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ORIGINAL PAPERS

Corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) population expansion influenced by endosymbiotic bacterial diversity along a gradient of maize management and climate conditions

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Abstract

In the present study, corn leaf aphid population expansion along a gradient of 200,000 square kilometers was tested under four different maize management systems and climatic regions, and the influence of these factors on the aphids' endosymbiotic bacterial community composition (both obligate and facultative) assessed. The central question posited was whether there was a correlation between corn leaf aphid distribution and variation in its endosymbiotic bacterial species composition due to climate change. After a detailed symbiont assessment, it was found that climate was the major factor in determining endosymbionts distribution (>80%). A significant positive linear correlation was shown to exist between the infestation rate (number of infected maize fields) and increasing frequency of two facultative endosymbiotic bacteria, *Serratia symbiotica* and *Wolbachia*, both under high and low temperature conditions, whilst the opposite trend was observed in the case of the obligate endosymbiont, *Buchnera* aphidicola. From these findings, we reveal for the first time that clear and strong relationships exist between the increase in corn leaf aphid populations along a large geographic area in relation to climatic change and the increase of *S. symbiotica* and *Wolbachia*, yet at the same time, with a concomitant decrease of *B. aphidicola* populations. We conclude that this scenario may well explain the recent rapid expansion of the corn leaf aphid in– and damage to– European maize fields.

Keywords Rapid distribution, Large-scale crops · Total bacterial diversity · Primary symbionts · Secondary symbionts · 16S rRNA gene

1 Introduction

Insect pests population expansion under climate change is one of the major question in modern agriculture and food production, a scenario requiring new monitoring technologies, but also new and modern methods to test the mechanisms behind the rate of expansion and possible management of these pests (Subedi et al. 2023a). Abiotic factors have major influences on insect population dynamics by modifying their reproduction (i.e. no overwintering as

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eggs in the case of aphids), survival, fecundity, voltinism, host plant and predator interactions and dispersal. Among the abiotic factors, temperature is the most crucial factor affecting insect population flow in different landscapes and ecological regions (Sunil et al. 2023).

Several previous studies have followed the expansion of invasive insects under different abiotic factors (Hartl et al. 2024), but only a few such studies have actually followed the expansion of aphid pests species under climate change (Wu et al. 2020; Subedi et al. 2023). Aphids (Hemiptera: Aphididae) play a major role as major pests of agriculture, horticulture and forestry, both by causing physical damage and transmitting plant pathogenic viruses; their expansion under climate change has not been sufficiently studied until now (Liu et al. 2019; Guo et al. 2017; Zepeda-Paulo and Lavandero 2021). Some important pest aphids such as the corn leaf aphid, *Rhopalosiphum maidis* (Fitch), a global pest of cereals, especially maize (*Zea mays* L.) in North America, have been found to increase significantly in terms of its populations in European maize fields during the last five years (Csorba et al. 2021, 2022). Significant population increase and damage were detected in Germany, Poland, Hungary and from 2019 onwards, in Romania (Banat region) (Blackman and Eastop 2000; Emden and Harrington 2007, 2017). Because maize is the major cereal host of the corn leaf aphid, the largest agricultural crop in several European counties, expansion of this pest aphid and the damage it causes is likely to cost billions of Euros before an effective monitoring system and satisfactory control method is developed. Moreover, hitherto, no technologies were– or indeed are– available to control this particular serious pest other than the application of synthetic insecticides and use of hymenopterous parasitoids as biological control agents in European maize fields, as in the USA (Van Emden and Harrington 2017).

Several factors need to be identified in order to help understand the rapid expansion of certain pest aphid species, including the corn leaf aphid, including the effect of microbial symbionts on aphids adaptations. Aphids are closely associated with bacterial symbionts (Douglas 2016). Of these, some are obligate, usually referred to as a primary symbiont, i.e. *Buchnera aphidicola.* There are also several facultative or secondary species too such as *Serratia symbiotica*, *Wolbachia* spp., *Hamiltonella defensa*, *Regiella insecticola*, and others, that are harboured and shared between divergent aphid host lineages by undergoing both vertical and horizontal transfer among asexual female (parthenogenetic) aphid matrilines and which might confer additional adaptation mechanisms (Zepeda-Paulo and Lavandero 2021).

Only a few earlier studies have discussed the possibility that these endosymbiotic bacterial communities may well be– or indeed are– involved in the expression of different traits related to aphid adaptations, including tolerance to high and low temperature (Montllor et al. 2002) and host plant adaptation (Tsuchida et al. 2010). Overall, the functional role of these bacterial symbionts can be classified in relation to two main traits: 1) their **protective role**, traits that may confer advantages to the aphid under specific ecological conditions (high and low temperatures, natural enemies i.e. parasitoids; and 2) their **metabolism role**, traits that confer advantages in specific nutrients decomposition when this is required after a new host plant invasion, either to an existing host or to a novel one (Douglas 2016).

Previous studies involving heat exposure experiments have revealed the role of *B. aphidicola* in aphid fitness parameters. Studies that are more detailed show that aphid fecundity decreases due to a mutation suppressing transcriptional response of a gene encoding a small heat shock protein. The absence of this heat shock gene conferred by *B. aphidicola* seems to explain the heat sensitivity of some aphids (i.e. the black bean aphid, *Aphis fabae* Scopoli) (Zhang et al. 2019).

Of the studies performed to date, the presence of *S. symbiotica* in ant populations has been investigated, especially on species with mutualistic interactions with aphids although the diversity of strains was found to be similar within the gut of aphids and ants since ants feed on the honeydew of aphids (Renoz et al. 2019). It has also been reported that *S. symbiotica* is involved in defense against heat stress and potentially in aphid nutrition, being capable of producing proteases such as metalloproteases, which may facilitate aphid development, help in plant protein digestion, as well as suppressing plant defenses (Skaljac et al. 2019). Other studies have further revealed that both obligate and facultative symbiont strains are e transmitted maternally (Montllor et al. 2002).

The endosymbiont *Wolbachia* was first detected in aphids (e.g. in *Cinara cedri* Mimeur) by Gómez-Valero et al. in 2004a and reported to have importance in the prevalence and persistence of asexual lineages. Later studies showed that *Wolbachia* is one of the most frequently found microorganisms in aphids, i.e. in 82 species (Zytynska and Weisser 2016), these symbiotic relationships protecting aphids and other arthropods against several pathogens and abiotic stresses (Oliver et al. 2003; Gómez-Valero et al., 2004a).

The role of the other facultative endosymbionts such as *Hamiltonella defensa* and *Regiella insecticola* in aphid adaptation to abiotic and biotic factors should be reconsidered. Studies report that the presence of *Hamiltonella* serves as a defense against hymenopterous parasitoid wasps (braconids) by inhibiting the development of wasp larvae inside the body of their aphid host e.g. the pea aphid, *Acyrthosiphon pisum* Harris (Oliver et al. 2005). In contrast,, aphids infested with *R. insecticola* were observed to be more predated and did not improve defenses against ladybird (Coleoptera: Coccinellidae) predators; also such -infected aphids showed increased performance on wheat, while the opposite was true on barley (Ramírez-Cáceres et al. 2019).

1.1 Objectives of the present study

In light of the various factors outlined above, in the present study three central questions, important both from a fundamental as well as potentially an applied point of view (i.e. aphid pest control), were posited: (1) Do corn leaf aphid populations expand significantly along a gradient of 200,000 square kilometer, which includes different maize management systems and climatic regions?; (2) Do the aforementioned factors, especially climatic conditions, influence corn leaf aphid endosymbiotic (both obligate and facultative) bacterial community composition? If so, (3) can these factors be correlated with rapid corn leaf aphid distribution and population increase with time, as currently observed in many European agroecosystems?

