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# Survey on the aphid communities in Northern Italy open field tomato crops

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## Abstract

Aphids are phytophagous insects that damage a wide range of plant species and may act as vectors of several pathogens, including Cucumber Mosaic Virus (CMV). CMV seriously affects tomato crops but, although earlier studies have described CMV in northern Italy, little is known about the aphid species in the area that could be involved in its transmission in tomato fields. This three-year survey (2021–2023) focused on monitoring aphid populations in open field tomato crops in the northern Italian provinces of Piacenza, Cremona and Mantua. Sampling included both tomato plants and nearby weeds like *Solanum nigrum*, *Convolvulus arvensis*, *Abutilon theophrasti* or crop like *Medicago sativa*, which may serve as CMV reservoirs. DNA barcoding of the COI gene, along with morphological analysis where necessary, was used to identify the aphids. A total of 28 aphid taxa were identified from the collected samples, and 89.8% of all identified samples were known CMV vectors. *Aphis fabae* and *Macrosiphum euphorbiae* were the most frequently identified species. The greatest number of aphid specimens and species diversity were recorded during the first sampling of each year, whereas the highest sampling diversity was recorded in Piacenza. The current study provides a comprehensive qualitative assessment of the diverse aphid species associated with open field tomato crops in one of the most important districts for their cultivation in Italy.

## Key Words

Cucumber Mosaic virus, field survey, vectors, weeds

## Introduction

Aphids (Hemiptera: Aphididae) are phytophagous insects that can cause serious economic damages not only through their feeding activities but also by acting as virus vectors (Brault et al. 2010; Leybourne 2024; Ohlson et al. 2024). Several aphid species are polyphagous and may feed on both the crops and weeds commonly found in fields and surrounding areas, with the latter acting as secondary feeding sources and refuges for those insects (Ingwell et al. 2017; Liu et al. 2017; Szabó et al. 2020; Van Helden et al. 2021). Weeds can negatively affect crop production both directly, by competing for space and other natural resources, and indirectly, by acting as reservoirs for plant viruses (Ingwell et al. 2017; Eigenbrode et al. 2018; Maachi et al. 2022; Szabó et al. 2022; Clark et al. 2023). It is therefore necessary to know the plant and aphid species that are present in agricultural ecosystems to develop effective control strategies and so to limit the proliferation of pathogens (Clark et al. 2023; Mazzitelli et al. 2023; Harelimana et al. 2024; Paiva et al. 2024).

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Among the viruses affecting the tomato plant, Cucumber Mosaic Virus (CMV) is one of the most dangerous. It is transmitted by various aphid species through non-persistent transmission (Gildow et al. 2008; Jacquemond 2012) and can infect a wide range of plants belonging to over 100 families (Hobbs et al. 2000). The presence in the field of multiple weeds that can serve as a virus reservoir may increase CMV transmission to crops (Cunniffe et al. 2021). One of the suggested strategies to limit the spread of viral diseases is the use of chemical treatments that target insect vectors (Jones 2006; Rubio et al. 2020). However, it is very difficult to timeously and effectively target very mobile pests, like aphids, that can spread CMV with just probing assays. Furthermore, even if insecticides could be able to reduce the abundance of pest populations, their use could lead to the selection of resistant strains, to environmental pollution in crop systems and to the presence of agrochemical residues in the final product (Panini et al. 2021; Pandit et al. 2022). Alternative control methods could focus on the management of CMV reservoir weeds (Jacquemond 2012) or the exploitation of natural antagonists, such as predators and parasitoids which could successfully reduce aphid populations on secondary hosts (Cock et al. 2016; Garzón et al. 2016; Harelimana et al. 2024). To implement these control methods effectively, it is essential to study the vector species present in the agro-ecosystem (Mazzitelli et al. 2023; Paiva et al. 2024) and identify the network of potential natural antagonists.

Industrial tomato crop grown in open fields plays a significant role in the local economy of several northern Italian provinces, such as Cremona, Mantua and Piacenza, with the latter achieving the second highest tomato production in Italy in 2023 (ISTAT 2024). Traditionally, several pest species affect tomato crops in these areas but, in recent years, farmers have expressed growing concerns about the incidence of CMV, especially in fields located close to cucurbitaceous crops such as melons, pumpkins or other similar species.

There is currently little information on the aphid species colonising open-field tomato crops and the surrounding weeds in northern Italy. In this study, we collected tomato plants and weeds from fields located in the above-mentioned provinces to determine the aphid species present on these plants, which could act as potential vectors for CMV.

The aim of this research was to improve the available knowledge on the aphid fauna that colonise tomato fields and surrounding weeds and crops in northern Italy and which serve as potential CMV vectors and virus reservoirs.

# Materials and methods

## Study area and plant sampling

This study was conducted in 11 tomato fields located in the Italian provinces of Piacenza (PC), Cremona (CR) and Mantua (MN) (Po Valley, Northern Italy) from 2021 to 2023 (Suppl. material 1: table S1). During each sampling, in each tomato field, or in the nearest surrounding, the epigeal part of tomato plants, of some cultivated species and of common weeds were randomly collected, stored in plastic bags and delivered to the laboratory within a few hours.

Plant species identification was later confirmed in the laboratory using the identification keys of Pignatti and colleagues (2017). The samplings in each field were carried out three times per year, between the second half of May and the first half of July.

## Aphid collection and storage

In the laboratory, the plants were examined for aphid presence. Any aphids were then gently collected using a fine brush and stored in 70% ethanol at -20 °C until further analysis. They were grouped and stored separately considering the year, the sampling round (first, second or third), the field and the host plant species from which they were collected.

