	(f = 29; m = 18)	(f = 14; m = 13)		
Amblyseius andersoni	(n = 63)	(n = 65)	(n = 57)	(n = 49)	(n = 60)	(n = 58)	(n = 49)
Survival to adult	0.95 ± 0.59 a	0.94 ± 0.49 a	0.89 ± 0.43 a	0.94 ± 0.60 a	0.87 ± 0.38 a	–	–
Sex ratio	0.58 ± 0.26	0.62 ± 0.26	0.63 ± 0.29	0.59 ± 0.29	0.63 ± 0.29	–	–
	(f = 35; m = 25)	(f = 38; m = 23)	(f = 32; m = 19)	(f = 27; m = 19)	(f = 19; m = 33)		

Table 1. Juvenile survival rate and sex ratio observed for *Kampimodromus aberrans*, *Typhlodromus pyri* and *Amblyseius andersoni* on different food sources and in the control. Different letters indicate significant differences (*P*=0.05).

	Pollen	*E. carpini*	*P. ulmi*	*Col.vitis*	GDM
Kampimodromus aberrans	(n=25)	(n=12)	(n=10)	(n=18)	(n=21)
Egg (h)	48.48 ± 2.35	47.00 ± 2.32	48.00 ± 2.53	48.67 ± 2.05	50.29 ± 1.58
Larvae (h)	12.96 ± 0.96	13.00 ± 2.32	10.80 ± 2.15	14.00 ± 1.45	12.57 ± 1.01
Protonymph (h)	56.64 ± 1.63 b	61.00 ± 2.32 b	79.20 ± 2.65 a	59.33 ± 2.05 b	76.00 ± 2.53 a
Deutonymph (h)	54.72 ± 1.22 c	59.00 ± 1.78 bc	62.40 ± 2.99 b	57.33 ± 1.55 bc	86.86 ± 1.14 a
Egg-Adult (h)	172.80 ± 1.83 c	180.00 ± 2.09 c	200.40 ± 2.56 b	200.40 ± 2.56 c	225.71 ± 3.27 a
Preoviposition (h)	66.24 ± 1.97 a	66.00 ± 2.34 a	56.40 ± 2.56 b	67.33 ± 2.59 a	–
Egg-Egg (h)	239.04 ± 2.18 b	246.00 ± 3.13 b	256.80 ± 3.67 a	246.67 ± 3.24 ab	–
	(n=19)	(n=10)	(n=10)	(n=18)	(n=13)
Fecundity (eggs/female)	22.84 ± 0.61 a	14.90 ± 1.19 b	13.70 ± 0.68 b	16.11 ± 0.86 b	–
Adult longevity (days)	32.47 ± 1.19 a	25.80 ± 1.03 b	27.55 ± 1.16 ab	30.42 ± 0.76 ab	10.35 ± 0.96 c
Typhlodromus pyri	(n=17)	(n=11)	(n=15)	(n=19)	(n=13)
Egg (h)	47.29 ± 1.25	46.91 ± 1.95	46.40 ± 1.98	47.37 ± 1.71	47.08 ± 2.13
Larvae (h)	12.71 ± 1.25	13.09 ± 1.09	12.80 ± 0.80	13.26 ± 0.87	13.85 ± 1.25
Protonymph (h)	56.47 ± 2.00	58.91 ± 1.09	59.20 ± 1.42	57.47 ± 1.15	59.08 ± 1.64
Deutonymph (h)	55.77 ± 1.43 b	55.64 ± 1.83 b	56.80 ± 1.42 b	54.94 ± 1.39 b	66.46 ± 1.73 a
Egg-Adult (h)	172.24 ± 1.43 b	174.55 ± 1.89 b	175.20 ± 2.57 b	173.05 ± 1.67 b	186.46 ± 1.73 a
Preoviposition (h)	66.35 ± 3.11 ab	67.64 ± 3.34 a	68.00 ± 3.45 a	66.95 ± 2.12 a	55.39 ± 2.89 b
Egg-Egg (h)	238.59 ± 3.23	242.18 ± 3.90	243.20 ± 4.61 a	240.00 ± 1.84	241.85 ± 3.29
	(n=13)	(n=11)	(n=10)	(n=18)	(n=10)
Fecundity (eggs/female)	25.31 ± 0.66 a	21.50 ± 0.90 ab	20.46 ± 0.95 b	22.50 ± 0.84 ab	6.31 ± 0.35 c
Adult longevity (days)	30.92 ± 0.71 a	28.10 ± 1.20 a	28.42 ± 0.80 a	28.97 ± 0.44 a	18.77 ± 0.77 b
Amblyseius andersoni	(n=21)	(n=13)	(n=11)	(n=16)	(n=16)
Egg (h)	34.29 ± 1.71	35.08 ± 1.64	34.91 ± 1.95	35.25 ± 2.04	36.75 ± 1.33
Larvae (h)	22.86 ± 1.41	23.08 ± 1.64	24.00 ± 1.62	20.25 ± 1.81	22.50 ± 1.02
Protonymph (h)	34.86 ± 1.41	38.77 ± 2.41	37.09 ± 1.09	35.25 ± 1.72	38.25 ± 1.63
Deutonymph (h)	34.29 ± 1.50	36.00 ± 2.35	39.27 ± 1.69	37.50 ± 1.85	39.00 ± 1.34
Egg-Adult (h)	126.29 ± 1.96 b	132.92 ± 2.13 ab	135.27 ± 1.69 a	129.00 ± 2.04 b	136.50 ± 1.50 a
Preoviposition (h)	46.29 ± 2.08	48.00 ± 2.72	48.00 ± 3.24	46.50 ± 2.66	51.00 ± 2.32
Egg-Egg (h)	172.57 ± 2.11 b	180.92 ± 3.45 ab	183.27 ± 3.65 ab	175.50 ± 2.87 b	187.50 ± 2.42 a
	(n=11)	(n=10)	(n=10)	(n=11)	(n=11)
Fecundity (eggs/female)	25.73 ± 0.45 a	24.20 ± 0.55 a	22.20 ± 0.61 a	25.55 ± 1.00 a	9.27 ± 0.71 b
Adult longevity (days)	35.55 ± 1.01 a	33.95 ± 1.29 a	33.85 ± 1.07 a	33.95 ± 0.92 a	19.55 ± 0.91 b

Table 2. Effects of food sources on the development, fecundity and longevity (means ± SE) of *Kampimodromus aberrans*, *Typhlodromus pyri* and *Amblyseius andersoni* females. Different letters indicate significant differences (p=0.05).

	Pollen	E. carpini	P. ulmi	Col. vitis	GDM
Kampimodromus aberrans	(n=23)	(n=13)	(n=11)	(n=19)	(n=18)
Egg (h)	46.96 ± 0.72	45.23 ± 1.99	46.91 ± 3.01	48.63 ± 2.15	50.67 ± 1.55
Larvae (h)	13.04 ± 0.72	12.00 ± 1.92	13.09 ± 1.95	14.53 ± 1.15	12.00 ± 0.97
Protonymph (h)	55.83 ± 1.22 c	61.85 ± 2.29 bc	78.55 ± 2.97 a	58.74 ± 1.81 c	70.67 ± 2.89 ab
Deutonymph (h)	55.83 ± 1.22 b	60.00 ± 1.92 b	57.82 ± 2.72 b	58.74 ± 1.81 b	77.33 ± 2.61 a
Egg-Adult (h)	171.65 ± 1.59 d	179.08 ± 2.13 cd	196.36 ± 1.83 b	180.63 ± 1.94 c	210.67 ± 3.25 a
	(n=23)	(n=13)	(n=11)	(n=11)	(n=12)
Adult longevity (days)	20.78 ± 0.79 a	20.31 ± 1.15 a	19.64 ± 1.58 a	19.06 ± 0.76 a	7.33 ± 0.62 b
Typhlodromus pyri	(n=13)	(n=11)	(n=11)	(n=10)	(n=10)
Egg (h)	47.08 ± 1.64	45.82 ± 2.18	46.91 ± 1.95	48.00 ± 1.79	45.60 ± 2.40
Larvae (h)	12.92 ± 0.92	13.09 ± 1.95	13.09 ± 1.09	13.20 ± 1.20	13.20 ± 1.20
Protonymph (h)	55.39 ± 1.69	55.64 ± 1.83	57.82 ± 1.46	55.20 ± 1.96	57.60 ± 1.60
Deutonymph (h)	54.46 ± 2.20	55.64 ± 1.83	52.36 ± 2.44	54.00 ± 2.68	54.00 ± 2.00
Egg-Adult (h)	169.85 ± 2.29	170.18 ± 1.46	170.18 ± 2.72	170.40 ± 2.40	170.40 ± 3.49
	(n=13)	(n=10)	(n=11)	(n=10)	(n=10)
Adult longevity (days)	12.85 ± 0.66	12.91 ± 0.84	13.40 ± 0.81	12.50 ± 0.77	12.60 ± 0.99
Amblyseius andersoni	(n=19)	(n=9)	(n=10)	(n=13)	(n=12)
Egg (h)	35.37 ± 1.44	34.67 ± 3.71	34.80 ± 2.15	34.15 ± 1.25	36.00 ± 0.00
Larvae (h)	22.74 ± 0.87	21.33 ± 1.76	21.60 ± 1.60	21.23 ± 1.46	23.00 ± 1.78
Protonymph (h)	33.47 ± 1.15	37.33 ± 2.40	36.00 ± 2.53	33.23 ± 1.46	35.00 ± 1.78
Deutonymph (h)	30.95 ± 1.40	32.00 ± 2.00	37.20 ± 2.15	34.15 ± 1.85	36.00 ± 2.09
Egg-Adult (h)	122.53 ± 1.47	125.33 ± 2.11	129.60 ± 1.60	122.77 ± 2.77	130.00 ± 2.00
	(n=17)	(n=9)	(n=10)	(n=10)	(n=10)
Adult longevity (days)	8.71 ± 0.53	8.67 ± 0.58	8.70 ± 0.52	8.80 ± 0.64	8.90 ± 0.82

Table 3.: Effects of food sources on the development and longevity (means ± SE) of *Kampimodromus aberrans*, *Typhlodromus pyri* and *Amblyseius andersoni* males. Different letters indicate significant differences (p=0.05).

	n	Dt (day)	λ (female/female/day)	r_m (female/female/day)	R_0 (female/female)	T (day)
Kampimodromus aberrans						
Pollen	19	4.518 ± 0.086 c	1.166 ± 0.003 a	0.153 ± 0.003 a	11.300 ± 0.303 a	15.827 ± 0.186 a
E. carpini	10	5.141 ± 0.213 b	1.144 ± 0.006 b	0.135 ± 0.006 b	7.621 ± 0.607 bc	15.809 ± 0.298 a
P. ulmi	10	6.113 ± 0.177 a	1.120 ± 0.004 c	0.113 ± 0.003 c	6.165 ± 0.308 c	16.058 ± 0.420 a
Col. vitis	18	5.4152 ± 0.1542 b	1.1364 ± 0.0041 b	0.1279 ± 0.0036 b	7.9702 ± 0.4268 b	16.237 ± 0.3132 a
GDM	13	–	–	–	–	–
Typhlodromus pyri						
Pollen	13	4.126 ± 0.047 c	1.183 ± 0.002 a	0,168 ± 0.006 a	14.274 ± 0.374 a	15.827 ± 0.186 a
E. carpini	10	4.242 ± 0.146 bc	1.177 ± 0.007 ab	0.163 ± 0.002 ab	12.197 ± 0.509 b	15.315 ± 0.537 a
P. ulmi	13	4.597 ± 0.088 b	1.163 ± 0.003 b	0.151 ± 0.003 b	10.624 ± 0.494 c	15.683 ± 0.288 a
Col. vitis	18	4.1846 ± 0.0695 c	1.1801 ± 0.0033 a	0.1656 ± 0.0028 a	12.834 ± 0.4776 b	15.414 ± 0.2301 a
GDM	13	8.801 ± 0.440 a	1.082 ± 0.004 c	0.079 ± 0.004 c	2.980 ± 0.164 d	13.917 ± 0.306 b
Amblyseius andersoni						
Pollen	11	3.934 ± 0.098 b	1.193 ± 0.005 a	0.176 ± 0.004 a	14.176 ± 0.247 a	15.050 ± 0.383 a
E. carpini	10	3.834 ± 0.121 b	1.198 ± 0.007 a	0.181 ± 0.006 a	14.046 ± 0.330 a	14.614 ± 0.549 a
P. ulmi	10	3.9491 ± 0.095 b	1.192 ± 0.005 a	0.175 ± 0.004 a	12.448 ± 0.343 b	14.370 ± 0.313 a
Col. vitis	11	3.7149 ± 0.085 b	1.205 ± 0.0051 a	0.1865 ± 0.0042 a	14.117 ± 0.5614 a	14.195 ± 0.2784 a
GDM	11	5.331 ± 0.1806 a	1.139 ± 0.005 b	0.130 ± 0.004 b	5.082 ± 0.392 c	12.544 ± 0.348 b

Table 4. Effects of food source on life-table parameters (means ± SE) of *Kampimodromus aberrans*, *Typhlodromus pyri* and *Amblyseius andersoni*. Different letters indicate significant differences (p=0.05).
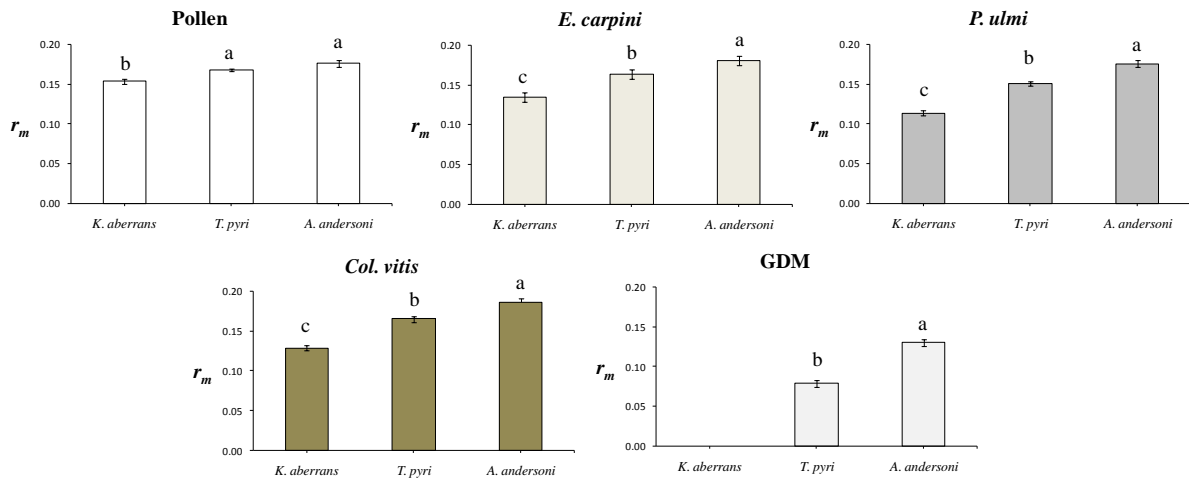
Figure 1. Intrinsic rate of increase (r_m) of *Kampimodromus aberrans*, *Typhlodromus pyri* and *Amblyseius andersoni* on different food sources. Different letters indicate significant differences among species(p=0.05).

Discussion

The biological and demographic parameters of the three predatory mites were affected by food source. Data on *K. aberrans* is of particular interest, since the biology of this predatory mite species has been poorly investigated, despite its importance in European vineyards and orchards (Ivancich Gambaro, 1973; Duso, 1989; Kreiter et al., 2002; Duso et al., 2009). This is mainly due to problems encountered in rearing successfully a number of *K. aberrans* strains in the laboratory. Schausberger (1991) reared an Austrian strain of *K. aberrans* collected on apple on pollen and *P. ulmi*, which is an important pest in fruit orchards and vineyards. In other studies *K. aberrans* life history parameters were estimated by rearing predatory mites on the tetranychids *Tetranychus urticae* Koch and *Amphitetranychus viennensis* (Zacher) important in some apple growing areas (Kasap, 2005), or on *Phytoptus avellanae* (Nalepa), an eriophyoid mite damaging hazelnut trees (Ozman-Sullivan, 2006). Among these species, only *T. urticae* can be a pest of grapevines in Europe. Broufas et al. (2007) evaluated the effect of temperature on *K. aberrans* life history providing cattail pollen as food. In the present study we have compared the fitness of *K. aberrans* on the two most important European mite pests in vineyards (*P. ulmi* and *E. carpini*), on another phytophagous species (*Col. vitis*) and on other food sources potentially available in vineyards (pollen, *T. caudatus*, grape downy mildew). Among these potential foods, *T. caudatus* was not suitable for *K. aberrans*, while GDM was a supplementary food as the predator completed its development on the plant pathogen mycelium but juvenile survival and adult longevity were reduced compared to parameters reported for tetranychids, *Col. vitis* or pollen. Since females reared on GDM did not reproduce, *K. aberrans* population cannot increase and persist for long periods feeding on this plant pathogen exclusively. For *K. aberrans*, pollen can be considered an optimal food confirming the results obtained in previous investigations (Kasap, 2005). Our studies also suggest that *E. carpini* is a more suitable prey than *P. ulmi* for this predator. Suitability of *Col. vitis* is comparable with *E. carpini*. In release experiments *K. aberrans* proved to be successful in keeping populations of both mite pests at low levels (Duso, 1989; Duso et al., 1991; Duso and Vettorazzo, 1999). In these investigations *K. aberrans* showed to be more effective than *T. pyri* and *A. andersoni* in controlling *E. carpini* populations. The importance of *E. carpini* is increasing in south-European vineyards (Castagnoli et al., 2009) and, on the base of previous findings, *K. aberrans* was considered the first option in release experiments (Castagnoli et al., 2009).

Typhlodromus pyri did not exploit *T. caudatus* as food source in contrast with previous studies where *T. pyri* completed its development feeding on this tydeid mite (Calis et al., 1988). The other

food sources resulted adequate for the development and reproduction of *T. pyri* but at different levels. A higher performance was obtained on pollen and *Col. vitis* rather than on *P. ulmi* while *E. carpini* was associated to intermediate values. The role of *T. pyri* in controlling *P. ulmi* in vineyards has been emphasized in several studies (Boller, 1988; Schruft, 1985; Maixner, 1990; Engel and Onhesorge, 1994a, b). A number of release experiments conducted in northern Italy showed that *T. pyri* was able to control *P. ulmi* and *E. carpini* but also that the performance of this predatory mite was affected by a number of environmental and cultural factors (Duso and Pasqualetto, 1993; Camporese and Duso, 1996). Tetranychid mites were more suitable than GDM in terms of development and reproduction. However, GDM can support population increase of *T. pyri* in absence of other food sources (Duso et al., 2003; Pozzebon and Duso, 2008). Moreover, the occurrence of GDM can mitigate the impact of pesticides and favor the re-colonization of treated plots by predatory mites (Duso et al., 2009; Pozzebon and Duso, 2010).

Amblyseius andersoni did not survive by feeding on *T. caudatus* confirming the results obtained by Calis et al. (1988). Pollen confirmed to be an optimal food for *A. andersoni* (Overmeer and van Zon, 1983; Duso et al., 1991 etc) but predators reared on *E. carpini* and *Col. vitis* exhibited similar biological values. A comparison of demographic parameters of predatory mites reared on *E. carpini* or on *P. ulmi* would suggest a higher performance of *A. andersoni* on the first prey. These results agree with those obtained by Duso and Camporese (1991). However, field studies showed the opposite (Duso, 1989; Duso and Vettorazzo, 1999) and thus *A. andersoni* was less considered than *K. aberrans* and *T. pyri* in release experiments and IPM tactics (Duso, 2005).

GDM confirmed to be an alternative food for *A. andersoni* (Pozzebon and Duso, 2008). Demographic parameters exhibited by *A. andersoni* on this plant pathogen suggest that GDM has a major role for *A. andersoni* compared to *T. pyri* and *K. aberrans*. Field data are consistent with this assumption (Duso et al., 2003; Pozzebon et al., 2010).

Our results provide an overview of some potential food sources for three generalist predatory mites occurring on grapevine in Europe. Tydeids cannot contribute to sustain populations of these predatory mite species in absence of main prey. Among predatory mites inhabiting European vineyards only *Paraseiulus talbii* Athias-Henriot has proved to exploit *T. caudatus* as food source (Camporese and Duso, 1995; Duso et al., 2005). Since tydeids can prey upon eriophyids in vineyards (Schruft, 1972; Duso and de Lillo, 1996) predation by *P. talbii* could affect biocontrol of these herbivore mites.

The results of the present study provide a step forward in the knowledge on the role of plant pathogens for predatory mite population dynamics in vineyards. This study confirm previous

investigations where GDM was an alternative food source that meet the nutritional requirement for *T. pyri* and *A. andersoni* population persistence and increase (Pozzebon and Duso, 2008). Nevertheless, GDM can be considered as low quality alternative food source for these phytoseiids if compared with pollen. GDM resulted as supplementary food source for *K. aberrans* that meet the nutritional requirement only for development. A similar role is played by the grape powdery mildew *Uncinula necator* (Schw.) Burr., which results a supplementary food source for *T. pyri* and *A. andersoni* (Pozzebon et al., 2009). In these cases plant pathogens can be of importance for short term persistence in absence of more suitable food sources.

Among tested resources, *Typha* spp. pollen resulted a high quality food, more suitable than prey for *K. aberrans* and *T. pyri* and this phenomenon has been observed for other predatory mite species (e.g. McMurtry and Johnson, 1965). The higher value of pollen over the prey in terms of demographic parameters opens a discussion on the profile of *K. aberrans* and *T. pyri* in the framework of life-style types suggested by McMurtry and Croft (1997) as the latter authors considered both species as belonging to the life-style II. The response of generalist predatory mites to pollen can depend on pollen taxa and features, as well as experimental procedures. In previous investigations where *Mesembryanthemum criniflorum L.* pollen was used, the reproductive response of *T. pyri* to pollen or tetranychid mites (*P. ulmi* and *E. carpini*) was comparable while that of *A. andersoni* was higher on pollen than on *P. ulmi* (Duso and Camporese 1991). In another study no differences in the performance of *T. pyri* was observed between *Typha* pollen and *T. urticae* while the latter was the best food source for *A. andersoni* (Pozzebon et al., 2009). Engel and Ohnesorge (1992) tested different pollen taxa for their potential for population increase evidencing that not all types of pollen were suitable for population increase for *T. pyri*.

Different foods can co-occur on grapevine with possible influence on the performance of predatory mites as biocontrol agents. Predation pressure on the prey can be reduced in presence of alternative food sources as apple powdery mildew or *T. latifolia* pollen (Wei and Walde, 1997; Zemek, 2005). Optimal foraging theory would predict that a predator should prefer to forage on a more profitable food and switch to a less profitable food when the first is scarce, increasing the probability of predator persistence (Murdoch, 1969; van Baalen *et al.,* 2001). In this context, the relative quality and quantity of different food sources can influence the outcome of biological control (Srinivasu et al., 2007). In our study we used an *ad libitum* supply of different food resources focusing on their qualitative aspects. Life history parameters can provide an estimation of the relative quality/suitability of different food sources for predatory mites (Sabelis, 1985; Dicke et al., 1990). According to our results some problems for the predation of both tetranychids by *K.*

aberrans and *P. ulmi* by *T. pyri* may arise if *Typha* spp. pollen (or another valuable pollen taxon) is present at high levels on grapevine leaves. However, it should be stressed that the availability of fresh pollen at comparable levels of those used in this study is unlikely to occur in the field. Considering GDM, it should be noted that a positive effect of plant-pathogen infection was observed on herbivore mites in other crops (Filajdic et al., 1991; Reding et al., 2001; Fournier et al., 2004). Based on the comparison between life history parameters, results indicate that at high levels of herbivore mites, predatory mites should prefer to prey, switching to GDM only at low level of tetranychids.

The quality/suitability of different food sources can also be implied in interspecific interactions among predatory mites (e.g., Zhang and Croft 1996; Schausberger and Croft 2000). Predatory mites involved in this study can engage in competition and some of them can be displaced by others (e.g., Duso et al 1991; Duso and Vettorazzo, 1999). In vineyards in North-eastern Italy, *K. aberrans* resulted often dominant over *T. pyri* and *A. andersoni* (Duso, 1989; Duso and Pasqualetto, 1993; Duso and Vettorazzo, 1999; Camporese and Duso, 1996). Regarding the interactions among *T. pyri* and *A. andersoni*, the former competed successfully on grapevine varieties with pubescent leaves while the opposite occurred on varieties characterized by non-pubescent leaves (Duso and Pasqualetto, 1993: Camporese and Duso 1996; Duso and Vettorazzo, 1999). Laboratory experiments evidenced that *A. andersoni* was the more aggressive among the three predatory mite species (Schausberger and Croft 1999 and 2000). Leaf characteristics have been indicated as important factor in mediating intraguild interactions among predatory mites (Seelman et al., 2007). Indeed, trichome rich leaves provide favorable habitats for *K. aberrans* and *T. pyri* retention on plants but this factor seems to be less important for *A. andersoni* (Duso 1992; Duso et al., 2003; Kreiter et al., 2002; Loughner et al., 2008 and 2009; Duso et al., 2009). Phytoseiids with different efficiency in food conversion into egg biomass can exhibit a differential demographic response to the same resource. *A. andersoni* evidenced higher population increase capacity than other phytoseiids on *E. carpini*, *P. ulmi* and GDM, while this parameter was comparable to that of *T. pyri* when pollen was considered. In terms of r_m, *T. pyri* was always superior compared to *K. aberrans*. These results confirm previous field observations where GDM spread was associated to the competitive displacement of the other two species by *A. andersoni* (Duso et al., 2003; Duso et al., 2009). Predatory mite population abundance was associated to pollen availability (e.g., Engel and Ohnesorge, 1994; Duso et al., 1997 and 2004) and spider mites in vineyards (e.g., Camporese and Duso 1996; Duso and Pasqualetto, 1993; Duso and Vettorazzo, 1999) but not clear effects on

interspecific interactions among predators were observed. Further investigation are required to clarify the effect of supplemental food on intraguild interactions among predatory mites.

The information provided by this work can contribute to explain factors affecting population dynamics of predatory mites in vineyards with important implications on biological control of herbivore mites in vineyards (Duso and de Lillo, 1996; Schruft, 1985; Kreiter et al., 2000; Marshall and Lester, 2001). The persistence is the key factor explaining the success of these predatory mites, and they have to cope with a fluctuating availability of different food sources. The peculiar successful strategy of generalist predatory mites in perennial plant systems implied various factors that include alternative foods, leaf traits, spatial aggregation and metapopulation structure (Ivancich-Gambaro, 1973; Walde et al., 1992; Nyrop et al., 1998; Engel and Ohnesorge, 1994b; Duso et al., 1997; Lester et al., 2005). Capacity of population increase is crucial to the success of predatory mites (Sabelis, 1985). The knowledge of the relative quality/suitability of different food sources is of basic importance to understand the complex of interaction that shape the population dynamic of generalist predatory mites in response to the different resources.