2 Materials and methods

2.1 Study area and sampling methods of corn leaf aphids

Maize is the largest cash crop in Romania, with an average yield of 18.6 million tonnes *per annum* (*p.a*), making the country the ninth largest maize producer in the world (Şimon et al. 2023).

In our study, a comprehensive field assessment of corn leaf aphid colonies and sampling were performed in Romania during a four-year period (2020-23) and involving the most important agricultural regions in the country. The variation of corn leaf aphid population density were followed starting from the 2020 vegetative propagation season, and 16 maize plots inside eight fields were periodically visited, and aphid populations assessed from the four climate areas. As aphid populations started to increase, comprehensive field collection was begun with aphids collected from the same eight fields (and two plots inside each one, so 16 plots in total) during the same time period (June and July) when all plots were in flowering stages R2 to R3.

Since about 2 million ha of maize is cultivated in Romania annually, the eight maize fields were selected along a large geographic gradient of 200,000 square kilometers, taking into consideration two main aspects, each representing the Romanian and the European maize cultivation systems. These were the geographic locations, and maize management systems, with climatic zones were defined according to Marcu and Borz (2013) (Marcu and Borz 2013). Accordingly, corn leaf aphid infested maize fields ranging from south, south-western and east-western Romania (Timiş, Olt, Călăraşi, Brăila sitest) were sampled from both wet-warm and wet-temperate climate regions of maize cultivation and high size and input management systems (abbreviated as HIFw = high input fields and wet warm climate and HIFt = high input fields and wet temperate climate, respectively), together representing some 60% of the total Romanian maize production p.a. Fields from eastern and mid-western parts of Romania (Iaşi, Cluj) with a temperate continental climate and medium size and input maize management systems (abbreviated as MIF = medium impact fields), represented about 20% of Romanian maize production *p a*. Lastly, fields from Central-Romania (Braşov, Harghita) with cool continental climate regions between mountainous areas and low area and input management maize systems (abbreviated as LIF = low impact fields), also represented about 20% of Romanian maize production p.a. (Fig. 1). All fields and plots were managed using seed treatments, fertilizers and insecticides against corn rootworm beetle, *Diabrotica virgifera* LeConte (Coleoptera: Chryomelidae) in conventional management regimes (Table 1).

The infestation rate during the four-year assessment were made in each field and plot by choosing 50 plants per plot (16 plots inside the 8 fields) in a transect of 10 × 5 m from the margin to the field interior and the presence of the aphids colonies were searched for. If all 50 plants had colonies, the infestation was determined as severe, if up to 25 plants, as high, while if only ten plants were colonised, as low (Fig. 2).

Inside each of the eight fields, two smaller similar plots were defined and sampled from. Aphids were collected from maize plants inside these smaller plots to minimize the possible effects of field margins, and the same sampling method was used in all sites. Asexual wingless (apterous) aphids were searched for inside the colonies, choosing ten maize plants per smaller plots. From each colony, first instar aphid nymphs (five from each colony / plant) were collected and stored in 0.5 mL Eppendorf tubes containing 99% ethanol prior to DNA analysis. Because at the beginning of the study, not all the sites were infected, the rate of infestation in each of the 16 plots was assessed by searching for the colonies. If a colony was detected, samples were collected, but also the start of any plant infections were noted and used in the results. The increased infestation rate from 2021, and especially in 2022 and 2023, enabled thorough sampling (all sites and plots having aphid colonies), all samples collected being used for genetic analyses. From each sample of ten plants (i.e. with an aphid colony per small plot sampled as earlier stated and the procedure replicated biweekly three-times each year in June and July), five samples were selected and used for symbiont genetic assessment. In such cases,

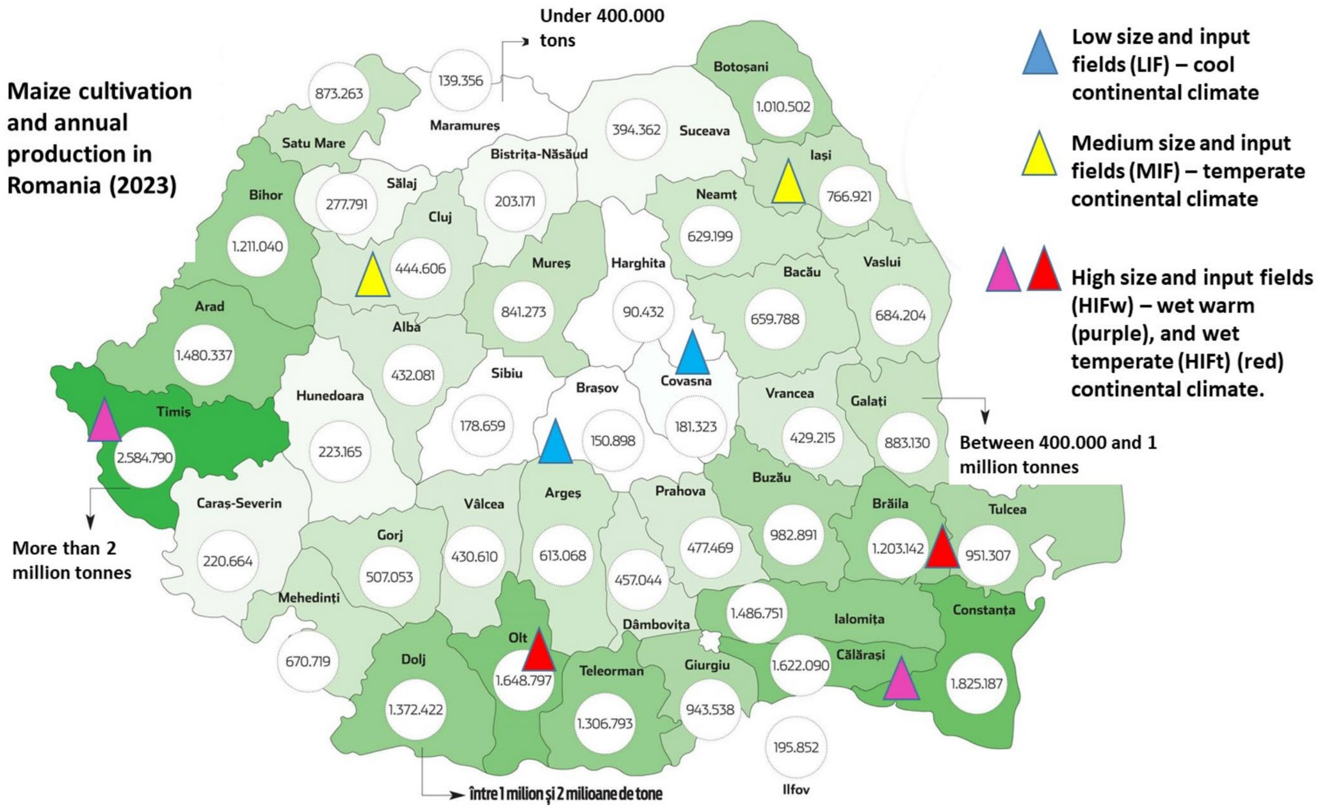

Fig. 1 Locations of maize cultivation and corn leaf aphid sample collections in Romanian maize management in relation to field size systems and climate conditions

a number of 20 samples per three collection dates in 2020 ($n=60$), 30 in 2021($n=90$), 65 in 2021 ($n=195$) and 80 in 2023 ($n=240$) were analyzed genetically In total, 585 corn leaf aphid samples were analyzed, but because there was no continuous infestation in 2020, only data from 2021 to 2023 (525 samples) were tested statistically from the eight maize fields and four climate areas.