## DNA extraction

For aphid species identification, a DNA barcoding approach was used (Cocuzza et al. 2008). From each of the above defined groups, up to 6 specimens were analysed, considering, also, within each group, any visually evident morphological differences, to detect the presence of multiple species infesting the same host even if, at this step, no identification at genus or species level, based on morphology was done. Aphid DNA was extracted from every specimen following a slightly modified "salting-out" protocol (Panini et al. 2017) that sought to keep the exoskeleton as intact as possible (Santos et al. 2018). The process involved puncturing each specimen in the ventral part of the thorax and abdomen using a sterile stainless-steel pin. The exoskeleton was then transferred to a 2 mL Eppendorf tube containing 300 µL of TNES buffer (50 mM Tris, 400 mM NaCl, 20 mM EDTA, 0.5% SDS, pH 7.5) and 3 µL proteinase K (final concentration 100 µg/mL). The tubes were incubated for 24 hours at 55 °C, after which the exoskeleton was removed and stored at -20 °C for further morphological identification, depending on the DNA barcoding results. Subsequently, 85 µL of NaCl 5 M were added to each tube. After vortexing for 15 seconds, the tubes were centrifuged at 16,000 g for 5 minutes. The DNA was then isolated from the supernatant via overnight ethanol precipitation at -20 °C and resuspended in 50 µL of sterile water.

## COI amplification and sequencing

The extracted DNA was used to amplify a portion of the cytochrome oxidase 1 (COI) gene (Hebert et al. 2003). The PCR reactions (25 µL) were prepared as follows: 12 µL of DreamTaq Green PCR Master Mix 2X (Thermo Scientific™), 1 µL (10 nM) each of the primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994), 1 µl of sample DNA and molecular-grade water to volume. The thermal cycling conditions were: initial denaturation at 95 °C for 3 minutes, followed by 5 cycles each of 95 °C for 30 s, 45 °C for 30 seconds and 72 °C for 50 seconds, and then 34 cycles each of 95 °C for 30 seconds, 50 °C for 30 seconds and 72 °C for 50 seconds, and, finally, an elongation step at 72 °C for 5 minutes. The PCR products were purified with NucleoSpin® Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany) and Sanger sequenced in forward and reverse strands and a consensus sequence was built.

### Sequence analysis and morphological identification

The resulting sequences were compared with those stored in Bold Systems and NCBI GeneBank databases. When sequence quality was too low or there was amplification of parasitoid DNA, the exoskeleton of the corresponding specimen was slide-mounted and identified using the keys of Blackman and Eastop (Blackman and Eastop 2000, 2006).

### Statistical analysis

The Chi-square goodness of fit test, using IBM SPSS Statistics for Microsoft Windows (version 29.0.1.0. IBM Corp., Armonk, NY, United States), was applied to compare the temporal and spatial distribution of insect species.

We also analysed matrices of the composition of the aphid community. Sample identifiers encoded the accompanying metadata. In the first dataset, the identifiers contained province, site, year, sampling round, and host plant; in the second dataset, the identifiers contained province, site, year, and sampling round only. From these identifiers we extracted the factors used as constraints in the ordination: Plant, Year, and Province for the first dataset; Province and Year for the second.

Before analysis, samples with zero total abundance were removed. Each sample vector was then converted to relative abundances and transformed using the Hellinger transformation (square root of row-wise proportions), a standard approach that preserves zeros and makes Euclidean methods appropriate for community data. The transformed matrix was column-centred.

We performed redundancy analysis (RDA) to quantify and visualise the portion of community variation that is linearly explained by the specified constraints. Categorical predictors were represented with indicator variables and combined with an intercept. The response matrix was projected onto the space spanned by the constraints, and the principal axes were extracted from the fitted values to obtain the site and species scores and the percentage of total (Hellinger-scale) variance accounted for by each canonical axis. For the first dataset, the RDA model included Plant, Year, and Province; for the second dataset, it included Province and Year. All analyses and figures were carried out using the scikit-learn python library (Pedregosa et al. 2011).

## Results

### Plant samples

A total of 601 samples were collected and distributed across 18 species plus *Solanum lycopersicum* L. (Table 1). After *S. lycopersicum*, the most common collected species were *Solanum nigrum* L., *Abutilon theophrasti* Medik and *Convolvulus arvensis* L.. In contrast, species such as *Avena sativa* L., *Echinochloa crus-galli*

**Table 1.** Number of samples of plant species collected per year and province (*: species known from literature to be host for Cucumber Mosaic Virus; CR: Cremona, MN: Mantua, PC: Piacenza).

| Plant species | Total | 2021 | 2022 | 2023 | CR | MN | PC |
|---|---|---|---|---|---|---|---|
| *Abutilon theophrasti* Medik. (Malvales: Malvaceae) (*) | 74 | 22 | 27 | 25 | 7 | 46 | 21 |
| *Amaranthus retroflexus* L. (Caryophyllales: Amaranthaceae) (*) | 59 | 19 | 20 | 20 | 6 | 34 | 19 |
| *Avena fatua* L. (Poales: Poaceae) | 3 | 0 | 3 | 0 | 0 | 3 | 0 |
| *Avena sativa* L. (Poales: Poaceae) | 1 | 0 | 0 | 1 | 0 | 1 | 0 |
| *Carduus* sp. L. (Asterales: Asteraceae) (*) | 3 | 0 | 0 | 3 | 0 | 0 | 3 |
| *Chenopodium album* L. (Caryophyllales: Amaranthaceae) (*) | 61 | 22 | 18 | 21 | 6 | 28 | 27 |
| *Convolvulus arvensis* L. (Solanales: Convolvulaceae) (*) | 76 | 29 | 25 | 22 | 6 | 38 | 32 |
| *Datura stramonium* L. (Solanales: Solanaceae) (*) | 4 | 3 | 1 | 0 | 0 | 4 | 0 |
| *Echinochloa crus-galli* (L.) (Poales: Poaceae) (*) | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| *Equisetum* sp. (L.) (Equisetales: Equisetaceae) | 41 | 15 | 8 | 18 | 0 | 22 | 19 |
| *Hordeum murinum* L. (Poales: Poaceae) | 2 | 1 | 0 | 1 | 0 | 2 | 0 |
| *Medicago sativa* L. (Fabales: Fabaceae) (*) | 49 | 20 | 17 | 12 | 0 | 24 | 25 |
| *Rumex* sp. L. (Caryophyllales: Polygonaceae) (*) | 3 | 0 | 2 | 1 | 0 | 2 | 1 |
| *Solanum lycopersicum* L. (Solanales: Solanaceae) (*) | 98 | 32 | 33 | 33 | 9 | 52 | 37 |
| *Solanum nigrum* L. (Solanales: Solanaceae) (*) | 79 | 26 | 27 | 26 | 9 | 37 | 33 |
| *Sorghum halepense* (L.) (Poales: Poaceae) | 35 | 13 | 13 | 9 | 4 | 10 | 21 |
| *Triticum aestivum* (L.) (Poales: Poaceae) (*) | 4 | 1 | 2 | 1 | 2 | 1 | 1 |
| *Vicia sativa* L. (Fabales: Fabaceae) (*) | 7 | 0 | 1 | 6 | 0 | 0 | 7 |
| *Xanthium* sp. L. (Asterales: Asteraceae) | 1 | 0 | 0 | 1 | 1 | 0 | 0 |
| Total | 601 | 203 | 198 | 200 | 50 | 304 | 247 |

