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Airborne pollen can affect the abundance of predatory mites in vineyards: implications for conservation biological control strategies

Valeria Malagnini,^a [©] Alberto Pozzebon,^b [©] Paolo Facchin,^b Arturo Paganelli^{c†} and Carlo Duso^{b*} [©]

Abstract

BACKGROUND: The importance of pollen as alternative food for generalist phytoseiid mites occurring in vineyards has been investigated in northeastern Italy. We compared pollen and phytoseiid abundance in four vineyards and in plots located at different distance from flowering hop plants. Pollen (*Carpinus betulus* and *Typha* spp.) was sprayed onto the foliage to evaluate the potential impact of this food source on predatory mite abundance. Finally, grass management was investigated to analyze the effect of a reduced mowing frequency on predatory mite population densities.

RESULTS: Arboreal pollen was found mostly during the spring and the grapevine blossoming period. Nonarboreal pollen dominated throughout the growing seasons. In vineyards, the abundance of *Amblyseius andersoni*, *Kampimodromus aberrans*, *Phytoseius finitimus*, *Typhlodromus pyri* eggs and motile forms increased after a phase of large pollen availability. Hop pollen promoted *K. aberrans* population increases in vineyards. Pollen applications increased predatory mite egg and motile form densities and similar effects were obtained by reducing mowing frequency in vineyards.

CONCLUSION: Pollen availability positively affects the biology of four phytoseiid species, promoting stable predatory mite populations in vineyards. However, natural pollen availability and predatory mite abundance often decrease in summer, and pollen supply can mitigate this trend. A higher pollen availability could be guaranteed by inserting hedges comprising species having scalar bloom, reducing mowing of inter-row groundcover and spraying pollen. The presence of flowering plants surrounding vineyards and in their inter-rows should be considered as a relevant factor to enhance the success of biocontrol tactics against phytophagous mites in viticulture.

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Keywords: Acari Phytoseiidae; Kampimodromus aberrans; Typhlodromus pyri; Amblyseius andersoni; Phytoseius finitimus; airborne pollen; grapevine

1 INTRODUCTION

European vineyards are dominated by generalist predatory mites belonging to the family Phytoseiidae.^{1–5} The importance of alternative foods – mainly pollen – for the survival, development and reproduction of a number of generalist phytoseiids has been shown in the laboratory.^{6–13} Common phytoseiids in Italian vineyards are *Typhlodromus pyri* Scheuten, *Kampimodromus aberrans* (Oudemans), *Amblyseius andersoni* (Chant) and *Phytoseius finitimus* Ribaga.⁹ Moreover, also it has been shown that the demographic parameters of generalist phytoseiids can be affected by different palynological types.^{14–16}

The positive role of pollen in enhancing the potential of phytoseiids as biocontrol agents in field conditions has been less explored. McMurtry and Scriven¹⁷ offered one of the clearest demonstrations that pollen could improve the ability of *Euseius* (= *Amblyseius*) *hibisci* (Chant) to suppress spider mite populations. Other investigations showed that pollen can promote the persistence of generalist phytoseiids in apple and¹⁸ citrus orchards^{18,19} as well in vineyards.^{4,20}

The possibility of improving spider mite control using plants that produce large amounts of pollen was emphasized by McMurtry,²¹ Grout and Richards²² and Grafton-Cardwell *et al.*²³

- * Correspondence to: C Duso, Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Viale dell'Università 16, 35020 Legnaro Padova, Italy. E-mail: carlo.duso@unipd.it
- [†] Deceased
- a Center for Technology Transfer, Edmund Mach Foundation, San Michele all' Adige (Trento), Italy
- b Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Legnaro, Italy
- c Department of Biology, University of Padova, Padova, Italy

© 2022 The Authors. *Pest Management Science* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made. In addition, the effect of pollen applications on predatory mite abundance and on their performance in field conditions has been investigated in a few cases. The densities of *E. hibisci* increased after cattail pollen treatments on citrus trees.²⁴ In California vineyards, pollen applications increased tydeid mite abundances (Acari Tydeidae). Because these mites were preyed upon by phytoseiids, phytoseiid populations also increased.²⁵ Results obtained by Calvert and Huffaker²⁶ were consistent with these observations. In Europe, pollen application in summer reduced the natural decline of *T. pyri* populations on grapevines.²⁷

Pollen management in field conditions requires a detailed knowledge of its diversity and seasonal abundance. In previous studies, a positive relationship between pollen abundance and phytoseiid population numbers was found in European vineyards using different experimental approaches and methods, in particular techniques to estimate pollen diversity and abundance.^{16,28–30}

In this paper we summarize the results of experiments aimed at investigating the influence of airborne pollen and grass management on the dynamics of generalist phytoseiid mites in vineyards. In particular we studied: (i) the relationship between pollen availability and phytoseiid seasonal abundance; (ii) the impact of pollen produced by hop plants close to a vineyard on predatory mites occurring on vines; (iii) the effects of pollen application on phytoseiids in field conditions; and (iv) the effect of grass management on phytoseiid seasonal abundance.