2.2 Bacterial symbionts identification and bioinformatics assessments

Amplified partial sequences of the 16 S rRNA gene from aphid DNA extracts were performed using primer pairs designed for the hypervariable regions of the genes V2-4-8 and V3-6, 7–9. The resulting PCR products targeting specific regions of the 16 S rRNA gene were purified with Agencourt AMPure beads (Beckman Coulter™). Subsequently, libraries were constructed using the Ion Plus Fragment Library kit (Applied Biosystems) and their concentration determined with the Ion Universal Library Quantitation kit (Cat no. A26217). Template preparation was completed using the ION PGM Hi-Q View OT2 kit-400, followed by sequencing of the amplicon libraries on a 318-chip using the Ion Torrent PGM system (Figure S1).

The sequence reads obtained were filtered by the Ion Reporter PGM software to eliminate polyclonal and low-quality reads. The resulting sequencing data were analyzed using Quantitative Insights Into Microbial Ecology (QIIME). Operational Taxonomic Units (OTUs) of the 16 S rRNA gene were defined at a threshold of ≥97% sequence homology to calculate downstream diversity measures. Classification of all reads was performed utilizing reference datasets (Curated Greengenes v13.5; Curated MicroSEQ(R) 16 S Reference Library v2013.1).

2.3 Data analysis

The community composition of the most frequent bacterial symbionts for each aphid colony and site were analyzed by year and 16 plots separately. The data were paired by year, and because there were generally only low infestations by aphids in the plots, the frequency of the bacterial endosymbionts were only compared between plots with infections. Because within fields and years (i.e. HIFw Timis and years) there were no differences found in terms of symbiotic bacterial community composition and frequency in those plots where colonies were discovered, and also no differences between collection periods, these samples were pooled after genetic analysis, and clumped together for statistical

Table 1 Management plan for sample areas in different maize fields in Romania. Each treatment has been presented in the order of application during the crop vegetation period

County	Treatment	Maize crop management plan
Timis	High size and input fields (HIF)– wet warm continental climate.	75,000 grains/ha, Seed treatment included fungicide and Nuprid 10 l/t, Fertilization at seedbed preparation urea 250 kg/ha, Fertilization at sowing 14:28:14 300 kg/ha, Herbicide Adengo 0.4 l/ha pre-emergent, Herbicide Nicogan 1.5 l/ha + Concordia 0.5 l/ha post emergent
Calarasi	High size and input fields (HIF)– wet warm continental climate.	75,000 grains/ha, Seedbed fertilization NPK 20:20:0 150 kg/ha, Seeded fertilization ammonium nitrate 100 kg/ha, Herbicide at 2–4 leaves Arigo 0.33 g/ha + adjuvant Vivolt 0.25 l/ha, Insecticide Scato 0.5 l/ha at 1–2 leaf emergence and repeated at 2–4 leaves, Herbicide Samson Extra 60D 1 l/ha at 6–8 leaves
Braila	High size and input fields (HIF)– wet temperate continental climate.	78.000 grains/ha, Seed treated with fungicide and Nuprid AL600 FS 8 l/t and Trichoderma and Mycorrhiza biostimulator, Glyphocover herbicide 2.5 l/ha myrtle, Fertilization at sowing Cx. 14.28.14 + 5.5% S 250 kg/ha and Urea 100 kg/ha, Inazuma 0.2 kg/ha insecticide at emergence + Spraygard 0.1 l/ha adjuvant, Lumax 537.5 SE herbicide + Spraygard 0.1 l/ha adjuvant,
Olt	High size and input fields (HIF)– wet temperate continental climate.	78,000 grains/ha, Seedbed preparation fertilization NPK 20:20:0 250 kg/ha, Pre-emergent Dual Gold herbicide, Elumis herbicide, Ammonium nitrate fertilization 250 kg/ha.
Cluj	Medium size and input fields (MIF)– temperate continental climate	68,000 grains/ha, Seed treatment: Prothioconazole + Metalaxyl. Herbicide pre-emergent: Isoxaflutole + Thiencarbazone-methyl. Fertilizers: 50 t/ha organic fertilizer at seeding. At 12 leaf stage: Inorganic fertilizer, $NH_4NO_3 + CaMg(CO_3)$ 2200 kg/ha.
Iasi	Medium size and input fields (MIF)– temperate continental climate	68,000 grains/ha, Fertilized NPK 16:16:16 200 kg/ha at seedbed preparation, Fertilized at sowing Urea 90 kg/ha, Herbicide Leo Green Plus 7 l/ha, Macro- and micro-element fertilizer Corona N 1 kg/ha; Herbicide Mustang 0.6 l/ha; Insecticide Inazuma 0.2 l/ha.
Brasov	Low size and input fields (LIF)– cool continental climate	68,000 grains/ha, Seed treatment: Prothioconazole + Metalaxyl. Herbicide pre-emergent: Isoxaflutole + Thiencarbazone-methyl. Fertilizers: 50 t/ha organic fertilizer at seeding. At 12 leaf stage: Inorganic fertilizer $NH_4NO_3 + CaMg(CO_3)$ 2200 kg/ha.
Covasna	Low size and input fields (LIF)– cool continental climate	68,000 grains/ha, Seed treatment: Prothiocanazole + Metalaxyl. Herbicide pre-emergent: Isoxaflutole + Thiencarbazone-methyl. At 4–6 leaf stage Nicosulfuron. Fertilizers:70 t/ha organic fertilizer at seeding. At 12 leaf stage: Inorganic fertilizer $NH_4NO_3 + CaMg(CO_3)$ 2200 kg/ha.

analyses. Alignment of all bacterial genetic sequences was performed using ClustalW, and the results visualized in EMPeror (Vázquez-Baeza et al. 2013). Non-metric multidimensional scaling (NMDS) based on beta diversity analysis using 3-D principal coordinates analysis was then performed involving the Bray–Curtis dissimilarity index. This was followed by bacterial community classifications in terms of family, genus and species level using data from 2021 to 2023, when all sites were infested), and the most dominant bacterial taxa from each community were presented at these three levels. Hence at family level, the 15 most frequent endosymbiont taxa were analyzed and presented, while at genus and species level, the 20 most frequent taxa.

Principal Coordinate Analyses (PCoA) was used to test the effect of climate conditions and site on bacterial symbionts abundances at species level for the eight maize fields, where locations were considered as main components and endosymbiont species abundances as variables. In the following analyses, heat-map matrices were constructed to compare bacterial symbionts abundances between sites. In this approach, blue colours of matrices represent low abundance and red high abundances. For this analysis, the most frequent 14 endosymbiont species were used for a better representation in a heat-map matrix. Bray-Curtis similarity indices were computed and cluster analyses used to compare the composition of bacterial endosymbionts species in the eight sites. Data analyses and visualization were made using the computer program PAST 4.02. (https://past.en.lo4d.com/windows).

To improve the reliability of the statistics performed using PCoA, heat map and Bray-Curtis analyses, complete data from 2021to 2023 were used. Correlations between the infestation rate of the maize fields visited between 2020 and 2023 (when all fields had aphid colonies), and the frequency of the most abundant endosymbionts (2021to 2023) were compared using linear relation factors.