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Chapter II

Feeding habits of overwintered predatory mites inhabiting European vineyards

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Introduction

In European temperate regions, plant mites enter into diapause before winter. In European vineyards located in these regions, predatory mites belonging to the family Phytoseiidae overwinter frequently under the bark, in crevices or other sites, sometimes together with tetranychids (e.g. *Eotetranychus carpini* (Oudemans)), tydeids (e.g. *Tydeus caudatus* Dugés), eriophyids (e.g., *Calepitrimerus vitis* Nalepa), other mites or insects (e.g. the thrips *Drepanothrips reuteri* Uzel). From late winter to early spring diapause terminates and predatory mites resume their activity feeding on various prey, pollen and other kinds of food (Veerman, 1992). Regarding spider mites, *E. carpini* females also overwinter in large numbers under bark crevices of vines. At vine sprouting *E. carpini* females move from these shelters to young shoots where they cause severe damage. Generalist predatory mites belonging to the family Phytoseiidae are key biocontrol agents of *E. carpini* in European vineyards. In some vineyards located in Northeastern Italy predation on spider mites (*E. carpini*) by phytoseiids was also observed in warmer days of late winter and eggs laid by overwintered predatory mites were found under the bark some days before sprouting (Duso, 1990). Predation on overwintering *E. carpini* females by predatory mites could accelerate the termination of diapause, reduce spider mite damage at sprouting and improve their response to spider mite populations in spring. Other foods, like pollen or fungi could have a role in diapause termination of predatory mites. Flowering of a number of arboreal plants also occurs in early spring and windborne pollen can spread over long distances from plant sources becoming potentially available for

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overwintered predatory mites (Boller et al. 1988; Weidmer and Boller, 1990). Knowledge on this topic is required to manage better these biocontrol agents.

Kampimodromus aberrans (Oudemans), *Amblyseius andersoni* (Chant) and *Typhlodromus pyri* Scheuten are the most frequent phytoseiid mites in vineyards in Northern Italian as well as in other temperate regions (Schruft, 1985; Duso, 1989; Kreiter et al., 2000; Duso et al., 2009). Some aspects of their overwintering in vineyards (e.g., migration to overwintering sites, preference for these sites, termination of diapause, feeding behaviour) have been matter of preliminary investigations (Duso, 1990). In the present work, the feeding habits of *K. aberrans*, *A. andersoni* and *T. pyri* on *E. carpini* overwintered females were investigated. Biological parameters of predatory mites fed with *E. carpini* or pollen were studied in the laboratory; prey consumption and prey conversion into egg biomass were also investigated.

Materials and Methods

Mite sources and rearing methods

Two-year old branches were collected in late winter from some vineyards located in the Veneto region (Treviso and Verona districts) and inhabited by *K. aberrans, A. andersoni* or *T. pyri*. Branches were brought in the laboratory where overwintering predatory mite females were transferred into rearing units. The latter were realized using a piece of sponge placed in a middle of a plastic box containing water and covered with cotton. Portions of grapevine leaves were put on the cotton layer. Wetted paper strips and a glue barrier surrounded leaf portions to avoid mite escape. Leaf portions were renewed every week. *Eotetranychus carpini* overwintered females were collected from branches or bark portions removed from infested vineyards. Pollen of *Carpinus betulus* L. was considered as a reference treatment. A group of predatory mite females were maintained without food as a control.

Increasing prey densities (1, 2, 4 and 8 *E. carpini* females) were offered to predators daily. Each treatment comprised at least 40 predatory mite females. Observations were carried out daily at the same hour and females were checked until their death. Those escaped from rearing units or drowned were excluded from the statistical analysis. All the experiments were performed at 25±2°C R.H. 70-90 %, photoperiod 16L : 8D. The numbers of prey consumed and eggs laid by predatory females were recorded. Eggs and dead spider mites were removed at each observation. The first laid

egg was considered as a sign of diapause termination. Aged pollen was periodically removed to avoid fungi and fresh pollen was added twice per week (about 0.2 g for application).

Statistical analysis

We analyzed the effects of different foods and prey densities on prey consumption (Number of *E. carpini* per female), time required to terminate diapause (days), longevity (days), fecundity (eggs/female and eggs/female/day) and conversion of prey into egg biomass (number of prey per egg) with one-way ANOVA using the GLM procedure of SAS (SAS Institute Inc., 1999) and applying the Tukey–Kramer's test (a = 0.05) to evaluate the differences among means.

We analyzed the effects of treatments on the percentage of ovipositing on total females applying a logit model and using the GENMOD procedure of SAS (SAS Institute Inc., 1999).

Results

Kampimodromus aberrans

Predatory mite longevity differed among treatments ($F_{5, 135}$=668.47; P<0.001). Predators kept without food showed the lowest longevity, those fed with *E. carpini* the longest (Fig. 1). The longevity on pollen was frequently lower than that on prey. Among *E. carpini* treatments longevity was higher when two or four prey were offered instead of one.

Predators kept without food did not lay eggs. A high proportion of ovipositing predators were found in pollen treatment than on *E. carpini* treatments (χ^2 = 127.55; df = 5; P < 0.001; Fig. 2).

Food affected the time required to terminate the diapause ($F_{4, 97}$=49.83; P<0.001). The effect of pollen and the highest prey offer (4 or 8) were similar (Fig. 3). Lower prey supplies resulted in longest times.

The oviposition times differed among treatments ($F_{4, 97}$ =22.08; P<0.001). They were shorter on the minimal prey supply or pollen treatments than on the remaining ones (Fig. 4).

The total and daily fecundity were affected by food ($F_{4, 97}$=110.07; P<0.001; $F_{4, 97}$=65.00; P<0.001; respectively). The total fecundity increased with prey offer (Fig. 5). The same trend was found for daily oviposition (Fig. 6). Predators fed with pollen exhibited a fecundity similar to those receiving 4 or 8 prey (Fig. 5, 6).

Total prey consumption ($F_{3, 79}$=181.25; P<0.001) increased with prey supply (Fig. 7). A similar pattern occurred with daily prey consumption in the predator life span ($F_{3, 79}$=165.69;

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$P<0.001$; fig. 8). Prey consumption during preoviposition followed similar trends but there no differences when 2 or 4 prey were offered (total consumption: $F_{3, 79}=9.11$; $P<0.001$; daily consumption: $F_{3, 79}=22.34$; $P<0.001$) (fig 9, 10). Prey consumption during oviposition reflected the tendencies seen for total prey consumption (total consumption: $F_{3, 79}=204.80$; $P<0.001$; daily consumption $F_{3, 79}=115.21$; $P<0.001$) (fig 11, 12).

The proportion of prey consumed over the number of prey offered decreased with prey density ($F_{3, 79}=173.91$; $P<0.001$; Fig. 13).

The ratio between the total number of prey and the total number of eggs differed among treatments ($F_{3, 79}=7.96$; $P<0.001$). The lowest figures were obtained at the lowest prey supply and vice versa (Fig. 14). When one prey was offered predatory mites consumed about 1.54 prey to lay one egg; when eight prey were offered these figures increased to about 1.96 prey per egg (fig. 13).

Typhlodromus pyri

Longevity was affected by different treatments ($F_{5, 146}=823.33$; $P<0.001$) and in particular was higher when predators were fed with pollen or with 4 prey per day. The lowest figures were seen for predators kept without food followed by those receiving one prey per day. The remaining treatments were intermediate (Fig. 1).

The percentage of ovipositing females did not differ among pollen or *E. carpini* treatments ($\chi^2 = 107.71$; df = 5; $P < 0.001$; Fig. 2). Predators kept without food did not lay eggs.

Food affected diapause termination ($F_{4, 114}=24.48$; $P<0.001$). Times decreased with prey offer and pollen values were intermediate (Fig. 3).

Oviposition times were influenced by food ($F_{4, 116} =30.02$; $P<0.001$). They were low at the lowest prey offer and increased with prey offer. The highest times were recorded when pollen or 4 prey were supplied (Fig. 4).

The total and daily fecundity were affected by food ($F_{4, 114}=172.72$; $P<0.001$; $F_{4, 114}=146.28$; $P<0.001$; respectively). They increased with prey (except from 4 to 8 prey in total fecundity) offer while pollen showed intermediate values (Fig. 5, 6).

Total prey consumption and daily prey consumption followed similar trends ($F_{3, 87}=479.55$; $P<0.001$; $F_{3, 87}=469.01$; $P<0.001$; respectively, Fig. 7, 8). Prey consumption in pre-oviposition (total consumption: $F_{3, 87}=45.21$; $P<0.001$; daily consumption: $F_{3, 87}=79.65$; $P<0.001$) (fig 9, 10), and oviposition (total consumption: $F_{3, 87}=417.46$; $P<0.001$; daily consumption $F_{3, 87}=310.18$; $P<0.001$) reflected these tendencies with some variation (Fig. 11, 12).

The proportion of prey consumed over the total number of offered prey decreased with prey density ($F_{3, 87}$=184.88; P<0.001). There were no differences between treatments where 1 or 2 prey were supplied (Fig. 13).

The ratio between the total number of prey and the total number of eggs differed among treatments ($F_{3, 87}$=5.33; P=0.002). Figures were lower when 1, 2 or 4 prey were supplied compared to the highest prey supply (Fig. 14). In this case predators consumed about 2.02 prey per egg while at 1, 2, or 4 prey densities this parameter declined to 1.81, 1.73 and 1.70 respectively.

Amblyseius andersoni

Longevity of *A. andersoni* overwintered females was affected by food ($F_{5, 67}$=201.68; P<0.001). Longevity reached the highest values when pollen or 2 prey per day were provided and the lowest in predators kept without food or with 1 prey per day (Fig. 1). The remaining treatments gave intermediate results.

Predatory females kept without food did not oviposit. Food type had no effects on the percentage of ovipositing females (χ^2 = 18.51; df = 5; P = 0.002; Fig. 2).

Time required to terminate diapause declined when the highest prey offers or pollen were considered ($F_{4, 64}$=25.27; P<0.001) (Fig. 3).

Oviposition times were affected by food ($F_{4, 64}$=48.38; P<0.001). They were longer with pollen or when 4 prey were supplied and shorter when one prey was provided. Results were similar when 2, 4 or 8 prey were supplied (Fig. 4).

Total fecundity as well as daily fecundity were affected by treatments ($F_{4, 64}$=189.83; P<0.001; $F_{4, 64}$=101.18; P<0.001; respectively). They were higher in predators fed with pollen or 4-8 prey. Fecundity declined significantly reducing prey numbers (Fig. 5, 6).

Total and daily prey consumption differed among treatments increasing with prey density ($F_{3, 40}$=202.74; P<0.001; $F_{3, 40}$=131.40; P<0.001, respectively; Fig. 7, 8). Trends in prey consumption during pre-oviposition (total consumption: $F_{3, 40}$=5.43; P=0.003; daily consumption: $F_{3, 40}$=16.77; P<0.001; Fig. 9, 10) and oviposition were similar (total consumption: $F_{3, 40}$=160.56; P<0.001; daily consumption ($F_{3, 40}$=99.17; P<0.001; Fig. 11, 12). In some comparisons differences were not significant when 2 or 4 prey were provided.

The proportion of prey consumed over prey offered declined with prey density ($F_{3, 40}$=166.81; P<0.001) but there were no differences when 1 or 2 prey were provided (Fig. 13).

The number of consumed prey per egg differed among treatments ($F_{3, 40}$=47.59; P<0.001). The lowest values (about 2.17) were calculated when 4 prey was provided and the highest (about

4.97) when 1 prey was supplied. The remaining treatments gave intermediate values (3.84 and 2.50 at 2 and 8 prey offer, respectively; Fig. 14).
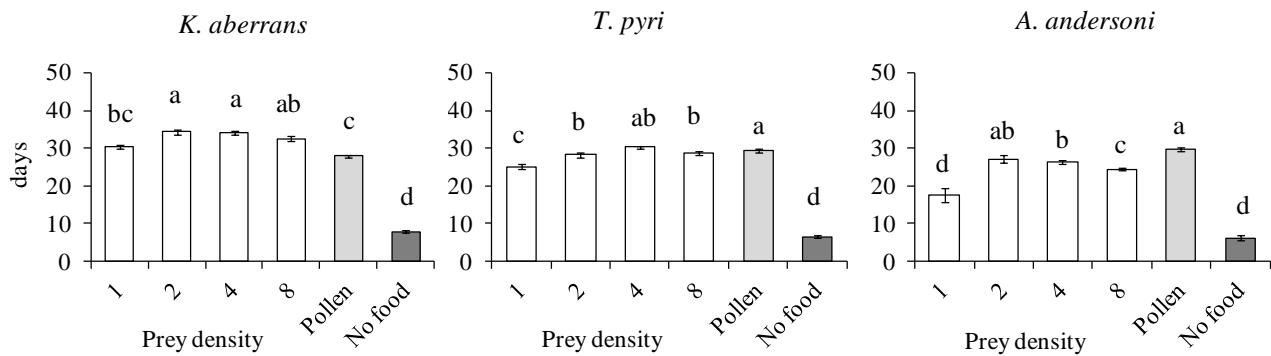
Figure 1. Longevity (mean±S.E.) of predatory mites on different foods and prey density. Different letters indicate significant differences (*P*=0.05).
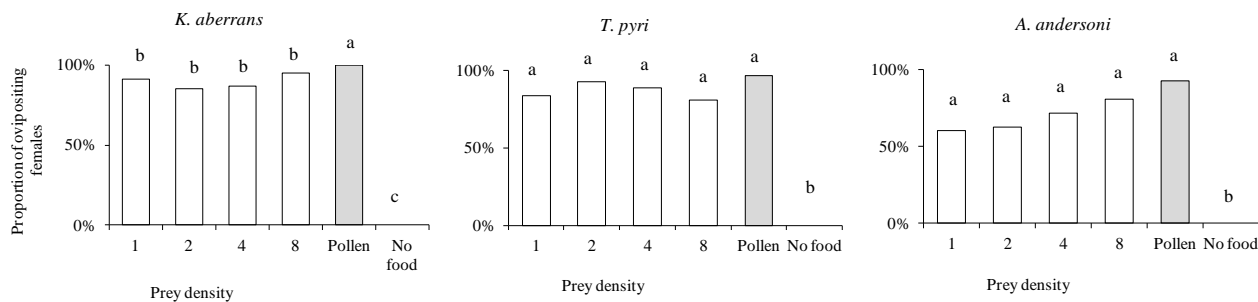
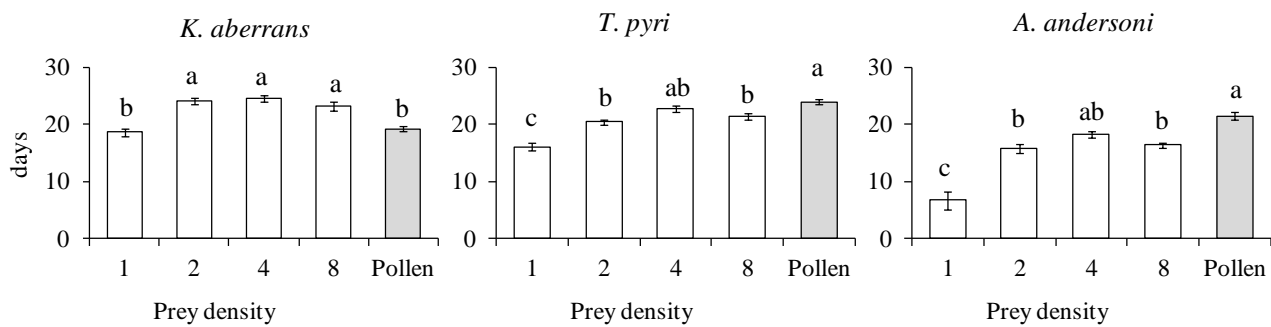**Figure 2.** Percentage of ovipositing predatory mites (on the total number) on different treatments. Different letters indicate different differences (*P*=0.05).
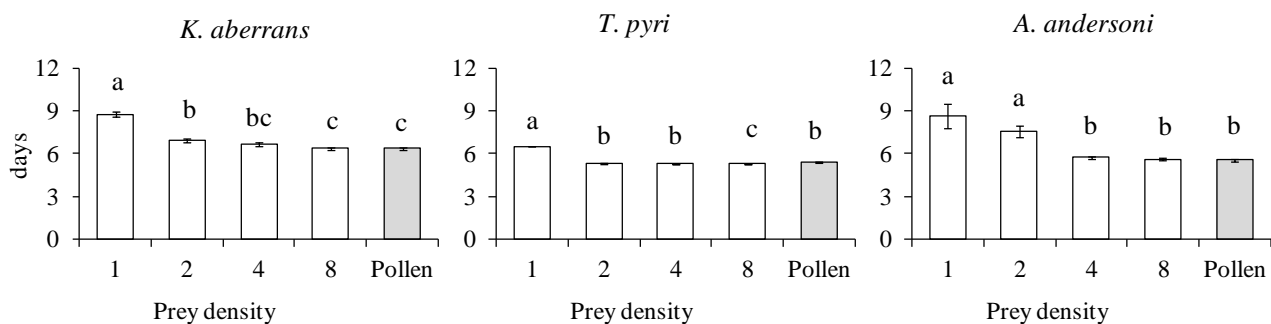
Figure 3. Time required for diapause termination (mean±S.E.) in predatory mites fed with different foods and prey densities. Different letters indicate significant differences (*P*=0.05).

Figure 4. Oviposition times (mean±S.E.) observed in predatory mites fed with different foods and prey densities. Different letters indicate significant differences (*P*=0.05).
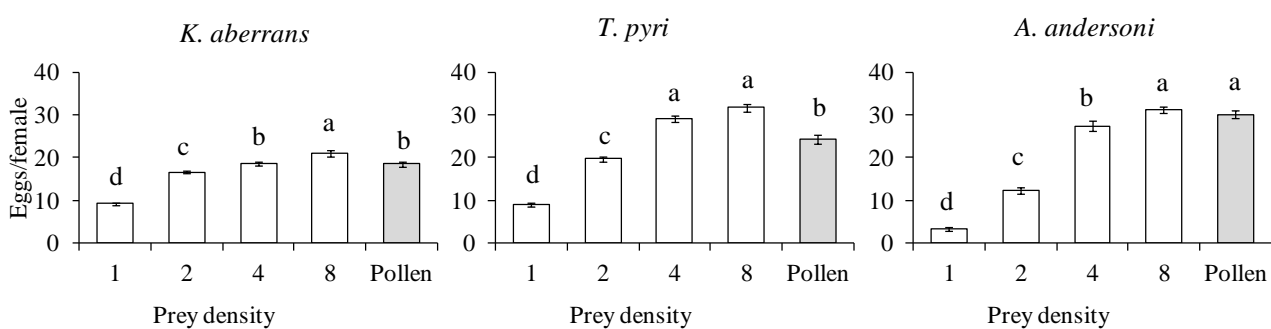
Figure 5. Fecundity (mean±S.E.) of overwintering females on different foods and prey density. Different letters indicate significant differences (*P*=0.05).
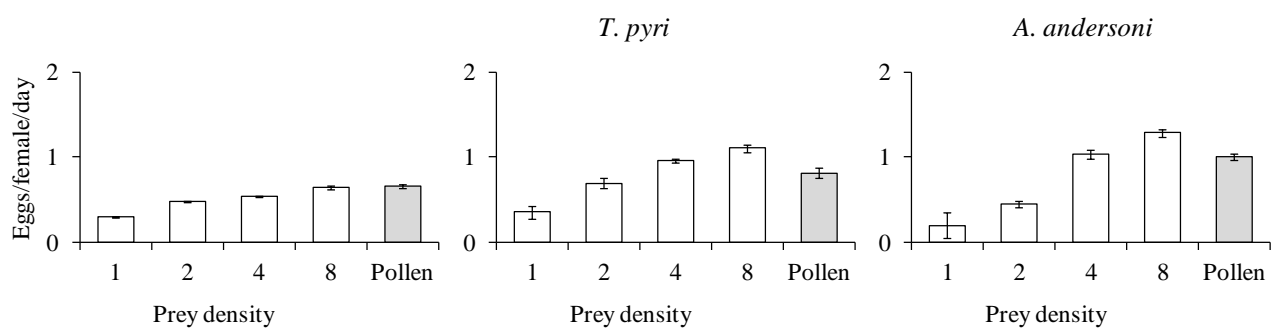
Figure 6 Daily fecundity (mean±S.E.) of overwintering females on different foods and prey density. Different letters indicate significant differences (*P*=0.05).

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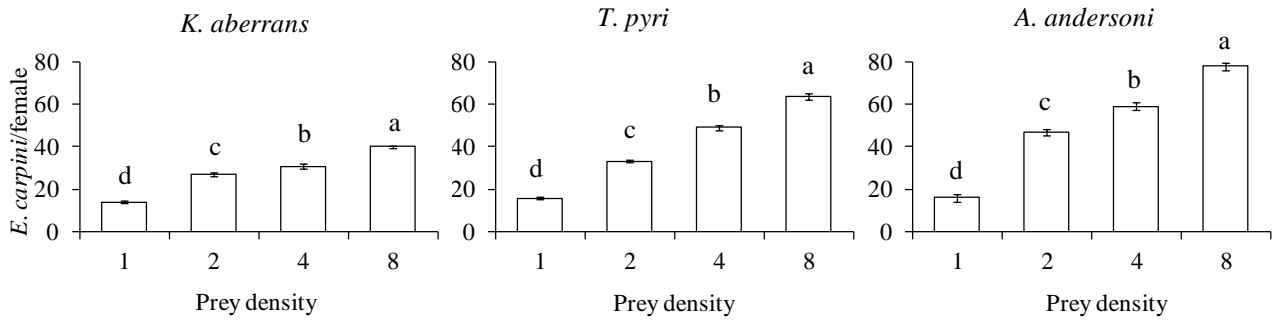
Figure 7. Total prey consumption (mean±S.E.) during life span by predators reared at different prey densities. Different letters indicate significant differences (*P*=0.05).
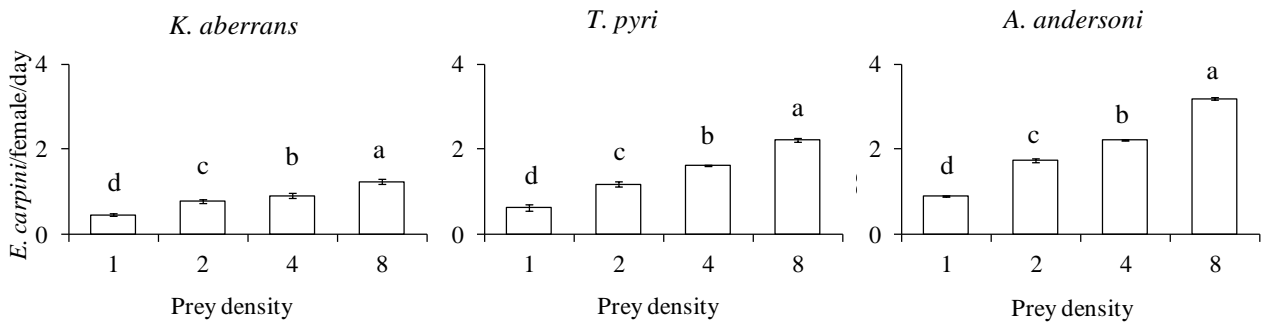
Figure 8. Daily prey consumption (mean±S.E.) during life span by predators reared at different prey densities. Different letters indicate significant differences (*P*=0.05).
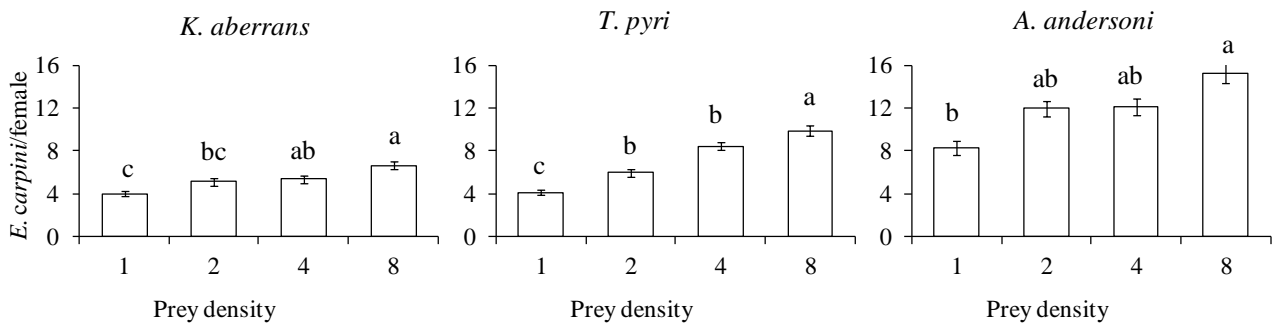
Figure 9. Total prey consumption (mean±S.E.) during pre-oviposition by predators reared at different prey densities. Different letters indicate significant differences (*P*=0.05).
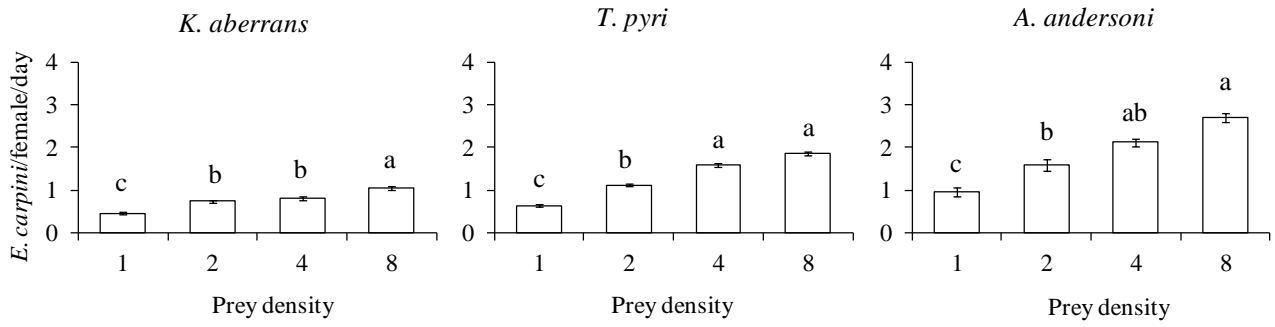
Figure 10 Daily prey consumption (mean±S.E.) during pre-oviposition by predators reared at different prey densities. Different letters indicate significant differences (*P*=0.05).
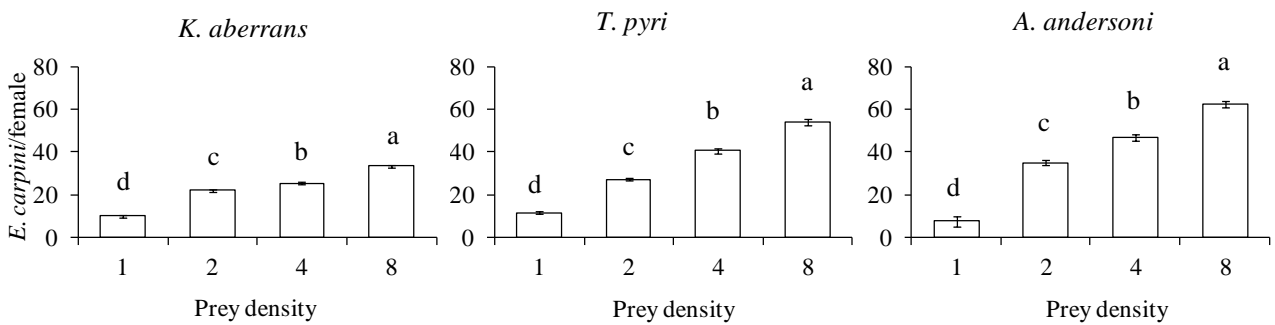
Figure 11 Total prey consumption (mean±S.E.) during oviposition by predators reared at different prey densities. Different letters indicate significant differences (*P*=0.05).
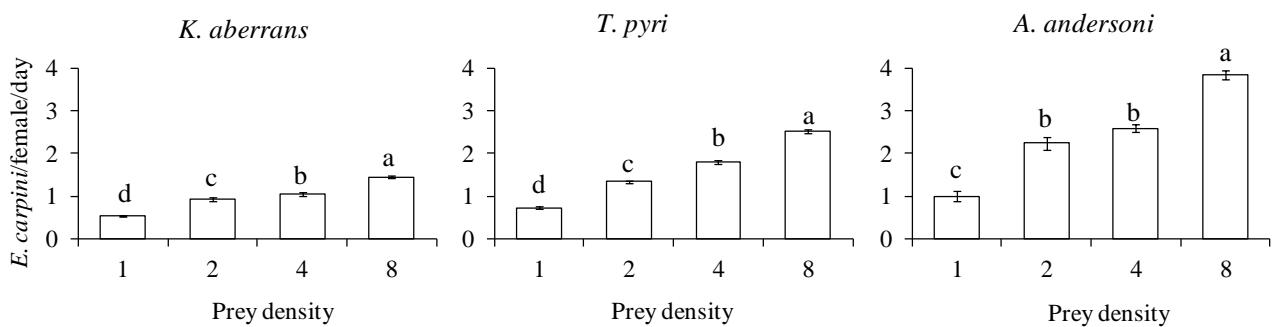
Figure 12. Daily prey consumption (mean±S.E.) during oviposition by predators reared at different prey densities. Different letters indicate significant differences (*P*=0.05).