(L.) and *Xanthium* sp. L. were each collected only once. Most of the collected plant species are known from the literature to be host for CMV (Douine et al. 1979; Yoon et al. 2019; Palukaitis et al. 2025).

## Aphid species

Aphid presence was detected on approximately one third of the collected plants samples (n = 201). From these samples, 3411 aphid specimens were collected but a great abundance variation was observed among samples (Fig. 1). In some cases only a few specimens were present on the collected plants: on *A. theophrasti* or *Equisetum* sp. (L.) only 0.08 and 0.07 aphids per sample were collected, respectively. In other cases, colonies with tens of adults and pre-imaginal instars were detected: e.g. from *Carduus* sp., *A. sativa* or *Rumex* sp. L. the mean number of aphids/sample collected was 43.3, 39 and 27.3. On tomato the mean number of collected aphids was slightly lower: 12.4 aphids/sample. No aphids were found on *Avena fatua* L., *E. crus-galli* and *Xanthium* sp.

DNA was extracted from 685 specimens out of 3411 (~ 20%). PCR amplicons from these DNA samples were sequenced: 526 (76.8%) specimens were successfully identified as aphid taxa (mostly at the species levels and a small portion at genus level). The exoskeletons of the remaining 159 specimens were slide mounted for morphological identification either because they were identified as parasitoids through DNA barcoding or because they failed to produce any amplification. The morphological identification was successful for 119 samples (17.4%) but it was not reliable for 40 specimens (5.8%) because only pre-imaginal instars were available for those samples and it was not possible to produce an unambiguous identification. A total of 28 aphid taxa were identified and among them 14, that represent 89.8% of all identified samples, are known CMV vectors (Table 2).

Despite the number of collected plants varying across sampling rounds, the species diversity consistently peaked during the first sampling in each year and decreased in the following ones (Suppl. material 1: table S3). The number of aphid species ranged between 18 and 13 in May decreasing to 11 – 4 in July (Fig. 2A). Considering

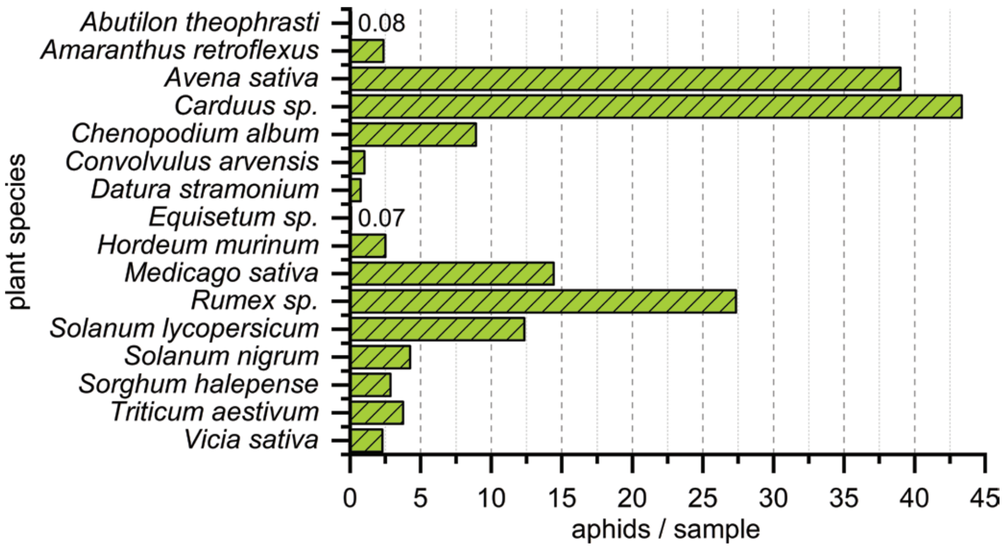


**Figure 1.** Mean number of aphid specimens collected on the plant species sampled during the survey. No aphids were collected from *Avena fatua*, *Echinochloa crus-galli* and *Xanthium* sp.

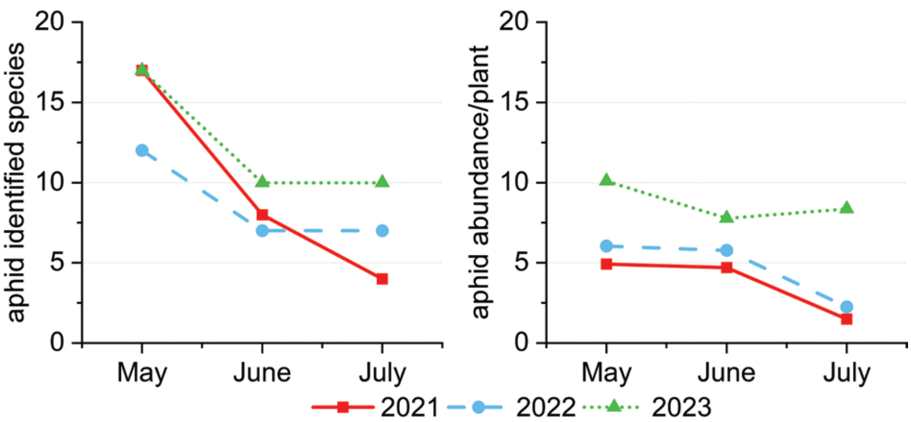


**Figure 2. A.** Number of aphid species identified each year, in the first, second and third samplings; **B.** Abundance of aphids collected each year, in the first, second and third samplings.