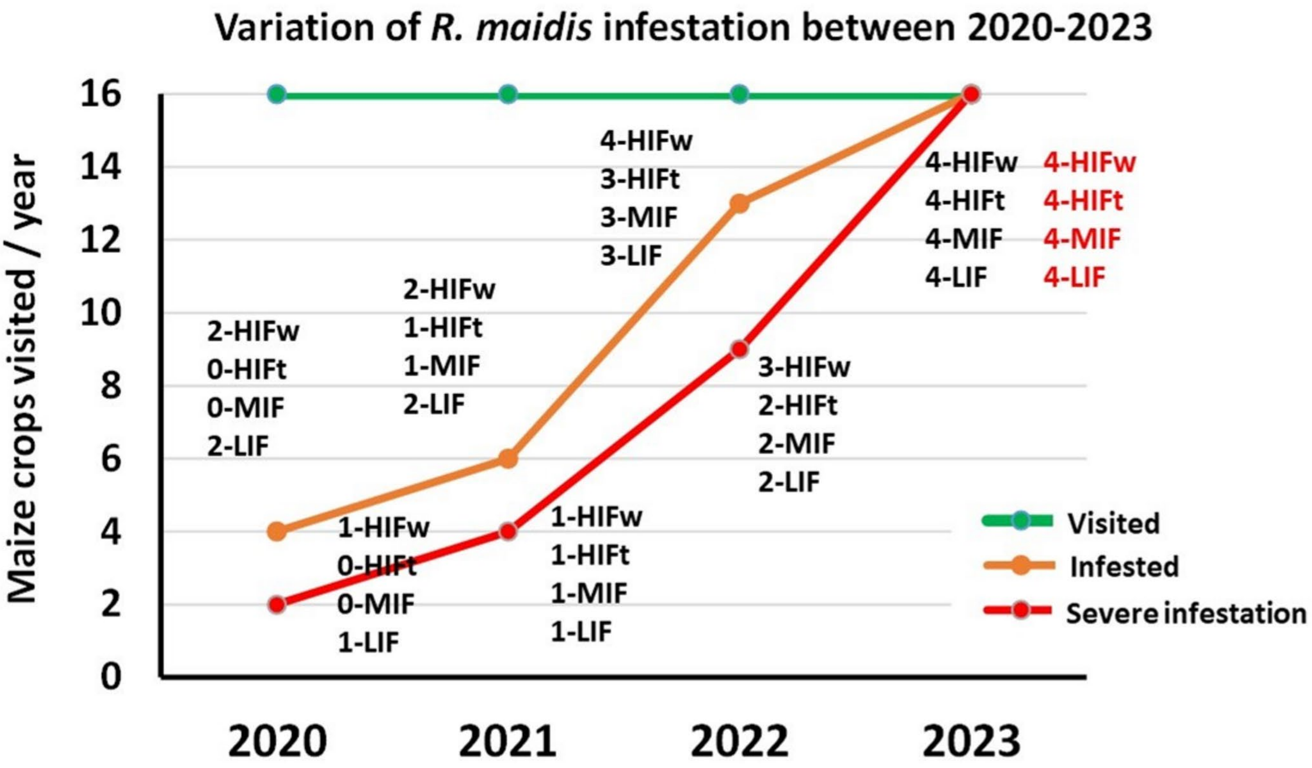

Fig. 2 The rate of infestation and severe infestation of corn leaf aphids in the visited 16 maize fields between 2020–2023

3 Results

An increasing number of the infested maize fields were detected from 2020 onwards, when only four fields were infested at low level with corn leaf aphids, and thereafter an increasing tendency was observed until 2024, when all maize fields and inside them all plots assessed were infested. Severe infestation (almost each plant starting from the field margins) with well-developed aphid colonies and concomitant crop damage were detected in each eight fields and 16 plots (Fig. 2).

The community composition analyses of the aphid bacterial endosymbionts clearly represent a snapshot of the frequency of the microbiomes at the level of family, genus and species. At family level, the 15 taxa represent 95% of the total families identified; high size plots with a wet warm continental climate (HIFw) were clearly dominated by endosymbiont taxa Enterobacteriaceae followed by Anaplasmataceae (Figure S2A). High size plots with wet temperate climate (HIFt) were likewise dominated by Enterobacteriaceae; however here endosymbionts belonging to the following taxa, Microbacteriaceae, Moraxellaceae, Vibrionaceae, Pseudomonadaceae and Sphingomonadaceae were also frequent at sites from Braila (Figure S2B). Medium size and input fields at temperate continental climate (MIF) had higher bacterial symbiont diversity with dominance of Enterobacteriaceae, Pseudomonadaceae, Moraxellaceae and Vibrionaceae (Figure S2C). Corn leaf aphid microbial symbionts at low size and input field with cool continental climate (LIF) were similar with those from HIFw, and dominated by Enterobacteriaceae followed by Anaplasmataceae (Figure S2D).

At the genus level, the 20 endosymbiont taxa represented > 90% of the total taxa identified. HIFw were clearly dominated by endosymbionts belonging to the genus *Serratia*, followed by *Wolbachia* and *Buchnera* (Figure S3A), whereas both HIFt and MIF were dominated by *Buchnera* at high frequency, followed by *Serratia*, *Wolbachia* and *Shigella* at a lower frequency (Figure S3B, C). The microbial symbiont composition of LIF were again very similar to HIFw and were dominated by a high frequency of *Serratia*, but a much lower frequency of *Buchnera*. At HIFw, the frequency of *Wolbachia* was higher than that of *Buchnera* (Figure S3D).

The same general trends were detected at the species level. Thus, *Serratia symbiotica* dominated HIFw and LIF (fields under extreme climate conditions), whereas in contrast, *S. marcescens* and *S. ureilytica* were detected, whilst a low frequency of *Buchnera* and high frequency of *Wolbachis* under HIFw conditions were again observed (Fig. 3A, D). The clear domination of *B. aphidicola* and a much lower frequency of *S. symbiotica* were seen under both HIFt and MIF conditions. Lastly, the presence of *Sigella* spp, and