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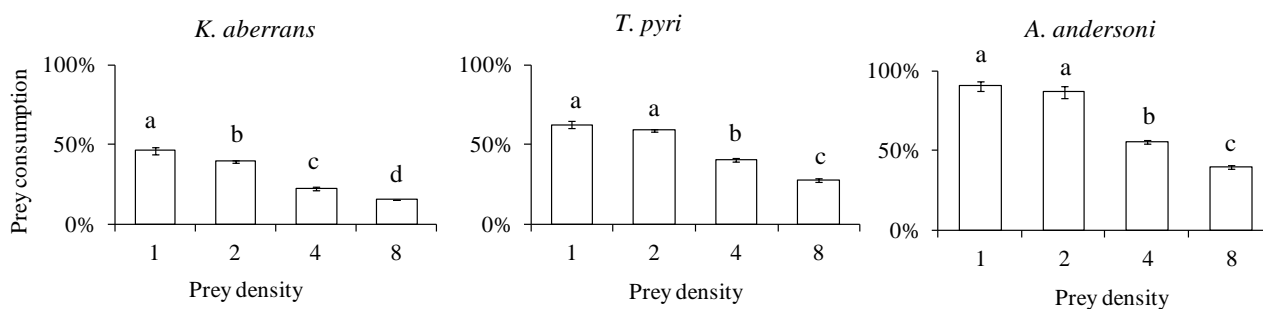
Figure 13. Proportion (mean±S.E.) of consumed prey over the number of prey offered to predators reared at different prey densities. Different letters indicate significant differences (*P*=0.05).
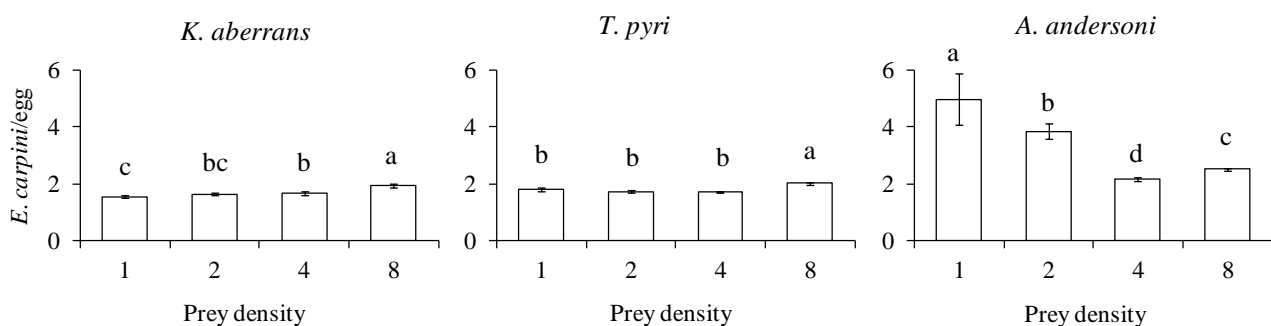
Figure 14. Ratio between the number of prey consumed and eggs laid by predators reared at different prey densities (mean±S.E.). Different letters indicate significant differences (*P*=0.05).

Discussion

In early spring overwintered predatory mites terminate their diapause. At the grapevine sprouting they leave their shelters and move to young leaves. Environmental conditions or agricultural practices affecting this delicate phase of phytoseiid colonization can produce severe effects on their persistence and subsequently on the outcome of biological control. The fate of overwintered phytoseiids also depends on food availability. Food sources tested in this study (*E. carpini* and hornbeam pollen) are realistically available for overwintered predatory mites in North-Italian vineyards. In these regions overwintered *E. carpini* females move to the new vegetation in April and hornbeam flowering takes place in the same period. In the present study we tried to simulate these conditions by transferring predatory mites to the laboratory in conditions of temperature and day length favorable to the termination of diapause. We provided pollen or prey and simulated different prey/predator ratios in order to compare the response by different predatory

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mite species to *E. carpini* different densities. Both food sources proved to be optimal for overwintered predatory mites. On the other hand longevity dramatically decreased and oviposition did not occur in predatory mites kept without food. This result stresses about overwintered predatory mite requirements in early spring.

Several predatory mite parameters were affected by food sources with some variation among the three predatory mite species. Pollen confirmed to be an optimal food for *K. aberrans*, *A. andersoni* and *T. pyri* (e.g., Dosse, 1961; Overmeer, 1985; Addison et al., 2000; Duso et al., 1997, 2004; Kasap, 2005; Roda et al., 2003). In most comparisons pollen induced performances comparable to those reported when predatory mites were supplied with moderate to high (4-8) prey numbers. *Typhlodromus pyri* and *A. andersoni* females survived longer when fed on pollen and the percentage of *K. aberrans* ovipositing females was higher when pollen was supplied instead of spider mite prey. For *K. aberrans* and *T. pyri*, times to terminate diapause were comparable when pollen or 8 prey were provided. Oviposition times were also high for predators fed with pollen. Total fecundity of *A. andersoni* was comparable when pollen or 8 prey were supplied while prey offer obtained better results than pollen on *K. aberrans* and *T. pyri*. However in *K. aberrans* daily fecundity was similar when predators were fed with pollen or 8 prey. The amount of pollen supplied was considered to be *ad libitum* and thus a different outcome could be observed when pollen availability is low in field conditions. Moreover, we tested a single pollen type while there is a variability in the effect of pollen types on predatory mites (Engel and Onhesorge, 1994a,b; Boller and Frey, 1990; Bouras and Papadoulis, 2005; Bermudez et al., 2010). We can conclude that overwintered predatory mites can overcome problems associated with prey scarcity if pollen is largely available on grapevine leaves. In this framework, hedgerows or stand margins contiguous to vineyards can act an important role. The latter should be considered when new hedgerows are planned in farms, in particular those involved in organic agriculture.

The response of predatory mites to different prey offer depended on species. It showed common trends among predatory mites but also some differences. Increasing prey density prolonged the longevity of *A. andersoni* and *T. pyri* but this effect was not clear for *K. aberrans*. The percentage of ovipositing females over the total number of tested females was not affected by prey offer. However, increasing prey offer reduced times to terminate the diapause in predatory mite species. Regarding *K. aberrans* this result was obtained even with a 4 prey offer. Oviposition times increased with prey offer in all species but for *K. aberrans* this effect was seen already at 2 prey offer. Total and daily fecundity increased with prey density following the same trend for the

three predatory mites. A similar experiment conducted providing increasing *E. carpini* numbers to summer females of *T. pyri* and *A. andersoni* showed similar results (Duso and Camporese, 1991). The total fecundity of *A. andersoni, T. pyri* and *K. aberrans* overwintered predators observed at 8 prey density appeared greater than that observed for summer females of the same species kept with a surplus of summer *E. carpini* females (this thesis, chapter I). Indeed only 24.20 eggs were laid by summer females of *A. andersoni* during total life span rater than 31.34 eggs of overwintered females. Similar differences were found in total fecundityof *T. pyri* that reached 21.50 eggs in case of summer females and 31.75 eggs in case of overwintered females. An increase has been observed even for *K. aberrans* that showed a fecundity of 14.90 eggs/female in summer experiments in contrast with 21 eggs/female of overwintered female. In all cases increase has been of about 30% of eggs per female. Furthermore the oviposition time was longer in case of summer young female. Rearing conditions of two experiments were almost the same and cannot explain the differences in fecundity rate. The only difference, further than type of phytoseiids females, was the prey phenologic phase.The females of *E. carpini* in dormant phase could have a greater nutritional value than summer females. The latter appear to defend themselves better from predators that save energy.

The number of prey consumed during the lifespan had the same tendency among the three predatory mites. There were some differences when prey consumption during pre-oviposition was considered because only *T. pyri* consumed more prey at the maximum prey offer. However, daily prey consumption during pre-oviposition and oviposition followed trends reported for the overall life span. The proportion of consumed over total prey declined with prey offer as expected. For *A. andersoni* and *T. pyri* an effect of satiation was observed when 4 prey were supplied while for *K. aberrans* this occurred at 2 prey offer. The ratio between the number of prey consumed during oviposition and eggs laid by predators can reflect the efficiency to convert prey in egg biomass. In this case trends differed among predators. *Kampimodromus aberrans* showed the lowest values at the lowest prey offer. *Typhlodromus pyri* did the same but there were no differences between 1, 2 and 4 prey densities. Regarding *A. andersoni* the highest values were associated with the lowest prey offer and the optimal ratio was obtained at the 4 prey offer.

The results obtained in these experiments can help in understanding some aspects of the behavior of the three predatory mites in vineyards. They confirm that *K. aberrans, T. pyri* and *A. andersoni* can survive and reproduce on pollen when prey is scarce; at the same time their phenology can reflect that of windborne pollen (Duso et al., 1997, 2000). Interactions between predatory mites and *E. carpini* in field conditions showed different outcomes. *Kampimodromus aberrans* and *T. pyri*

responded to spider mite population increases and proved to be effective in controlling its densities. The response by *A. andersoni* to *E. carpini* was less clear and failures in controlling the mite pest have been reported (Duso and Vettorazzo, 1999; chapter IV of this thesis). The three predatory mites can respond to volatiles emitted by *E. carpini* infested leaves. Moreover, their voracity and fecundity increased with prey offer. The scarcity of *E. carpini* (one prey offer) reduced drastically predatory mite oviposition but this effect was greater for *A. andersoni*. This can explain the low persistence of *A. andersoni* populations when prey diminishes in vineyards (Duso et al., 2009). *Kampimodromus aberrans* required a low amount of prey to lay eggs confirming its capacity to survive and reproduce in conditions of prey scarcity. *Typhlodromus pyri* requirements were slightly higher than the latter. The ratio between the number of prey consumed by phytoseiids and the number of laid eggs can be used as an index of conversion of food into egg biomass. *Amblyseius andersoni* has a large body size compared to *K. aberrans* and *T. pyri*. Therefore it requires more energy for maintenance than other species and thus has to search food more continuously. Food scarcity represents a great limit for the persistence of this predator in perennial crops.

Predation activity of phytoseiids in early spring has been poorly investigated despite its importance for population establishment in vineyards and perennial crops. Moreover, damage caused by *E. carpini* in spring can be serious because overwintered females are concentrated on few leaves. Shoot growth is affected, leaves are reduced in size and mesophyll tissues altered. The use of acaricides is frequent in these occasions. However, the role of generalist predatory mites can reduce E. carpini populations effectively. Results of the current study can improve the knowledge of these biocontrol agents in order to preserve their role in conservation biological control tactics.

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Chapter III

Olfactory response of the predatory mites *Amblyseius andersoni*, *Kampimodromus aberrans* and *Typhlodromus pyri* to different volatiles

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Introduction

Arthropods can use volatile cues to interact with the environment where they live in. A large variety of functions are piloted by volatile cues, for example they can lead herbivores in host plant searching (de Kogel et al., 1999; Choh and Takabayashi, 2007) or can permit the aggregation for mating or defense (Eller et al., 1994; Barnes and Capatos, 1989; van Tol et al., 2004). In case of carnivores, chemicals produced by prey or host plants absolve the important function of prey detecting guide (Shimoda et al., 1997; Venzon et al., 1999; van Tol et al., 2002; Milonas et al., 2009; Moayeri et al., 2006a,b, 2007; Mochizuchi and Yano, 2007; Yoneya et al., 2009).

The ability of arthropods to detect and recognize volatile cues is the consequence of evolution. This is clearly understandable when food source searching is considered: individuals that find and consume a high quantitative and qualitative level of food can reach a higher fecundity; food volatile cues detection can increase the probability of success in food searching and consequently get up the reproduction rate. How much arthropods are able to discriminate between different volatile sources and choose for suitable foods so much their reproductive success increases. In case of carnivores, they can face different prey on the same plant or environment. If one of prey species allows higher performance than others in term of intrinsic rate of population increase, individuals choosing such one can take more advantage. Probably during their evolution herbivores evolved the ability to minimize volatiles production to reduce the predator detection. At the same time, plants produce

cues in response of prey injures that can attract carnivores and, in this way plants ensure themselves a indirect protection (Dicke and Sabelis, 1988b; Dicke and Vet, 1999; Janssen et al., 2002).

Cues produced by plants are synomones, substances favorable to both receiver and emitter, instead prey cues are kairomones, substances favorable to receiver but not to the emitter. Another category of volatiles are the allomones which are favorable only to the emitter, while pherormones are used in intraspecific communication (Dicke and Sabelis, 1988a).

The capacity of predators to detect substances emitted by herbivores and/or by their infested plants has been recorded even for some predatory mites belonging to the family Phytoseiidae. A large number of studies have focused on predatory mites more or less specialized on *Tetranychus urticae* Koch, e.g. *Phytoseiulus persimilis* Athias-Henriot (e.g., Dicke and Sabelis, 1988; Sabelis and van der Weel, 1993; Morgalies et al., 1997; Shimoda and Dicke, 1999; Takabayashi et al., 2000; Pels and Sabelis 2000; Horiuchi et al., 2003; van Wijk et al., 2006; van Wijk et al., 2008; Yano and Osakabe, 2009; Sznajder et al., 2010) and *Neoseiulus californicus* (McGregor) (Shimoda et al., 2005; Shimoda, 2010). Both species were attracted by volatiles getting from plants infested by *T. urticae*. A few papers deal with generalist predatory mites. Information is available for *Amblyseius andersoni* (Chant), *Typhlodromus pyri* Scheuten and *Euseius finlandicus* (Oudemans). In Y-tube olfactometer experiments, the three species showed a positive response to volatiles coming from mite infested leaves, either in case of tetranychid mites *Panonychus ulmi* (Koch) and *T. urticae* or of the rust mite *Aculus schlechtendali* (Nalepa). Predatory mites were even able to choose among volatile cues and select the more suitable prey when volatiles of two different prey were contemporary offered them even if mite diet, starvation time and prey density level affected this ability (Dicke, 1988; Dicke and De Jong, 1986, 1988; Dicke et al., 1986).

Some volatile compounds attracting predators have been identified and tested in olfactometer experiments (Dicke et al., 1993; Takabayashi et al., 1994; De Boer and Dicke, 2004a,b; De Boer et al., 2004, 2008; Shimoda et al., 2005; Munn et al., 2008; Kappers et al., 2010). The most important among them appeared to be volatiles produced by mite infested plants. Indeed in some experiments predators were not attracted by prey alone as well as by infested plants (Sabelis and Van de Baan, 1983; Sabelis et al., 1984).

Plants can produce so called herbivore induced plant volatiles (HIPVs). They can also produce similar compounds when they are mechanically damaged, so-called green leaf volatiles (GLVs). However, the response by carnivores to them is low or absent (Sabelis and Van de Baan, 1983). Knowledge on chemical compounds produced by fungi and/or their host infected plant is limited,

and little is known on the relationships between plant-pathogens and predatory mite olfactory response.

The attracting volatiles vary in relation to herbivores species on the same plant species, in fact predatory mites can discriminate between different prey and choose the more suitable one (Fadini et al., 2010). Hence volatiles can been considered as a result of the interaction between plants and their pests (Dicke et al., 1988). Generally attraction is determined by a group of molecules while each single chemical component does not have a high attractive effect to predators (van Wijk et al., 2006). However response can be different among predators and even among populations of the same species (Maeda et al., 2001; Gardiner et al., 2005). Thus the prediction needs studies performed on specific predator-plant-pest combinations. Comprehension of such relation has clear consequences even on biological control efficiency. Predators able in detecting prey have more possibilities to find a food source that allows reproduction and offspring development, and to displace less effective competitors that share the same prey.

Knowledge on the role of volatile cues in grape tritrophic interactions involving herbivore and predatory mites is limited. Studies conducted on *A. andersoni* and *T. pyri* in relation to mites occurring in apple orchards as well in vineyards (e.g. *P. ulmi*) can provide some suggestions (Sabelis and Van de Baan, 1983; Dicke et al., 1986; Dicke, 1986, 1988; Koveos et al, 1995). However, no studies have been conducted on important predatory mites such as *Kampimodromus aberrans* (Oudemans) or grape mite pests such as *Eotetranychus carpini* (Oudemans). Generalist phytoseiids can feed on alternative foods other than their main prey, such as pollen or fungi (Duso and Camporese, 1991; Kasap 2005; Bermudez et al., 2010; Zemek and Prerenova, 1997; Pozzebon and Duso, 2008; Pozzebon et al., 2009) but little is known on the possibility that alternative food sources can produce stimuli attracting predatory mites. The results of experiments aimed at evaluating the olfactory response by important predatory mites occurring in European vineyards to different stimuli coming from leaves infested by spider mites or infected by Grape downy mildew (*Plasmopara viticola* Berk. et Curtis ex. de Bary) are here reported.

Materials and Methods

Mite rearing

The predatory mites *A. andersoni, K. aberrans* and *T. pyri* were collected in commercial vineyards located in the Veneto region (North-Eastern Italy) and transferred to the laboratory to be

reared. For mite rearing grape leaves were placed on a sponge covered with a layer of wetted cotton in a plastic box. Leaves were surrounded with paper strips and glue to avoid mite escape and pollen was used as food source. Rearing units were kept at constant climatic conditions (25 ± 10 °c and 80 ± 10 % R.H.).

Y tube olfactometer experimental procedure

A glass Y tube olfactometer similar to that used by Sabelis and Van de Baan (1983) was used to test the response of the three predatory mite species to volatiles produced by different sources. The olfactometer was composed by a Y shape glass tube within a metal wire of Y shape passing in the middle of the tube, and parallel to the tube walls. The Y tube was placed at the center of a black table, and a lamp illuminated the Y junction of olfactometer to reduce disturbs. Vacuum was placed at the end of the base of the olfactometer in order to create a laminar air flow in the two arms. Arms were connected with glass bottles using plastic tubes. Glass bottles were used as containers of leaf samples. Air suction was positioned outside of laboratory room and connected to a filter where air passed through distillated water. From the filter the air flow was split in two tubes connected with a glass bottle. Airflow was tested with a flow meter positioned between the end of olfactometer and the vacuum. Two leaves of the same treatment were placed in each bottle, they were similar in size and age and represented the source of volatile cues.

Before starting with the tests some coetaneous and conspecific predatory mite females (about one week old) where isolated on a rearing unit and maintained in starvation for 24 h. At the start of the tests, predatory females were positioned with a fine brush on the iron wire at the base of Y tube and then the tube was closed and connected to vacuum. Predatory mites movements were monitored, in particular the response was considered as positive when they pass over at least four centimeters after the divergence and run along one of two wire branches. Individuals which did not choose between the two arms within five minutes were removed and they were not considered in the statistical analysis. After each run the Y-shaped wire was extracted and washed with acetone. Y tube glass was also cleaned using acetone. After 10 runs the location of cue sources was switched. Leaves were replaced after 20 runs.

Volatile sources

Predatory mites were tested for the following olfactory cues: *E. carpini* and *P. ulmi* infested leaves and leaves showing sporangia of *P. viticola*. Healthy and clean grape leaves were used as white. All leaves used in experiments belonged to the cultivar (Glera)

Leaves infested by *E. carpini* or *P. ulmi* were collected in infested vineyards and used for experiments in the same day of collection or one day later. Leaves inhabited by about 40 spider mite motile stages were selected. The presence of predators was a criterion to discard leaves.

Leaves infected by *P. viticola* were collected from vines where mites were virtually absent. They were inspected under a dissecting microscope to confirm this assumption. Leaves with fresh sporulating sporangia of *P. viticola* were selected for experiments.

The following comparisons were tested:

1. *E. carpini* infested leaves **versus** clean leaves;
2. *P. ulmi* infested leaves **versus** clean leaves;
3. *P. ulmi* infested leaves **versus** *E. carpini* infested leaves;
4. *P. viticola* infected leaves **versus** clean leaves;
5. *E. carpini* infested leaves **versus** *P. viticola* infected leaves;
6. *P. ulmi* infested leaves **versus** *P. viticola* infected leaves;

Each comparison was repeated with 60 predatory mites.

Statistical analysis

Data were analyzed with the logistic regression model using the GENMOD procedure of S.A.S. program version 9.1. Wald chi-square test was used to analyze the differences in the choice distributions of predators across treatments (p = 0.05). Predators that did not make a choice were excluded from the analysis.

Results

All predatory mite species showed a significant preference for leaves infested by *E. carpini*(Fig. 1). *Amblyseius andersoni* and *T. pyri* responded positively to volatiles emitted by leaves infested by *P. ulmi* in contrast with *K. aberrans* (Fig. 2). When *P. ulmi* and *E. carpini* infested leaves were placed simultaneously in the opposite arms of olfactometer, *K. aberrans* moved significantly to the arm containing *E. carpini* infested leaves while *A. andersoni* and *T. pyri* did not exhibit a clear preference between the two arms (Fig. 3).

Plasmopara viticola infected leaves were attractive for *A. andersoni* and *T. pyri* while *K. aberrans* did not exhibit a clear response (Fig. 4). When *P. viticola* infected leaves and *E. carpini* infested leaves were compared a significantly higher proportion of *K. aberrans* preferred to move in

direction of spider mites, while the remaining predatory mite species did not exhibit any preference (Fig. 5). In the comparison between *P. viticola* infected leaves and *P. ulmi* infested leaves, *A. andersoni* and *T. pyri* showed a preference for the mite prey. In contrast, *K. aberrans* females did not choose one of these treatments (Fig.6).

Response to *E. carpini*

Mites choosing clean leaves

| 100% | 50% | 0% |

K. aberrans   n = 40; χ^2 = 27.74; P < 0.001**

T. pyri   n = 40; χ^2 = 30.63; P < 0.001**

A. andersoni   n = 40; χ^2 = 14.99; P < 0.001**

| 0% | 50% | 100% |

Mites choosing *E. carpini* infested leaves

■ *E. carpini*   □ *Clean leaves*
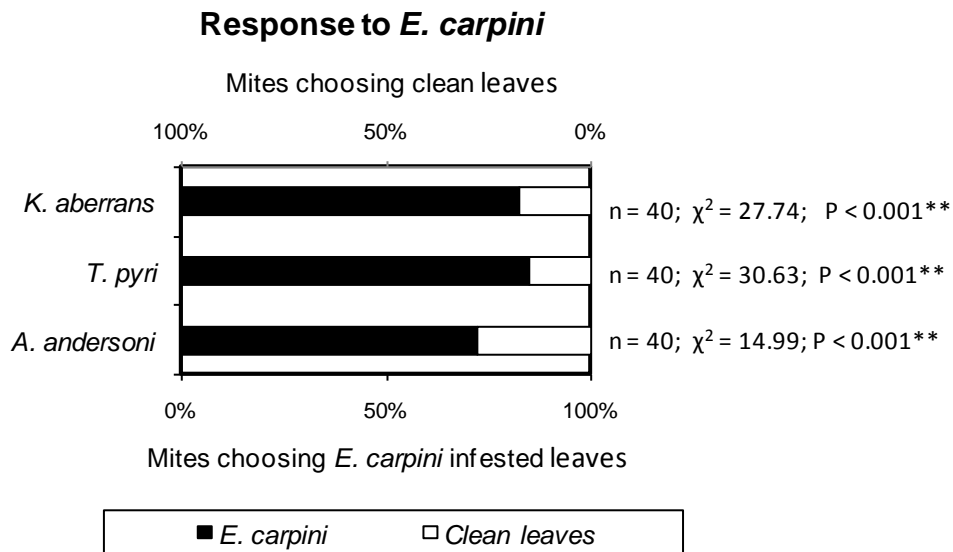
Figure 15 Percentage of *K. aberrans*, *T. pyri* and *A. andersoni* females choosing between *E. carpini* infested leaves and clean healthy leaves.