**Table 2.** Relationship between aphid and plant species collected: number of cases and mean number of aphid specimens per plant sample. *Avena fatua*, *Echinochloa crus-galli* and *Xanthium* sp. are not reported as no aphids were collected from these plants. (#: aphid species known from literature to be CMV vector; §: plant species known from literature to be CMV host).

| | *Abutilon theophrasti*§ | *Amaranthus retroflexus*§ | *Avena sativa* | *Carduus* sp.§ | *Chenopodium album*§ | *Convolvulus arvensis*§ | *Datura stramonium*§ | *Equisetum* sp. | *Hordeum murinum* | *Medicago sativa*§ | *Rumex* sp.§ | *Solanum lycopersicum*§ | *Solanum nigrum*§ | *Sorghum halepense*§ | *Triticum aestivum*§ | *Vicia sativa*§ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Acyrthosiphon malvae* | 1/0.04 | 1/0.02 | | | | | | | 1/1.50 | | | | 1/0.01 | | | |
| *Acyrthosiphon pisum*# | | | | | | | | | | 13/2.06 | | 9/0.69 | 1/0.05 | | | 2/1.71 |
| *Anoecia* sp. | | | | | | | | | | | | 1/0.01 | | | | |
| *Aphis craccivora*# | 2/0.04 | 2/0.75 | | | | | | | | 21/11.24 | | 4/0.05 | | | | |
| *Aphis fabae*# | | 6/1.24 | | | 24/8.92 | 4/0.51 | 1/0.75 | | | 2/0.06 | 1/13.67 | 14/0.48 | 17/3.16 | 1/0.06 | | |
| *Aphis frangulae* | | | | | | | | | | | | 2/0.05 | | | | |
| *Aphis gossypii*# | | | | | | 1/0.01 | | | | | | 2/0.02 | 2/0.05 | | | |
| *Aphis rumicis*# | | | | | | | | | | | 1/13.67 | | | | | |
| *Aphis spiraecola*# | | | | | | 1/0.01 | | | | | | | | | | |
| *Aulacorthum solani* | | | | | | 1/0.04 | | | | | | | 2/0.08 | | | |
| *Brachycaudus cardui* | | 1/0.02 | | 2/30.33 | | 1/0.01 | | | | | | | | | | |
| *Brachycaudus harmalae* | | 1/0.29 | | | | | | | | | | | | | | |
| *Hyalopteroides humilis* | | | | | | | | | | | | | | | 1/0.25 | |
| *Hyperomyzus lactucae*# | | | | | | 1/0.01 | | | | | | 2/0.02 | | | | |
| *Macrosiphum euphorbiae*# | | 1/0.02 | | | | | | | | 1/0.20 | | 35/10.20 | 4/0.76 | | 1/0.25 | |
| *Macrosiphum rosae* | | | | | | | | | | | | 1/0.02 | | | | |
| *Metopolophium dirhodum*# | | | | | | | | | | | | | | | 1/0.25 | |
| *Metolophium carnosum* | | | | | | | | 1/0.07 | | | | | | | | |
| *Myzus persicae*# | | | | | | 7/0.41 | | | | 2/0.18 | | 14/0.42 | 2/0.10 | | | |
| *Pemphigus bursarius* | | 1/0.02 | | | | | | | | | | | | | | |
| *Rhopalosiphum maidis*# | | | | | | | | | | 1/0.02 | | | | 9/1.51 | | |
| *Rhopalosiphum padi*# | | | 1/39.00 | | | | | | 1/1.00 | | | 3/0.11 | | 1/0.09 | 2/2.00 | 1/0.57 |
| *Schysaphis graminum* | | | | | | | | | | | | | | 1/0.09 | | |
| *Sitobion avenae*# | | | | | | | | | | | | 3/0.12 | 1/0.01 | 5/1.00 | 1/1.00 | |
| *Tetraneura nigriabdominalis* | | 1/0.02 | | | | | | | | | | | | | | |
| *Tetraneura ulmi* | | | | | | | | | | | | 2/0.08 | | 1/0.11 | | |
| *Therioaphis trifolii*# | | | | | | | | | | 4/0.65 | | 3/0.04 | | | | |
| *Uroleucon* sp. | | | | 1/13.00 | | | | | | | | 1/0.02 | 1/0.01 | | | |

the abundance, per plant sample, a small decrease was observed between the first and the second sampling rounds and a more evident reduction between the second and the third, but only in 2021 and 2022; meanwhile, in 2023 the abundance remained constant from June to July (Fig. 2B). Some differences in the number of species were also observed across provinces with the lowest diversity in samplings recorded in Cremona and the highest in Piacenza (Suppl. material 1: tables S2, S4).

When the diversity of aphid species across the years was considered, the total number of collected species was quite similar (Suppl. material 1: table S5). The Chi square goodness of fit test confirms that the number of identified aphid species was significantly different among locations ($\chi^2_{(2,\ N = 83)}$ = 9.656, $p$ = 0.008) while no significant differences in this parameter have been detected among the years ($\chi^2_{(2,\ N = 83)}$ = 0.291, $p$ = 0.864).

In Fig. 3, the total number of aphid species collected from the sampled hosts, splitted into vector and non-vector species, is plotted (Fig. 3).

