

# Biological control of spider mites in North-Italian vineyards using pesticide resistant predatory mites

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## ABSTRACT

The success of phytoseiid mite releases to control spider mites [*Eotetranychus carpini* (Oudemans) and *Panonychus ulmi* (Koch)] on grapevines can be influenced by pesticide use and competition with local predatory mites. In field experiments we evaluated the effect of the release of *Kampimodromus aberrans* (Oudemans) and *Typhlodromus pyri* Scheuten strains showing field resistance to organophosphates and dithiocarbamates. Predatory mites were released in two vineyards infested by spider mites despite the occurrence of *Amblyseius andersoni* (Chant) and/or *Phytoseius finitimus* Ribaga. Single or mixed releases were planned. Spider mite populations were not effectively controlled by local predatory mites while successful control was achieved by released species. The effects of releases were higher in the second experimental year. In most cases *A. andersoni* densities were reduced by *T. pyri* and *K. aberrans* releases. *Ph. finitimus* suffered less than *A. andersoni* from intraguild predation. Among released species, the effect of the presence of a competitor was higher on *T. pyri* than on *K. aberrans*. Results suggest that the outcome of intraguild predation is prey-mediated. The equilibrium level between *K. aberrans* and *T. pyri* may depend on which spider mite species is the shared prey. The implications in management of spider mites on grapevines are discussed.

**Keywords** grapevine, biological control, predatory mites release, intraguild predation

## 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 been demonstrated to be effective in the 1980s (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 predatory insects (Duso, 1992; Duso and Pasqualetto, 1993; English-Loeb *et al.* 2002; Duso *et al.* 2012). In Integrated Pest Management (IPM) programs these factors and predatory mite species attributes should be considered to maximize their performance. Mechanisms of intraguild competition may enhance or disrupt biological control (e.g. Croft and McRae, 1993; Rosenheim *et al.* 1995; Seelmann *et al.* 2007).

Studies conducted in vineyards in North-eastern Italy have shown that predatory mite populations sometimes disappear or get reduced to low levels because of pesticide use leading to spider mites outbreaks (Ivancich Gambaro, 1973; Girolami, 1981; Duso *et al.* 1983). 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). Certain 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). Experimental

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releases of *K. aberrans* and *T. pyri* proved to be 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 decades outbreaks of *Eotetranychus carpini* (Oudemans) have been reported frequently in North-eastern Italy. Pesticides misuse, in particular that of ethylene-bis-dithiocarbamates (EBDCs) and organophosphates (OPs) has been claimed to be the key factor in this phenomenon. EBDCs are used to control Grape downy mildew *Plasmopara viticola* while OPs are employed to control leafhoppers (e.g., *Scaphoideus titanus* Ball.) and berry moths (e.g., *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 where spider mites infestations were previously observed, 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 resistant 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).

## Materials and methods

Experiments were carried out in vineyards in 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 province. Four vineyards of the cultivar Glera, planted in the same year but with different canopy management and 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 to releases 40 two-years old branches were collected during winter and moved to the laboratory. Here the branches were analyzed under a stereomicroscope using a forceps to remove the bark on the internodes and for bud dissection. Overwintering predatory mites were counted and identified. Their identity was confirmed by mounting adult mites on slides in Hoyer's medium, and by identifying them using a phase contrast microscope and morphological keys (e.g., Tixier *et al.* 2012). the predatory mites *A. andersoni* and *Paraseiulus talbii* (Athias-Henriot) were found on these branches (respectively 1.51 and 0.12 mean overwintering females per internode and a bud).

Farm B is located at Meolo (45°38'06" N; 12°27'19" E; 0 m a.s.l.), Venice district. A single vineyard of the Verduzzo trevigiano cultivar (Sylvoz pruning system) was considered. High population densities of *E. carpini* were recorded in the year preceding experiment. Samples collected during 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

The *K. aberrans* and *T. pyri* strains released 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 Felleto) where it was completely dominant among phytoseiid species. *Kampimodromus aberrans* and *T. pyri* strains proved to be resistant to OPs and EBDCs (Posenato, 1994; Moret, 2006; Pozzebon *et al.* 2010; Tirello *et al.* 2012).

In January, before releases, 40 two-year old branches were collected from each farm and brought to the laboratory. Here the branches were analyzed as described above, and the identity and density of overwintered phytoseiids were 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 (Jolliffe, 2000) that allows us to test the effect of predator diversity and density without confounding between the two factors (Table 1). In particular, the effect of predatory mite diversity was tested by keeping constant the total density in mixed and single species releases, and the effect of predatory mites density was tested by compared two levels of release density (0.5 and 1 predatory mites per bud; Table 1). A control without predatory mite releases was also included (Table 1). Each replicate comprised three continuous vines, separated from the subsequent plot by 10-30 vines, depending on vineyard size. Releases were carried out only in February of 2009 and observations were conducted over the vegetative seasons of 2009 and 2010. The 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 April – May. Regarding insecticides, chlorpyrifos was applied in July of 2009 and thiamethoxam in July of 2010.

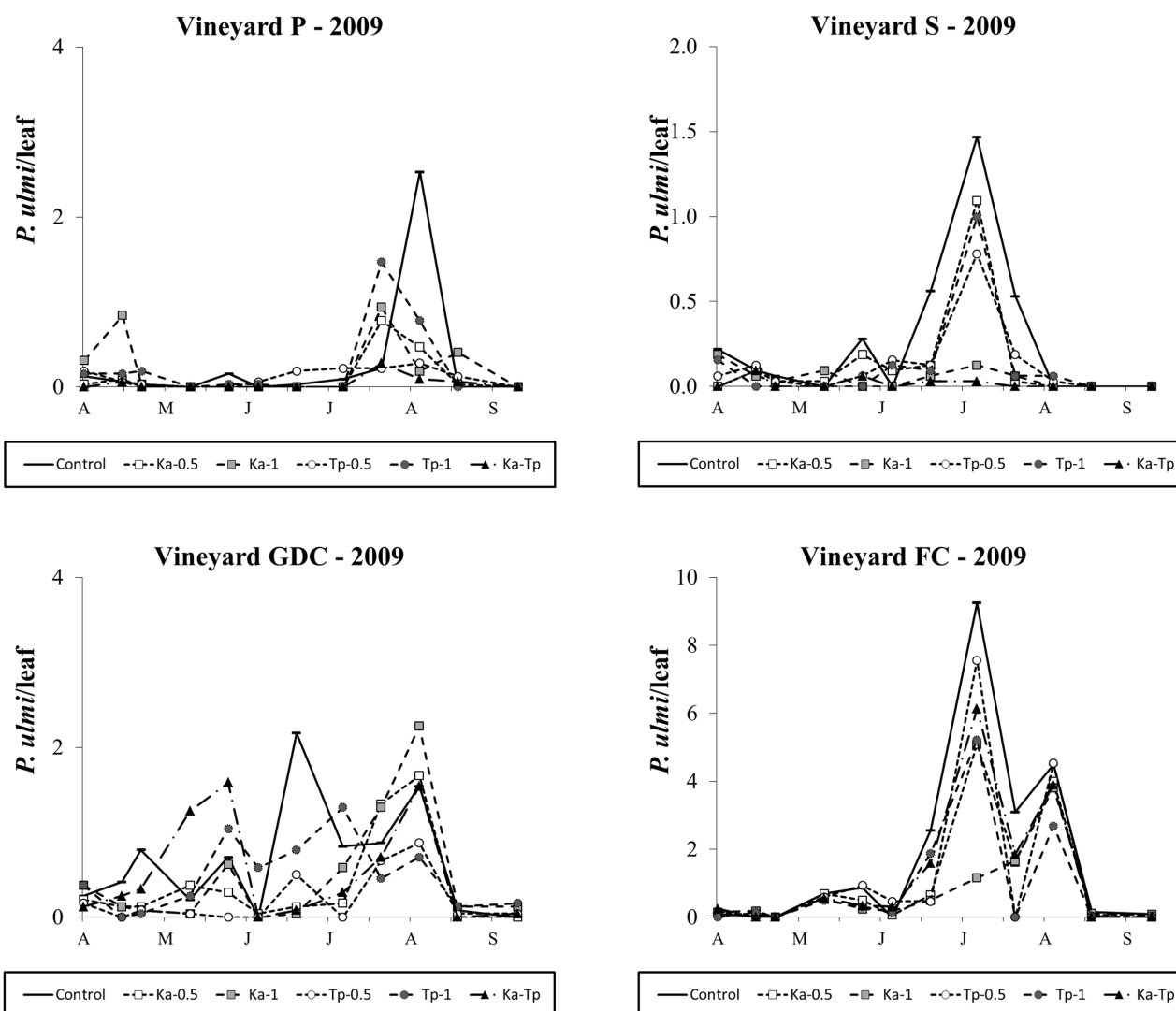
In farm B six release or non-release treatments randomly replicated were compared using a similar experimental design to farm B (Table 1). Here the release density was increased compared to Farm A (2 or 4 predatory mites per bud; Table 1) with the aim of obtaining a prompt control of spider mites. Six replicates per treatment were established and 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. Samplings started approximately two months after releases of predatory mites. At each sampling date a total of 48-64 leaves per treatment (Table 1) were collected (eight leaves per replicate) on mid shoots. Leaves were transferred to the laboratory and immediately observed by using a

**Table 1** Treatments and predatory mite release densities established in the two farms during the experiments.

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



**Figure 1** Seasonal abundance of *Panonychus ulmi* observed during 2009 (months are indicated in x-axis) on different treatments in vineyards of Farm A.

dissecting microscope in order to assess the identity and density of mites. Predatory mites identity was confirmed as described above.