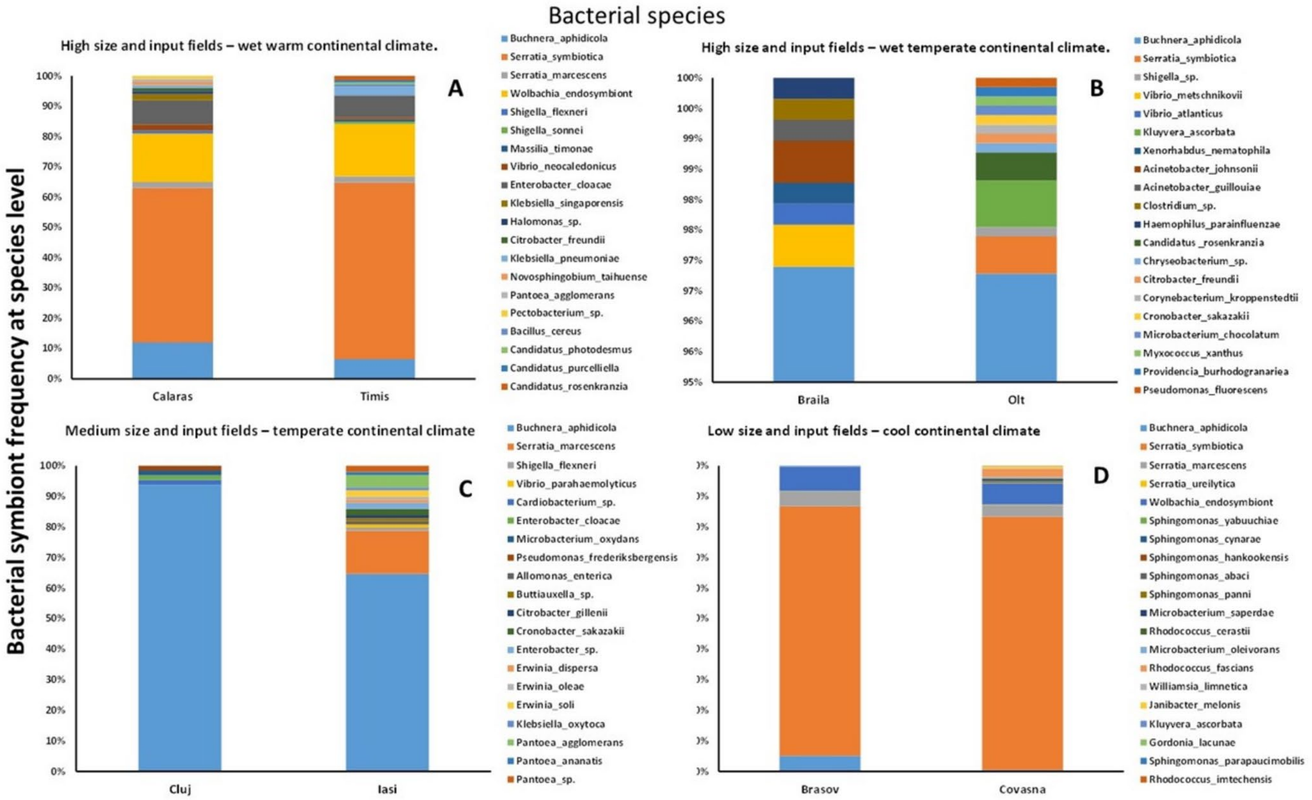

Fig. 3 Corn leaf aphid endosymbiont bacterial frequency at species level under different maize management systems and climate conditions. High size and input fields under wet warm climate (**A**), High size and input fields under wet temperate climate (**B**), Medium size and input fields under temperate continental climate (**C**), and Low size and input fields under cool continental climate (**D**). Only the most frequent 20 endosymbiont species are presented

Vibrio spp., in this instance *V. metschnikovii*, *V. atlanticus* and *V. parahaemolyticus*) were noted (Fig. 3B, C).

The Principal Coordinate Analyses revealed the effect of climate conditions on corn leaf aphid microbial endosymbionts distribution. The climate as factor determined > 88% of the endosymbionts distributions recorded, which in effect means that site effects were so dominant that no other factors were comparable, and no similar strong effect on endosymbionts distributions were detected (Fig. 4A). Heat map analyses also revealed that the abundance of the facultative endosymbionts *S. symbiotica* and *Wolbachia* were highest under LIF followed by HIFw conditions. The abundance of the obligate *B. aphidicola* was lower under LIF conditions, and was followed by HIFt (Fig. 4B). Bray-Curtis cluster analyses revealed leaf corn aphid endosymbiont taxa and frequency distributions to be similar between HIFw and LIF, as well as between MIF and HIFt (Fig. 4C).

Lastly, highly significant positive linear correlations ($P > 0.01$) were detected between the infestation rate (number of infected maize fields) and increasing abundance of *S. symbiotica* and *Wolbachia* under HIFw and LIF conditions, while their abundances under HIFt and MIF slowly decreased during the assessment period. The opposite trend was detected with *B. aphidicola*; this obligate endosymbiont was present, but its abundance decreased with the increasing rate of the maize field infection between 2021 and 2023 (Fig. 5A, B, C, D).

4 Discussion

In the present study, a comprehensive assessment of the population density and the endosymbiotic bacterial species variation of corn leaf aphids was performed. From this, it was found that a clear increase in aphid population density and increase in some endosymbionts were *detectable* during the four-year study period.

Previous studies have also reported that the primary symbionts *Buchnera* as well as a few facultative species (*Seratia*, *Wolbachia*) dominate corn leaf aphid communities (Csorba et al. 2022); however, variations in the abundance of these symbionts between crops, environmental factors and management can be more clearly explained. Moreover, our findings are the first to report correlations between environmental factors (climate change) and variation in endosymbionts harboured by corn leaf aphids infesting the same

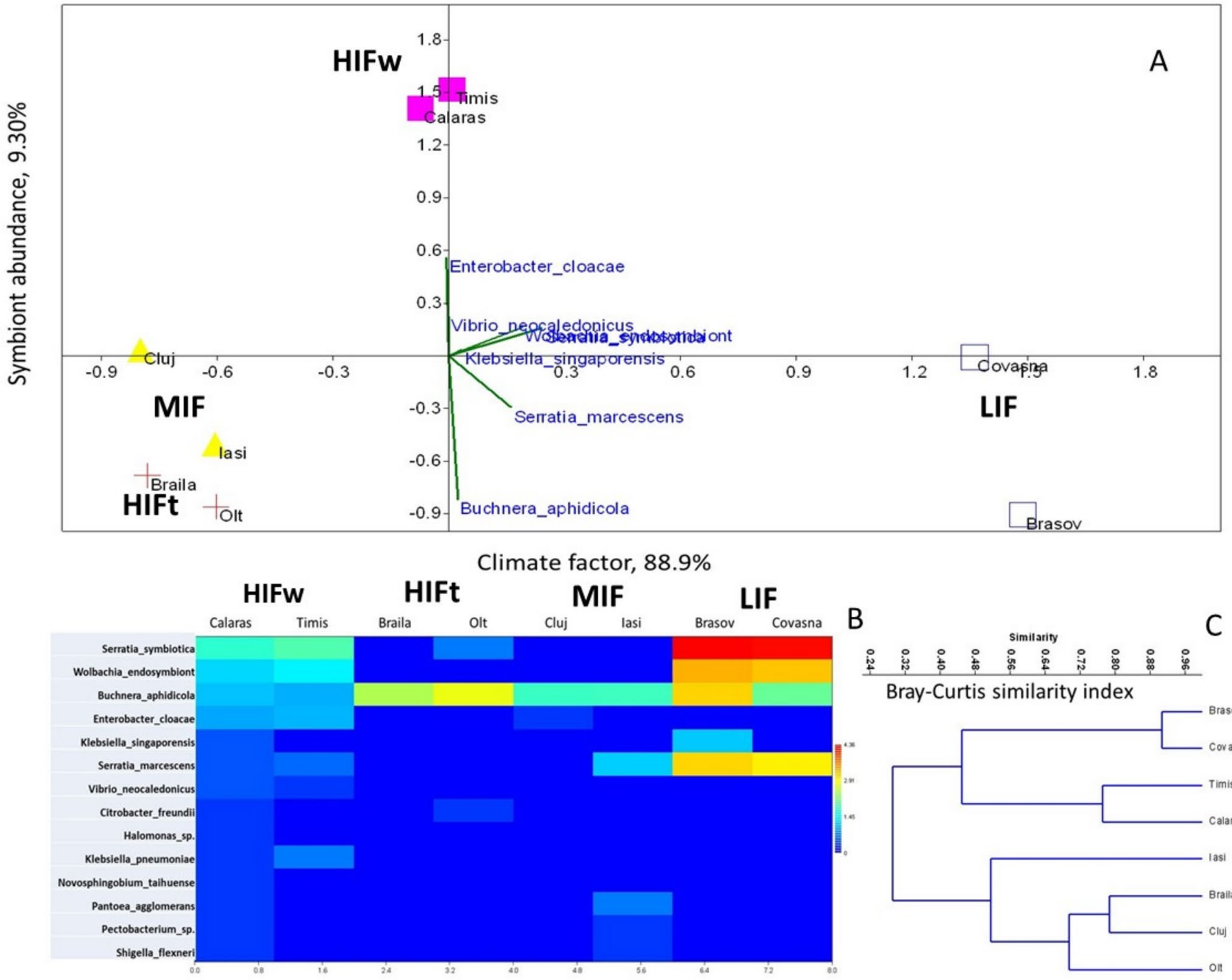

Fig. 4 (**A**). The Principal Coordinate effect of climate conditions on corn leaf aphid microbial symbionts distribution; (**B**) Heat map of the abundance of the most frequent 14 bacterial species under different management and climate conditions; and (**C**). Bray-Curtis similarity and cluster analyses of bacterial endosymbionts between the eight sites