Response to *P. ulmi*

Mites choosing clean leaves

| 100% | 50% | 0% |

K. aberrans   n = 44; χ^2 = 0.18; P = 0.6698

T. pyri   n = 40; χ^2 = 24.59; P < 0.001**

A. andersoni   n = 60; χ^2 = 20.99; P < 0.001**

| 0% | 50% | 100% |

Mites choosing *P. ulmi* infested leaves

■ *P. ulmi*   □ *Clean leaves*
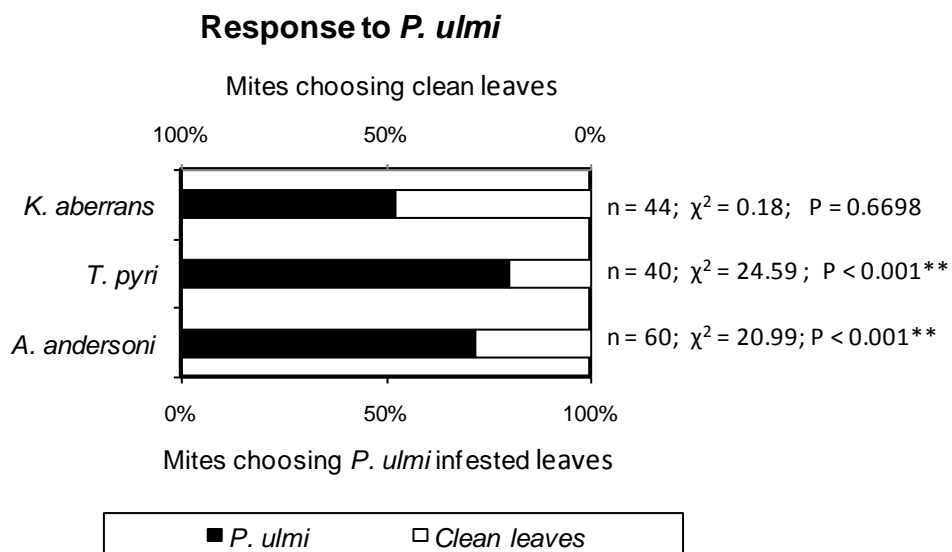
Figure 16 Percentage of *K. aberrans*, *T. pyri* and *A. andersoni* choosing between *P. ulmi* infested leaves and clean healthy leaves.

E. carpini versus P. ulmi

Mites choosing *E. carpini* infested leaves

| 100% | 50% | 0% |

K. aberrans   n = 40; χ^2 = 12.07; P = 0.0005**

T. pyri   n = 57; χ^2 = 0.04; P = 0.8514

A. andersoni   n = 60; χ^2 = 0.13; P = 0.7150

| 0% | 50% | 100% |

Mites choosing *P. ulmi* infested leaves
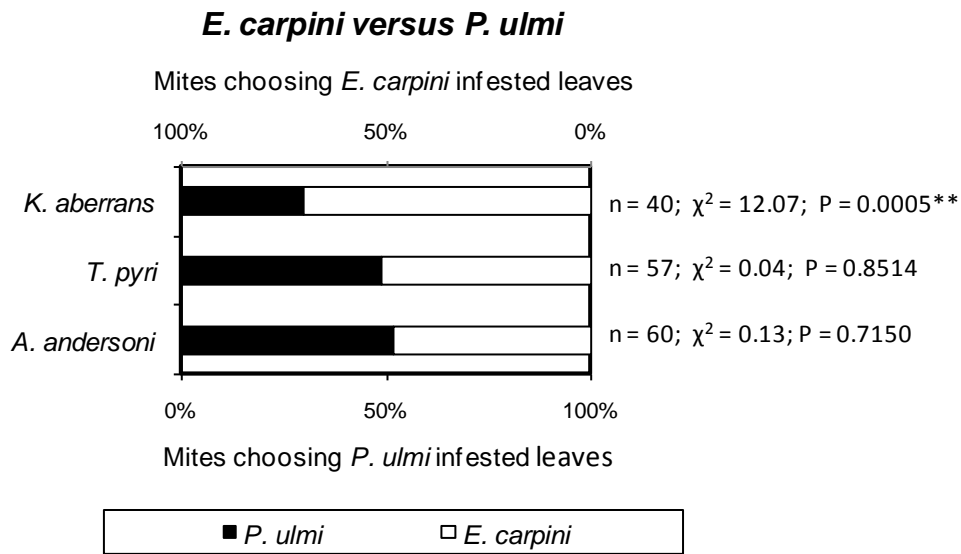
■ *P. ulmi*   □ *E. carpini*

Figure 17 Percentage of *K. aberrans*, *T. pyri* and *A. andersoni* choosing between *P. ulmi* and *E. carpini* infested leaves.

Response to *P. viticola*

Mites choosing clean leaves

| 100% | 50% | 0% |

K. aberrans   n = 40; χ^2 = 0.00; P = 1.0000

T. pyri   n = 40; χ^2 = 18.11; P < 0.001**

A. andersoni   n = 40; χ^2 = 30.63; P < 0.001**

| 0% | 50% | 100% |

Mites choosing *P. viticola* infected leaves

■ *P. viticola*   □ *Clean leaves*
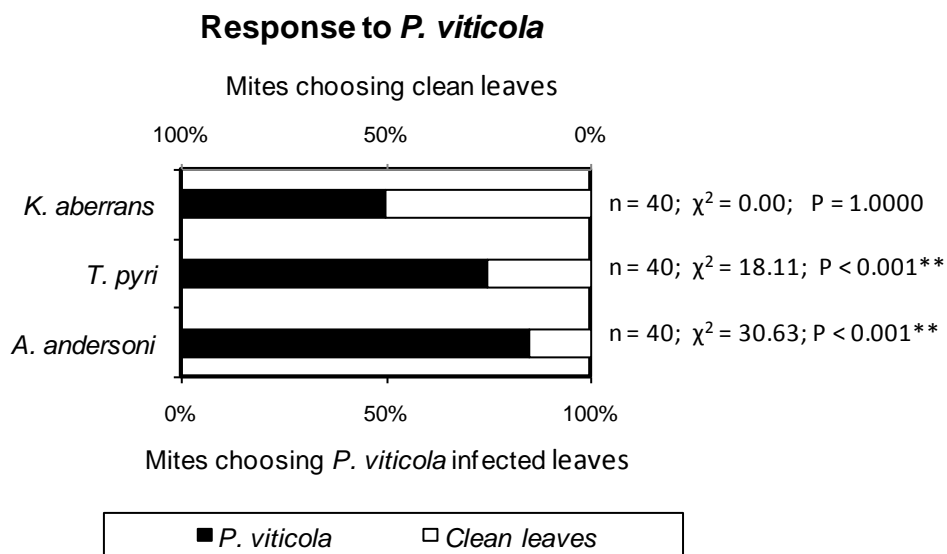
Figure 18 Percentage of *K. aberrans*, *T. pyri* and *A. andersoni* choosing between *P. viticola* infected leaves and clean healthy leaves.

E. carpini versus P. viticola

Mites choosing *E. carpini* infested leaves

n = 40; χ^2 = 21.33; P = 0.0005**

n = 40; χ^2 = 0.80; P = 0.3717

n = 70; χ^2 = 0.11; P = 0.7353

Mites choosing *P. viticola* infected leaves

■ *P. viticola*   □ *E. carpini*
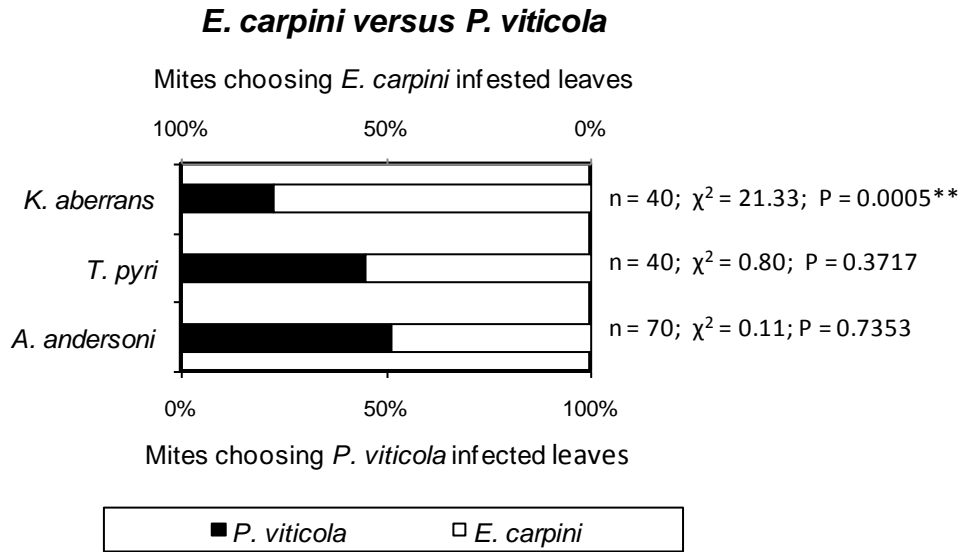
Figure 19 Percentage of *K. aberrans*, *T. pyri* and *A. andersoni* choosing between *E. carpini* infested leaves and *P. viticola* infected leaves.

P. ulmi versus P. viticola

Mites choosing *P.ulmi* infested leaves

n = 50; χ^2 = 0.16; P = 0.6892

n = 60; χ^2 = 10.46; P = 0.0012**

n = 40; χ^2 = 9.38; P = 0.0022**

Mites choosing *P. viticola* infected leaves
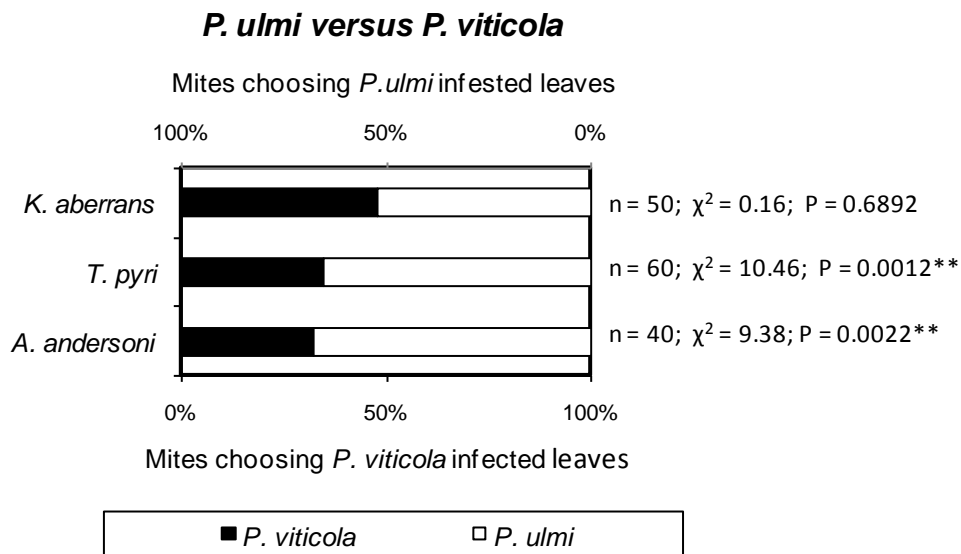
■ *P. viticola*   □ *P. ulmi*

Figure 20 Percentage of *K. aberrans*, *T. pyri* and *A. andersoni* choosing between *P. ulmi* infested leaves and *P. viticola* infected leaves.

Discussion

Results of experiments stress on the ability of predatory mites to detect volatiles emitted by grape spider mites. The three predatory mites species responded to volatiles emitted by leaves

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infested by *E. carpini* and two of them (*A. andersoni* and *T. pyri*) exhibited a preference for volatiles emitted by *P. ulmi* infested leaves. At our knowledge, this is the first evidence of the capacity to detect volatiles by *K. aberrans*, a key predatory mite in south-European vineyards (Ivancich Gambaro, 1973; Girolami, 1987; Duso, 1989; Duso et al., 2009).

We also demonstrate that pathogen infected leaves can attract predatory mite species (*A. andersoni* and *T. pyri*) when compared to clean leaves. Nevertheless, volatiles emitted by leaves infested by spider mites were more attractive than those infected by *P. viticola*. This behavior can have important consequences for biological control activity of predatory mite species.

The response by *A. andersoni* and *T. pyri* to *P. ulmi* confirms results obtained in previous studies applied to apple systems (Dicke et al., 1988). Apparently, both predatory mite species have evolved the ability to detect both *E. carpini* and *P. ulmi*, which are very common and suitable prey (Duso e Camporese, 1991) in vineyards. In contrast, *K. aberrans* responded to *E. carpini* but not to *P. ulmi*. The lack of a clear response to *P. ulmi* infested leaves by the latter species was confirmed when predators had a chance to choose between cues emitted by leaves infested by the two mite prey. Difficulties in detecting *P. ulmi* by *K. aberrans* could affect its effectiveness as biocontrol agent. Experiments conducted at ascertain the impact of different diets on *K. aberrans* and *T. pyri* showed that *E. carpini* guaranties higher performance in terms of development and reproduction of both predatory mites (this thesis). The absence of a response to *P. ulmi* cues could limit the efficiency of *K. aberrans* against this pest in vineyards. However, field studies showed that *K. aberrans* can control *P. ulmi* in vineyards (Duso, 1989; Girolami et al. 1992; Camporese and Duso, 1996; Duso and Vettorazzo, 1999). Probably other attributes, such as the persistence in conditions of prey scarcity and the intra-plant distribution can compensate the low ability by *K. aberrans* to detect the prey.

Predatory mites can feed on the mycelium of some fungi such as grape downy mildew or powdery mildew (Zemek and Prerenova, 1997; English-Loeb and Norton, 2007; Pozzebon and Duso, 2008; Pozzebon et al., 2009). Taking into consideration that alternative food has a crucial importance for persistence and biological control efficiency of predatory mites, our studies provide useful information. Field studies demonstrated the relationship between *P. viticola* symptoms spread in vineyards and *A. andersoni* and *T. pyri* abundance (Duso et al., 2003). In the laboratory the same species could feed, develop and reproduce on pathogen mycelium (Pozzebon and Duso, 2008). Current study showed that *A. andersoni* and *T. pyri* can detect volatiles associated to *P. viticola* infection. Likely evolutionary process brought them to be attracted by different food sources for the same reason hypothesized for the attraction by spider mite infestation. Individuals

which detect food cues have more probability to find it and take advantage. Sudden increase of phytoseiids on symptomatic plants (see Duso et al., 2003; Pozzebon and Duso, 2010) could be due to the immigration from healthy plants and volatile infochemicals could lead to those stimuli source.

Kampimodromus aberrans did not respond to *P. viticola* volatiles. In laboratory experiments *K. aberrans* survived by feeding on *P. viticola* mycelium but did not lay eggs. Hence the nutritional value of this food is very low and does not allow the population increase in absence of other food sources (Lorenzon et al., in press). Thus *P. viticola* sporangia and spores are not a suitable alternative food for this species that does not confound volatiles emitted by infected leaves with those emitted by prey (*E. carpini*) and choose the most suitable food.

When *A. andersoni* and *T. pyri* were obligated to choose between volatiles associated with prey or fungi, *P. ulmi* was preferred to *P. viticola* in contrast with *E. carpini*. Hence *P. viticola* stimuli could reduce or mask the effects of prey volatiles. The presence of pathogen symptoms in vineyards could distract predatory mites from prey location. Indeed in a laboratory experiment *T. pyri* consumed half *T. urticae* eggs when powdery mildew mycelium was supplied compared with non-mycelium supplying treatment (Zemek, 2005). Data would suggest consequence for biological control. Nevertheless, fungi maintain their beneficial role for predatory mites when prey is absent or rare.

Typhlodromus pyri and *A. andersoni* showed a similar response to food sources. Present results are consistent with those of previous studies on predatory mites that preferentially select food sources to maximize reproductive success (Sabelis 1985; Dicke et al. 1990). *Typhlodromus pyri* and *A. andersoni* evidenced a different hierarchy in food sources preferences compared to *K. aberrans* that can be explained by differences in life table parameters (this thesis). These results have a clear implication in biological control since a predator should prefer to forage on a more profitable food (energy content divided by handling time) and switch to a less profitable food when the first is scarce, allowing predator persistence (Murdoch, 1969; van Baalen et al., 2001). The reproductive potential induced by a food is an estimator of its intrinsic nutritional value and thus profitability. These aspects evidenced different strategies among generalist predatory mites and highlight their role in biological control.

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Chapter IV

Biological control of *Eotetranychus carpini* in North-Italian vineyards: a summary of experiments on predatory mite releases

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Introduction

The possibility to use phytoseiid mites to control grape mites in European vineyards has been suggested first in the 1970s (Ivancich-Gambaro, 1973) and then demonstrated (Girolami, 1981; Baillod et al., 1982; Schruft, 1985). Several factors can affect the success of predatory mites as biological control agents, e.g. pesticide application, cultivar features, presence of alternative food, intraguild competition, activity of macropredators (Duso and Pasqualetto, 1993). In biological control programs these factors and predatory mite species attributes should be considered to maximize their performance. Mechanisms of intraguild competition have attracted a number of researchers in order to see if intraguild predation may enhance or disrupt biological control (e.g. Croft and McRae, 1993; Rosenheim *et al.*, 1995).

Studies conducted in vineyards in North-eastern Italy have shown that predatory mite populations sometimes disappear or get to low levels because of pesticide use leading to spider mites outbreaks (Ivancich Gambaro, 1973; Girolami, 1981). In other situations vineyards can be inhabited by predatory mite populations that can tolerate pesticides but disappear when food is scarce or climatic conditions are unsuitable (Duso, 1989; Duso et al., 1991). Some predatory mites such as *Kampimodromus aberrans* (Oudemans) and *Typhlodromus pyri* Scheuten have shown a high persistence in vineyards when prey is scarce (Ivancich Gambaro, 1973; Baillod et al., 1982; Engel and Ohnesorge, 1994). The spread of these populations is very slow and thus release techniques have been pointed out. Experimental releases of *K. aberrans* and *T. pyri* proved to be

(*)Mauro Lorenzon contributed to all parts of the experimental work, data analysis and paper writing

successful in terms of spider mite control while those of *Amblyseius andersoni* (Chant) were ineffective (Duso et al., 1983; Girolami, 1987; Duso, 1989; Duso et al., 1991; Girolami et al., 1992; Duso and Pasqualetto, 1993). Moreover, *K. aberrans* showed a high competitiveness towards *T. pyri*, *A. andersoni* and *Phytoseius finitimus* Ribaga (Duso and Vettorazzo, 1999).

In the last decade outbreaks of *Eotetranychus carpini* (Oudemans) have been reported frequently in North-eastern Italy. Pesticide misuse, in particular that of ethylene-bis-dithiocarbamates (EBDCs) and organophosphates (OPs) has been claimed as key factor in these phenomenon. EBDCs are used to control Grape downy mildew *Plamopara viticola* while OPs are employed to control leafhoppers (*Scaphoideus titanus* Ball.) and berry moths (*Lobesia botrana* Den. & Schiff.). Moreover, native predatory mite populations (*A. andersoni* or *Ph. finitimus*) appear to be ineffective in keeping *E. carpini* densities under economic threshold levels. In two farms involved in these problems releases of *K. aberrans* and *T. pyri* were planned to compare their effectiveness in controlling *E. carpini*. At the same time we evaluated the effects of competition between released and native predatory mite species. In contrast with similar experiments carried out in the past (e.g. Duso et al., 1991; Duso and Pasqualetto, 1993; Duso and Vettorazzo, 1999) predatory mites used for releases were known to be low susceptible to EBDCs and OPs, two pesticide categories frequently involved in the disruption of biological control in vineyards (Ivancich Gambaro, 1973; Duso et al., 1983; Girolami, 1981). In one of this farm we tested predatory mite releases in vineyards of the same age and cultivar but trained with different systems in order to improve knowledge of potential effects of pruning on prey-predator interactions.

Materials and Methods

Experiments were carried out in vineyards belonging to two farms (A and B) located in the Veneto region, North-eastern Italy.

Farm A is located at Spresiano (45°47'49" N; 12°15'05" E; 64 m a.s.l.), Treviso district. Four vineyards comprising the cultivar Glera, planted in the same year but trained with different pruning systems (i.e. Pergola, Sylvoz, G.D.C. and Free cordon) were selected. Spider mite (*P. ulmi* and *E. carpini*) infestations were frequently observed in the seasons preceding the experiments. Prior of releases 40 two-years old branches were collected during winter and analyzed using a dissecting microscope. Among predatory mites *A. andersoni* and *Paraseiulus talbii* (Athias-Henriot) were found (1.51 and 0.12 overwintered females per internode).

Farm B is located at Meolo (45°38'06'' N; 12°27'19" E; 0 m a.s.l.), Venice province. A single vineyard comprising the Verduzzo trevigiano cultivar and trained with Sylvoz pruning system was considered. High population densities of *E. carpini* were recorded in the year preceding experiment. Analysis of samples collected during the winter showed the presence of moderate densities of *Ph. finitimus* and *A. andersoni* (2.78 and 0.43 overwintered females per internode).

Strains used for releases

Kampimodromus aberrans and *T. pyri* strains utilized for releases were collected from two farms located in the Veneto region, North-eastern Italy. *Kampimodromus aberrans* was collected from a farm located in the Verona province (Monteforte d'Alpone) where it was the dominant phytoseiid species. In previous studies the release of this strain in vineyards proved to be successful (Facchin, 1996). The *T. pyri* strain was collected from a farm located in the Treviso province (San Pietro di Feletto) where it was completely dominant among phytoseiid species. Both strains proved to be poorly susceptible to OPs and EBDCs (Posenato, 1994; Moret, 2006; Pozzebon et al., 2010).

In January, before releases, 40 two-year old branches were collected from each farm and brought to the laboratory to be analyzed by using a dissecting microscope. The identity and density of overwintered phytoseiids were assessed and the presence of other arthropods was recorded. Densities of 7.21 *K. aberrans* females per internode and of 11.34 *T. pyri* females per internode were calculated. These figures were considered in order to assess the number of predatory mites to be released per bud.

Release procedures are described in Duso (1989) and Duso et al. (1991). The number of overwintered females released on each vine was calculated considering the mean number of buds per vine.

Experimental design

In each vineyard of farm A, six release or non release treatments randomly replicated four times were compared. Treatments were settled according to addition series design that allows us to test the effect of predator diversity and density without confounding between the two factors (Table 1). Each replicate comprised three continuous vines, separated by ten-thirty vines from the subsequent plot. Releases were carried out in February of 2009 and observations were conducted over the vegetative seasons of 2009 and 2010. Among fungicides EBDCs (mixed with dimethomorph), copper hydroxide, wettable sulphur were used to control Grape downy mildew and

Grape powdery mildew. EBDCs were used four times in early season. Regarding insecticides, chlorpyriphos was applied in July of 2009 and thiamethoxam in July of 2010.

In farm B six release or non release treatments randomly replicated six times were compared (Table 1). Each replicate comprised two subsequent vines separated by about ten vines from the subsequent plot. Releases were carried out in February 2010 and observations were conducted from April to September of the same year. EBDC fungicides, copper and wettable sulphur were used as fungicides. No insecticides or acaricides were used.

Sampling methods

Seasonal mite abundance was monitored by taking leaf samples every two weeks. In each sampling date a total of 48-64 leaves per treatment were collected (eight leaves per replicate). Leaves were transferred to the laboratory and immediately analyzed by using a dissecting microscope in order to assess the identity and density of mites. The occurrence of macropredators was also estimated.

		Release density (Phytoseiids/bud)	
Treatment		Farm A	Farm B
Control		0	0
K. aberrans	(Ka)	0.5	2
	(Ka)	1	4
T. pyri	(Tp)	0.5	2
	(Tp)	1	4
K. aberrans + *T. pyri*	(Ka-Tp)	1 (0.5+0.5)	4 (2+2)

Table 2. Treatments and predatory mite release densities.

Canopy's features

In Farm A the structure of the canopy of each vineyards was assessed by applying the "point quadrat" method (Wilson, 1963; Bertamini *et al.,* 1994). Following this method we used a square plastic panel measuring 1 m^2 in size with pre-drilled holes at 10 cm intervals. The panel is placed on the vegetation. A pointed metal pole approximately 1 m length was inserted in the holes in the panel and used to penetrate the canopy. The number of contacts of the metal pole with leaves and bunches were recorded. The following parameters were calculated: (1) percentage of empty spaces in the canopy, obtained by calculating the ratio between the number of gaps detected and the total number of penetrations; (2) number of leaf layers, i.e. the number of leaf contacts divided by the number of penetrations; (3) percentage of leaves inside canopy, by dividing the number of leaves (except the first and last of each penetrations) by the total number of leaves detected.

Statistical analysis

Data were analyzed using a Restricted Maximum Likelihood Repeated Measures model with the Proc MIXED of SAS (SAS Institute Inc., 1999). Treatments, time and their interactions were considered as fixed effect and were evaluated with F test ($\alpha = 0.05$). The Kenward-Roger method was used for degrees of freedom estimation (Littell *et al.,* 1996). We tested the effects of experimental factors on mite populations using contrasts with F test ($\alpha = 0.05$). The parameters of canopy structure were analyzed using a one-way ANOVA and means separated by Tukey test ($\alpha = 0.05$).

Results

Farm A

Effects of predatory mite releases on P. ulmi densities

In 2009, *P. ulmi* populations reached relatively low densities in vineyards trained with Pergola (P), Sylvoz (S) and G.D.C. (GDC) systems; peaks of 2.53, 1.47 and 2.17 motile forms per leaf were observed in the control plots of these vineyards. European red spider mite populations reached higher levels (peak of 9.25 motile forms per leaf in the control plots) in the vineyard trained with free cordon system (FC) (fig. 1).

Regarding *P. ulmi* abundance in vineyard P, the effect of treatment was significant ($F_{5, 76.5}$=2.50; P=0.038). *K. aberrans* releases affected significantly *P. ulmi* densities ($F_{1, 76.5}$=7.82; P=0.006) but this result was obtained with the highest release densities ($F_{1, 76.5}$=8.43; P=0.005; Fig. 1). In contrast *T. pyri* releases did not affect significantly *P. ulmi* abundance ($F_{1, 76.5}$=0.86; P=0.357; Fig. 1). Therefore, *K. aberrans* releases were more effective than *T. pyri* releases in controlling *P. ulmi* ($F_{1, 76.5}$=5.24; P=0.025). *Panonychus ulmi* densities were not reduced significantly in Ka-Tp treatment compared to the control ($F_{1, 76.5}$=0.05; P=0.820). The effect of density in predatory mite releases was not significant ($F_{1, 76.5}$=0.09; P=0.768). At the same release density, *K. aberrans* releases proved to be more effective than mixed releases (Ka-Tp) in reducing *P. ulmi* numbers ($F_{1, 76.5}$=7.16; P=0.009; Fig. 1).