### Canopy's features

In Farm A, different canopy managements were applied in different vineyards, with potential effect on mite's population dynamics (e.g., Prischmann *et al.* 2006). For this reason we assessed the structure of the canopy of each vineyard by applying the "point quadrat" method (Wilson, 1963, Bertamini *et al.* 1994). Following this method we used a square plastic panel measuring 1 m<sup>2</sup> 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 on predatory mites populations were analyzed using a Restricted Maximum Likelihood Repeated Measures model with the Proc MIXED of SAS® ver. 9.4. 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 one-way ANOVA and means were separated by Tukey test ( $\alpha = 0.05$ ).

## Results

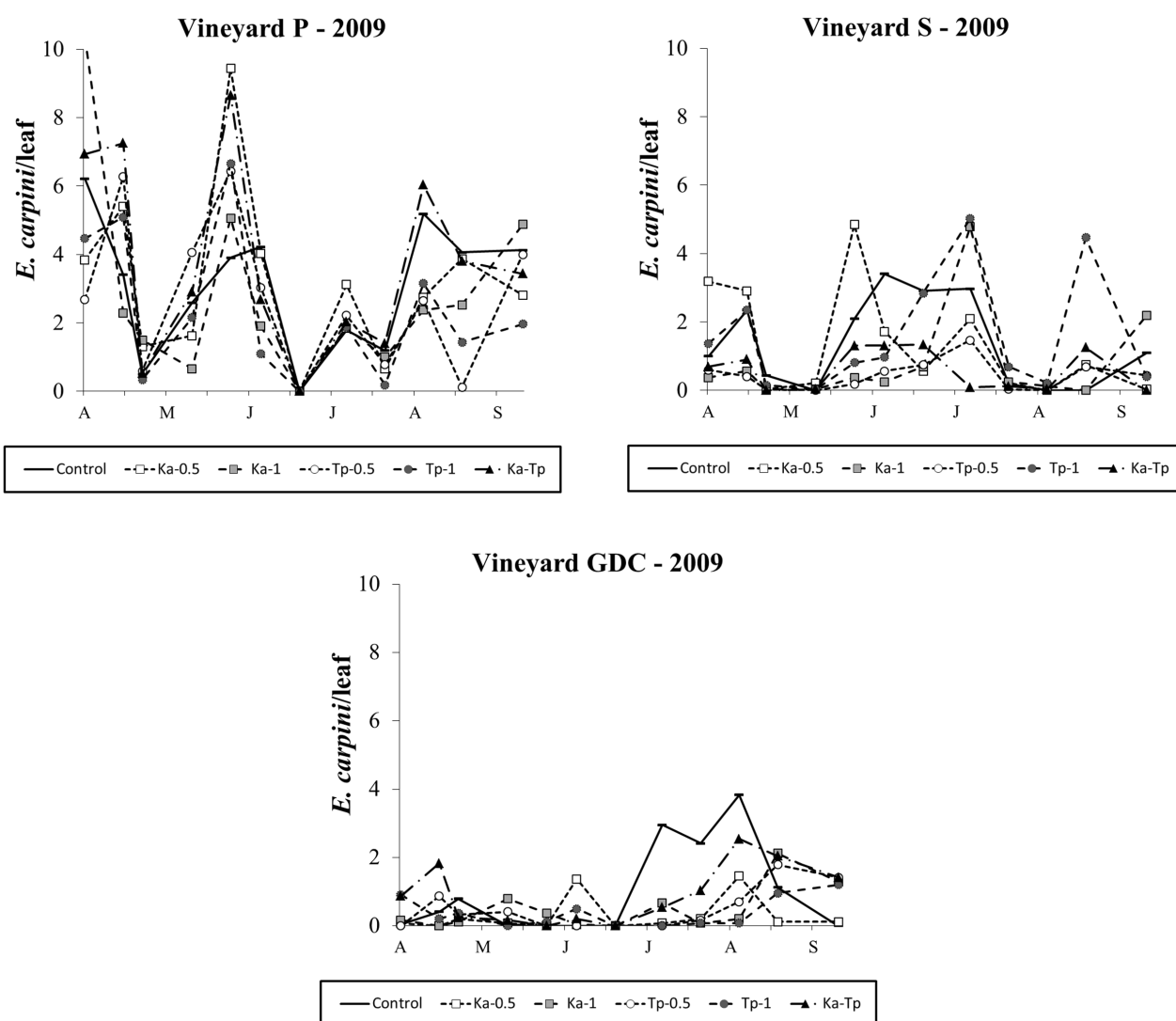
### Farm A

#### 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 plots without predatory mites release. 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) (Figure 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 significantly affected *P. ulmi* densities ( $F_{1, 76.5}=7.82$ ;  $P=0.006$ ) but this result was obtained with the highest release densities ( $F_{1, 76.5}=8.43$ ;  $P=0.005$ ; Figure 1). In contrast, *T. pyri* releases did not affect significantly *P. ulmi* abundance ( $F_{1, 76.5}=0.86$ ;  $P=0.357$ ; Figure 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 significantly reduced 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 ( $F_{1, 76.5}=0.09$ ;  $P=0.768$ ). In single releases, at the same release density, *K. aberrans* releases proved to be more effective than mixed releases (Ka-Tp) in reducing *P. ulmi* numbers ( $F_{1, 76.5}=7.16$ ;  $P=0.009$ ; Figure 1). The effect of treatment was not significant in vineyard S ( $F_{5, 49.6}=2.03$ ;  $P=0.091$ ; Figure 1), nor in vineyard GDC ( $F_{5, 56.9}=1.51$ ;  $P=0.202$ ; Figure 1). Nevertheless the contrasts' analysis showed a significant effect of *K. aberrans* releases (independently on the release density) on spider mite densities ( $p < 0.05$ ) in both vineyards. In vineyard FC the effect of treatment was significant ( $F_{5, 60.2}=2.90$ ;  $P=0.021$ ; Figure 1). *Kampimodromus aberrans* and *T. pyri* releases significantly reduced *P. ulmi* numbers ( $F_{1, 60.2}=6.42$ ;  $P=0.014$ ;  $F_{1, 60.2}=4.71$ ;  $P=0.034$ , respectively; Figure 4). There were no differences between predatory mite species in terms of spider mite abundance ( $F_{1, 60.2}=0.20$ ;  $P=0.659$ ) nor between their density levels ( $F_{1, 60.2}=0.28$ ;  $P=0.599$ ). *Panonychus ulmi* densities were significantly reduced 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 single 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; Figure 1). During 2010 *P. ulmi* reached negligible densities in the four vineyards.