2 MATERIALS AND METHODS

2.1 Pollen availability and phytoseiid seasonal abundance

2.1.1 Experimental sites

The relationship between pollen availability and phytoseiid population dynamics was studied in four experimental sites located in the Veneto region, northeastern Italy (Supporting information, Table S1). Vineyards were 6000 to 12 000 m² in area.

The first vineyard (named hereinafter as VI vineyard) planted with cv. Verduzzo vines was surrounded by annual crops (e.g. maize, wheat, soybean). Natural vegetation contiguous to this vineyard (northern site) comprised mainly elders and maples. Wild hops were growing close to the eastern border of the vineyard. In preliminary observations (C.D., personal communication) this vineyard was colonized mainly by *K. aberrans*. Grass was mown approximately every one to two months. Fungicides considered selective to phytoseiids were applied to control mildews (Table S1). No insecticides or acaricides were applied.

The second vineyard (VA, cv. Glera) was close to deciduous stands including mainly hornbeams and pubescent oaks. *Typhlo-dromus pyri* was dominant among the phytoseiids. Grass was mown three times during the growing season. Fungicides selective to phytoseiids usually were applied. No insecticides or acaricides were used (Table S1). The third vineyard (PA, cv. Pinot gris) was similar to the VI vineyard management and *A. andersoni* was the dominant species of phytoseiid. Most of the pesticides used were selective to phytoseiids (Table S1).

However, the fourth vineyard (PO, cv. Merlot) was managed organically. The grass was mown three times over the growing season. It was colonized mainly by *Ph. finitimus*. Fungicides selective to phytoseiids were applied but pyrethrins were sometimes used to control *Scaphoideus titanus* Ball, the main vector of the phytoplasma involved in Flavescence dorée disease (Table S1).

2.1.2 Sampling methods

The relationship between pollen availability and phytoseiid seasonal abundance was investigated for three growing seasons (Y1:1994, Y2:1995, Y3:1996) in the VI and VA vineyards, for two growing seasons (Y1:1995, Y2:1996) in the PA vineyard, and for one growing season (Y1:2004) in the PO vineyard.

Leaf sampling was carried out every 10–15 days, from April to September, to evaluate pollen diversity and abundance and predatory mite densities (eggs and motile forms) on the same leaves. Leaf samples (6–12 leaves), collected from three different experimental plots of six plants inside each vineyard, were transferred to the laboratory where the densities of phytoseiid mites and of the most common mite families (e.g. Tetranychidae, Eriophyidae, Tydeidae) were evaluated under a dissecting microscope. Phytoseiid females and males were identified at species level using a phase-contrast microscope and current keys.³¹

2.1.3 Pollen analysis

Leaves used for monitoring mite populations then were analyzed to assess pollen diversity and abundance. A disc measuring 0.8 cm² was removed from the central part of each leaf. In all of the vineyards and sampling dates, the leaf discs were pooled and treated using the acetolysis method;³² in total, 36 samples for VI, 29 for VA, 19 for PA and eight for PO vineyard were analyzed. For each sample, three slides containing three drops of glycerol solution were analyzed by microscope. Pollen identification was performed following Moore *et al.*³³; palynological types were defined according to Persano Oddo and Ricciardelli d'Albore,³⁴ and classified as arboreal pollen (AP) or nonarboreal pollen (NAP). The number of grains in each palynological category was counted.

We hypothesized that the leaf area close to the petiole could be more effective than central leaf areas in retaining pollen grains. This hypothesis came from the observation that in many grape cultivars the petiole area is characterized by cavities and a higher trichome density. Therefore, pollen grain numbers found in the central leaf surface (see above) or in a corresponding area located at the petiole were compared in the VI vineyard during two subsequent growing seasons. The comparison was carried out from May to September every 15 days for a total of ten sampling times per growing season.

In these studies, the effect of leaf position (central or petiole areas) on pollen grain density was evaluated using Friedman two-way ANOVA by ranks³⁶ with PROC FREQ in SAS (v9.4; SAS Institute Inc., Cary, NC, USA).³⁷ Spearman's correlation ($\alpha = 0.05$) was used for analyzing the relationship between pollen grains and predatory mites, motile forms and eggs using PROC CORR in SAS (v9.4; SAS Institute Inc.).