host plant (maize). These correlations also explain the exponential increase of severe infestation rate of this aphid species, which might have significances in aphid pest control. The presence of the obligate endosymbiont *B. aphidicola* has been definitively observed, and high variations in its frequency between fields from different climate conditions detected. This obligate endosymbiont is generally required for the survival of aphids and provides essential amino acids that are rare in their phloem sap diet (Shigenobu et al. 2000), as well as having a role in aphids heat tolerance (Zhang et al. 2019; Majeed et al. 2022).

Earlier researches have revealed that if a novel symbiont is found that physically replaces *B. aphidicola* (here in *Geopemphigus* aphids), and as such no longer adequately provided the essential functions to their hosts, the host may acquire additional symbiont species or even replace the old symbionts with new ones (Chong and Moran 2018). This change in endosymbionts was previously observed within the tribe *Cerataphidini* (subfamily *Hormaphidinae*) (Nováková et al. 2013; Wegierek et al. 2017). The *Cerataphidini* (and *Geopemphigus*) are adapted to warm climate conditions and because *B. aphidicola* is sensitive to both high and low temperatures, it is possible that aphids living in extreme climates are forced to acquire new, facultative endosymbionts that are tolerant of heat and cold (Chong and Moran 2018). In this study, the first evidence of the decrease of *B. aphidicola* abundance and increase in that of other, facultative endosymbionts has been demonstrated over a large geographic area, and four climatic conditions, which hence may explain the rapid expansion of the corn leaf aphid populations as observed do to the climate change.

The relative high abundance of the facultative endosymbiont *Serratia symbiotica* in both HIFw and LIF is also new evidence of the aphid's extreme climate adaptation

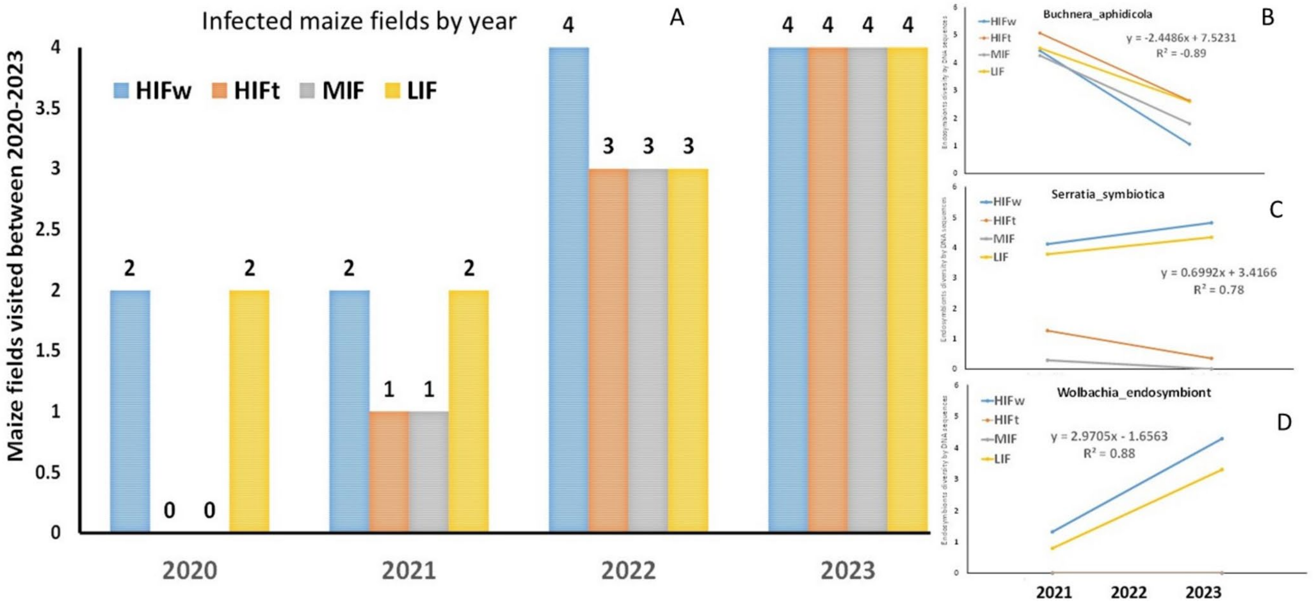

Fig. 5 (**A**). Correlations between the infestation rate of the visited maize fields between 2020 and 2023, and the frequency of the most abundant endosymbionts in these years. Infestation rate by year (number of fields) frequency of (**B**), *B. aphidicola* (**C**), *S. symbiotica* and (**D**), *Wolbachia* between 2021-23

mechanism. *S. symbiotica* is involved in defense against heat and potentially in aphid nutrition. In our study, *S. symbiotica* was dominant in HIFw, but also in LIF with cold climates. An earlier study describing the endosymbiotic bacterial diversity of two oligophagous aphids, *Melanaphis sacchari* (Zehntner) and *Neophyllaphis podocarpi* Takahashi, related with climate conditions, revealed that altitude was negatively correlated with the symbiont richness; even so, several facultative symbionts such as *Serratia* spp. were detected in high abundance and it was hence concluded that these symbionts protected pea aphids, *A. pisum*, from heat stress (in both directions) by regulating the aphid metabolome (Xu et al. 2021).

In this study, another *Serratia* species, *S. marcescens*, was also detected, but in lower frequency in HIFw, LIF as well as in MIF conditions. To our knowledge, there has hitherto been no previously published evidence of a clear effect of this symbiont on aphid adaptation. In previous studies, this opportunist symbiont has been isolated from rice roots and stems (Gyaneshwar et al. 2001). Also, it was shown that *S. marcescens* is highly resistant and can survive by utilizing a large variety of nutrients (Sleigh 1983). This property gives it the ability to survive under extreme conditions, and it can thus confer positive effects to its host, so its role in corn leaf aphid adaptations under extreme conditions is highly probable. Accordingly, additional studies are needed to confirm as well as elucidate the role of *S. marcescens* as a facultative endosymbiont of aphids.

Another *Serratia* species found in low density was *S. ureilytica* under LIF conditions; its presence can be explained due to the use of organic fertilizer, since this particular species can metabolize urea as a source of nitrogen (Bhadra et al. 2005). Again, as with the aforementioned *Serratia* species, its role in corn leaf aphid adaptation requires more research.

The facultative endosymbiont *Wolbachia* spp. was present in high frequency only under HIFw and LIF conditions. The presence of this particular endosymbiont was first reported by Gómez-Valero et al. (2004a) in *Cinara cedri* Mimeur aphids (Gómez-Valero et al., 2004b), and who also found that harbouring of this particular endosymbiont increased the prevalence of asexual lineages in the host. The role of *Wolbachia* is however diverse, previous studies reporting that it can also protect aphids (and other arthropods) against several pathogens and abiotic (extreme heat and cold) stresses (Brownlie et al. 2009; Iturbe-Ormaetxe et al. 2011). *Wolbachia* has also been reported as important for other arthropods (e.g. bed bugs, parasitic wasps and collembolan species), having a role in egg development (Hosokawa et al. 2010), as well as improving the fecundity of the female insect host (Ou et al. 2023). In our previous research involving preliminary exploration of corn leaf aphid symbiont diversity, *Wolbachia* was not only present in large-scale field corn crops under warm climate but also under colder conditions, so taken together with the findings of the present study, it is clear that the microorganism apparently has an important role in the thermal adaptation of corn leaf aphid.