#### Effects of predatory mite releases on *E. carpini* densities

During the 2009 season, *E. carpini* populations fluctuated at relatively high (i.e., more than 1 motile form per leaf as seasonal average; Figure 2) densities in P and S vineyards, moderate densities in GDC (i.e., 0.5 – 1 motile forms per leaf as seasonal average; Figure 2), and

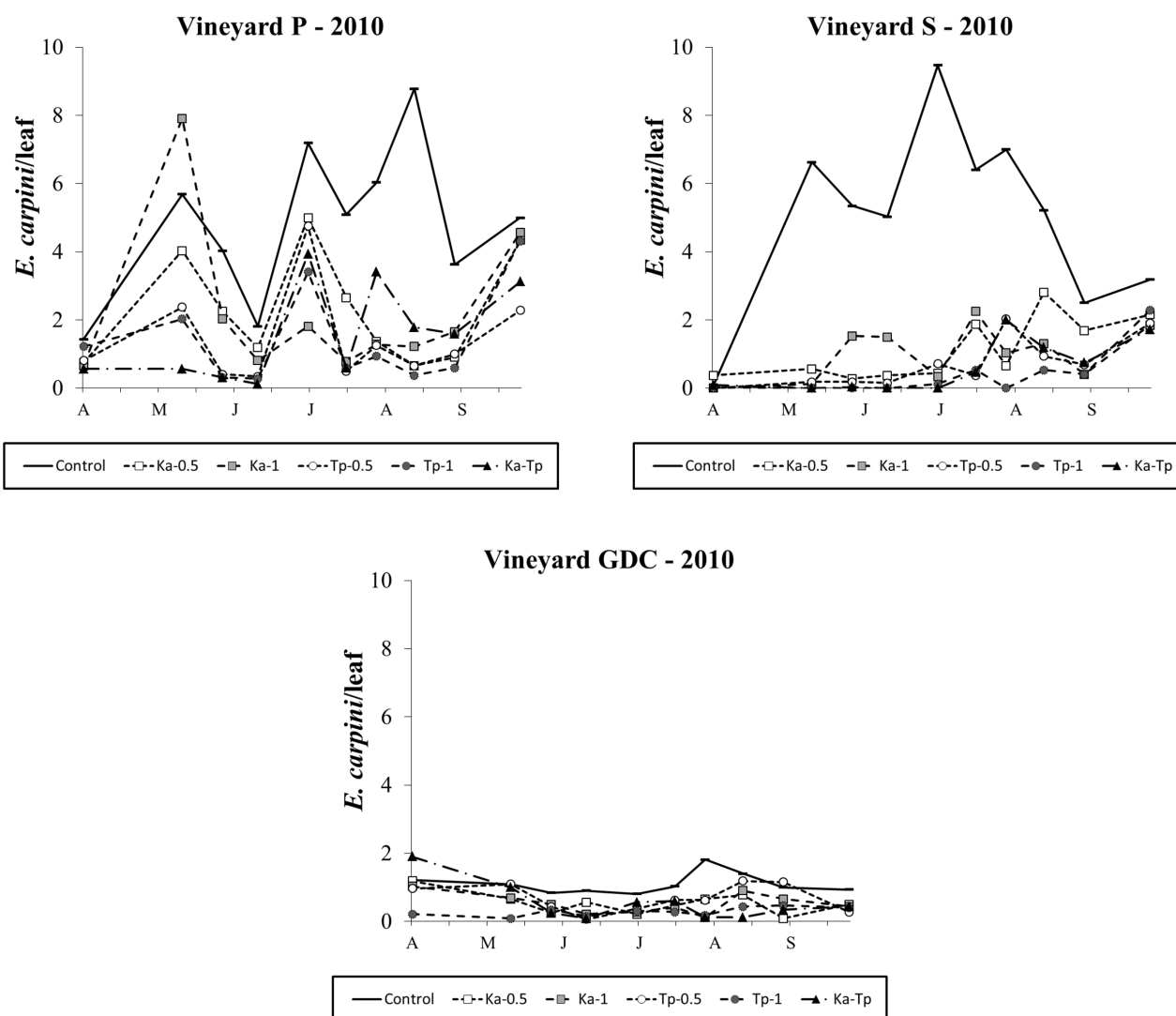


**Figure 2** Seasonal abundance of *Eotetranychus carpini* observed during 2009 (months are indicated in x-axis) on different treatments in vineyards of Farm A.

negligible levels in FC vineyard (i.e., less than 0.01 motile form per leaf as seasonal average; data not shown). The effect of treatment was not significant in vineyard P ( $F_{2, 52}=1.16$ ;  $P=0.339$ ; Figure 2) nor in vineyard S ( $F_{5, 36.6}=0.92$ ;  $P=0.482$ ; Figure 2). In contrast, it was significant in GDC vineyard ( $F_{5, 58}=2.68$ ;  $P=0.030$ ; Figure 2) where *T. pyri* releases significantly reduced *E. carpini* numbers, independently on release density ( $F_{1, 58}=9.51$ ;  $P=0.003$ ), 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 abundance of spider mites between release plots of the two predatory mites ( $F_{1, 58}=3.72$ ;  $P=0.087$ ). *Eotetranychus carpini* densities were significantly reduced in Ka-Tp treatment compared to the control ( $F_{1, 58}=4.79$ ;  $P=0.033$ ; Figure 2). The remaining comparisons were not significant.

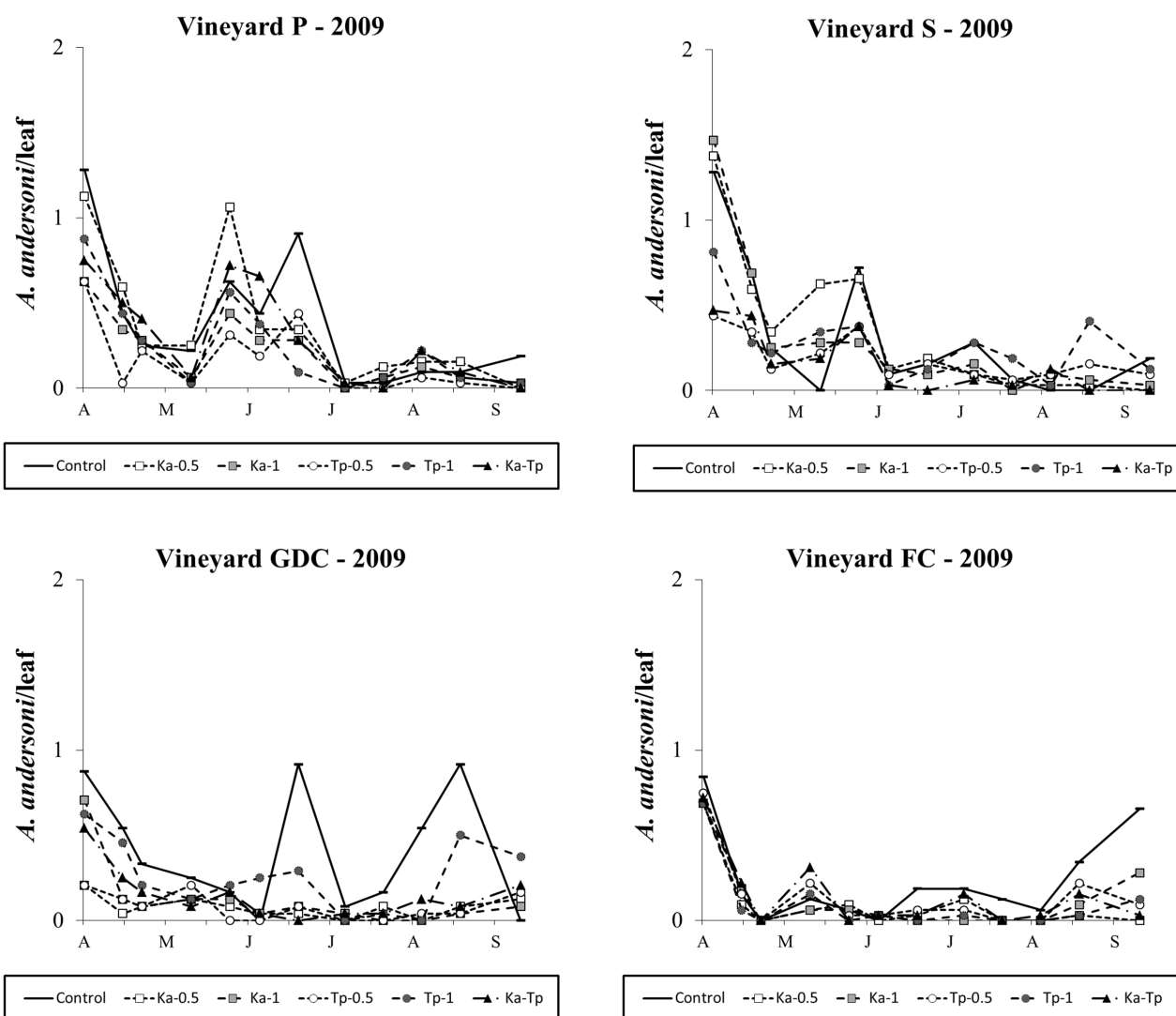
In 2010 *E. carpini* populations reached relatively high densities in vineyards P and S but low levels in vineyard GDC (Figure 3). The occurrence of this mite pest was still negligible in vineyard FC (data not shown). In the first vineyard differences among treatments





**Figure 3** Seasonal abundance of *Eotetranychus carpini* observed during 2010 (months are indicated in x-axis) on different treatments in vineyards of Farm A.