2.2 Impact of hop pollen on phytoseiid mites occurring on vines

Natural hops growing close to the eastern side of the VI vineyard were inhabited by *K. aberrans* in particular (>90% collected individuals in preliminary observations). We aimed at investigating the potential relationships between hop pollen and phytoseiid abundance on grape leaves by planning a sampling programme in six different positions into the vineyard rows. These positions, each comprising three vines, were equally spaced within the vineyard rows (Fig. S1). Leaf samples (six leaves per position) were collected every fivedays starting before hop flowering, across two growing seasons. In the first season (Y1:1997), the sampling was performed from 8 August (before hop flowering) until

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20 September, whereas in the second season (Y2:1998) it was performed from 1 August (before hop flowering) to 18 September. We evaluated predatory mite numbers (eggs and motile forms) on leaf samples under a dissecting microscope. Pollen abundance and diversity was evaluated following the protocol mentioned above.

The effect of hop pollen abundance on predatory mite seasonal abundance was tested using a Repeated Measures generalized linear model (GLM). We also tested the effect of pollen on mite population change over time. In this analysis we considered the population growth rate as dependent variable. Population growth rate is an estimation of the population change between consecutive observations and was calculated using the following formula:

$$rt = Ln (Nt/Nt - \tau)/\tau$$

where Nt and Nt $-\tau$ are the predatory mite densities observed on each plot on subsequent sampling dates and τ is the time lag in days between subsequent observations: if rt > 0, the population increased; if rt < 0, the population decreased; and it rt = 0, the population remained stable. Data on the population abundance and the population growth rate observed during the two years were plotted against the mean pollen density observed between consecutive samples. To control the spatial and temporal correlations that violate the independence of error assumption for GLMs, the analyses were adjusted by incorporating spatial and temporal covariance terms in the generalized linear mixed effects model (GLMM) using a Poisson distribution and log link function for count data (motile forms and eggs), whereas a normal distribution with identity link function was considered for data on population growth rate. The analyses were performed with PROC GLIMMIX in SAS (v9.4; SAS Institute Inc.).³⁷ We considered the time lag from flowering nested in year as a repeated-measures term, whereas the position in the vinevard was considered as a random effect term. First-order autoregressive temporal and power spatial covariance structures resulted in the minimizing of Pseudo Akaike's information criterion (Pseudo-AIC) and, hence, subsequently were used in the analyses.³⁸ The degrees of freedom were estimated using the Kenward-Roger method.

2.3 Effects of pollen application on phytoseiids in field conditions

Experiments were carried out in three vineyards characterized by frequent grass mowing and located in the Treviso province (northeastern Italy). The first vineyard (SPP) located at San Polo di Piave (lat. 45°79'N, long. 12°39'E) comprised two cultivars (Cabernet Sauvignon and Raboso) dominated by *K. aberrans* and *Ph. finitimus*, respectively (>90% of collected individuals from each species in preliminary observations). The second vineyard (CI) located at Cimadolmo (lat. 45°78'N, long. 12°35'E) comprised cv. Merlot colonized largely by *T. pyri* (>90% of collected individuals from each species in preliminary observations). The third vineyard (SPO) located at Spresiano (lat. 45°78'N, long. 12°96'E) also comprised cv. Merlot, but colonized by *A. andersoni* (>90% of collected individuals from each species in preliminary observations).

In these vineyards two treatments (pollen application and control), each made up of four replicates of four plants, were compared. Pollen of hornbeam (*Carpinus betulus* L.) was sprayed in the SPP and CI vineyard trials, whereas cattail (*Typha* spp.) pollen was applied in the SPO vineyard trial. Pollen was sprayed on four shoots per plant (~10 mg per shoot every three to four days) using an experimental applicator.³⁵ These experiments were performed in 1997 in SPP and CI vineyards, and in 2005 in SPO vineyard. Pollen applications were made from August to September when natural pollen availability usually is low. Sampling was carried out before the first pollen application and then every one to two weeks. Samples of 16 leaves per treatment were collected on every date. Leaf analyses were performed using the procedures mentioned above.

The effects of experimental pollen application on phytoseiid populations were analyzed utilizing a GLMM using a Poisson distribution, and log-link function with Laplace estimation via PROC GLIMMIX in SAS (v9.4; SAS Institute Inc.). We considered the number of predatory mites and their eggs observed on leaves as response variables with repeated measures made at different times (i.e. sampling dates). An *F*-test ($\alpha = 0.05$) was used to assess the effect of pollen application on phytoseiid and their egg numbers.