The highest number of species (n. = 15) was collected from tomato (Fig. 3). Among them *Macrosiphon euphorbiae* (Thomas) was the species with the highest abundance (10.2 specimens/sample) and the highest frequency of detection (35 cases), followed by *Acyrthosiphon pisum* (Harris) (0.69 specimens/sample; detected in 9 samples), *Aphis fabae* (Scopoli) (0.48 specimens/sample; detected in 14 samples) and *Myzus persicae* (Sulzer) (0.42 specimens/sample; detected in 14 samples). Among the aphid species recovered from tomato, only 3 (*Anoecia* sp. Koch, *Aphis frangulae* Kaltenbach e *Macrosiphon rosae* L.) were exclusively collected from this crop. The remaining 12 species were detected in variable abundance and frequency also on other plants (Table 2). Among them, *A. fabae* was recovered from the highest number of hosts (n = 9) followed by *Rhopalosiphum padi* (L.) (n = 6) and *M. euphorbiae* (n = 5). Fewer species were hosted by *S. nigrum*, *Amaranthus retroflexus* L., *C. arvensis* and *Medicago sativa* L., respectively 9, 8, 7 and 7 taxa, and only one aphid species was found on *A. sativa*, *Datura stramonium* L. and *Equisetum* sp. (Fig. 3, Table 2). Considering only those aphid species known to be vectors, their presence reflects the overall findings observed: these species were more abundant during the first sampling period and decreased

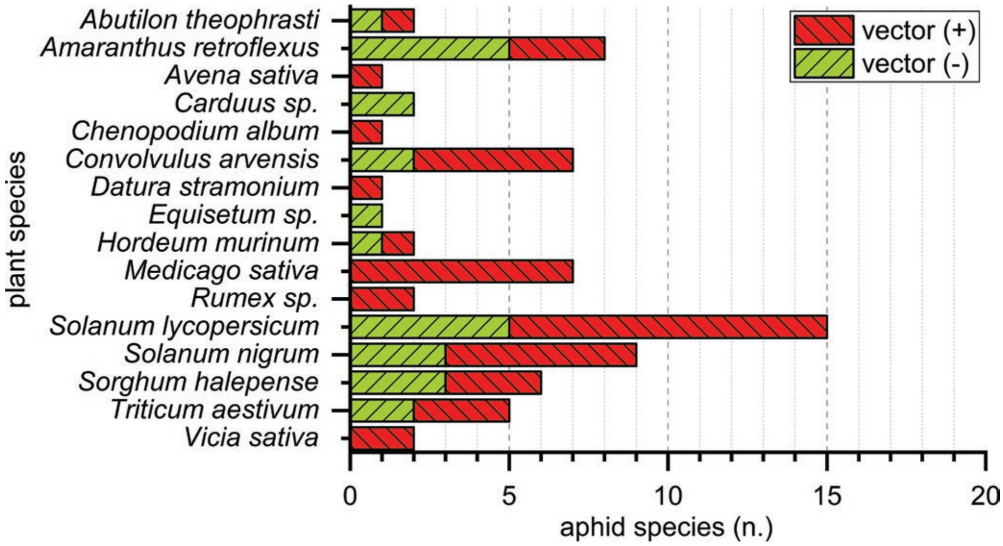


**Figure 3.** Number of aphid species (vector of CMV and not vector), detected on the plant species collected during the survey. No aphids were collected from *Avena fatua*, *Echinochloa crus-galli* and *Xanthium* sp.

in the subsequent ones. Their numbers varied from 14 to 9 species, while the number of plant species with positive collection of vector species were respectively 13, 7 and 8 (Suppl. material 1: tables S6–S8). *A. fabae* was detected on 9 different hosts in the first sampling, making it the species with the highest number of detected hosts among CMV vectors in this period.

The great aphid diversity observed in tomato was always detected across the three sampling periods, with 10, 9 and 4 aphid species respectively. The species *A. pisum*, *Aphis craccivora* Koch, *M. euphorbiae* and *Therioaphis trifolii* Monell were consistently collected, although *T. trifolii* was detected only once in each sampling. Two of the more abundant and frequent species, *A. fabae* and *M. persicae*, were not detected on tomato during the last sampling period (Suppl. material 1: table S8).

A few other aphid species reported in literature as pest of *S. lycopersicum* were detected on weeds or on crops but not on tomato: *Aphis spiraecola* Patch, *Aulacorthum solani* Kaltenbach, *Brachycaudus cardui* (L.), *Brachycaudus harmalae* Das (Perring et al. 2018), Only three known vector species were detected on just one plant host: *A. spirecola* on *C. arvensis*, *Aphis rumicis* (L.) on *Rumex* sp. and *Metopolophium dirhodum* Walker on *Triticum aestivum* (L.).

The black bean aphid, *A. fabae*, was the species that showed the highest polyphagy being collected from 9 different hosts. Its presence was highest in 2021, dropped in 2022 and increased again in 2023. In contrast, the number of *M. euphorbiae* identifications showed a continuous increase over the three years. *A. fabae* was the most represented species in all three provinces, while the second most common species was *M. euphorbiae* in Piacenza and Cremona and *Myzus persicae* Sulzer in Mantua (Suppl. material 1: table S4). Considering the seasonal distribution of the species, *A. fabae* was detected more frequently on plants collected in the first and second sampling rounds, *M. euphorbiae* had a similar distribution across sampling rounds, while *M. persicae* was mainly detected during the first sampling (Suppl. material 1: table S4).

Co-infestation by two or more aphid species on the same plant was recorded at least once on 8 of the surveyed hosts (Suppl. material 1: table S9). Of these, *S. lycopersicum* was the species with the highest observed number of co-infestations (23 combinations and 28 cases). In 2 cases, 4 different species of aphids were detected on the same sample, while 7 cases involving 6 different combinations of 3 species were observed. In addition, 15 combinations of 2 species were detected in 19 cases. Co-infestations involving three aphid species were also detected on *C. arvensis* and *S. nigrum*: on *C. arvensis*, combinations included *A. fabae*, *Hyperomyzus lactucae* (L.) and *M. persicae* or *A. fabae*, *Aphis gossypii* Glover and *M. persicae*; on *S. nigrum*, combinations included *A. fabae*, *A. solani* and *Uroleucon* sp. Mordvilko or *A. fabae*, *A. gossypii* and *M. euphorbiae* (Suppl. material 1: table S9).