The presence of the *Shigella flexneri* under HIFw, HIFt and MIF conditions was also detected. *Shigella* species are known to infect non-human primates such as the rhesus macaque, *Macaca mulatta* (Zimmermann) (Lee et al. 2011). *Shigella* has also been detected at different levels inside leaf samples of the mouse-ear cress plant, *Arabidopsis thaliana* (L.) Heynh., and is considered to act as an agent actively damaging plant cells (Jo et al. 2019). Its presence in aphids as a secondary symbiont was recorded in the present study, but also for the first time in our previous study with oak aphid symbionts (MS under preparation). *Shigella* is probably harbored by, and transferred via, plant cells, but further assessment is required to test its possible effect/s on aphid adaptations.

The presence of *Vibrio* species can be correlated with maize fields irrigations, in some cases (HIFt and HIFw fields) from rivers, and this particular microorganism is well known to be a sediment inhabiting bacterial species (Huang et al., n.d.). They are probably transmitted to aphids via plant phloem, but their role in aphid adaptations is as yet unconfirmed. In addition, whilst the presence of the other bacterial species found at low frequency cannot at present be associated with corn leaf aphid adaptations (e.g. *Massilia* at HIFw, *Kluyvera* at HIFt, *Cardiobacterium* at MIF or *Sphingomonas* at LIF), this possibility, as with *Shigella* mentioned above, requires further investigations and assessment.

In conclusion, we demonstrate for the first time that along a large geographic area and range of climatic conditions, a strong relationship exists between the decrease of the obligate endosymbiont *B. aphidicola*, and the increase of facultative endosymbionts *S. symbiotica* and *Wolbachia* spp. and the increase in corn leaf aphid populations, which we suggest explains this aphid pest's rapid expansion with concomitant damage in Europe. We also conclude that only the primary symbiont *Buchnera* and facultative symbionts *Serratia and Wolbachis* seemingly have a direct effect on aphid adaptations related to climate conditions and management systems. These findings are potentially important for future programmes to combat this aphid pest in maize fields via integrated pest management (IPM) scenarios.

Earlier studies have demonstrated that other factors could also influence the expansion of aphids. In the case of cereal aphids, the factors most strongly associated with the population dynamics have been found to be fertilizer input and mean winter temperature (Wang et al. 2015). In addition, other factors like tillage systems and biodiversity changes as well as different soil management and environmental factors must be considered when trying to tease apart which factors are indeed critical in the rise of corn leaf infestation of maize in Europe and further studies are needed to understand these complex interactions.

As no directed control as yet exists in European maize fields against corn leaf aphid, whereas more than ten synthetic pesticides are available in the USA to combat this pest, before using these compounds (which are all neurotoxins), the effect of symbionts on the distribution and abundance of this aphid species should be considered, and the possibility of using biochemical agents against their symbionts tested. Such a methodological approach will allow more effective methods of control to be developed, thereby providing better long term and environmentally-friendly solutions for controlling this economically important pest of maize under conditions of climate change, as are indeed currently ongoing world-wide.

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Data Availability The datasets used and/or analysed during the current study available at figshare, doi https://doi.org/10.6084/m9.figshare.26348230.

Declarations

Ethics approval and consent to participate Not applicable. The article does not contain any studies involving animals other than aphids as performed by the authors.

Consent for publication Not applicable.

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References

Bhadra B, Roy P, Chakraborty R (2005) Serratia ureilytica Sp. nov., a novel urea-utilizing Sp.cies. Int J Syst Evol Microbiol 55:2155–2158. https://doi.org/10.1099/ijs.0.63674-0

Blackman R, Eastop V (2000) Aphids on the World's Crops: An Identification and Information Guide, 2nd Edition

Brownlie JC, Cass BN, Riegler M, Witsenburg JJ, Iturbe-Ormaetxe I, McGraw EA, O'Neill SL (2009) Evidence for metabolic provisioning by a common invertebrate endosymbiont, Wolbachia Pipientis, during periods of nutritional stress. PLoS Pathog 5:e1000368. https://doi.org/10.1371/journal.ppat.1000368

Chong RA, Moran NA (2018) Evolutionary loss and replacement of Buchnera, the obligate endosymbiont of aphids. ISME J 12:898–908. https://doi.org/10.1038/s41396-017-0024-6

Csorba AB, Bálint J, Felfoldi T, Szabó A, Fora CG, Máthé I, Loxdale H, Balog A (2021) Endosymbiotic bacterial diversity associated with corn leaf aphid, Rhopalosiphum maidis Fitch (Hemiptera: Aphididae) populations under different maize management systems– preliminary study. North-Western Journal of Zoology

Csorba AB, Fora CG, Bálint J, Felföldi T, Szabó A, Máthé I, Loxdale HD, Kentelky E, Nyárádi I-I, Balog A (2022) Endosymbiotic bacterial diversity of corn leaf aphid, Rhopalosiphum maidis Fitch (Hemiptera: Aphididae) associated with maize management systems. Microorganisms 10:939. https://doi.org/10.3390/microorganisms10050939

Douglas AE (2016) How multi-partner endosymbioses function. Nat Rev Microbiol 14:731–743. https://doi.org/10.1038/nrmicro.2016.151

Emden HFV, Harrington R (eds) (2007) Aphids as Crop Pests. CABI

Emden HFV, Harrington R (eds) 2017. Aphids as Crop Pests, 2nd edition. CABI

Gómez-Valero L, Soriano-Navarro M, Pérez-Brocal V, Heddi A, Moya A, García-Verdugo JM, Latorre A (2004a) Coexistence of Wolbachia with Buchnera aphidicola and a secondary symbiont in the aphid Cinara cedri. J Bacteriol 186:6626–6633. https://doi.org/10.1128/JB.186.19.6626-6633.2004

Gómez-Valero L, Soriano-Navarro M, Pérez-Brocal V, Heddi A, Moya A, García-Verdugo JM, Latorre A (2004b) Coexistence of Wolbachia with Buchnera aphidicola and a secondary symbiont in the aphid Cinara cedri. J Bacteriol 186:6626–6633. https://doi.org/10.1128/jb.186.19.6626-6633.2004

Guo J, Hatt S, He K, Chen J, Francis F, Wang Z (2017) Nine facultative endosymbionts in aphids. A review. J Asia Pac Entomol 20:794–801. https://doi.org/10.1016/j.aspen.2017.03.025

Gyaneshwar P, James EK, Mathan N, Reddy PM, Reinhold-Hurek B, Ladha JK (2001) Endophytic colonization of rice by a diazotrophic strain of Serratia marcescens. J Bacteriol 183:2634–2645. https://doi.org/10.1128/jb.183.8.2634-2645.2001

Hartl T, Srivastava V, Prager S, Wist T (2024) Evaluating climate change scenarios on global pea aphid habitat suitability using species distribution models. Clim Change Ecol 7:100084. https://doi.org/10.1016/j.ecochg.2024.100084

Hosokawa T, Koga R, Kikuchi Y, Meng X-Y, Fukatsu T (2010) Wolbachia as a bacteriocyte-associated nutritional mutualist. Proceedings of the National Academy of Sciences 107, 769–774. https://doi.org/10.1073/pnas.0911476107