2.4 Effect of grass management on phytoseiid abundance

The effect of grass management on predatory mite abundance was studied in two experiments. The first experiment (MVA) was performed during 2010 in a vineyard (0.5 Ha; cv. Glera) located at Valdobbiadene (lat. 45°53'N, long. 11°57'E) that was colonized by K. aberrans. In this vineyard, groundcover vegetation was represented by several species (mainly Poaceae). Two grass management treatments were compared: 'no-mowing' and 'mowing'. In the 'no-mowing' treatment, grass on the inter-row was not mowed during the season, whereas in the 'mowing' treatment groundcover vegetation on the inter-row was cut five times (24 June, 6 July, 25 July, 7 August and 26 August). Each treatment was replicated on three plots of \approx 830 m² each and comprising five rows. Sampling was performed on nine plants located in the central part of the plots by collecting ten leaves per plot every 10-15 days. The second experiment (MGA) was performed during 2012 in a multivariety vineyard located at Gaiarine (lat. 45°53'N, long. 12°29'E; 1.5 Ha), comprising two blocks of cv. Glera, one block of cv. Cabernet Sauvignon and one block of cv. Merlot. Each block was divided into two parts, where the two grass management treatments ('no-mowing' and 'mowing') were applied. In the 'mowing' treatment, groundcover vegetation on the interrow was mowed three times (9 June, 28 July and 24 July). The two treatments were replicated four times with one replicate per block. In this vineyard, K. aberrans was released during winter by transferring 2-year-old branches (see Duso^{2,8} for details). Releases were performed on two plots of seven vines placed in the centre of each block part receiving different grass management treatments. Ten leaves collected from seven plants located in the central part of each replicate and receiving predatory mite releases were sampled every 10-15 days from 9 August until 30 September. Leaf samples were transferred to the laboratory where the numbers of predatory and of phytophagous mites were enumerated using a dissecting microscope. Predatory mites were slide-mounted and then identified at species level following Tixier et al.⁵

Data on mite abundance obtained from leaf sampling in the two experiments were analyzed separately by GLMM using a Poisson distribution, and log-link function by the Laplace estimation method via PROC GLIMMIX in SAS (v9.4; SAS Institute Inc.). In both analyses, the grass management treatment, time and their interaction were considered as sources of variation and tested using an *F*-test ($\alpha = 0.05$). The number of predatory mites observed on leaves was the response variable, with repeated measures made at different

times (i.e. sampling dates). In the analysis of data from the second experiment, the block was considered as a random effect term in the model.

3 RESULTS

3.1 Pollen availability and phytoseiid seasonal abundance

3.1.1 General trends on pollen diversity and densities over the seasons

Data regarding pollen diversity and abundance in the selected vineyards are reported in Table S2. Considering the total amount of samples, the following palynological types were found: VI vineyard, 26 AP and 20 NAP; VA vineyard, 27 AP and 19 NAP; PA vineyard, 24 AP and 16 NAP; and PO vineyard, 13 AP and 11 NAP. NAP were recorded more continuously than AP throughout the growing seasons. In most cases, pollen (in particular taxa belonging to AP and Poaceae) was relatively abundant from late April to late June (Fig. 1 for VI vineyard; Fig. 2 for VA vineyard; Fig. 3 for PA vineyard; and Fig. 4 for PO vineyard). Moreover V. vinifera pollen was recorded especially in June. Pollen abundance declined in mid-summer when temperatures increased, and RH decreased (Fig. S2 for VI vineyard; Fig. S3 for VA vineyard; Fig. S4 for PA vineyard; and Fig. S5 for PO vineyard) as the flowering of the groundcover vegetation dramatically declined under these conditions and during drought periods. Sometimes, there were moderate pollen peaks in late summer.

Regarding pollen diversity, Poaceae were recorded continuously over the growing seasons representing the main component of NAP. Plantaginaceae, Chenopodiaceae and Asteraceae T-form were found on several dates including those in late summer. *Humulus lupulus* L. pollen grains were detected in high densities in the VI vineyard in late summer, as expected. AP were abundant especially during May and June. This palnological group comprised pollen from grapevine, conifers (*Abies, Larix, Picea* and *Pinus* spp.), hornbeam (*C. betulus* and *O. carpinifolia*), ash (*Fraxinus* spp.), oak (*Quercus* spp.) and other deciduous plants.

3.1.2 VI vineyard

During the Y1 growing season, two peaks of pollen abundance were recorded, in early May and mid-June, respectively (Fig. 1); later, pollen densities declined dramatically. Phytoseiids and tydeids were the main components of the mite fauna occurring in this vineyard; phytophagous mites were rarely detected. *Kampimodromus aberrans* among the phytoseiids and *Tydeus caudatus* Dugès among the tydeids were completely dominant. Data on the seasonal abundance of tydeids are not reported, because they do not represent a suitable prey for *K. aberrans*.¹⁰ Phytoseiid densities and their oviposition increased in early May, in synchrony with the first peak of pollen grains. Predatory mite densities (juveniles and adults) increased again in June and peaked in July. Adult females were the most frequent stage until September, when they were still laying eggs.