## Aphid- host and aphid- province association

In the dataset that included the host plant, the constrained ordination revealed a plant-structured gradient in the community composition and the full constrained model accounted for roughly 30% of the variation. Samples clustered by host plant across RDA1 (18.8% of total variance) and RDA2 (10.7% of total variance), while differences associated with province and year were comparatively subtle. Species scores were consistent with this structure: *M. euphorbiae* loaded strongly along the positive side of RDA1 and aligned with samples on tomato, *A. fabae* pointed in the opposite direction and was most associated with *C. album*, and *A. craccivora* contributed chiefly along the lower-right quadrant. Other taxa showed shorter vectors near the origin, indicating weaker contributions to the constrained gradients (Fig. 4).

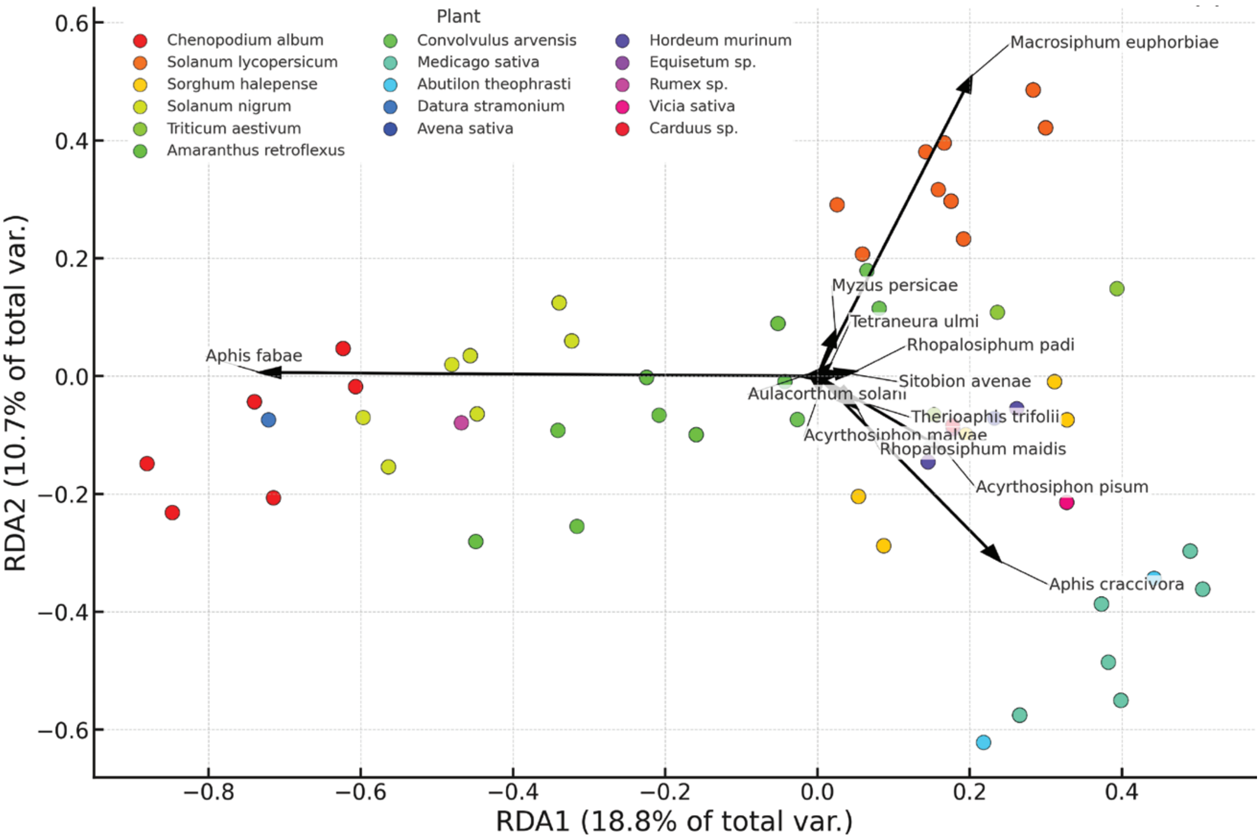


**Figure 4.** Constrained ordination of Hellinger-transformed abundances using "Plant + Year + Province" as constraints.

In the second dataset, which contained province and year but not the plant, the RDA recovered a more modest structure dominated by geographic differences. RDA1 and RDA2 explained about 11% and 4% of the total variance, respectively. Samples from CR and PC tended to score positively on RDA1, whereas MN samples were shifted toward negative values, indicating a broad provincial gradient with only minor temporal spread. Species vectors mirrored this pattern: *M. euphorbiae* aligned with the positive side of RDA1, while *A. fabae* loaded toward the negative side; other taxa contributed locally and with shorter arrows (Fig. 5).

Lastly, in 49 cases, DNA barcoding failed to identify the aphid species detecting parasitoids belonging to 11 species from 10 different aphid species (Suppl. material 1: table S10). Parasitoid detection was significantly correlated with the year ($\chi^2_{(2, N = 49)} = 6.945$, $p = 0.03$). Among the more common aphid species in our samples, *M. euphorbiae* was parasitised by wasps belonging to the genus *Aphelinus* (Hymenoptera: Aphelinidae), and by *Praon volucre* Haliday (Hymenoptera: Braconidae). Parasitoid species found attacking *A. fabae* were *Aphidius ervi* Haliday (Hymenoptera: Braconidae), *Aphidius matricariae* Haliday (Hymenoptera: Braconidae), *Binodoxys angelicae* Haliday (Hymenoptera: Braconidae) and two species of the genus *Lysiphlebus*. *A. pisum* was parasitised by 3 different species: *A. ervi*, *Binodoxys* sp. and *P. volucre*. No parasitoid species were detected on *Rhopalosiphum maidis* (Fitch) and *R. padi* (Suppl. material 1: table S10).