The effect of treatment was not significant in vineyard S ($F_{5, 49.6}$=2.03; P=0.091; Fig. 2), nor in vineyard GDC ($F_{5, 56.9}$=1.51; P=0.202; Fig. 3). Nevertheless the contrasts' analysis showed a significant effect of *K. aberrans* releases on spider mite densities ($p < 0.05$) in both vineyards.

In vineyard FC the effect of treatment was significant ($F_{5, 60.2}$=2.90; P=0.021; fig. 4). *Kampimodromus aberrans* and *T. pyri* releases reduced significantly *P. ulmi* numbers ($F_{1, 60.2}$=6.42; P=0.014; $F_{1, 60.2}$=4.71; P=0.034, respectively; Fig. 4). There were no differences in the performance of the two predatory mites ($F_{1, 60.2}$=0.20; P=0.659) nor between their density levels ($F_{1, 60.2}$=0.28; P=0.599). *Panonychus ulmi* densities were reduced significantly in Ka-Tp treatment compared to the control ($F_{1, 60.2}$=12.56; P<0.001). Considering the same release density, there were no differences between singly or mixed releases ($F_{1, 60.2}$=2.44; P=0.123; $F_{1, 60.2}$=1.16; P=0.285; for *K. aberrans* and *T. pyri* respectively; Fig. 4).

During 2010 *P. ulmi* reached negligible densities in the four vineyards.
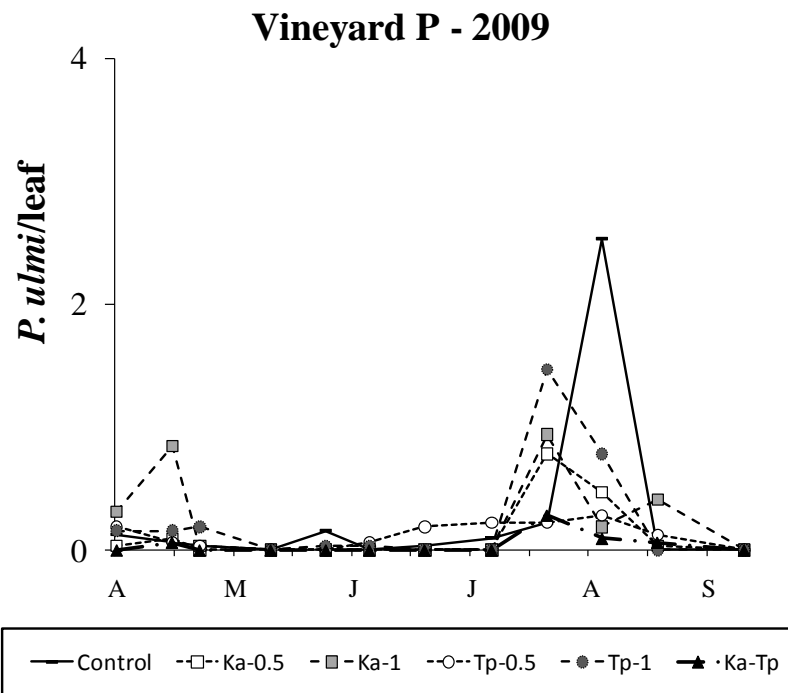
Figure 21. Seasonal abundance of *P. ulmi* on different treatments.
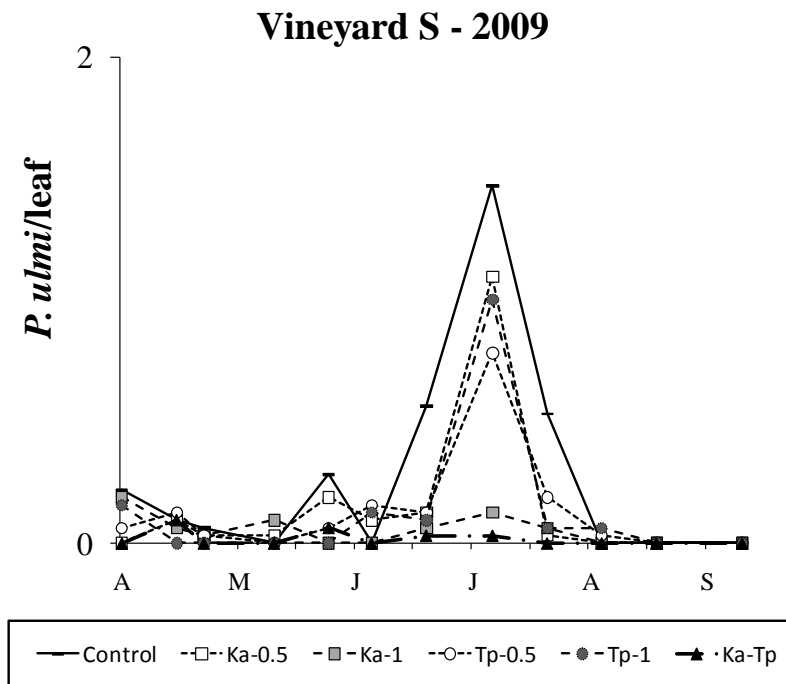
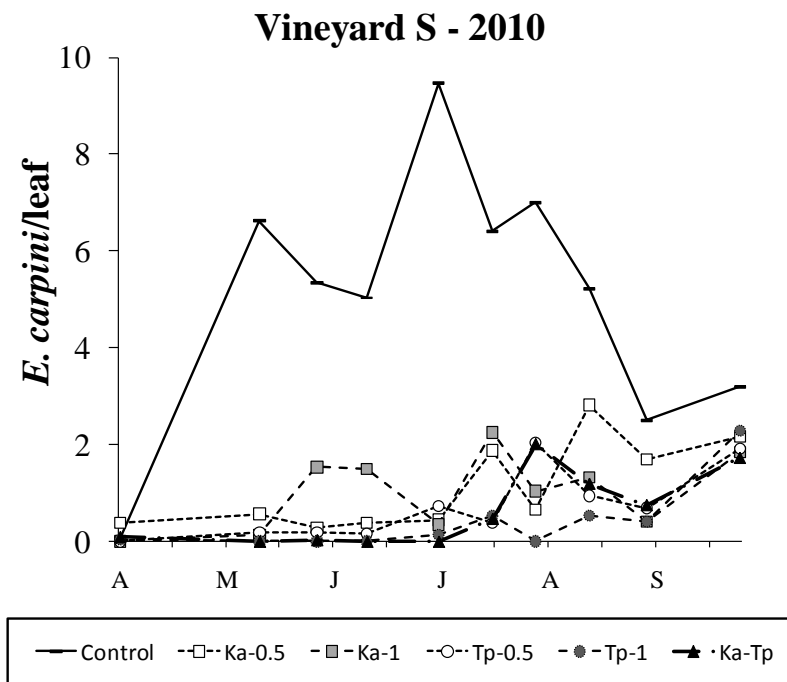**Figure 22.** Seasonal abundance of *P. ulmi* on different treatments..
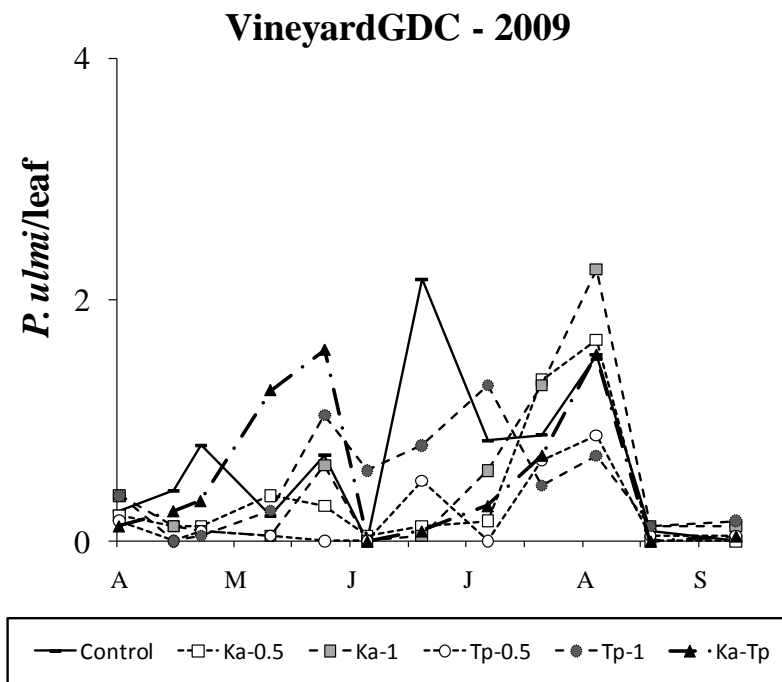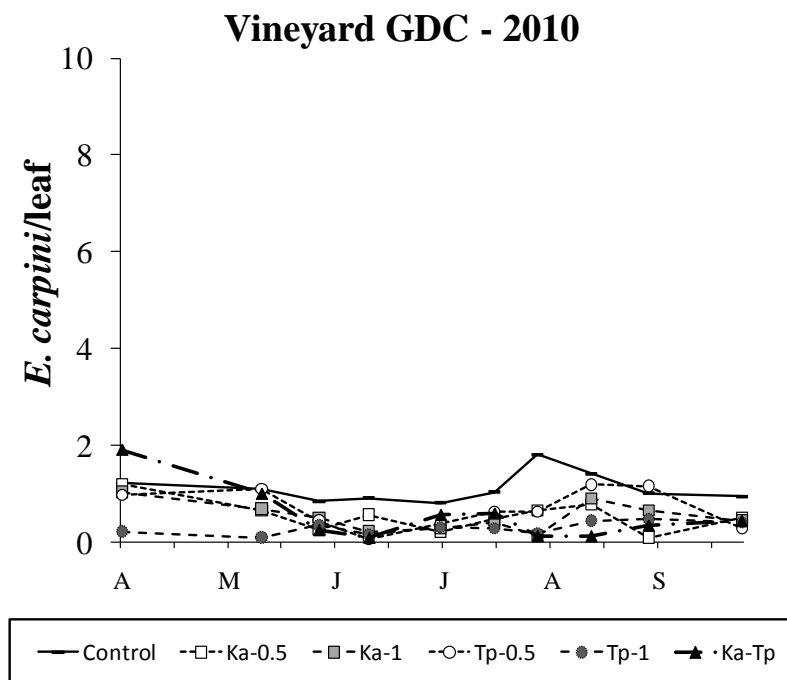
Figure 23. Seasonal abundance of *P. ulmi* on different treatments..
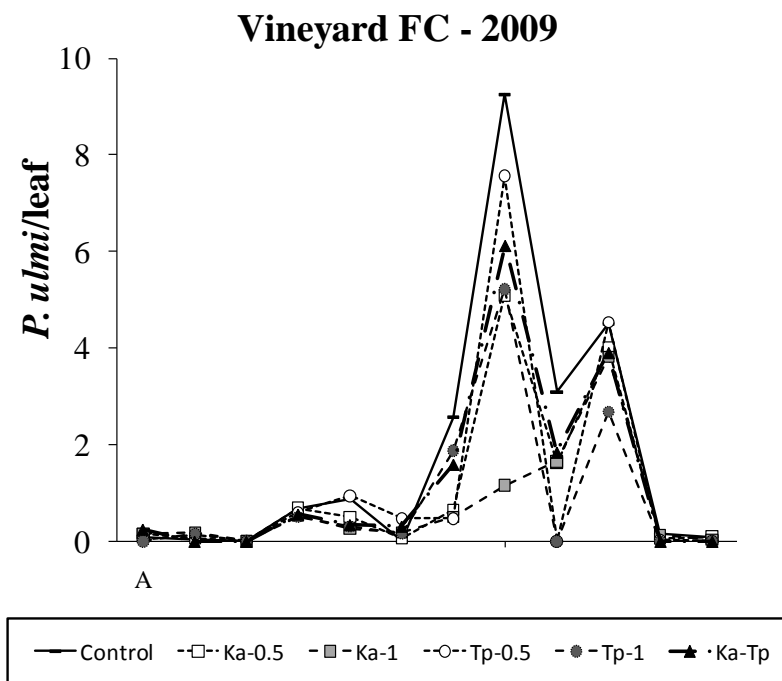
Figure 24. Seasonal abundance of *P. ulmi* on different treatments.

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Effects of predatory mite releases on E. carpini densities

During the 2009 season, *E. carpini* populations reached relatively high densities in P and S vineyards, moderate densities in GDC, and negligible levels in FC vineyard (data not analyzed).

The effect of treatment was not significant in vineyard P ($F_{2, 52}=1.16$; $P=0.339$; fig. 5) nor in vineyard S ($F_{5, 36.6}=0.92$; $P=0.482$; fig. 6). In contrast, it was significant in GDC vineyard ($F_{5, 58}=2.68$; $P=0.030$; fig. 7) where *T. pyri* releases reduced significantly *E. carpini* numbers ($F_{1, 58}=9.51$; $P=0.003$) in contrast with *K. aberrans* releases ($F_{1, 58}=2.28$; $P=0.136$). Only the lowest release densities of *K. aberrans* affected *E. carpini* densities ($F_{1, 58}=4.24$; $P=0.044$). There were no differences in the performance of the two predatory mites ($F_{1, 58}=3.72$; $P=0.087$). *Eotetranychus carpini* densities were reduced significantly in Ka-Tp treatment compared to the control ($F_{1, 58}=4.79$; $P=0.033$; Fig. 7). The remaining comparisons were not significant.
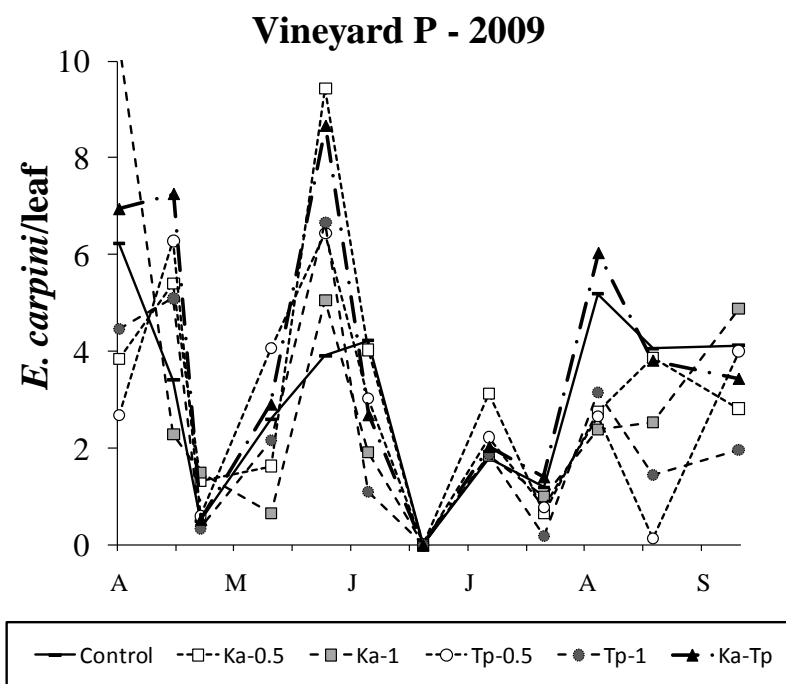
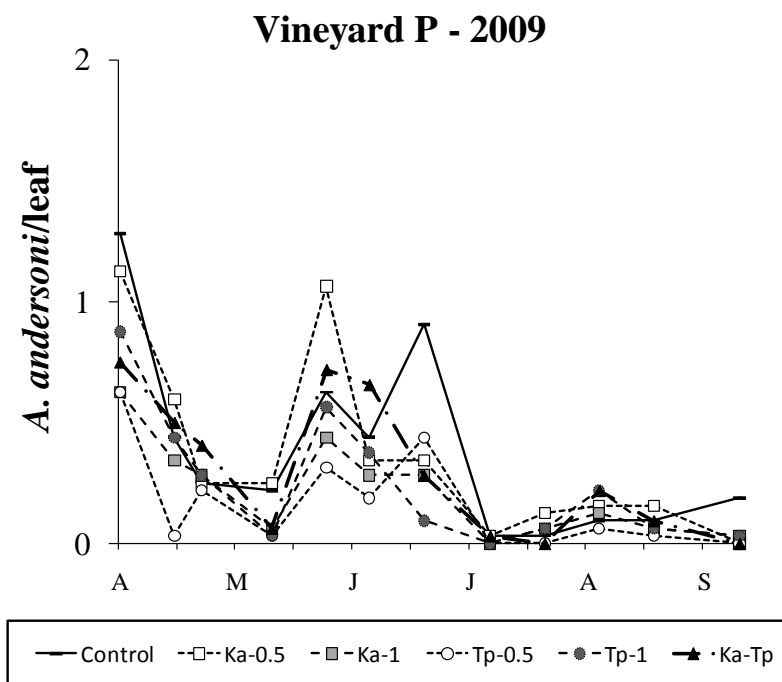**Figure 25.** Seasonal abundance of *E. carpini* on different treatments.
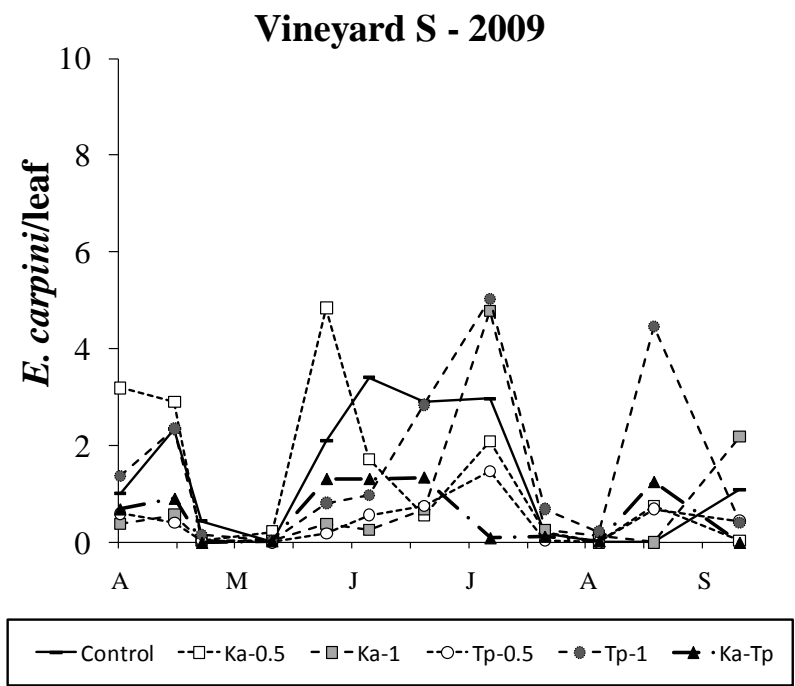
Figure 26. Seasonal abundance of *E. carpini* on different treatments.
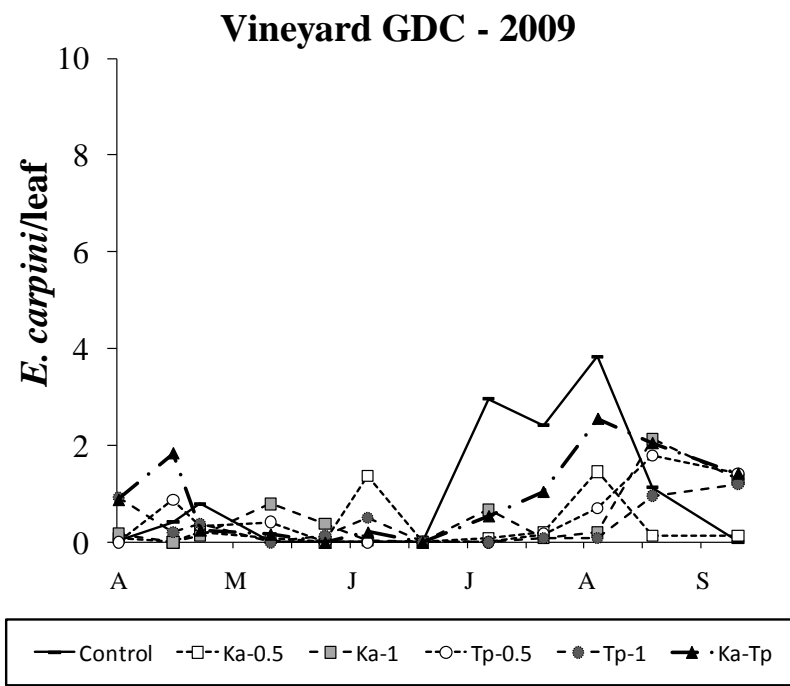
Figure 27. Seasonal abundance of *E. carpini* on different treatments.

In 2010 *E. carpini* populations reached relatively high densities in vineyards P and S but low levels in vineyard GDC. The occurrence of this mite pest was negligible in vineyard FC. In the first vineyard differences among treatments were significant ($F_{5, 53.3}$=15.45; P<0.001). Lower *E. carpini* densities were detected in release treatments than in control plots ($F_{1, 55.3}$=50.53; P<0.001; $F_{1, 55.3}$=70.92; P<0.001, respectively for *K. aberrans* and *T. pyri* treatments; Fig. 8). No differences were found among released species ($F_{1, 55.3}$=2.59; P=0.113) nor the densities of release ($F_{1, 55.3}$=1.75; P=0.191). Spider mite levels were reduced significantly in Ka-Tp treatment compared to the control ($F_{1, 55.3}$=32.33; P<0.001; Fig. 8).

In vineyard S, the effect of treatment was significant ($F_{5, 54.3}$=16.66; P<0.001; fig. 9) as spider mite populations were lower in release treatments than in the control ($F_{1, 54.3}$=8.20; P<0.001; $F_{1, 54.3}$=72.88; P<0.001, respectively for *K. aberrans* and *T. pyri* treatments; Fig. 9). No differences emerged between *K. aberrans* and *T. pyri* release treatments ($F_{1, 54.3}$=1.24; P=0.271) nor between the densities of release ($F_{1, 54.3}$=3.26; P=0.077). In Ka-Tp plots there were less spider mites compared to the control ($F_{1, 54.3}$=36.96; P< 0.001; Fig. 9).

Additional differences among treatments emerged in GDC vineyard ($F_{5, 39.5}$=2.59; P=0.04; fig. 10) where released predatory mites reduced *E. carpini* densities significantly compared to the control ($F_{1, 39.5}$=8.01; P=0.007; $F_{1, 39.5}$=8.86; P=0.005, respectively for *K. aberrans* and *T. pyri* treatments; Fig. 10). There were no differences between *K. aberrans* and *T. pyri* release treatments ($F_{1, 39.5}$=0.03; P=0.859), nor between the densities of release ($F_{1, 39.5}$=1.12; P=0.296). Spider mite numbers were lower in Ka-Tp treatment than in the control ($F_{1, 39.5}$=5.37; P=0.026; Fig. 10).
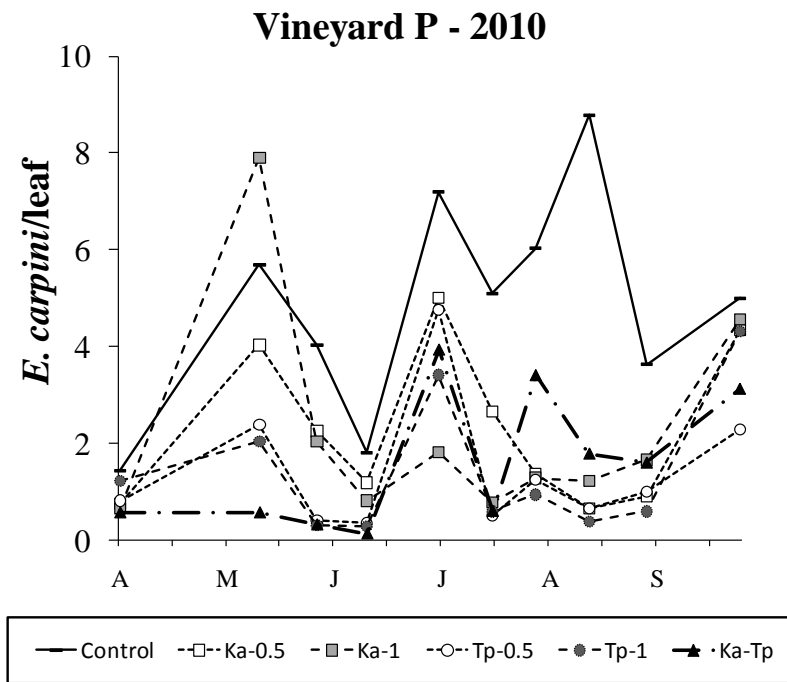
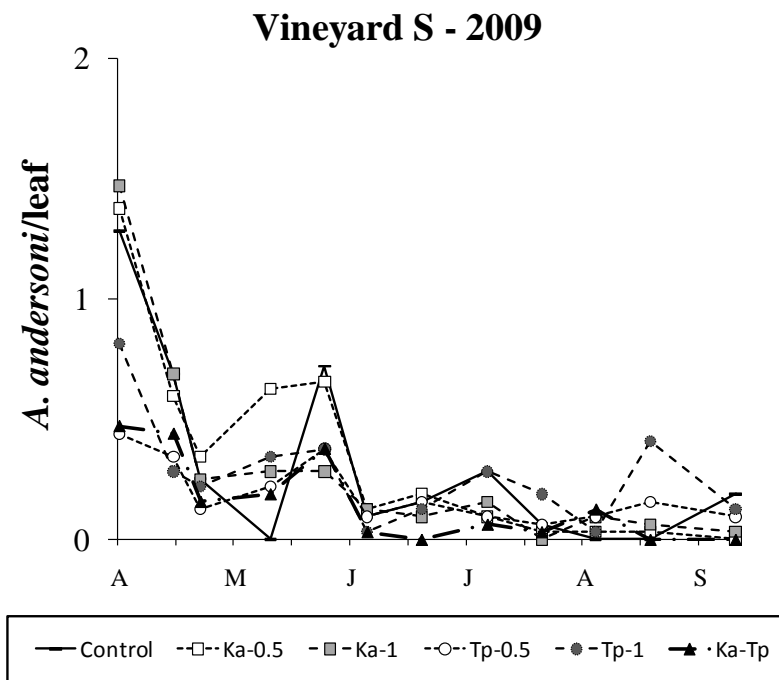**Figure 28.** Seasonal abundance of *E. carpini* on different treatments.