Kampimodromus aberrans



Figure 1. Average (±SE) *K. aberrans* densities and pollen abundance (upper part), and different stages (adult female, juveniles and eggs) of *K. aberrans* (lower part) during the three sampling years in VI vineyard.



Figure 2. Average (±SE) *T. pyri* densities and pollen abundance (upper part), and different stages (adult female, juveniles and eggs) of *T. pyri* (lower part) during the three sampling years in VA vineyard.

In the subsequent growing season (Y2), pollen densities (the leaf petiole area also was considered) reached relatively high levels in late May and early July (Fig. 1). Pollen abundance decreased to low levels from July onwards, but a new peak was recorded in early September. It should be mentioned that most of these pollen grains belonged to *H. lupulus*. Considering the overall season, pollen was more abundant in the leaf petiole area than in the central leaf area ($F_r = 394.94$; P < 0.0001). *Kampimo-dromus aberrans* oviposition attained maximum levels in early May whereas juveniles were recorded frequently until late July (Fig. 1). Motile forms peaked in early July. In September, juvenile numbers increased, following the same trend shown in the previous season.

In the third growing season (Y3), temperatures reached unusually high values in late May (Fig. S2). Pollen abundance showed two peaks in spring and a peak in September, as in the Y2 season (Fig. 1). Pollen was more abundant in the central leaf area than in the petiole area ($F_r = 517.51$; P < 0.001) but, in early September, most pollen was collected in the petiole area (598.67 ± 111.47 *versus* 164.33 ± 26.30 pollen grains). Pollen collected in September comprised mainly *H. lupulus* pollen. At vine sprouting, phytoseiid densities were lower than those recorded in the previous seasons. Nevertheless, populations increased in June when pollen was relatively abundant (Fig. 1). In this phase, oviposition and juvenile numbers reached relatively high levels. Juvenile densities increased again in September.

Considering the data collected during the three years, a significant positive correlation was observed between pollen grains and predatory mite eggs (Spearman's rho = 0.521, P = 0.001), whereas no association was found between pollen and motile forms (Spearman's rho = 0.115, P = 0.501).

3.1.3 VA vineyard

During the first growing season (Y1), pollen abundance, which was relevant at sprouting (mainly Poaceae), increased in June (mainly Poaceae and *V. vinifera*). *Typhlodromus pyri* population densities reached relatively high levels in May and late June. Pollen availability and phytoseiid numbers declined from July onwards (Fig. 2). The occurrence of phytophagous mites, in particular of *Colomerus vitis* (Pagenstecher) was very low.

One year later (Y2), pollen abundance (mainly Poaceae, hop and hornbeam) peaked in May, but declined soon afterwards, as a consequence of frequent rainfall (Fig. S3). In this period, phytoseiid numbers also declined. In late summer, population densities increased despite the low pollen availability. The occurrence of *Panonychus ulmi* (Koch) (~0.5–1 motile forms per leaf) probably influenced phytoseiid abundance.

During the third growing season (Y3), pollen numbers were found at high densities in mid-June (mainly Poaceae and *V. vinifera*) and late July (mainly Poaceae and Plantaginaceae). No significant association was found between motile forms of *T. pyri* and pollen (Spearman's rho = -0.148, *P* = 0.433), whereas a significant positive correlation was observed between pollen grains and predatory mite eggs (Spearman's rho = 0.429, *P* = 0.018).

Amblyseius andersoni



Figure 3. Average (±SE) *A. andersoni* densities and pollen abundance (upper part), and different stages (adult female, juveniles and eggs) of *A. andersoni* (lower part) during the two sampling years in PA vineyard.

3.1.4 PA vineyard

In the first growing season (Y1), pollen densities peaked in May (mainly Poaceae and Ostrya carpinifolia Scop.) and then decreased dramatically as a consequence of frequent rainfall. *Amblyseius* andersoni densities reached high levels in May, when oviposition peaked. Later, population densities decreased until August and then built up again in accordance with the occurrence of *P. ulmi* (0.6–2 motile forms per leaf as an average) and downy mildew *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni (maximum 7% of leaves showing symptoms) (Fig. 3).

In spring of the subsequent season (Y2), the abundance of *A. andersoni* was more clearly related to that of pollen (mainly Poaceae and *V. vinifera*). The predatory mite population increase in late summer probably was influenced by the presence of *P. ulmi* (approximately one motile form per leaf) and downy mildew (Fig. 3).