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; Figure 3). 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 significantly reduced in Ka-Tp treatment compared to the control ( $F_{1,55.3}=32.33$ ;  $P<0.001$ ; Figure 3). In vineyard S, the effect of treatment was significant ( $F_{5,54.3}=16.66$ ;  $P<0.001$ ; Figure 3) 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; Figure 3). There were no differences 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 ( $F_{1,54.3}=36.96$ ;  $P<0.001$ ; Figure 3). Additional differences among treatments emerged in GDC vineyard ( $F_{5,39.5}=2.59$ ;  $P=0.04$ ; Figure 3) where released predatory mites significantly reduced *E. carpini* densities compared to the control ( $F_{1,39.5}=8.01$ ;  $P=0.007$ ;  $F_{1,39.5}=8.86$ ;  $P=0.005$ ,



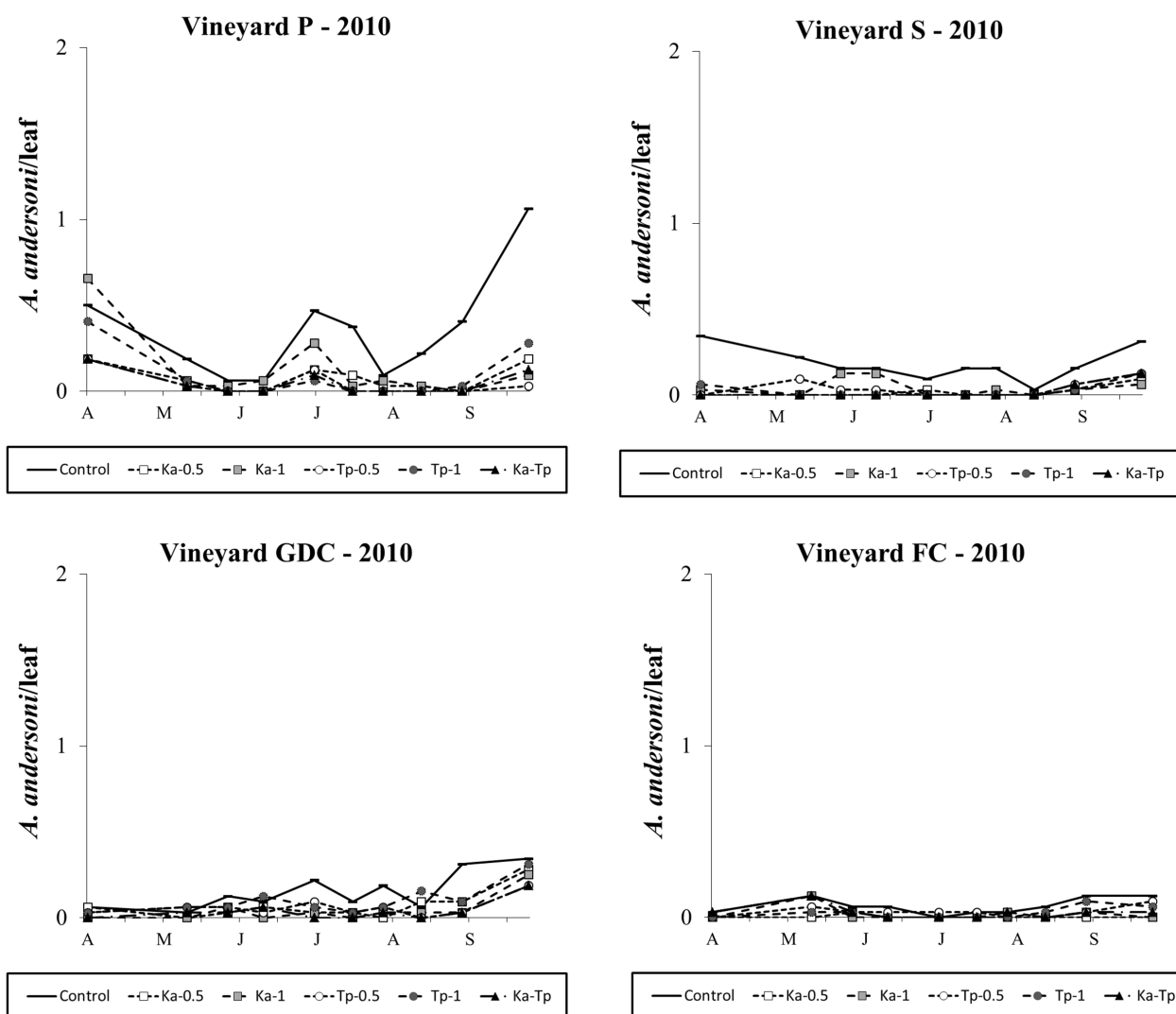
**Figure 4** Seasonal abundance of *Amblyseius andersoni* observed during 2009 (months are indicated in x-axis) on different treatments in vineyards of Farm A.

respectively for *K. aberrans* and *T. pyri* treatments; Figure 3). 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 ( $F_{1,39.5}=5.37$ ;  $P=0.026$ ; Figure 3).

### Effects of releases on predatory mite densities

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 ( $F_{5,63.4}=5.02$ ;  $P<0.001$ ; Figure 4). Compared to the control, *A. andersoni* numbers were significantly lower in *T. pyri* release plots ( $F_{1,63.4}=13.65$ ;  $P<0.001$ ) but not in *K. aberrans* plots ( $F_{1,63.4}=1.41$ ;  $P=0.24$ ). *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 ( $F_{1,63.4}=0.344$ ;  $P=0.559$ ; Figure 4). In vineyard S there were