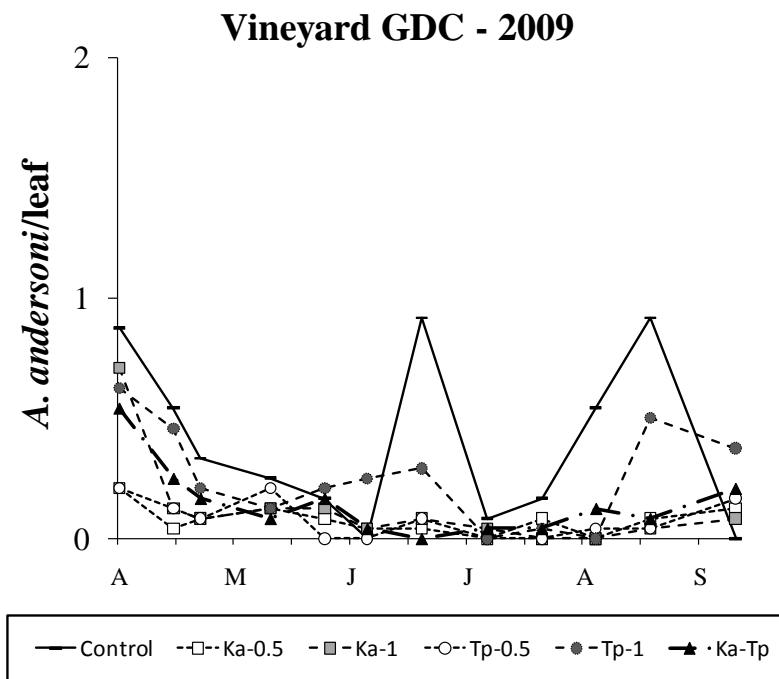**Figure 29.** Seasonal abundance of *E. carpini* on different treatments.

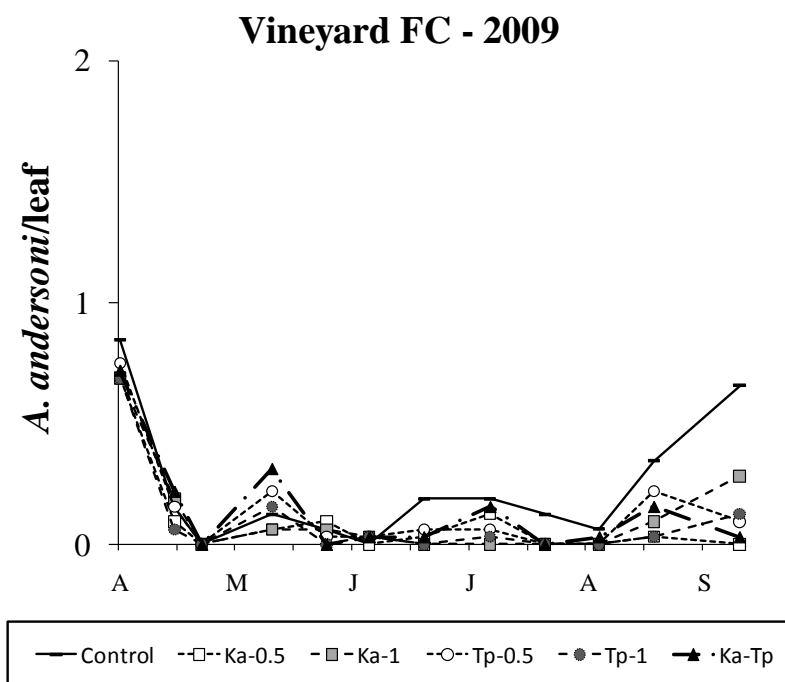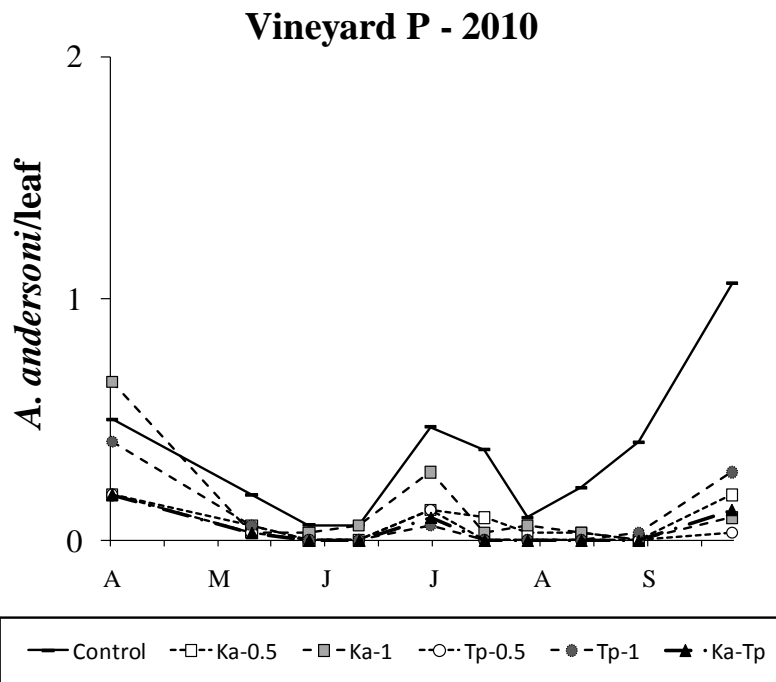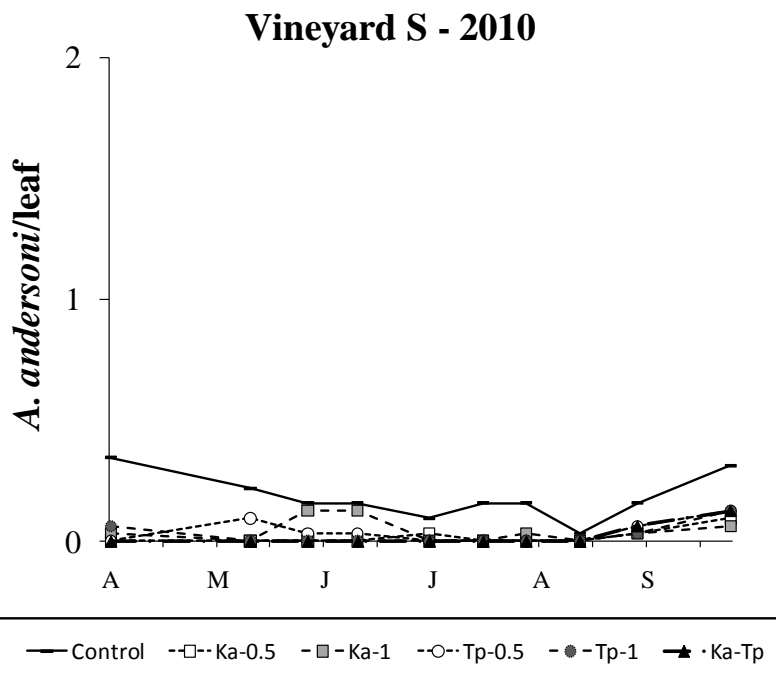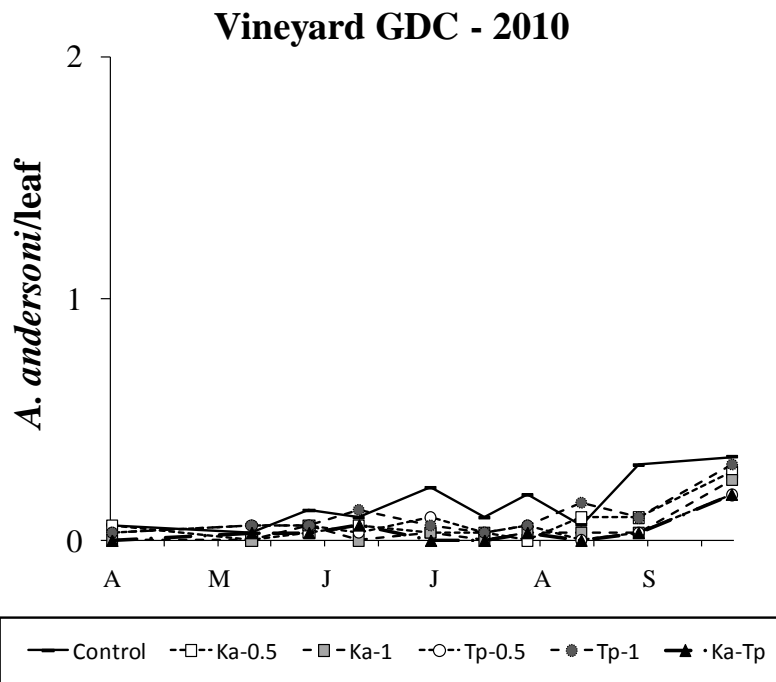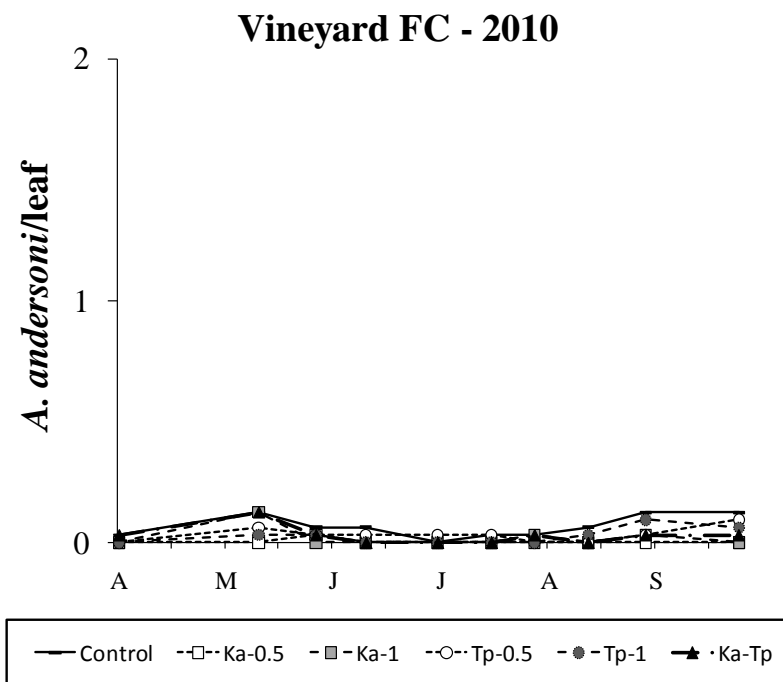**Figure 30.** Seasonal abundance of *E. carpini* on different treatments.

Effects of releases on predatory mite species

In 2009, native predatory mites (*A. andersoni*) reached moderate to low densities in the four vineyards. In vineyard P, the effect of treatment was significant ($F_{5,\ 63.4}$=5.02; P<0.001; fig. 11). *Amblyseius andersoni* numbers were significantly reduced in *T. pyri* release plots ($F_{1,\ 63.4}$=13.65; P<0.001) but not in *K. aberrans* plots compared to the control ($F_{1,\ 63.4}$=1.41; P=0.24). *Amblyseius andersoni* densities were lower in *T. pyri* than in *K. aberrans* release plots ($F_{1,\ 63.4}$=9.44; P=0.003) and in Ka-Tp plots compared to the control ($F_{1,\ 63.4}$=8.29; P=0.005). The effect of release density was not significant ($F_{1,\ 63.4}$=0.344; P=0.559; fig. 11).

In vineyard S there were no differences among treatments ($F_{5,\ 53.5}$=2.09; P=0.081; fig. 12). However, the contrasts' analysis showed that releases affected *A. andersoni* numbers ($F_{1,\ 53.5}$=4.09; P=0.048), in particular *K. aberrans* releases ($F_{1,\ 53.5}$=4.06; P=0.049; fig. 12).

In contrast, differences among treatments were significant in vineyard GDC ($F_{5,\ 50.7}$=11.97; P<0.001; fig. 13). *Amblyseius andersoni* densities were reduced in *K. aberrans* as well as *T. pyri* treatments ($F_{1,\ 50.7}$=41.89; P<0.001; $F_{1,\ 50.7}$=31.76; P<0.001, respectively) included Ka-Tp ($F_{1,\ 50.7}$=37.26; P<0.001). There were no differences in the effect of the two released species ($F_{1,}$

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$_{50.7}$=1.05; P=0.31). The effect of release density was significant ($F_{1,\,50.7}$=8.46; P=0.005; fig. 13) but there were less *A. andersoni* in plots with the lowest release density.

The effect of treatment was significant also in vineyard FC ($F_{5,\,63.2}$=4.56; P=0.001; fig. 14) where A. *andersoni* densities were significantly reduced by released predators ($F_{1,\,63.2}$=18.66; P<0.001). This effect was significant in *K. aberrans* as well as in *T. pyri* plots ($F_{1,\,63.2}$=13.14; P<0.01; $F_{1,\,63.2}$=16.64; P<0.001, respectively) included Ka-Tp plots ($F_{1,\,63.2}$=11.45; P<0.01). There were no differences between *T. pyri* and *K. aberrans* treatments ($F_{1,\,63.2}$=0.31; P=0.580). The effect of release density was not significant ($F_{1,\,63.2}$=0.00; P=0.961; fig. 14).
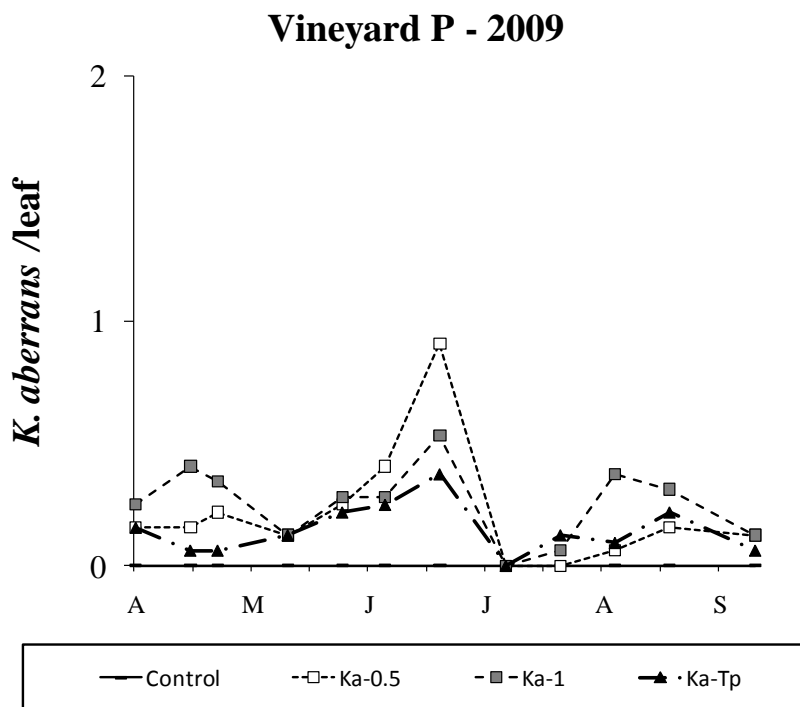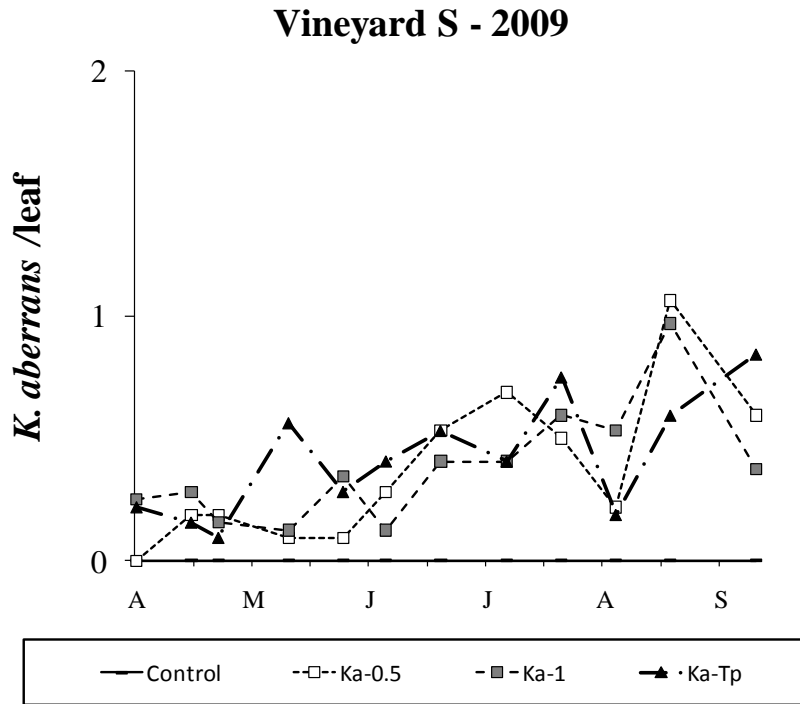

Figure 31. Seasonal abundance of *A. andersoni* on different treatments.

Figure 32. Seasonal abundance of *A. andersoni* on different treatments.

Figure 33. Seasonal abundance of *A. andersoni* on different treatments.

Vineyard FC - 2009

Figure 34 Seasonal abundance of *A. andersoni* on different treatments.

In 2010, *A. andersoni* populations appeared to be less abundant than in 2009. The effect of treatment was significant in all vineyards (vineyard P: $F_{5,\ 37.5}$=11.82; P<0.001; fig. 15; vineyard S: $F_{5,\ 35.4}$=8.96; P<0.001; fig. 16; vineyard GDC: $F_{5,\ 49.9}$=5.75; P<0.001; fig. 17; vineyard FC: $F_{5,\ 61.9}$=5.61; P<0.001; fig. 18). *Amblyseius andersoni* densities were reduced in *K. aberrans* (vineyard P: $F_{1,\ 37.5}$=45.31; P<0.001; vineyard S: $F_{1,\ 35.4}$=39.73; P<0.001; vineyard GDC: $F_{1,\ 49.9}$=21.28; P<0.001; vineyard FC: $F_{1,\ 61.9}$=22.37; P<0.001) as well as in *T. pyri* treatments (vineyard P: $F_{1,\ 37.5}$=50.23; P<0.001; vineyard S: $F_{1,\ 35.4}$=32.89; P<0.001; vineyard GDC: $F_{1,\ 49.9}$=13.95; P<0.001; vineyard FC: $F_{1,\ 61.9}$=13.14; P<0.001). There were no differences between the released species (vineyard P: $F_{1,\ 37.5}$=0.190; P=0.665; vineyard S: $F_{1,\ 35.4}$=0.48; P=0.491; vineyard GDC: $F_{1,\ 49.9}$=1.29; P=0.626; vineyard FC: $F_{1,\ 61.9}$=2.27; P=0.137) or the density of release (vineyard P: $F_{1,\ 37.5}$=0.01; P=0.912; fig. 15; vineyard S: $F_{1,\ 35.4}$=0.04; P=0.834; fig. 16; vineyard GDC: $F_{1,\ 49.9}$=0.09; P=0.766; fig. 17; vineyard FC: $F_{1,\ 61.9}$=0.74; P=0.392; fig. 18).

Figure 35. Seasonal abundance of *A. andersoni* on different treatments.

Figure 36. Seasonal abundance of *A. andersoni* on different treatments.

Figure 37. Seasonal abundance of *A. andersoni* on different treatments.

Figure 38. Seasonal abundance of *A. andersoni* on different treatments.

Releases of *K. aberrans* were successful even if predatory mite numbers did not reach high levels. In 2009, the effect of treatment was significant in all vineyards (vineyard P: $F_{3,46}$=18.85; P<0.001; fig. 19; vineyard S: $F_{3, 35.8}$=22.69; P<0.001; fig. 20; vineyard GDC: $F_{3, 37.5}$=33.89; P<0.001; fig. 21; vineyard FC: $F_{3, 38.8}$=18.70; P<0.001; fig. 22) and there were more *K. aberrans* in the respective release plots than in the control (vineyard P: $F_{1,46}$=47.62; P<0.001; vineyard S: $F_{1, 35.8}$=66.93; P<0.001; vineyard GDC: $F_{1, 37.5}$=86.55; P<0.001; vineyard FC: $F_{1, 38.8}$=35.72; P<0.001). In Ka-Tp treatments the presence of *T. pyri* affected *K. aberrans* densities in vineyards GDC and FC: ($F_{1, 37.5}$=4.32; P=0.044; fig. 21; $F_{1, 38.8}$=12.91; P<0.001; fig. 22; respectively) but not in vineyards P and S ($F_{1,46}$=2.02; P=0.162; fig. 19; $F_{1, 35.8}$=0.01; P=0.988; fig.20; respectively).
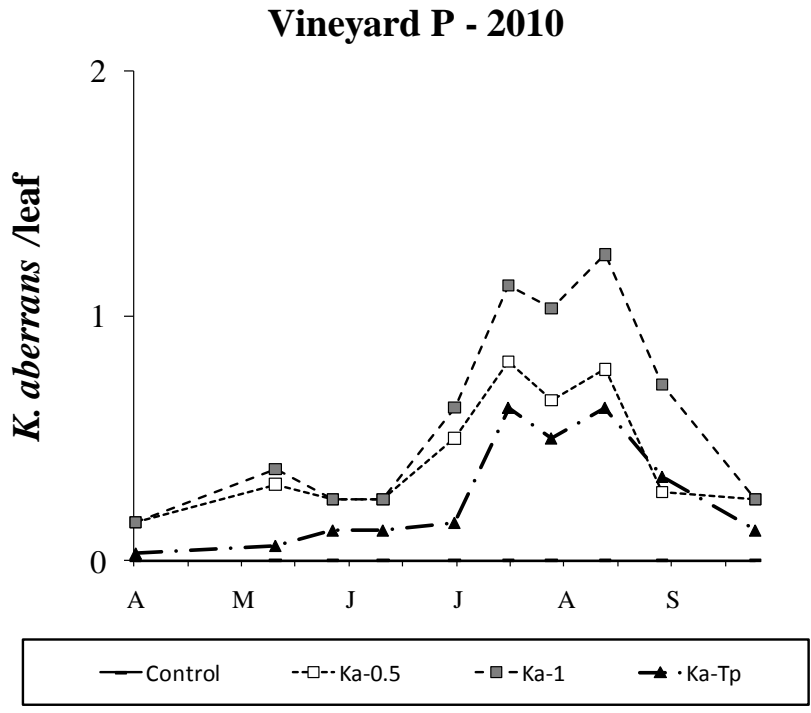

Figure 39. Seasonal abundance of *K. aberrans* on different treatments.
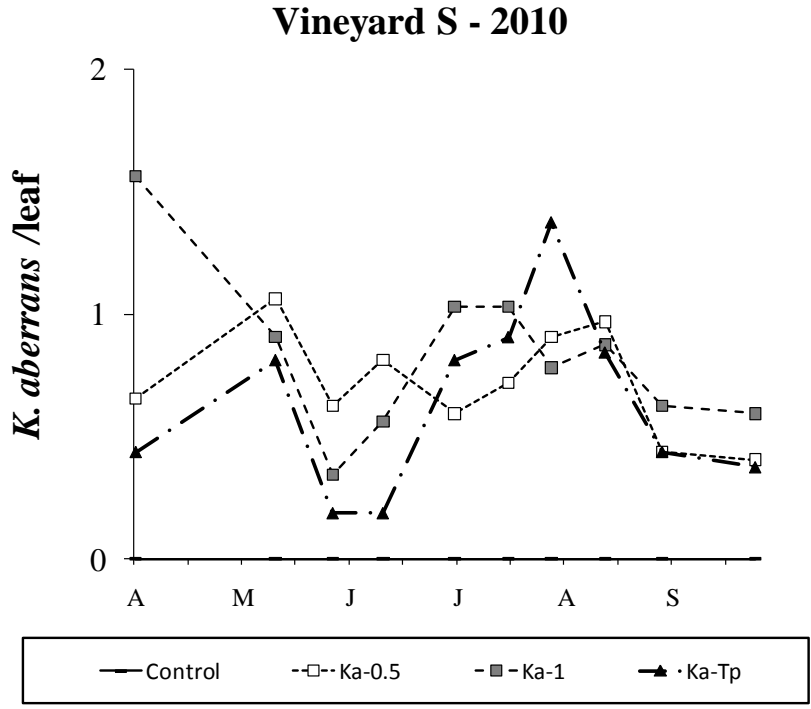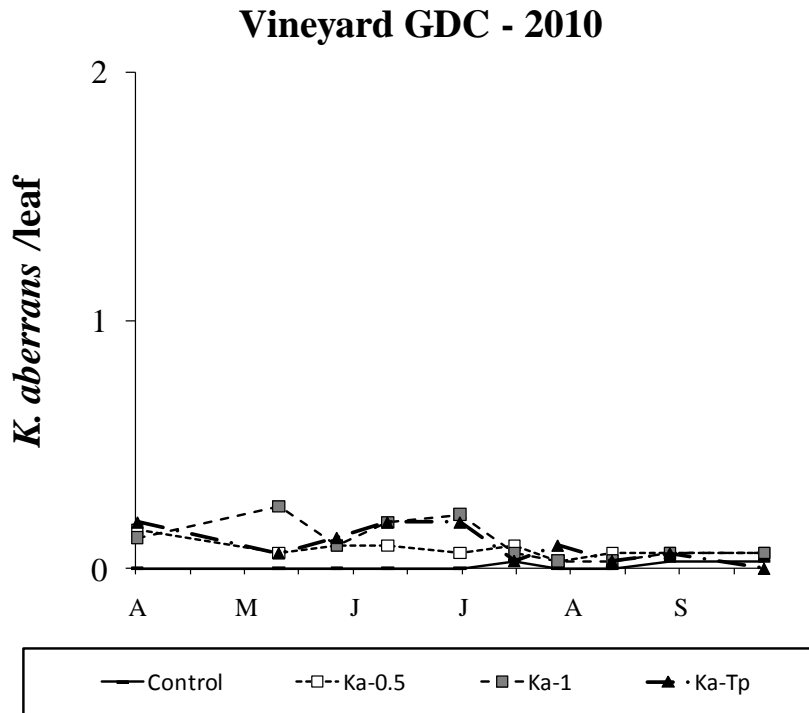
Figure 40. Seasonal abundance of *K. aberrans* on different treatments.
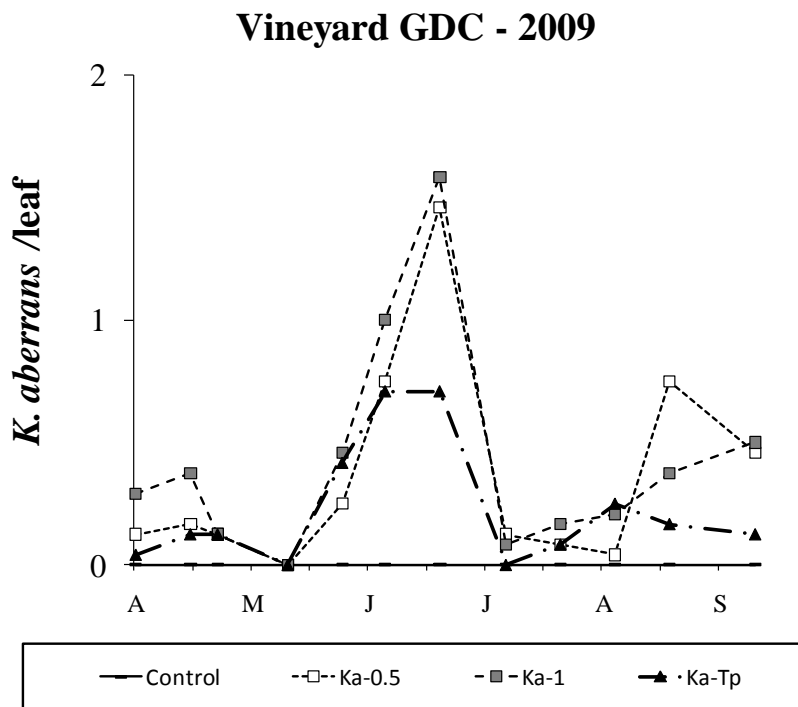
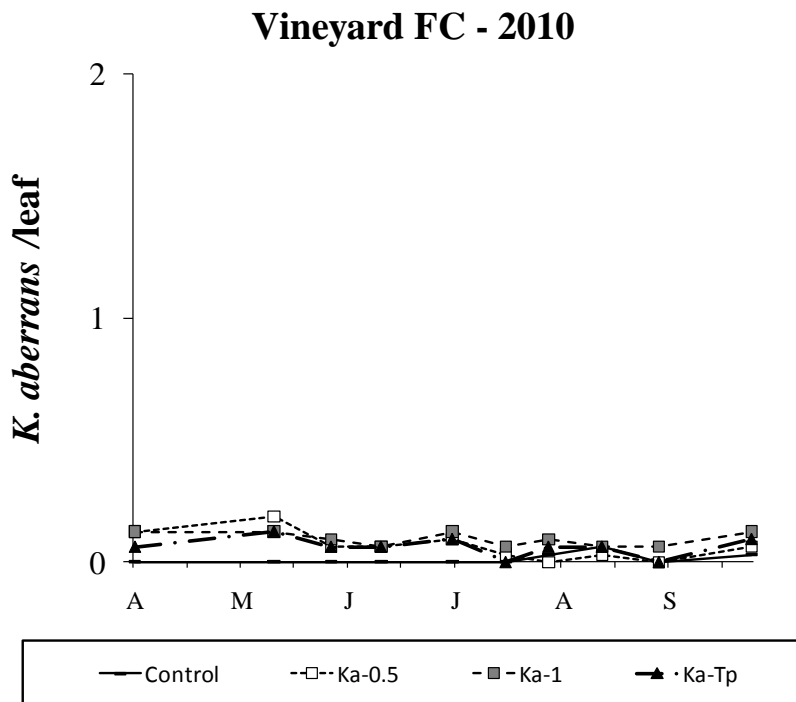**Figure 41.** Seasonal abundance of *K. aberrans* on different treatments.