No correlation was observed between pollen grains and predatory mite eggs (Spearman's rho = 0.151, P = 0.536), and motile forms (Spearman's rho = 0.177, P = 0.464).

3.1.5 PO vineyard

Pollen abundance revealed two peaks during the experimental season (Y1), at the end of May and of June (Fig. 4). Pollen types were mainly Poaceae and *V. vinifera. Phytoseius finitimus* was clearly dominant among phytoseiids. Its numbers fluctuated at

moderate levels during the first part of the season and increased at the beginning of July, after the pollen peak. Egg and juvenile dynamics also showed an increase in this phase (Fig. 4). Phytophagous mites were detected only rarely.

No correlation was observed between pollen grains and predatory mite eggs (Spearman's rho = 0.243, P = 0.560), and motile forms (Spearman's rho = -0.357, P = 0.385).

3.2 Impact of hop pollen on phytoseiid mites occurring on vines

In the two experimental growing seasons (Y1 and Y2), hop flowering started in the second half of August. Palynological analysis carried out on each leaf samples (data not shown) showed that hop pollen was dominant (>90% of total pollen grains). Pollen abundance, phytoseiid oviposition and their densities clearly increased in late August (Fig. S6). The presence of phytophagous mites was negligible.

There was a significant relationship between pollen densities and egg abundance on leaves ($F_{1,25,56} = 6.07$; P = 0.002; Fig. 5), whereas phytoseiid motile forms were not significantly related to the pollen trend ($F_{1,72,67} = 0.01$; P = 0.940; Fig. 6). However, pollen density showed significant effects on the *K. aberrans* population growth rate ($F_{1,27,68} = 31.34$; P < 0.001; Fig. 7), indicating that the change in the population abundance between two subsequent observations was positively correlated with the amount of pollen on leaves.



Figure 4. Average (±SE) *Ph. finitimus* densities and pollen abundance (upper part), and different stages (adult female, juveniles and eggs) of *Ph. finitimus* (lower part) during the sampling year in PO vineyard.



Figure 5. Relationship between number of eggs and pollen density observed on leaves. Black dots, raw data; lines, data predicted by a model that takes into account the spatial and temporal autocorrelation.

3.3 Effects of pollen application on phytoseiids in field conditions

Experiments involving pollen treatments were carried out in three vineyards during mid-summer. It should be mentioned that the SPP vineyard comprised two cultivars (Cabernet Sauvignon and Raboso) where *K. aberrans* or *Ph. finitimus* (respectively) were

found. Phytophagous mites were detected only rarely. On Cabernet Sauvignon, pollen treatments positively influenced *K. aberrans* abundance [pollen application: F = 15.48; d.f. = 1, 30; P < 0.001; Fig. 8(a)], and oviposition [F = 27.11; d.f. = 1, 30; P < 0.0001; Fig. 8(b)]. Similar results were obtained concerning *Ph. finitimus* abundance [F = 6.92; d.f. = 1, 30; P = 0.013; Fig. 8



pollen grains per slide

Figure 6. Relationship between number of motile forms and pollen density observed on leaves. Black dots, raw data; lines, data predicted by a model that takes into account the spatial and temporal autocorrelation.



Figure 7. Relationship between population growth rate (rt) and pollen amount observed on leaves. Black dots, raw data; lines, data predicted by a model that takes into account the spatial and temporal autocorrelation.

(c)] and oviposition [F = 7.66; d.f. = 1, 30; P = 0.009, Fig. 8(d)]. The CI vineyard was colonized by *T. pyri*. Pollen applications induced higher predatory mite densities [F = 27.29; d.f. = 1, 36; P < 0.0001; Fig. 9(a)] and promoted oviposition [F = 18.46; d. f. = 1, 36; P < 0.0001, Fig. 9(b)]. In the SPO vineyard pollen treatments increased *A. andersoni* motile forms [F = 22.07; d.f. = 1, 40; P < 0.0001; Fig. 9(c)], but no significant effect was observed on egg numbers [F = 0.26; d.f. = 1, 40; P = 0.612; Fig. 9(d)].

3.4 Effect of grass management on predatory mite abundance

The MVA vineyard was colonized by *K. aberrans*, and its abundance was influenced by grass management, being higher in the 'no-mowing' treatment (F = 4.47; d.f. = 1, 24; P = 0.045). The abundance of *K. aberrans* fluctuated over the season (F = 23.08; d.f. = 5, 24; P < 0.001), but the interaction between treatment and time was not significant [F = 0.50; d.f. = 5, 24; P = 0.776; Fig. 10(a)].