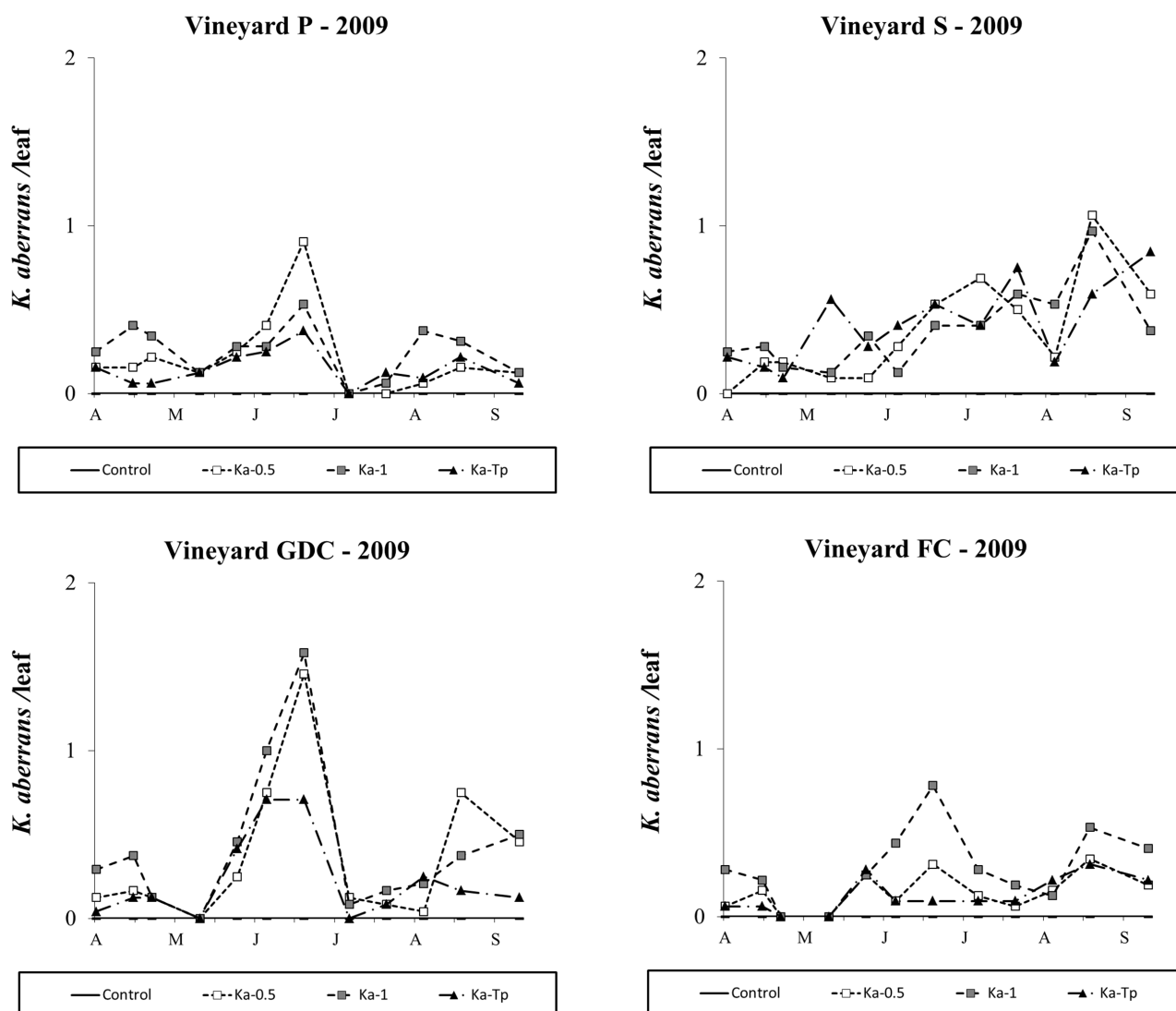


**Figure 5** Seasonal abundance of *Amblyseius andersoni* observed during 2010 (months are indicated in x-axis) on different treatments in vineyards of Farm A.

no differences among treatments ( $F_{5, 53.5}=2.09$ ;  $P=0.081$ ; Figure 4). 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 ( $F_{1, 53.5}=4.06$ ;  $P=0.049$ ; Figure 4).

In contrast, differences among treatments were significant in vineyard GDC ( $F_{5, 50.7}=11.97$ ;  $P<0.001$ ; Figure 4). *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 ( $F_{1, 50.7}=1.05$ ;  $P=0.31$ ). The effect of release density was significant ( $F_{1, 50.7}=8.46$ ;  $P=0.005$ ; Figure 4) but there were less *A. andersoni* in plots with the lowest release density.

The effect of treatment was significant also in vineyard FC ( $F_{5, 63.2}=4.56$ ;  $P=0.001$ ; Figure 4) 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 ( $F_{1, 63.2}=0.31$ ;  $P=0.580$ ).

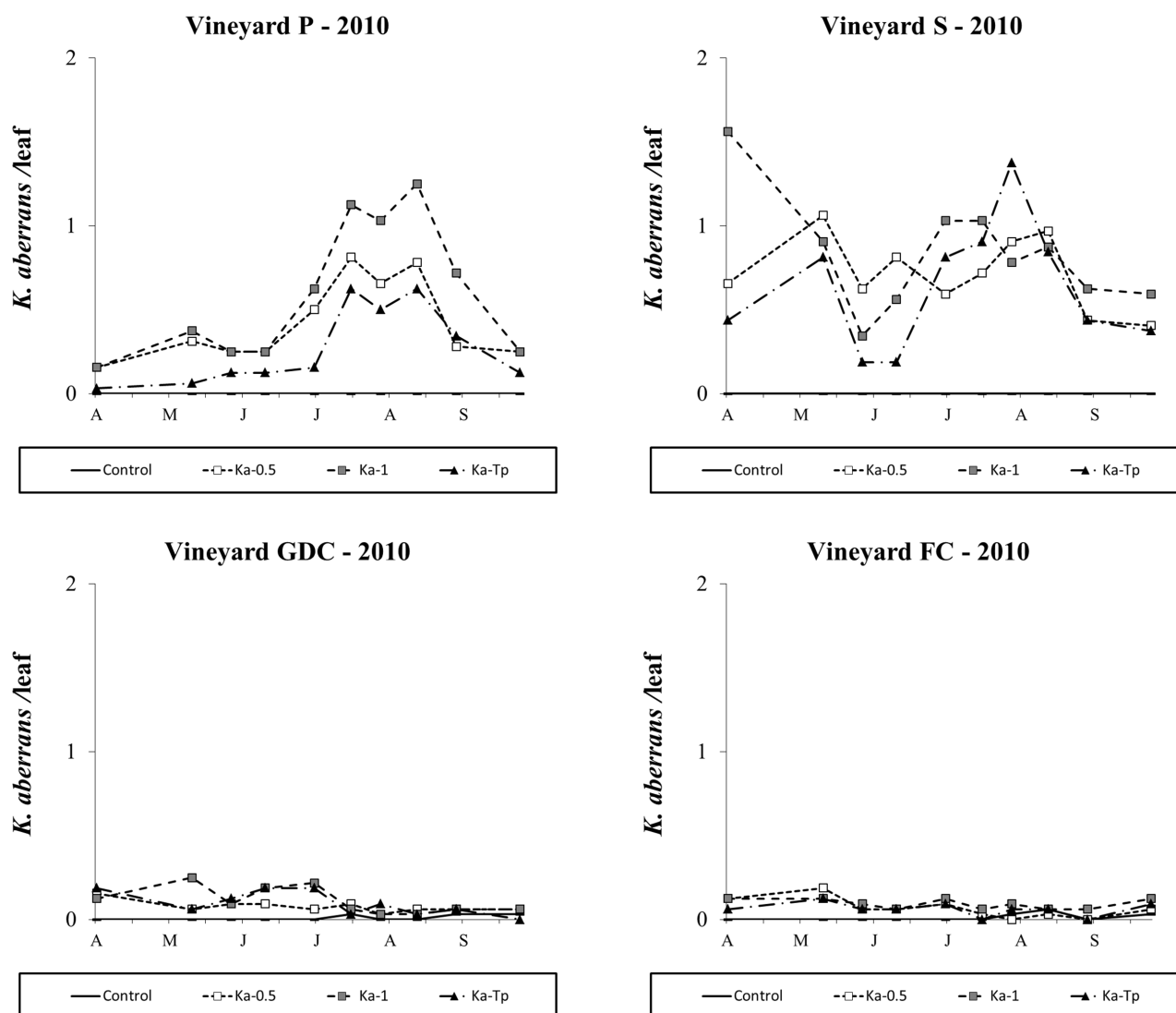


**Figure 6** Seasonal abundance of *Kampimodromus aberrans* observed during 2009 (months are indicated in x-axis) on different treatments in vineyards of Farm A.

The effect of release density was not significant ( $F_{1,63.2}=0.00$ ;  $P=0.961$ ; Figure 4).

In 2010, *A. andersoni* populations appeared to be less abundant than in 2009. The effect of treatment was significant in all vineyards (vineyard P:  $F_{5,37.5}=11.82$ ;  $P<0.001$ ; vineyard S:  $F_{5,35.4}=8.96$ ;  $P<0.001$ ; vineyard GDC:  $F_{5,49.9}=5.75$ ;  $P<0.001$ ; vineyard FC:  $F_{5,61.9}=5.61$ ;  $P<0.001$ ; Figure 5). *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:  $F_{1,37.5}=0.01$ ;  $P=0.912$ ; vineyard S:  $F_{1,35.4}=0.04$ ;  $P=0.834$ ; vineyard GDC:  $F_{1,49.9}=0.09$ ;  $P=0.766$ ; vineyard FC:  $F_{1,61.9}=0.74$ ;  $P=0.392$ ; Figure 5).