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Vineyard FC - 2009
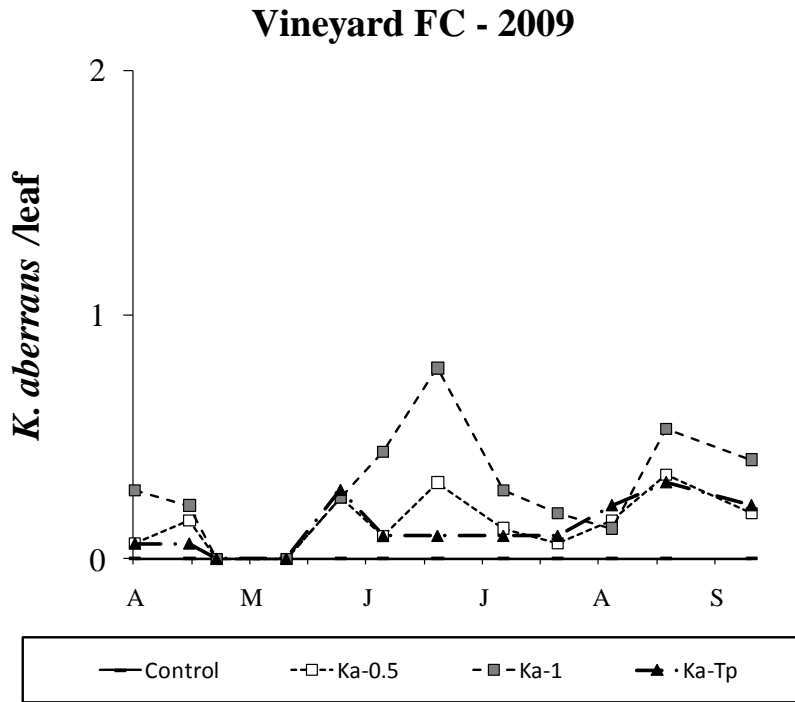
Figure 42. Seasonal abundance of *K. aberrans* on different treatments.

One year later, *K. aberrans* populations appeared more abundant in P and S vineyards than in GDC and FC ones. The effect of treatment was significant in all vineyards (vineyard P: $F_{3,31.2}$=6.05; P=0.002; fig. 23; vineyard S: $F_{3, 26.4}$=3.15; P=0.042; fig. 24; vineyard GDC: $F_{3, 33.6}$=27.58; P<0.001; fig. 25; vineyard FC: $F_{3, 36.7}$=21.2; P<0.001; fig. 26) and there were more *K. aberrans* in the respective release plots than in the control (vineyard P: $F_{1,31.2}$=14.87; P<0.001; vineyard S: $F_{1, 26.4}$=8.77; P=0.006; vineyard GDC: $F_{1, 33.6}$=80.12; P<0.001; vineyard FC: $F_{1, 36.7}$=47.14; P<0.001). In Ka-Tp treatments the presence of *T. pyri* did not affect *K. aberrans* densities ($F_{1,31.2}$=2.29; P=0.140; fig. 23; $F_{1, 26.4}$=0.68; P=0.417; fig. 24; $F_{1, 33.6}$=0.25; P=0.618; fig. 25; $F_{1, 36.7}$=3.15; P<0.084; fig. 26; P, S, GDC and FC respectively).

Figure 43. Seasonal abundance of *K. aberrans* on different treatments.

Figure 44. Seasonal abundance of *K. aberrans* on different treatments.

Vineyard GDC - 2010

Figure 45. Seasonal abundance of *K. aberrans* on different treatments.

Vineyard FC - 2010

Figure 46. Seasonal abundance of *K. aberrans* on different treatments.

Typhlodromus pyri releases appeared to be successful but its densities were low to moderate in 2009. The effect of treatment was significant in all vineyards (vineyard P: $F_{3,48.5}=22.99$; fig. 27;

$P<0.001$; vineyard S: $F_{3, 25}=3.96$; $P=0.019$; fig. 28; vineyard GDC: $F_{3, 33.6}=18.59$; $P<0.001$; fig. 29; vineyard FC: $F_{3, 30.4}=6.78$; $P=0.001$; fig. 30) and there were more *T. pyri* in the respective release plots than in the control (vineyard P: $F_{1,48.5}=43.79$; $P<0.001$; vineyard S: $F_{1, 25}=4.51$; $P=0.044$; vineyard GDC: $F_{1, 33.6}=33.49$; $P<0.001$; vineyard FC: $F_{1, 38.8}=18.18$; $P=0.001$). In vineyards P and GDC the presence of *K. aberrans* affected *T. pyri* densities where the two species were released in the same plots ($F_{1, 48.5}=10.11$; $P=0.003$;fig. 27; $F_{1, 33.6}=12.14$; $P=0.001$; fig. 29) but this phenomenon did not occur in vineyards S ($F_{1, 25}=2.52$; $P=0.125$; fig. 28) and FC ($F_{1, 38.8}=0.01$; $P=0.988$; fig. 29).
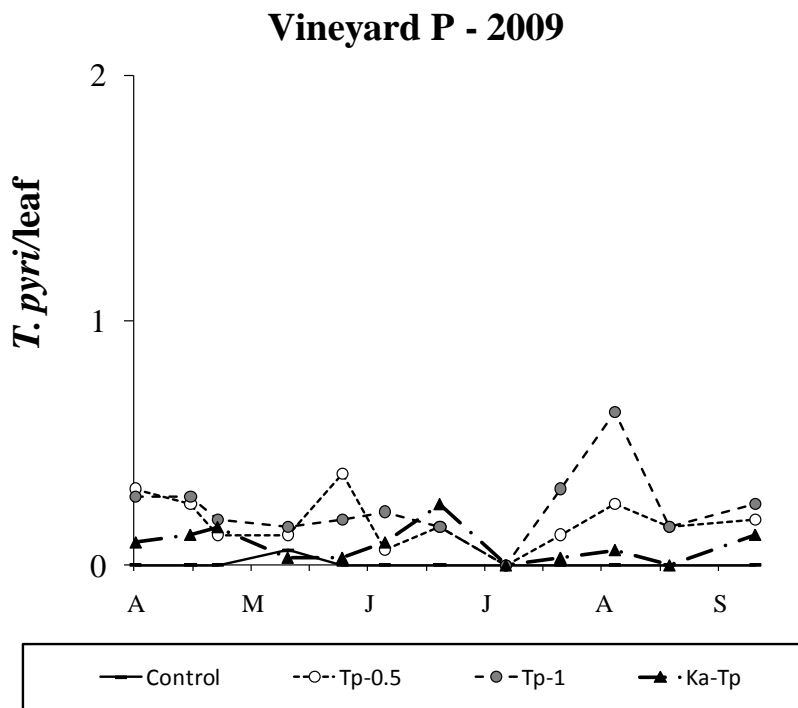
Figure 47. Seasonal abundance of *T. pyri* on different treatments.

Vineyard S - 2009
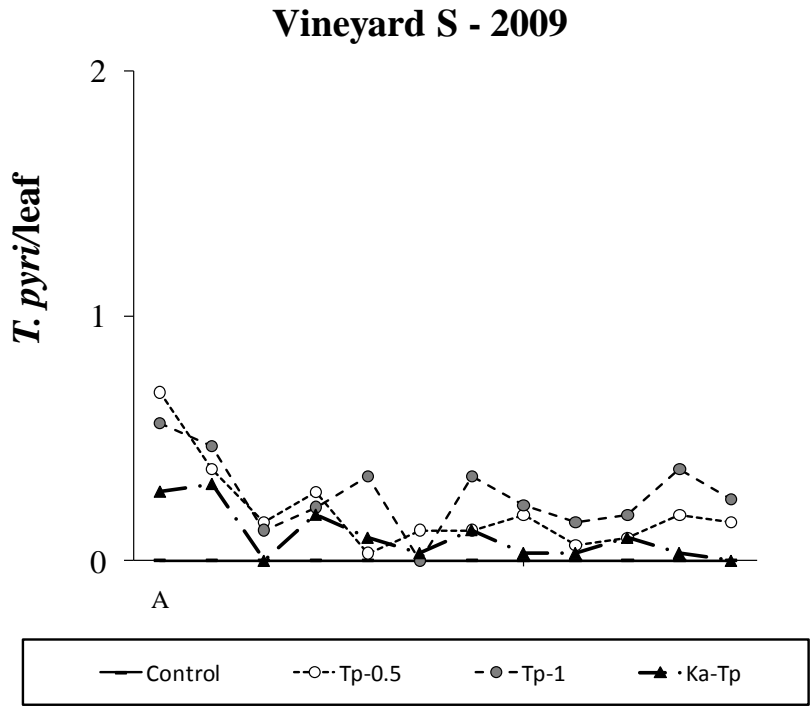
Figure 48. Seasonal abundance of *T. pyri* on different treatments.

Vineyard GDC - 2009
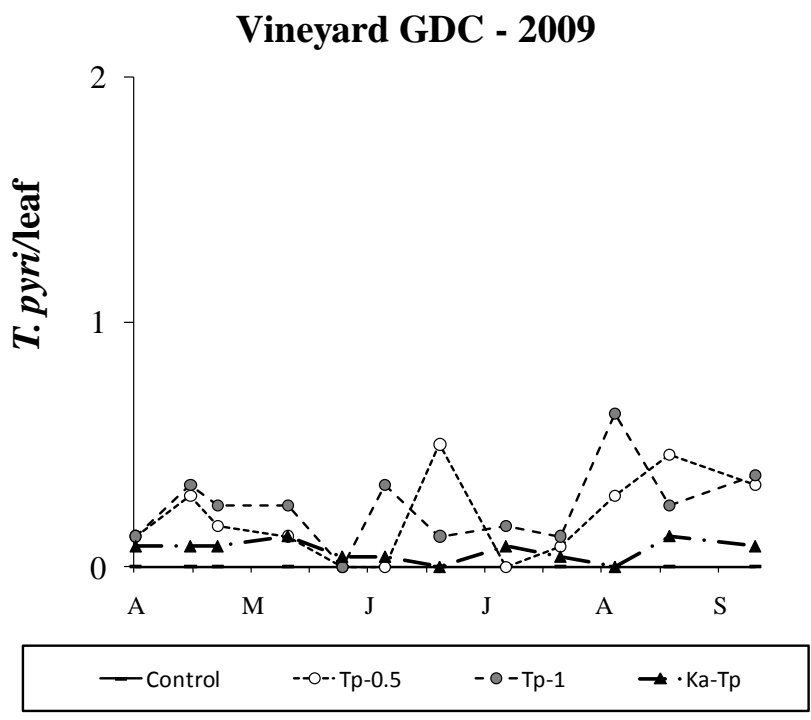
Figure 49. Seasonal abundance of *T. pyri* on different treatments.
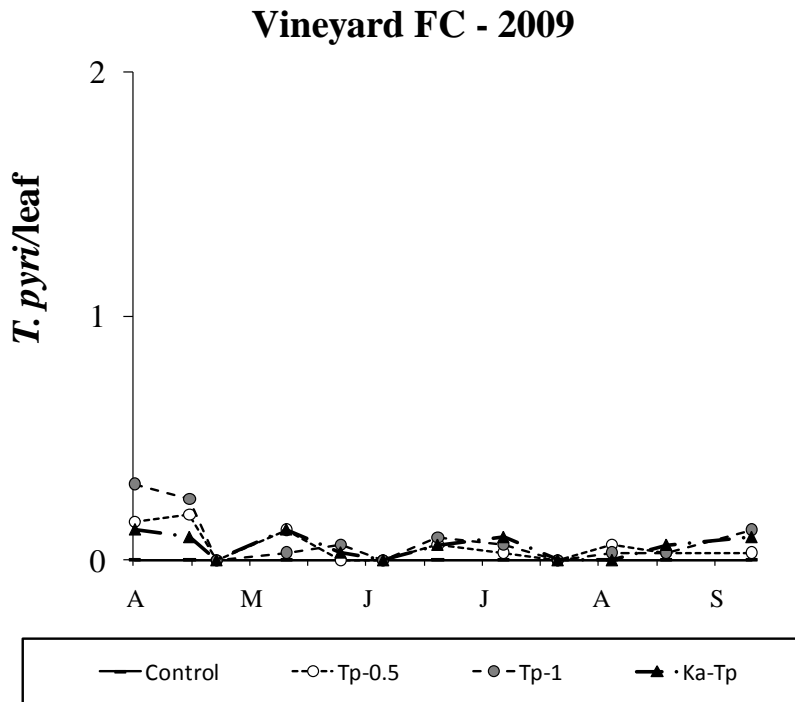
Vineyard FC - 2009

Figure 50. Seasonal abundance of *T. pyri* on different treatments.

In 2010, the effect of treatment confirmed to be significant in vineyards P ($F_{3,\ 45.7}$=29.57; P<0.001; fig. 31), S ($F_{3,\ 31}$=21.57; P<0.001; fig. 32), and GDC ($F_{3,\ 30}$=6.72; P=0.001; fig. 33) but not in vineyard FC ($F_{3,\ 15.7}$=21.2; P=0.425; fig. 34). *Typhlodromus pyri* densities were higher in the respective release plots than in the control plots of vineyards P ($F_{1,\ 45.7}$=59.61; P<0.001), S ($F_{1,\ 31}$=36.47; P<0.001) and GDC ($F_{1,\ 30}$=13.57.12; P=0.001); however this effect was not significant in vineyard FC ($F_{1,\ 15.7}$=2.42; P=0.140) where predatory mites reached low population densities. In Ka-Tp treatments the presence of *K. aberrans* reduced that of *T. pyri* in vineyards P ($F_{1,\ 45.7}$=20.12; P<0.001; fig. 31) and S ($F_{1,\ 31}$=19.32; P<0.001; fig. 32) in contrast with vineyards GDC ($F_{1,\ 30}$=2.91; P=0.09; fig. 33) and FC ($F_{1,\ 15.7}$=0.18; P=0.675; fig. 34).
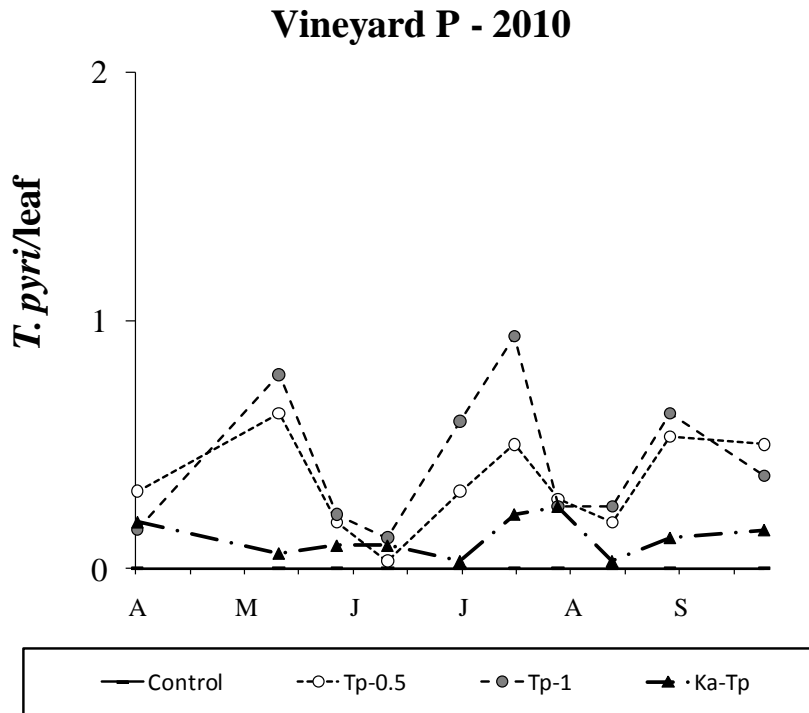
Vineyard P - 2010

Figure 51. Seasonal abundance of *T. pyri* on different treatments.

Vineyard S - 2010
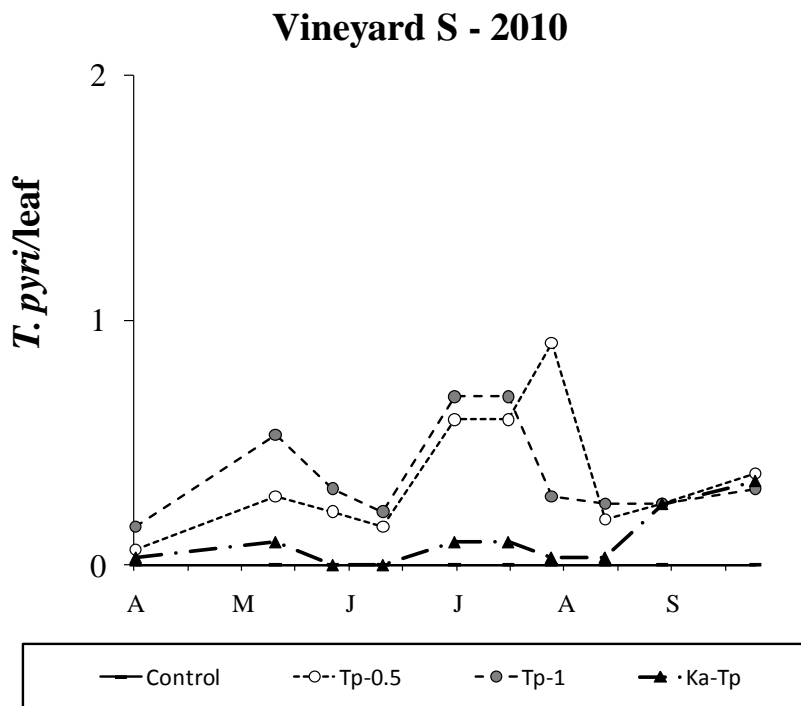
Figure 52. Seasonal abundance of *T. pyri* on different treatments.

Vineyard GDC - 2010
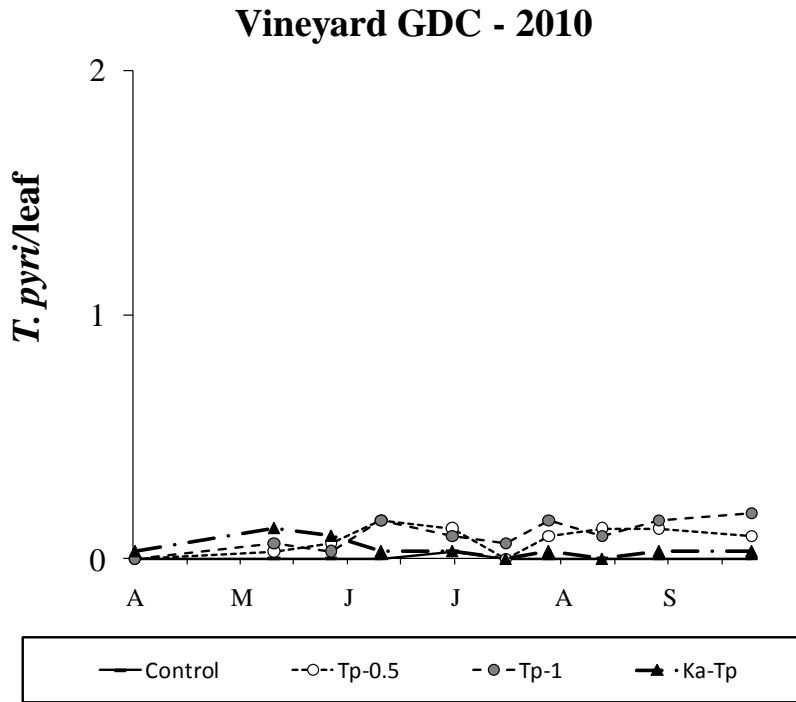
Figure 53. Seasonal abundance of *T. pyri* on different treatments.
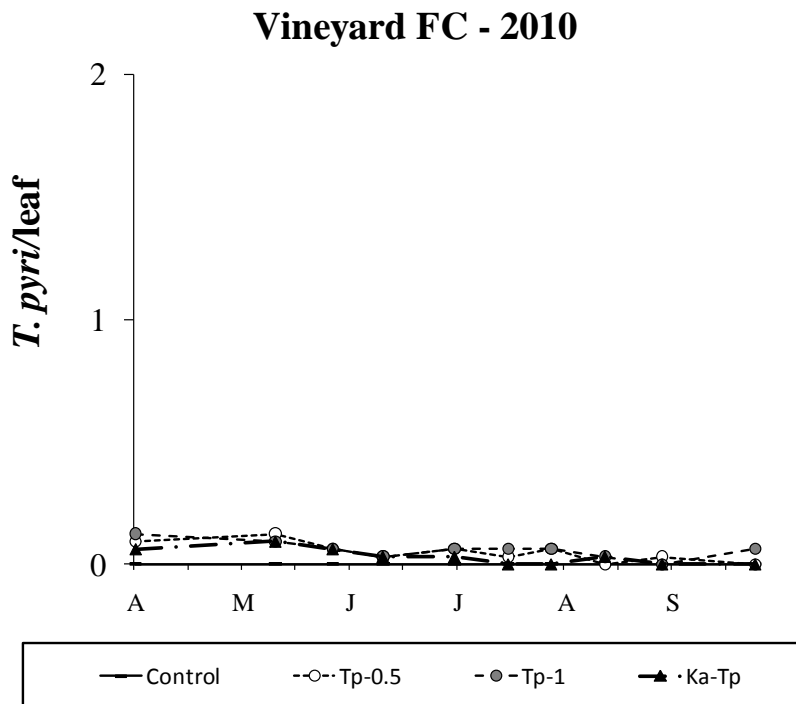
Vineyard FC - 2010

Figure 54. Seasonal abundance of *T. pyri* on different treatments.