# Discussion

The current study provides an overview of the diversity of aphid species detected in open field tomato crops in northern Italy over a three-year period and points out their relationships with tomatoes and other crops and weeds that aphids use as shelter and/or food source and potentially could also be a reservoir for Cucumber Mosaic Virus. Such data are a pre-requisite to evaluate the risk of spreading of such pests and disease in the considered environment. We observed some level of variation in the diversity and abundance of the aphid species among sampling periods with a higher diversity in the first part of the tomato growing season that, in the areas, is transplanted starting from the end of April. The aphid abundance was similar between the first (mid-May) and the second sampling period (mid-June) and clearly declined in the third period in 2021 and 2022. Such variations are known and, as reported in literature, fluctuations in the timing and intensity of aphid infestations are a key factor in the risk of CMV spreading strongly limiting or enhancing the spread of the virus between weed and crop species, thus increasing or decreasing the number of affected plants (Thackray et al. 2004).

Some of the observed annual variations in aphid presence could be due to several factors, such as adverse climatic conditions during the year that reduced the aphids' proliferation, typical year-to-year fluctuations in several aphid species (Bell et al. 2015), or even variations in agricultural practices in the studied fields (Benton et al.

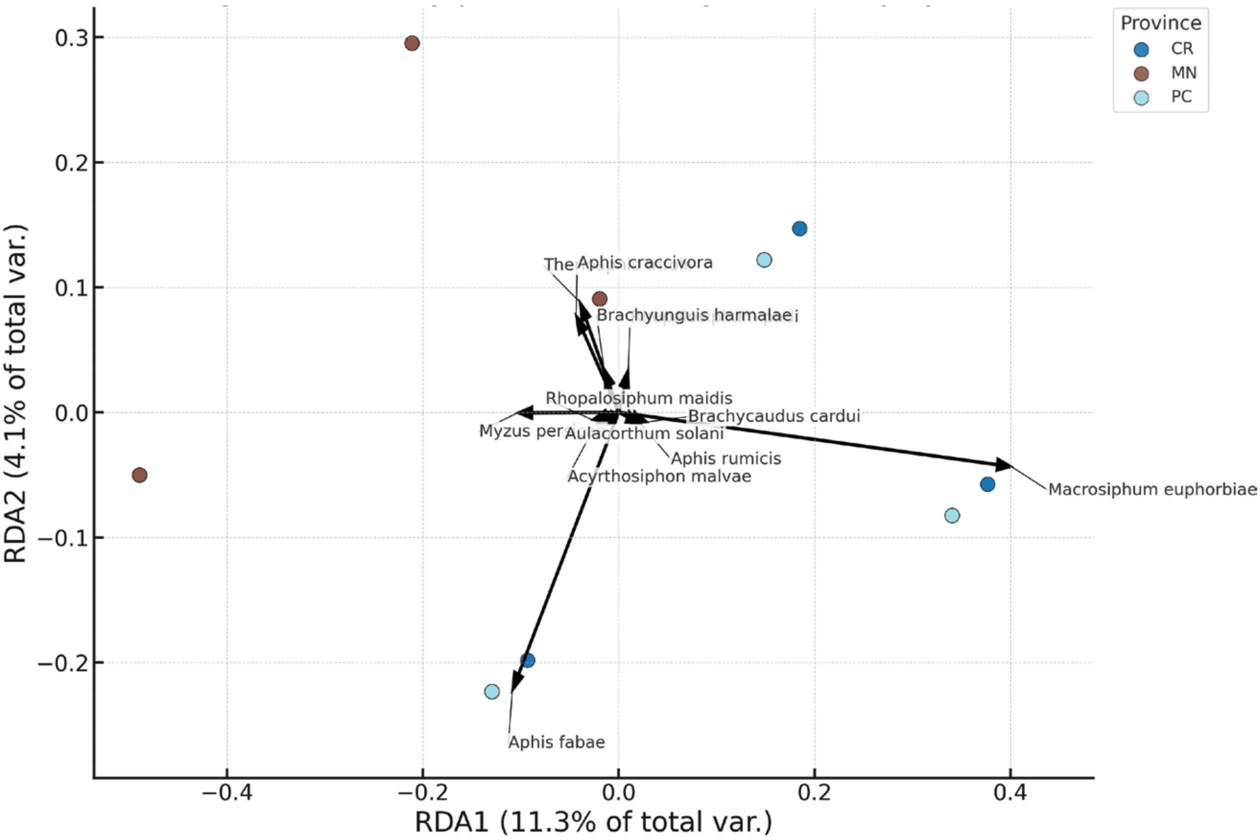


**Figure 5.** Constrained ordination of Hellinger-transformed abundances using "Province + Year" as constraints.

2002). Nevertheless, in this specific situation the association between year and aphid presence is quite weak, as pointed out by RDA. Overall, the ordination through RDA analysis depicts distinct plant-specific assemblages with limited geographic or interannual divergence at the scale sampled. Taken together, these ordinations show that when host identity is available it organizes the aphid communities most clearly, whereas in its absence the principal structure that remains is a weaker spatial gradient among provinces.

Considering the potential risk of virus spreading by aphid vectors, in the local situation it is to note that about half of the aphid species identified were known CMV vectors (Bradley 1964; Berlandier et al. 1997) and they represented almost 90% of the total identified samples.

The five most collected aphid species (*A. pisum*, *A. fabae*, *A. craccivora*, *M. euphorbiae* and *M. persicae*) are all virus vectors and the latter four are considered the most efficient vectors for CMV in tomato plants even if transmission efficiency can be influenced by several factors like vector species, virus strain and environmental conditions (Panno et al. 2021). In the investigated situation, differences in temporal and host distributions of these species were observed (Suppl. material 1: tables S3, S4).