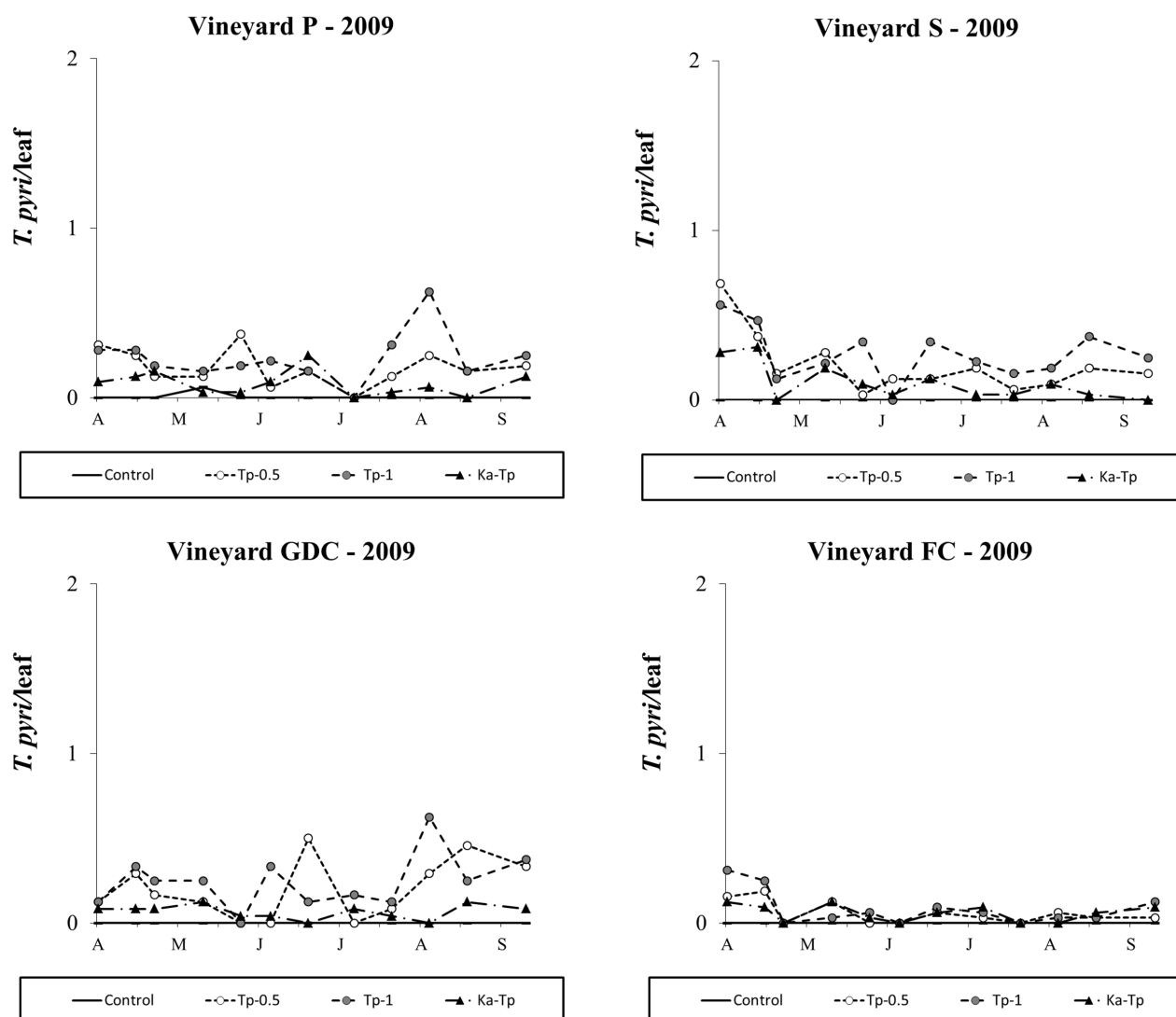
*Kampimodromus aberrans* populations successfully established after releases, even if



**Figure 7** Seasonal abundance of *Kampimodromus aberrans* observed during 2010 (months are indicated in x-axis) on different treatments in vineyards of Farm A.

predatory mite numbers did not reach high levels. In 2009, the effect of treatment was significant in all vineyards (vineyard P:  $F_{3,46}=18.85$ ;  $P<0.001$ ; vineyard S:  $F_{3,35.8}=22.69$ ;  $P<0.001$ ; vineyard GDC:  $F_{3,37.5}=33.89$ ;  $P<0.001$ ; vineyard FC:  $F_{3,38.8}=18.70$ ;  $P<0.001$ ; Figure 6) 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: (respectively:  $F_{1,37.5}=4.32$ ;  $P=0.044$ ;  $F_{1,38.8}=12.91$ ;  $P<0.001$ ; Figure 6) but not in vineyards P and S (respectively:  $F_{1,46}=2.02$ ;  $P=0.162$ ;  $F_{1,35.8}=0.01$ ;  $P=0.988$ ; Figure 6).

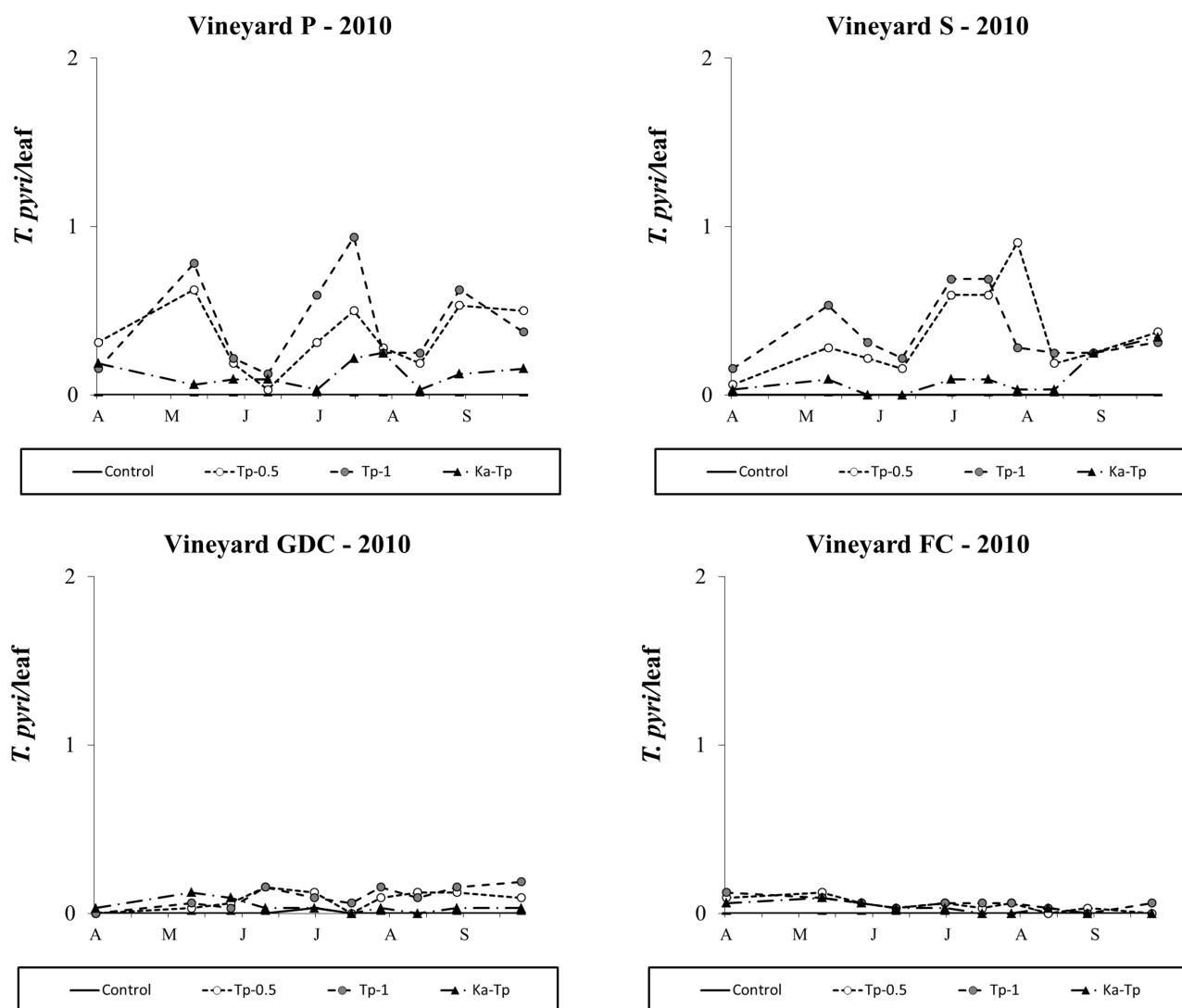
One year later, *K. aberrans* populations were more abundant in P and S vineyards than in GDC and FC ones. The effect of treatment was significant in all vineyards (vineyard P:  $F_{3,31.2}=6.05$ ;  $P=0.002$ ; vineyard S:  $F_{3,26.4}=3.15$ ;  $P=0.042$ ; vineyard GDC:  $F_{3,33.6}=27.58$ ;  $P<0.001$ ; vineyard FC:  $F_{3,36.7}=21.2$ ;  $P<0.001$ ; Figure 7) 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:



**Figure 8** Seasonal abundance of *Typhlodromus pyri* observed during 2009 (months are indicated in x-axis) on different treatments in vineyards of Farm A.