The analysis of canopy structure showed significant differences among vineyards in some parameters. Vineyards P and S had a lower incidence of empty spaces than vineyards GDC and FC ($F_{3, 164} = 98.78$; $P<0.001$; fig. 35). The number of leaf layers was higher in canopies of vineyards P and S than in those of GDC and FC ($F_{3, 164} = 76.28$; $P<0.001$; fig. 36). The proportion of leaves inside the canopy was higher in vineyard P than in vineyard S, while vineyards GDC and FC gave intermediate results ($F_{3, 164} = 3.72$; $P=0.013$; fig. 37).
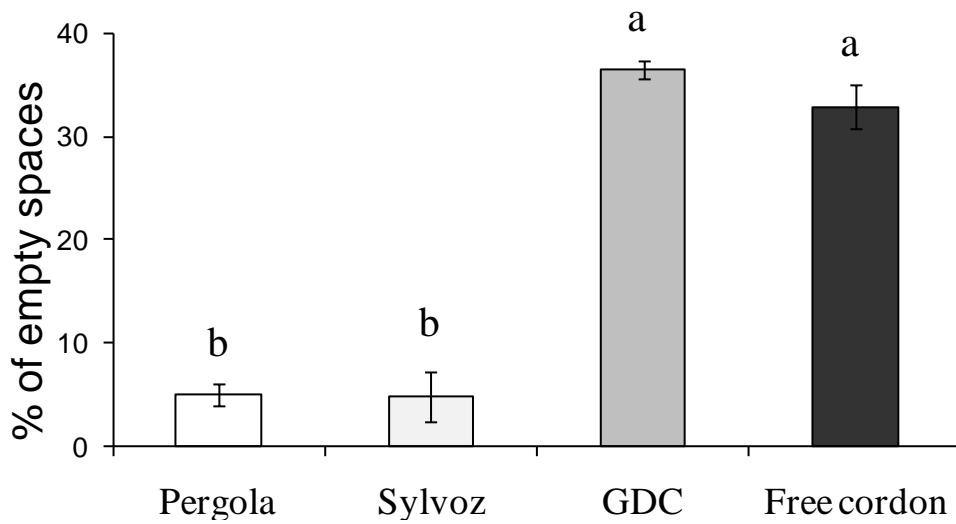

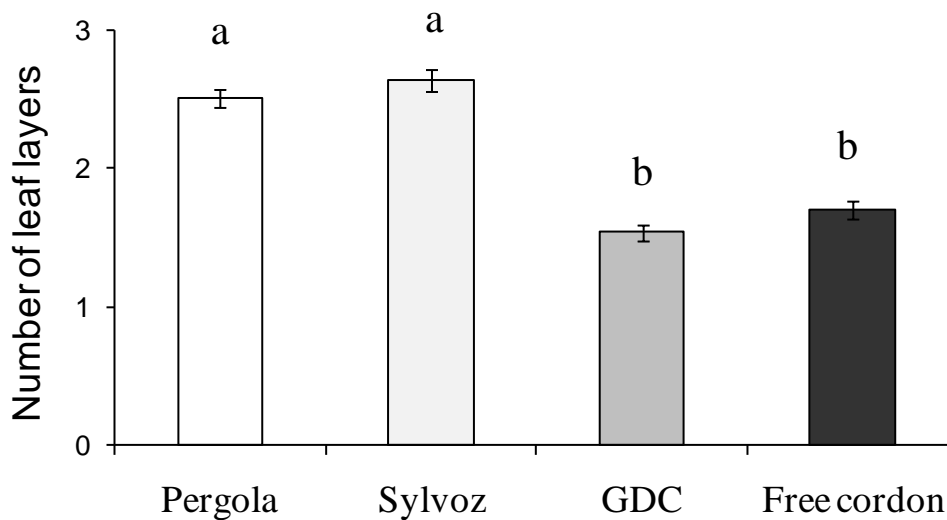**Figure 55.** Percentage of empty spaces in canopies of different vineyards.

Figure 56. Number of leaf layers of canopies of different vineyards.
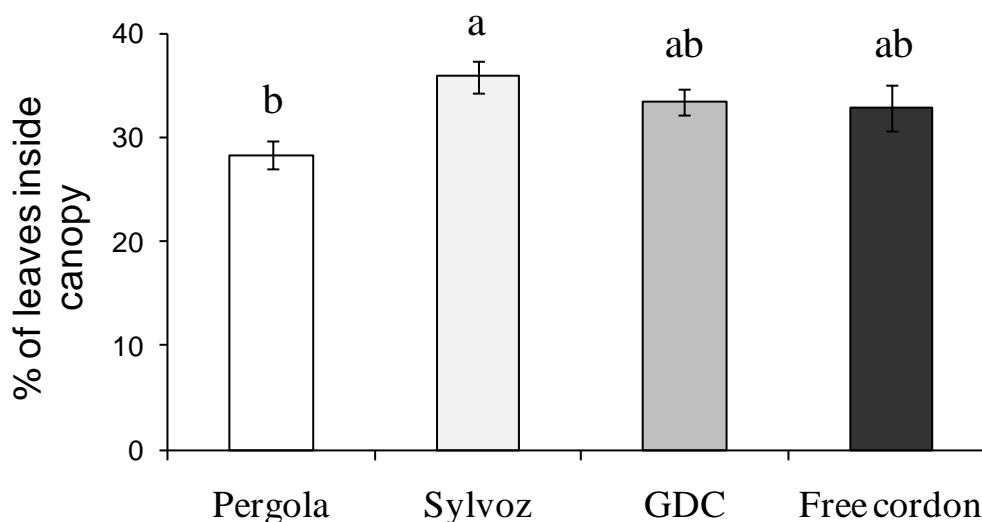
Figure 57 Percentage of leaves inside the canopy of different vineyards.

Farm B

Effect of predatory mite releases on E. carpini densities

In control plots *E. carpini* populations reached moderate densities over the season (Fig. 38) while in release plots they slightly increased in late season. *Panonychus ulmi* was not detected. The effect of treatment was significant ($F_{5,\ 59.6}$=11.01; P<0.001), and there were less spider mites in predatory mite release plots than in the control ones ($F_{1,\ 59.6}$=48.85; P<0.001). The effects of *K. aberrans* or *T. pyri* releases were both significant ($F_{1,\ 59.6}$=27.36; P<0.001; $F_{1,\ 59.6}$=49.09; P<0.001, respectively), and the latter resulted more effective ($F_{1,\ 59.6}$=4.73; P=0.034). Spider mites were reduced significantly even in Ka-Tp plots compare to control ($F_{1,\ 59.6}$=34.50; P<0.001). The effect of density in predatory mite releases was not significant ($F_{1,\ 59.6}$=0.69; P=0.41; fig. 38). All other comparisons were not significant.
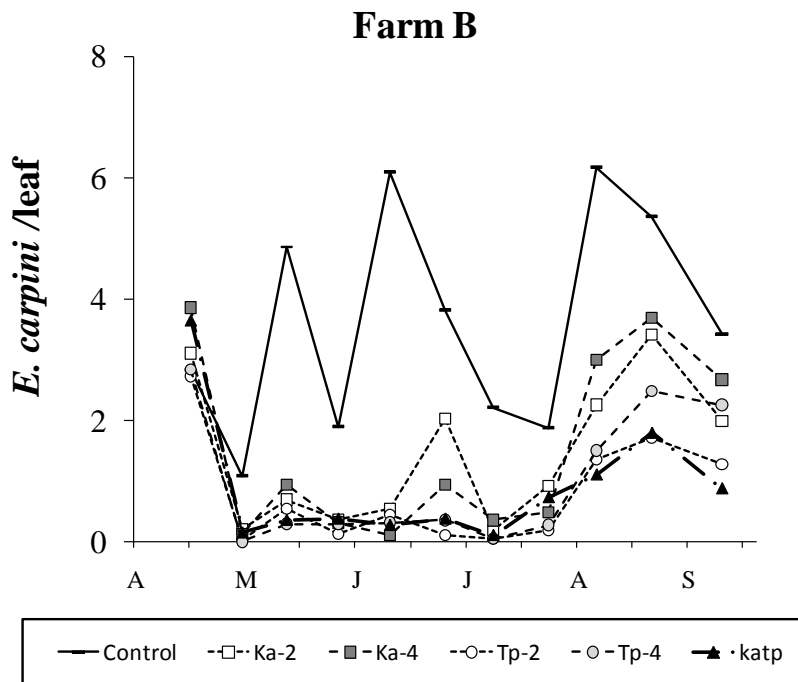
Figure 58. Seasonal abundance of *E. carpini* on different treatments.

Effects of releases on predatory mite species

Among native predatory mites the most frequent were *Ph. finitimus* and *A. andersoni*. Their populations fluctuated at low to moderate densities over the season (Fig. 39, 40). Regarding *Ph. finitimus* the effect of treatment was not significant ($F_{5,\ 56.5}$=1.39; P=0.243). In the contrasts' analysis *Ph. finitimus* was less abundant in release plots than in the control ($F_{1,\ 56.5}$=4.42; P=0.04). This result was due to the effect of *T. pyri* releases ($F_{1,\ 56.5}$=5.06; P=0.028; fig 39).

Regarding *A. andersoni*, the effect of treatment was significant ($F_{5,\ 96.4}$=3.13; P=0.012; fig. 40). *Amblyseius andersoni* numbers were significantly reduced in release plots ($F_{1,\ 96.4}$=11.90; P<0.001) and the effects of *K. aberrans* or *T. pyri* releases were both significant ($F_{1,\ 96.4}$=5.33; P=0.023; $F_{1,\ 96.4}$=13.18; P<0.001, respectively). There were no differences between *K. aberrans* or *T. pyri* treatments ($F_{1,\ 96.4}$=2.63; P=0.108). Additional differences were recorded between Ka-Tp and control plots ($F_{1,\ 96.4}$=9.44; P=0.003). The effect of release density was not significant ($F_{1,\ 96.4}$=0.52; P=0.473; fig. 40).

Kampimodromus aberrans releases were successful but predatory mite populations reached relatively low levels. The effect of treatment was significant ($F_{3,\ 45.9}$=19.61; P<0.001; fig. 41) and there were more *K. aberrans* in the respective release plots than in the control ($F_{1,\ 45.9}$=45.30;

P<0.001). In Ka-Tp treatments the presence of *T. pyri* did not affect *K. aberrans* densities S ($F_{1, 45.9}$=1.01; *P*=0.312). Higher *K. aberrans* numbers were recorded in plots receiving the highest release densities ($F_{1, 45.9}$=6.56; *P*=0.014; fig. 41).

   Typhlodromus pyri releases were also successful but also in these cases predatory mites did not exceed densities of one motile form per leaf. The effect of treatment was significant ($F_{3, 47.4}$=20.16; *P*<0.001; fig. 42) and *T. pyri* densities were higher in the respective release plots than in the control plots ($F_{1, 47.4}$=46.66; *P*<0.001). The presence of *K. aberrans* did not reduce that of *T. pyri* in Ka-Tp treatments ($F_{1, 47.4}$=2.98; *P*=0.09). The effect of release density was not significant ($F_{1, 47.4}$=3.95; *P*=0.053; fig. 42).

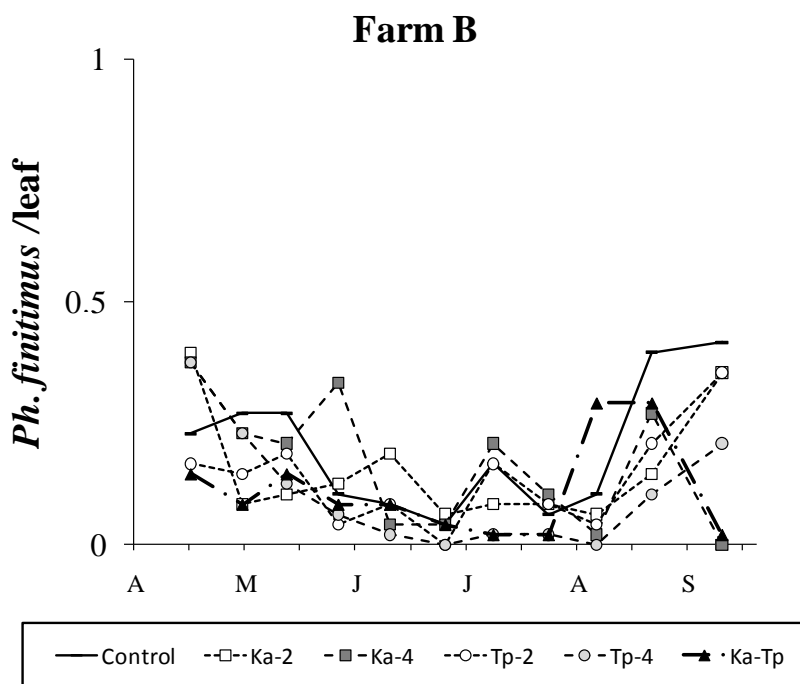**Figure 59.** Seasonal abundance of *P. finitimus* on different treatments.
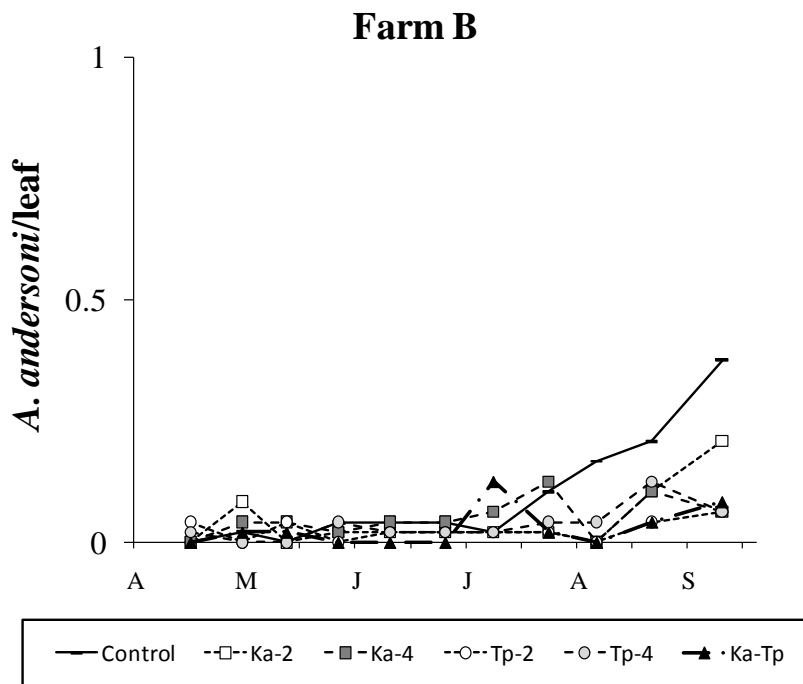
Farm B

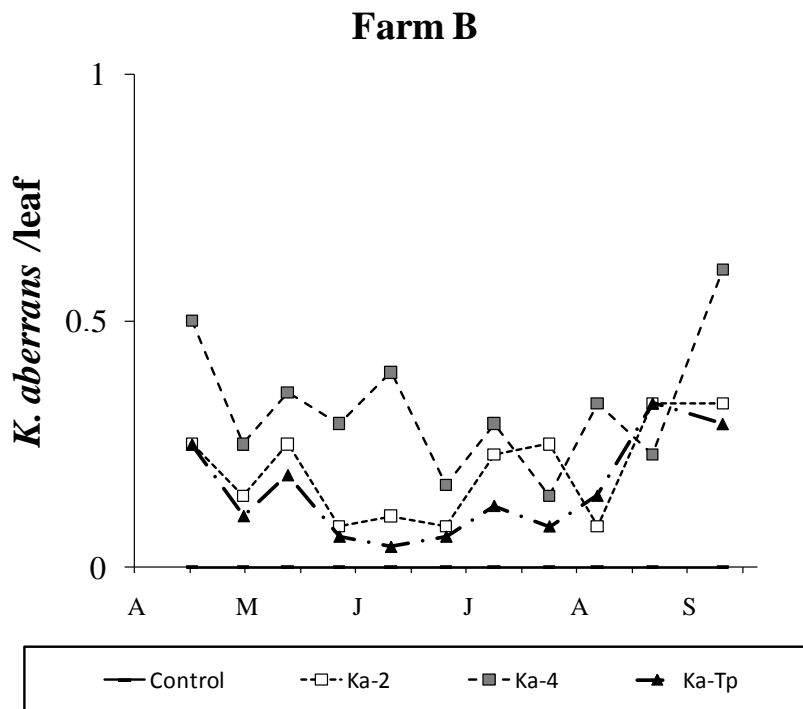**Figure 60.** Seasonal abundance of *A. andersoni* on different treatments.

Farm B

Figure 61. Seasonal abundance of *K. aberrans* on different treatments.
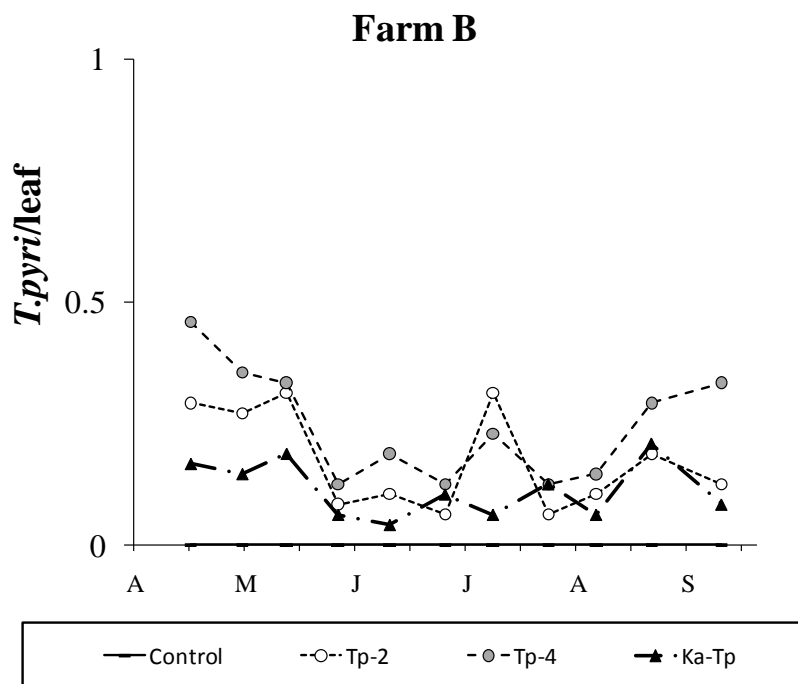
Figure 62. Seasonal abundance of *T. pyri* on different treatments.

Discussion

Mite seasonal abundance in farm A showed different patterns in selected vineyards. The most interesting differences concerned spider mite populations in 2009: *E. carpini* was dominant in vineyards P, S and GDC, *P. ulmi* in vineyard FC. Factors affecting the negligible occurrence of *E. carpini* in vineyard FC compared to vineyards trained with other systems should be investigated. Canopy of free cordon vineyard appeared to be less vigorous (lower leaf layers) and irregular (lower percentage of empty spaces) compared with Pergola and Sylvoz systems. Similar patterns were found in vineyard GDC where *E. carpini* was less abundant than in more vigorous P and S vineyards. Little is known on the ecology of *E. carpini* and thus this topic needs ad hoc studies. On the other hand *P. ulmi* was more abundant in vineyard FC than elsewhere. One could suggest that the low densities of *E. carpini* allowed *P. ulmi* populations to increase in these plots in 2009. Apparent competition between *P. ulmi* and *E. carpini* has been observed in other studies. High *E. carpini* densities in early season corresponded to relatively low *P. ulmi* populations in summer and vice versa (Duso and Pasqualetto, 1993). This topic also requires additional investigations.

In 2010 *P. ulmi* reached negligible densities but *E. carpini* persisted longer in three out four vineyards suggesting that this species is more difficult to be controlled by natural enemies. The low populations encountered in vineyard GDC confirm trends seen one year before.

In both farms spider mite populations were not effectively controlled by native predatory mites. In the control plots *E. carpini* and *P. ulmi* populations were not kept at low levels by *A. andersoni* (farm A). *Eotetranychus carpini* populations were not controlled by mixed populations of *A. andersoni* and *Ph. finitimus* in farm B. Natural control of *P. ulmi* by *A. andersoni* has been associated with successful results in some studies (e.g., Camporese and Duso, 1996) but not in others (e.g., Duso, 1989). The impact of macropredators seems to be more significant on *P. ulmi* than on *E. carpini* populations (Duso and Pasqualetto, 1993). Results of the present study confirm that problems with *E. carpini* persist despite the occurrence of *A. andersoni* (Duso and Vettorazzo, 1999) and *Ph. finitimus* (Duso, unpubl. data). The grape cultivars involved in our trials (Glera at Spresiano, Verduzzo trevigiano at Meolo) are characterized by pubescent leaf surfaces whereas *A. andersoni* shows a preference for smooth leaf surfaces (Camporese and Duso, 1996). This could explain its weak response to spider mite populations. The *A. andersoni* population increase in late summer observed in some treatments was probably due to the spread of grape downy mildew that represents an alternative food for this species (Duso et al., 2003; Pozzebon and Duso, 2008). This phenomenon seemed to have a limited importance for spider mite control. The impact of *Ph. finitimus* on *E. carpini* populations in farm B appeared to be not significant despite the preference of this predatory mite for grape pubescent leaves (Duso and Vettorazzo, 1999). Probably, pesticides affected the performance by *A. andersoni* and *Ph. finitimus* in both farms. EBDC fungicides were used more frequently in farm B than in farm A but OPs were also applied in the latter. The use of chlorpyriphos, wettable sulphur and other fungicides (cyprodinil/fludioxonil) in July of 2009 was associated with *A. andersoni* population decline in farm A. The repeated use of mancozeb in farm B could be involved in similar problems. The impact of wettable sulfur on the survival and fecundity of predatory mites has been demonstrated in several experiments (Bernard et al., 2010). In the same experiments cyprodinil/fludioxonil and mancozeb were highly toxic to predatory mites.

Native predatory mite colonization patterns appeared also different in the four vineyards of farm A. In vineyards P and S, *A. andersoni* appeared to be more abundant than in the remaining vineyards. Differences may be due to higher *E. carpini* populations in these vineyards but also to their canopy features.

The releases of *K. aberrans* and *T. pyri* obtained successful results with some variation among farms and experimental years. In 2009 (farm A), *P. ulmi* densities were reduced in vineyard P (*K.*

aberrans releases) and especially in vineyard FC (*K. aberrans* and *T. pyri* releases) where spider mites occurred at relatively high levels. In the same year and farm the impact of released predatory mites on *E. carpini* densities was less clear. Significant effects were obtained in vineyard GDC where *E. carpini* was not abundant. The weak response by *K. aberrans* and *T. pyri* to *E. carpini* contrasts with previous results obtained in the same area (e.g. Duso and Pasqualetto, 1993; Duso and Vettorazzo, 1999) and may be caused by the use of some pesticides. In fact in vineyards P, GDC and FC, *K. aberrans* and *T. pyri* populations declined in July after the use of above mentioned pesticides. Predatory mite strains used for releases are known to be low susceptible to mancozeb and chlorpyriphos in field conditions and it is likely that the early use of mancozeb as well as that of chlorpyriphos in summer had a low impact of their populations. At the same time, little is known about the effects of wettable sulphur and especially of cyprodinil/fludioxonil on these strains of *K. aberrans* and *T. pyri*. Laboratory studies will be addressed to investigate this topic.

Generally, increasing release densities had no effects on biological control in terms of spider mite seasonal abundance. However, the relatively high spider mite densities recorded in some experiments may suggest that an increase in release densities should attained a prompt control. The fast response of predatory mites to spider mites in farm B compared to their delayed response in farm A could be explained by this factor. Nevertheless, since grapevines can tolerate moderate spider mite densities without serious losses (Girolami, 1981, 1987), strategies based on several release points with relatively low release densities should be preferred to those based on few points and higher densities. This is also suggested by the limited mobility of predatory mites.

Intraguild competition showed some clear trends. Results suggest that competition effects among predatory mites were not symmetric. In both farms and most cases *A. andersoni* densities were significantly reduced in *K. aberrans* and *T. pyri* release plots. The latter result contrasts with conclusions taken from laboratory studies on interspecific predation between *A. andersoni* and *T. pyri* (e.g., Croft and Croft, 1996; Croft et al., 1996). In these trials competition favored *A. andersoni* and this outcome was explained to be associated with its larger body size. Other factors, such as prey density, climatic conditions or pesticide use can be involved in these interactions (Zhang and Croft, 1995; Croft and Croft, 1996; Croft *et al.*, 1996). Our results confirm trends reported in a previous paper where the role of leaf morphology in affecting interspecific competition was emphasized (Duso and Vettorazzo, 1999).

In farm B *Ph. finitimus* populations occurred at higher densities in early spring, decreased in summer but reestablished moderate levels in late season. Trials with *Ph. finitimus* suggest that this species suffered less than *A. andersoni* interspecific competition. Long term studies are required to

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understand the outcome of interspecific interactions involving this species (Duso and Vettorazzo, 1999). The role of EBDCs in affecting competition should be investigated more in depth.

The competition between *K. aberrans* and *T. pyri* in farm A gave interesting results. In 2009, the presence of *T. pyri* in mixed release plots (Ka-Tp) affected *K. aberrans* densities in two out four vineyards (GDC and FC). On the other hand, the presence of *K. aberrans* also affected *T. pyri* numbers in two out four vineyards (P and GDC). In 2010 the presence of *K. aberrans* reduced that of *T. pyri* in vineyards P and S but not in vineyards GDC and FC where predatory mites reached low population densities. In contrast the occurrence of *T. pyri* had no effects on that of *K. aberrans*.

Results also suggest that interspecific interaction are prey mediated: *K. aberrans* outcompeted *T. pyri* where *E. carpini* was abundant (vineyards P in 2009 and P and S in 2010), while *T. pyri* outcompeted *K. aberrans* where *P. ulmi* was more abundant (vineyard FC in 2009). GDC vineyard represents an interesting case study because both spider mite species were lowered by interspecific interaction. On GDC both spider mites were found at moderate levels. This aspect can be related to intraguild predation between predatory mites. Theoretical studies predict that coexistence and thus persistence of intraguild predators are likely at intermediate level of productivity and when the intraguild prey is a superior competitor for the shared resource than the intraguild predator (Polis et al. 1989; Holt and Polis 1997; Diehl and Feissel 2001; Mylius et al. 2001). In this case it is not clear which predator is the superior competitor for the shared resources. Moreover we didn't observe the extinction of one of the two predators, but we observed fluctuations in equilibrium level. According to life table parameter as a measure of prey conversion into offspring, *T. pyri* appeared superior competitor on both prey, but if we consider behavioral response to volatiles *K. aberrans* respond better to *E. carpini* than *P. ulmi* (Lorenzon et al., this thesis). When the two preys were offered *K. aberrans* clearly choose *E. carpini* while *T. pyri* did not show any preferences. Behavioral studies can help in understanding field results. When *E. carpini* is the main pest, *K. aberrans* is likely favored because of its capacity in prey detection, while when *P. ulmi* is main pest *K. aberrans* appears to be an inferior competitor compared to *T. pyri*. An intermediate levels of both prey none of these predators is favored and both persist at a lower level.

However, interspecific interaction results confirm the capability of *K. aberrans* to compete with heterospecific predatory mites reported in other studies (Duso, 1989; Duso and Camporese, 1991; Duso and Pasqualetto, 1993; Duso and Vettorazzo, 1999). In the latter *K. aberrans* displaced *T. pyri* after 2-4 years from their releases, in particular when spider mite densities declined. The persistence of spider mites in the second year of the present study (see farm A) could have slow down this process. Body size and feeding specialization are considered important factors in

interspecific competition (Croft and Croft, 1993, 1996; Croft et al., 1996). Laboratory experiments showed that *T. pyri* females survived longer than *K. aberrans* females in conditions of prey scarcity (Schausberger 1997) but we showed different results (Lorenzon et al., this thesis). Studies on interspecific predation between *T. pyri* and *K. aberrans* did not suggest a definite trend (Schausberger 1997, 1999). Factors affecting the success of *K. aberrans* over *T. pyri* in north-east Italian vineyards and orchards remains in part unexplained (Duso et al. 2009). *Kampimodromus aberrans* could be more able than *T. pyri* to find shelters to escape to competitors. This topic will be investigated in the next future.

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