The abundance of *K. aberrans* turned out to be influenced by grass management also in the MGA vineyard as predatory mite numbers were higher in the 'no-mowing' treatment than in the

'mowing' treatment (F = 11.75; d.f. = 1, 6; P = 0.014). An effect of time also was found (F = 7.32; d.f. = 5, 79; P < 0.001), but no significant interaction between time and treatment emerged [F = 3.65; d.f. = 5, 6; P = 0.0734; Fig. 10(b)].

The occurrence of phytophagous mites, in particular of *Col. vitis*, was very low in both MVA and MGA vineyards.

4 **DISCUSSION**

Vitis vinifera leaves could be considered as effective pollen traps, as we detected several palynological types and high pollen grain numbers on their surface. Pollen densities on leaves fluctuated according to the flowering status of plants located in the ground-cover vegetation and the environment surrounding the vine-yards. The quality of pollen produced by the groundcover can seriously impact the dynamics and the abundance of phytoseiids.^{39,40} In our work we found a large variety of palynological types, and a number of them have been used as alternative food in laboratory studies on phytoseiid mites.^{41–43} The most frequent NAP belonged to Poaceae, Plantaginaceae, Chenopodiaceae, Asteraceae T-form and Cannabaceae (*H. lupulus*), whereas AP



Figure 8. Average (\pm SE) densities of motile forms of *K. aberrans* (a) and *Ph. finitimus* (c) in the control and in the pollen treatment (upper part). Average of eggs (\pm SE) of *K. aberrans* (b) and *Ph. finitimus* (d) in the control and in the pollen treatment (lower part).

were represented by Betulaceae, Pinaceae, Oleaceae and, of course, *V. vinifera*. On the one hand, pollen grain densities sometimes declined, as a consequence of frequent rains that washed them off and/or reduced pollen flows. On the other, hot and windy conditions alter the pollenkitt (adhesive material present around pollen grains) causing a decrease in the pollen grain adhesiveness to leaf surfaces.⁴⁴ Detailed analyses of the seasonal abundance of pollen grains on grapevine leaves showed that AP and Poaceae dominated before grape flowering, *V. vinifera* during grape flowering, and Poaceae, Chenopodiaceae, Plantaginaceae after grape flowering. NAP trends reflected multiple flowering events, in contrast with AP. In some cases, pollen grains (e.g. those of *V. vinifera*) persisted on leaf samples until the end of the season, but only fresh pollen is considered an appropriate source of food for generalist predatory mites.¹⁷

Wiedmer and Boller²⁹ recorded similar data using artificial pollen traps in Switzerland. AP (Pinaceae, Betulaceae and Fagaceae) dominated from May to early June, grapevine pollen in late June–early July, NAP (mainly Poaceae, Plantaginaceae and Urticaceae) from late June to August. In Germany, Eichhorn and Hoos²⁸ provided additional information on the most abundant pollen type using the count of pollen grains on trapes and leaves. They confirmed the dominance of Pinaceae, Betulaceae, Fagaceae and grapevine among AP, and Poaceae, Urticaceae and Chenopodiaceae among NAP.

The observation of several phytoseiid eggs laid all around the petiole area led us to investigate the importance of this leaf area

in retaining pollen grains. In the VI vineyard, petiole areas retained more pollen than central leaf areas after *H. lupulus* flowering (from late August onwards). The estimation of pollen abundance through the analysis of leaves or leaf regions (petioles) was useful to delineate trends in pollen diversity and availability over the growing seasons. Why pollen grains were so abundant in the petiole areas is not clear, yet leaf morphology (trichome density and domatia) probably plays a key role in this phenomenon.^{45–47}

The positive role of pollen as food source for generalist predatory mites occurring in vineyards has been emphasized.^{14,15,27} However, most of these studies were carried out in the laboratory and thus relationships between pollen availability and predatory mite abundance in field conditions remains poorly explored. An interesting approach to improve our knowledge of this phenomenon was adopted by Engel and Ohnesorge,¹⁶ who used electrophoresis analysis of the gut contents to identify palynological types in predatory mites collected in vineyards. These analyses revealed mainly Fagus spp. and Pinus spp. pollen in predatory mites collected before grape flowering. Vitis vinifera pollen was durina detected grape flowering and weed pollen (e.g. Alopecurus sp. and Urtica dioica L.) in summer. These findings supported the hypotheses that predatory mite seasonal abundance is related to pollen flows and that different types of pollen can contribute to support predatory mite population in different periods of the growing season.