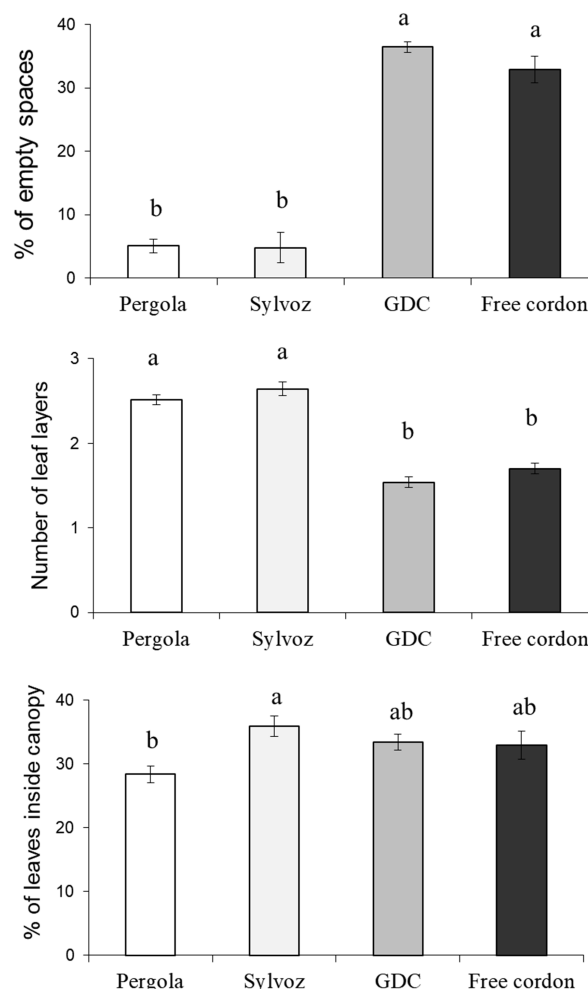
$F_{1,26.4}=8.77$ ;  $P=0.006$ ; vineyard GDC:  $F_{1,33.6}=80.12$ ;  $P<0.001$ ; vineyard FC:  $F_{1,36.7}=47.14$ ;  $P<0.001$ ). In Ka-Tp treatments the presence of *T. pyri* did not affect *K. aberrans* densities ( $F_{1,31.2}=2.29$ ;  $P=0.140$ ;  $F_{1,26.4}=0.68$ ;  $P=0.417$ ;  $F_{1,33.6}=0.25$ ;  $P=0.618$ ;  $F_{1,36.7}=3.15$ ;  $P<0.084$ ; P, S, GDC and FC respectively; Figure 7).

*Typhlodromus pyri* releases appeared to be successful but its densities were always lower than 1 motile form per leaf in 2009. The effect of treatment was significant in all vineyards (vineyard P:  $F_{3,48.5}=22.99$ ;  $P<0.001$ ; vineyard S:  $F_{3,25}=3.96$ ;  $P=0.019$ ; vineyard GDC:  $F_{3,33.6}=18.59$ ;  $P<0.001$ ; vineyard FC:  $F_{3,30.4}=6.78$ ;  $P=0.001$ ; Figure 8) 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 ( $F_{1,48.5}=10.11$ ;  $P=0.003$ ;  $F_{1,33.6}=12.14$ ;  $P=0.001$ ; Figure 8) but this phenomenon did not occur in vineyards S ( $F_{1,25}=2.52$ ;  $P=0.125$ ; Figure 8) and FC ( $F_{1,38.8}=0.01$ ;  $P=0.988$ ; Figure 8).



**Figure 9** Seasonal abundance of *Typhlodromus pyri* observed during 2010 (months are indicated in x-axis) on different treatments in vineyards of Farm A.

In 2010, the effect of treatment confirmed to be significant in vineyards P ( $F_{3,45.7}=29.57$ ;  $P<0.001$ ; Figure 9), S ( $F_{3,31}=21.57$ ;  $P<0.001$ ; Figure 9), and GDC ( $F_{3,30}=6.72$ ;  $P=0.001$ ; Figure 9) but not in vineyard FC ( $F_{3,15.7}=21.2$ ;  $P=0.425$ ; Figure 9). *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 ( $F_{1,45.7}=20.12$ ;  $P<0.001$ ; Figure 9) and S ( $F_{1,31}=19.32$ ;  $P<0.001$ ; Figure 9) in contrast with vineyards GDC ( $F_{1,30}=2.91$ ;  $P=0.09$ ; Figure 9) and FC ( $F_{1,15.7}=0.18$ ;  $P=0.675$ ; Figure 9).



**Figure 10** Canopy's feature parameters observed in different vineyards of Farm A. Different letters indicate significant differences at Tukey test ( $\alpha = 0.05$ ).

### Canopy's features

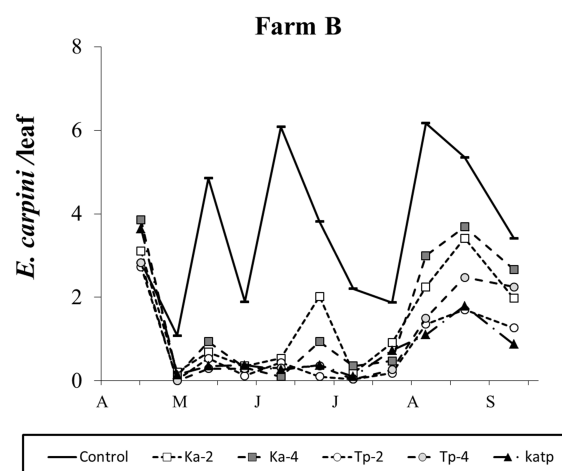
The analysis of canopy structure showed significant differences among vineyards in certain parameters. Vineyards P and S had a lower incidence of empty spaces than vineyards GDC and FC ( $F_{3,164} = 98.78$ ;  $P < 0.001$ ; Figure 10). The number of leaf layers was higher in canopies of vineyards P and S than in those of GDC and FC ( $F_{3,164} = 76.28$ ;  $P < 0.001$ ; Figure 10). The proportion of leaves inside the canopy was higher in vineyard P than in vineyard S, while vineyards GDC and FC gave intermediate results ( $F_{3,164} = 3.72$ ;  $P = 0.013$ ; Figure 10).

### Farm B

#### Effect of predatory mite releases on *E. carpini* densities

Here *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 were more effective ( $F_{1,59.6} = 4.73$ ;  $P = 0.034$ ). Spider mites were reduced significantly even in Ka-Tp plots compared to control ( $F_{1,59.6} = 34.50$ ;  $P < 0.001$ ). The effect of density in predatory mite





**Figure 11** Seasonal abundance of *Eotetranychus. carpini* observed during 2010 (months are indicated in x-axis) on different treatments in Farm B.

releases was not significant ( $F_{1, 59.6}=0.69$ ;  $P=0.41$ ; Figure 11). All other comparisons were not significant.