*M. euphorbiae* and *M. persicae* are considered global tomato pests (Perring et al. 2018) and, in our study, both were primarily found on tomato plants, but with different temporal distributions. *M. euphorbiae* was consistently detected during all three samplings so confirming its primary role as tomato pest (Powell et al. 2006; Perring et al. 2018). On tomato, *M. persicae* was identified principally in the first sampling period. The reduced number of *M. persicae* samples, mainly from *C. arvensis* and *S. lycopersicum*, may be linked to population dynamics, as also reported by Summers and colleagues (2010). However, considering the extreme polyphagy of the green peach aphid, it was expected to be found on a wider range of hosts, including *A. retroflexus*, *S. nigrum* and *C. album* (Weber 1985; Fernandez-Quintanilla et al. 2002).

As expected, *A. fabae*, known for its wide host range (Akca et al. 2015), was identified on the greatest number of plant species during the first and second samplings of each year, particularly on *C. album* and *S. nigrum*, which aligns with a previous study by Fernandez-Quintamilla and colleagues (2002). However, in contrast to these earlier results, no preferential colonisation of *A. retroflexus* was observed.

The cowpea aphid, *A. craccivora*, a pest of various leguminous crops (Berberet et al. 2009), was the fifth most important species among those collected from *S. lycopersicum* during all our samplings. Together with *A. pisum*, it was detected primarily on *M. sativa*. There was an increase in detection frequency during the second and third samplings of the year, linked also to an expansion in the host species colonised, which included tomato. The growth over time of *A. craccivora* populations, as reported by Summers and colleagues (2010), may have triggered the observed migration from its preferred exploited host, *M. sativa*, to tomato plants and other new plant species.

Winged morphs of a few other species, not reported to be pest of *S. lycopersicum*, have been detected occasionally: *Anoecia* sp., *A. frangulae*, *H. lactucae*, *M. rosae*, *R. padi*, *Sitobion avenae* (Fabricius), *Tetraneura ulmi* (L.) and *Uroleucon* sp. In several cases, these aphids were detected also in the presence of colonies or specimens of other species.

Indeed, the simultaneous presence of two or more species on the same plant was observed several times, and approximately half of these cases were detected in tomato plants. Interference between different aphid species affects host selection, as aphids tend to choose plants with less likely future competition (Mehrparvar et al. 2014). This may explain the relatively low number of sampled plants where co-infestations were recorded. Furthermore, most of the recorded combinations were unique because one or more adults were captured while randomly moving or resting on plants already colonised by other species.

Lastly, the identification of parasitoids was a side effect of the molecular identification of aphids. Total DNA extraction is not a novel approach in parasitoid species identification, but it allows for their rapid identification without the need for rearing or dissecting collected aphids (Derocles et al. 2012; Ye et al. 2017). DNA was extracted from morphologically normal adult aphids, but the higher concentration of parasitoid DNA probably led to preferential amplification. Certainly further investigation on this topic is needed to clearly outline the network of relationships in the studied areas and to quantify the role of antagonists in reducing the impact of aphids. All the aphid-parasitoid interaction such as those between *A. ervi* and *M. euphorbiae* (Digilio et al. 2010), *P. volucre* and *M. euphorbiae* (Lins et al. 2011) or *Lysiphlebus fabarum* Marshall and *A. craccivora* (Ali 2014) have been previously observed. Some of these parasitoids are species reared for agricultural purposes; as such, their potential application in the control of aphid pests in open-field tomato cultivation could be further analysed.

The current study provides a comprehensive qualitative assessment of the aphid species composition detected in open-fields tomato crops in northern Italy over a three-year period and shows the relationships of the aphids with the surrounding plant species. It emerges from the survey that most of the detected aphid species can be CMV vectors and they were found on both tomato and at least one other sampled plant species that in several cases are known to act as virus reservoirs (Freeman and Aftab 2011).

For this, tomato cultivation in northern Italy, in the investigated areas, could be at risk of CMV spreading because both vectors and hosts are present. Currently is increasing, among the agriculture practices applied by tomato growers, the use of insecticides against aphids but it must be considered that such control strategies can be quite inefficient being compromised by the difficulty to foresee the presence of aphid pests on tomato, above all for those species non forming colonies whose specimens, moving from a plant to another, through feeding probes, could spread non-persistently transmitted, stylet-borne viruses as CMV and by the great genetic plasticity of aphids that easily select insecticide resistant populations, as it is already well known for some of the most important vector collected (Panini et al. 2014; Müller et al. 2023; Cominelli et al. 2024).

## Authors' contributions

Conceptualization, E.M.; methodology, A.A and E.M.; validation, F.C., R.H and G.C.; investigation, G.C., F.C. and R.H.; resources, E.M. and A.A.; data curation, E.M.; statistical analysis, M.M.; writing—original draft preparation, F.C. R.H. and M.M.; writing—review and editing, E.M., R.H. and F.C.; supervision, E.M. and A.A.; project administration, E.M. All authors have read and agreed to the published version of the manuscript. The authors declare no conflicts of interest.

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# Supplementary material 1

## Suppl. tables S1–S10

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Data type: docx

Explanation note: **table S1.** Farm localities where samples were collected; **table S2.** Number of aphid species identified on sampled plants per year and province; **table S3.** Number of aphid taxa uniquely identified on sampled plants during the three sampling periods; **table S4.** Number of times the aphid taxa were identified in the surveyed provinces; **table S5.** Numbers of cases when an aphid species was collected from sampled plants in 2021, 2022 and 2023; **table S6.** Number of times an aphid species, known to be a CMV vector, was collected from a plant species during the first sampling period; **table S7.** Number of times an aphid species, known to be CMV vector, was collected from a plant species during the second sampling period; **table S8.** Number of times an aphid species, known to be CMV vector, was collected from a plant species during the third sampling period; **table S9.** Co-infestations of two or more aphid species on each plant; **table S10.** Parasitoid species identified, number of identified samples and aphid species parasitised.



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