In our studies the relationship between pollen grain numbers and phytoseiid population abundance appeared to be significant



Figure 9. Average (±SE) densities of motile forms of *A. andersoni* (a) and *T. pyri* (c) in the control and in the pollen treatment (upper part). Average of eggs (±SE) of *A. andersoni* (b) and *T. pyri* (d) in the control and in the pollen treatment (lower part).

in spring when NAP and AP reached their highest levels. We observed a significant correlation between pollen abundance and the number of eggs of *K. aberrans* and *T. pyri* as these species were studied for three years in vineyards poorly colonized by potential prey. Similar relationships were observed in previous studies carried out on *T. pyri* in Germany and Switzerland.^{27,28} Regarding *A. andersoni* population dynamics, the trends suggest that additional food types, such as spider mites and downy mildew, potentially were involved in phytoseiid dynamics.^{48,49} No correlation was reported for *Ph. finitimus* but data were collected in a single season but longer studies will be needed to confirm this. Observations conducted on the effect of hop plants contiguous to the VI vineyard provided further evidence of the relationships between pollen and *K. aberrans*, as predatory mite oviposition significantly increased after hop flowering.

Pollen applications showed that the phytoseiid species considered in this study increased oviposition and, consequently, motile form densities. Frequently, generalist phytoseiid mites show similar life-table paramenters when fed with pollen or mite prey^{41,42,50} and this fits for *K. aberrans*, *T. pyri* and *Ph. finitimus*.^{10,13,27} Several studies carried out in glasshouses showed that adding pollen to a crop can enhance pest control by predatory mites^{51–58} and recently this was validated in commercial greenhouse roses.^{59,60} Fewer studies have been carried out in open field conditions, mainly in orchards, where pollen can be dusted directly on the crop.^{24,52,61,62} The summer pollen application carried out in citrus and avocado orchards increased

the abundance of phytoseiid mites even if climate conditions could reduce these positive effects.^{24,52,62} Summer applications of pollen slowed, but did not stop, the decline of *T. pyri* on grape-vines in autumn.²⁷

Pollen can be provided by different types of groundcover management and hedges.^{30,47,63–66} Studies carried out in vineyards showed that the densities of predatory mites (mainly *T. pyri* and *K. aberrans*) were usually higher in vineyards with cover crops than in vineyards without.⁶⁷ In the present work we underline how the presence of hop plants near a vineyard can improve phytoseiid abundance and oviposition in late summer, when grass cover flowering is low.

Pollen dynamics frequently were characterized by a decline in grain numbers in summer. This phenomenon can be influenced by the frequency of grass mowing, as shown in previous studies.⁶⁴ Field experiments carried out in this work showed that the reduction in mowing frequency increased *K. aberrans* population densities, confirming the results of preliminary studies carried out in apple orchards and vineyards.^{35,68–70} Our results are consistent with those reported by Sáenz-Romo *et al.*⁷¹ even if in that work the authors underlined the importance of the choice of flowering plants that could provide a great amount of pollen during the overall growing season. The reduction of grass mowing allows for the flowering of the groundcover plants and therefore increases the availability of pollen for phytoseiid mites. It should be considered that groundcover plants can host the phytophagous mite *Tetranychus urticae* Koch, potentially increasing the





Figure 10. Motile forms (average \pm SE) of *K. aberrans* in the mowing and in nonmowing treatment in MVA (a) and MGA (b) vineyards.

risks of infestation of grapevines,⁷² yet in the present study the presence of spider mites on grapevine leaves was observed only rarely.

The protection and enhancement of phytoseiid mite populations is an important aspect for the success of integrated pest management strategies. We can suggest that the persistence of phytoseiid mites in vineyards, in the absence of prey, is largely the consequence of pollen availability^{10,20} and that groundcover management is crucial in this framework.

5 CONCLUSION

The conservation of generalist phytoseiid mites in vinevards allows control of phytophagous mites without using acaricides. This phenomenon, which is sustained largely by pollen, is threatened by hot and dry periods occurring in summer. In our work we have shown how pollen availability, and in particular NAP, obtained by spraying it on the vegetation or by the flowering of groundcover grasses, has a positive effect on phytoseiid mite populations. This effect is more evident in late summer when phytoseiid mite abundance tends to decrease. An adequate pollen availability on grapevine leaves can be achieved by increasing plant diversity in vineyard agroecosystems with the addition of hedges with scalar blooms that keep flowering even in late summer. The same objective can be reached by reducing inter-row mowing frequency. The artificial application of pollen also can be adopted, but further research on this technique is needed with a particular reference to outdoor conditions. In conclusion, the presence of flowering plants surrounding vineyards and in the

inter-rows is a significant factor that can enhance the success of biocontrol tactics against phytophagous mites in viticulture.

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CONFLICT OF INTEREST

The authors state that there are no conflicts of interest with any parties.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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