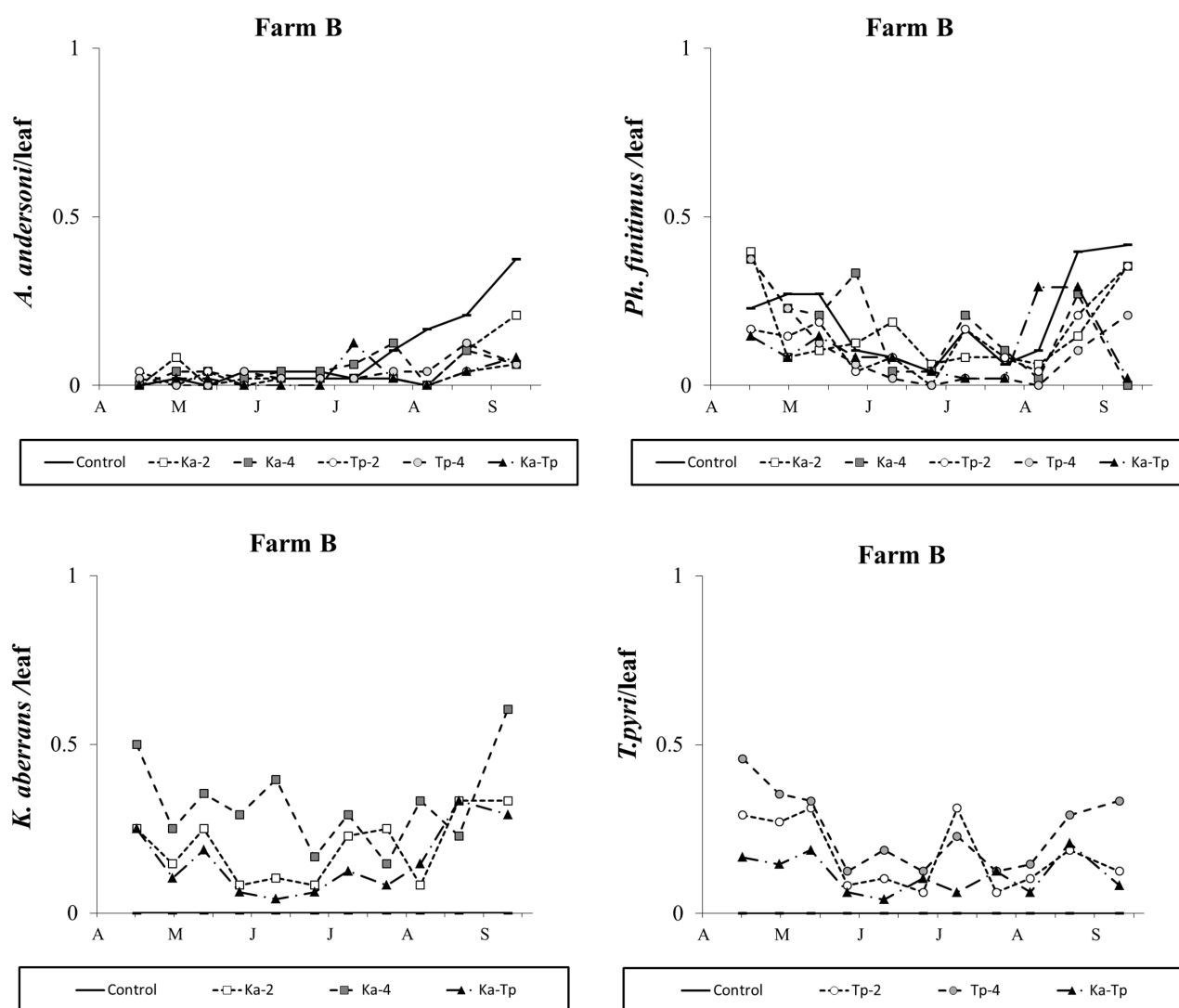
### Effects of releases on predatory mite species

Among native predatory mites the most frequent were *Ph. finitimus* and *A. andersoni*. Their populations fluctuated at low to moderate densities (i.e.,  $< 0.5$  motile forms per leaf) over the season (Figure 12). 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 ( $F_{1, 56.5}=5.06$ ;  $P=0.028$ ; Figure 12).

Regarding *A. andersoni*, the effect of treatment was significant ( $F_{5, 96.4}=3.13$ ;  $P=0.012$ ; Figure 12). *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 ( $F_{1, 96.4}=2.63$ ;  $P=0.108$ ). Significant 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 ( $F_{1, 96.4}=0.52$ ;  $P=0.473$ ; Figure 12).

*Kampimodromus aberrans* releases were successful but predatory mite populations reached relatively low levels. The effect of treatment was significant ( $F_{3, 45.9}=19.61$ ;  $P<0.001$ ; Figure 12) 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 ( $F_{1, 45.9}=1.01$ ;  $P=0.312$ ). Higher *K. aberrans* numbers were recorded in plots receiving the highest release densities ( $F_{1, 45.9}=6.56$ ;  $P=0.014$ ; Figure 12).

*Typhlodromus pyri* releases were also successful but also in these cases predatory mites did not exceed densities of 1 motile form per leaf. The effect of treatment was significant ( $F_{3, 47.4}=20.16$ ;  $P<0.001$ ; Figure 12) 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 ( $F_{1, 47.4}=3.95$ ;  $P=0.053$ ; Figure 12).



**Figure 12** Seasonal abundance of predatory mites observed during 2010 (months are indicated in x-axis) on different treatments in Farm B.

## Discussion

Mite seasonal abundance in farm A showed different patterns in the selected vineyards. The most interesting differences concerned spider mite populations in 2009: *E. carpini* was dominant in vineyards P, S and GDC, while *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. The canopy of free cordon vineyard appeared to be less vigorous (lower number of 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* fluctuated at lower density than in the more vigorous P and S vineyards. Little is known on the ecology of *E. carpini* and the effect of canopy management on this spider mite needs further investigations. On the other hand *P. ulmi* was more abundant in vineyard FC than elsewhere. One could suggest that *P. ulmi* populations were allowed to increase in this vineyard because of the relatively low densities of *E. carpini* in 2009. 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 GDC vineyard confirm trends seen one year before. In both farms highest densities of spider mites were reached where only native predatory mites (i.e., *A. andersoni* and *Ph. finitimus*) were present. Spider mite population levels were, in some cases, close to or above the action thresholds (i.e., 5 and 10 motile forms per leaf for *E. carpini* and *P. ulmi* respectively; see Duso *et al.* 2012). 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 predatory insects 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 glabrous 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 occurrence of Grape Downy Mildew that represents an alternative food for this species and can sustain population increase and persistence on plants in absence of prey (Duso *et al.* 2003; Pozzebon and Duso, 2008; Pozzebon *et al.* 2009, 2010). This phenomenon had 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 pubescent grape 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 chlorpyrifos, 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 the above mentioned pesticides. Resistance to OP insecticides and EBDC fungicides has been reported for *T. pyri* and *K. aberrans* (Van den Baan *et al.* 1985; Auger *et al.* 2004; Bonafos *et al.* 2007; Tirello *et al.* 2012; Cassanelli *et al.* 2015). Predatory mite strains used for releases are known to have low susceptibility to mancozeb and chlorpyrifos. It is likely that the early use of mancozeb as well as that of chlorpyrifos in summer had an impact on their populations due to possible sub-lethal effects induced by these products (Tirello *et al.* 2013; Duso *et al.* 2014; Pozzebon *et al.* 2011, 2014). 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 conducted to investigate this topic.

Generally, increasing release densities had no significant effects on biological control in terms of spider mites abundance. However, in farm B where higher release densities were used, spider mites were controlled in the same season of the predator releases while in farm A, spider mites reached the lowest densities in the second season after predator release. Nevertheless, since grapevines can tolerate a certain level of spider mite densities without serious losses (Girolami, 1981, 1987), strategies based on several release points within vineyards, with relatively low release densities should be preferred to those based on few points and higher densities. This could help a faster vineyard colonization by the released 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, leaf architecture or pesticide use can be involved in these interactions (Zhang and Croft, 1995; Croft and Croft, 1996; Croft *et al.* 1996; Pozzebon *et al.* 2015a,b). 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). Differential effects of pesticides (particularly EBDCs and OPs) on predatory mites likely favored released predatory mites populations in contrast with *A. andersoni*. Moreover, recent studies showed that *A. andersoni* females have a less favorable conversion rate of food into eggs compared to the other predatory mites involved here, and this can explain the results obtained here (Lorenzon *et al.* 2015; Ahmad *et al.* 2015).

In farm B *Ph. finitimus* populations occurred at higher densities in early spring, decreased in summer but re-established moderate levels in late season. Trials with *Ph. finitimus* suggest that this species suffered less from interspecific competition than *A. andersoni*. Long term studies are required to 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 of four vineyards (GDC and FC). On the other hand, the presence of *K. aberrans* also affected *T. pyri* numbers in two out of 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 interactions 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 densities of both spider mite species were reduced. In 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 common prey availability 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 we did not observe the extinction of one of the two predators, but we observed fluctuations in equilibrium level. According to life-history traits as a measure of prey conversion into offspring, *T. pyri* appeared to be a superior competitor on both prey (Lorenzon *et al.* 2012). 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 slowed down this process. Body size and feeding specialization are considered important factors in interspecific competition (Croft and Croft, 1993, 1996; Croft *et al.* 1996). Studies on interspecific predation between *T. pyri* and *K. aberrans* did not suggest a definite trend (Schausberger, 1997, 1999; Lorenzon *et al.* 2012, 2015; Ahmad *et al.* 2015). Factors affecting the success of *K. aberrans* over *T. pyri* in north-east Italian vineyards and orchards remain partly unexplained (Duso *et al.* 2009). *Kampimodromus aberrans* could be more effective than *T. pyri* to find shelters to escape to competitors. Indeed the exploitation of food and spatial resources can play an interactive role in shaping the outcome of intraguild predation among predatory mites (Pozzebon *et al.* 2015; Calabuig *et al.* 2018). This topic should be further explored in the future to clarify the competition between *T. pyri* and *K. aberrans*.

We can conclude that releases of predatory mites resistant to pesticides are successful spider mites control tactics that could be included in IPM strategies in vineyards. Released predatory mites were able to outcompete native species improving spider mites control.

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