Huang Z, Yu K, Lan R, Glenn Morris J, Xiao Y, Ye J, Zhang L, Luo L, Gao H, Bai X, Wang D n.d. Vibrio metschnikovii as an emergent pathogen: analyses of phylogeny and O-antigen and identification of possible virulence characteristics. Emerg Microbes Infect 12, 2252522. https://doi.org/10.1080/22221751.2023.2252522

Iturbe-Ormaetxe I, Walker T, O' Neill SL (2011) Wolbachia and the biological control of mosquito-borne disease. EMBO Rep 12:508–518. https://doi.org/10.1038/embor.2011.84

Jo SH, Lee J, Park E, Kim DW, Lee DH, Ryu CM, Choi D, Park JM (2019) A human pathogenic bacterium Shigella proliferates in plants through adoption of type III effectors for shigellosis. Plant Cell Environ 42:2962–2978. https://doi.org/10.1111/pce.13603

Lee J-I, Kim S-J, Park C-G (2011) Shigella flexneri infection in a newly acquired rhesus macaque (Macaca mulatta). Lab Anim Res 27:343–346. https://doi.org/10.5625/lar.2011.27.4.343

Liu X-D, Lei H-X, Chen F-F (2019) Infection pattern and negative effects of a facultative endosymbiont on its insect host are environment-dependent. Sci Rep 9:4013. https://doi.org/10.1038/s41598-019-40607-5

Majeed MZ, Sayed S, Bo Z, Raza A, Ma C-S (2022) Bacterial symbionts confer thermal tolerance to cereal aphids Rhopalosiphum padi and Sitobion avenae. Insects 13:231. https://doi.org/10.3390/insects13030231

Marcu MV, Borz S (2013) Global Warming and Extreme Meteorological Phenomena Recorded in the First 12 Years of the 21st Century in Romania

Montllor CB, Maxmen A, Purcell AH (2002) Facultative bacterial endosymbionts benefit pea aphids Acyrthosiphon pisum under heat stress. Ecol Entomol 27:189–195. https://doi.org/10.1046/j.1365-2311.2002.00393.x

Nováková E, Hypša V, Klein J, Foottit RG, von Dohlen CD, Moran NA (2013) Reconstructing the phylogeny of aphids (Hemiptera: Aphididae) using DNA of the obligate symbiont Buchnera aphidicola. Mol Phylogenet Evol 68:42–54. https://doi.org/10.1016/j.ympev.2013.03.016

Oliver KM, Russell JA, Moran NA, Hunter MS (2003) Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. Proc Natl Acad Sci U S A 100:1803–1807. https://doi.org/10.1073/pnas.0335320100

Oliver KM, Moran NA, Hunter MS (2005) Variation in resistance to parasitism in aphids is due to symbionts not host genotype. PNAS 102:12795–12800. https://doi.org/10.1073/pnas.0506131102

Ou D, Qiu J-H, Su Z-Q, Wang L, Qiu B-L (2023) The phylogeny and distribution of Wolbachia in two pathogen vector insects, Asian citrus psyllid and Longan psyllid. Front Cell Infect Microbiol 13. https://doi.org/10.3389/fcimb.2023.1121186

Pons I, Scieur N, Dhondt L, Renard M-E, Renoz F, Hance T (2022) Pervasiveness of the symbiont Serratia symbiotica in the aphid natural environment: distribution, diversity and evolution at a multitrophic level. FEMS Microbiol Ecol 98:fiac012. https://doi.org/10.1093/femsec/fiac012

Ramírez-Cáceres GE, Moya-Hernández MG, Quilodrán M, Nespolo RF, Ceballos R, Villagra CA, Ramírez CC (2019) Harbouring the secondary endosymbiont regiella insecticola increases predation risk and reproduction in the cereal aphid Sitobion avenae. J Pest Sci 92:1039–1047. https://doi.org/10.1007/s10340-019-01090-z

Renoz F, Pons I, Vanderpoorten A, Bataille G, Noël C, Foray V, Pierson V, Hance T (2019) Evidence for Gut-Associated Serratia symbiotica in wild aphids and ants provides new perspectives on the evolution of bacterial mutualism in insects. Microb Ecol 78:159–169. https://doi.org/10.1007/s00248-018-1265-2

Shigenobu S, Watanabe H, Hattori M, Sakaki Y, Ishikawa H (2000) Genome sequence of the endocellular bacterial symbiont of aphids Buchnera Sp. APS Nat 407:81–86. https://doi.org/10.1038/35024074

Șimon A, Moraru PI, Ceclan A, Russu F, Chețan F, Bărdaș M, Popa A, Rusu T, Pop AI, Bogdan I (2023) The impact of Climatic factors on the development stages of maize crop in the Transylvanian plain. Agronomy 13:1612. https://doi.org/10.3390/agronomy13061612

Skaljac M, Vogel H, Wielsch N, Mihajlovic S, Vilcinskas A (2019) Transmission of a Protease-Secreting bacterial symbiont among pea aphids via host plants. Front Physiol 10. https://doi.org/10.3389/fphys.2019.00438

Sleigh JD (1983) Antibiotic resistance in Serratia marcescens. Br Med J (Clin Res Ed) 287:1651–1652

Subedi B, Poudel A, Aryal S (2023) The impact of climate change on insect pest biology and ecology: implications for pest management strategies, crop production, and food security. J Agric Food Res 14:100733. https://doi.org/10.1016/j.jafr.2023.100733

Sunil V, Majeed W, Chowdhury S, Riaz A, Shakoori FR, Tahir M, Dubey VK (2023) Insect population dynamics and climate change, in: climate change and insect biodiversity. CRC

Tsuchida T, Koga R, Horikawa M, Tsunoda T, Maoka T, Matsumoto S, Simon J-C, Fukatsu T (2010) Symbiotic bacterium modifies aphid body color. Science 330:1102–1104. https://doi.org/10.1126/science.1195463

Van Emden HF, Harrington R (eds) (2017) Aphids as crop pests, Second edition. ed. CABI, Wallingford, Oxfordshire, UK; Boston, MA

Vázquez-Baeza Y, Pirrung M, Gonzalez A, Knight R (2013) EMPeror: a tool for visualizing high-throughput microbial community data. GigaScience 2:2047–217X. https://doi.org/10.1186/2047-217X-2-16

Wang L, Hui C, Sandhu HS, Li Z, Zhao Z (2015) Population dynamics and associated factors of cereal aphids and armyworms under global change. Sci Rep 5:18801. https://doi.org/10.1038/srep18801

Wegierek P, Michalik A, Wieczorek K, Kanturski M, Kobiałka M, Śliwa K, Szklarzewicz T (2017) Buchnera aphidicola of the Birch blister aphid, Hamamelistes betulinus (Horváth, 1896) (Insecta, hemiptera, Aphididae: Hormaphidinae): molecular characterization, transmission between generations and its geographic significance. Acta Zool 98:412–421. https://doi.org/10.1111/azo.12186

Wu Y, Li J, Liu H, Qiao G, Huang X (2020) Investigating the impact of climate warming on phenology of aphid pests in China using Long-Term historical data. Insects 11:167. https://doi.org/10.3390/insects11030167

Xu S, Chen J, Qin M, Jiang L, Qiao G (2021) Geography-dependent symbiont communities in two oligophagous aphid species. FEMS Microbiol Ecol 97. https://doi.org/10.1093/femsec/fiab132

Zepeda-Paulo F, Lavandero B (2021) Effect of the Genotypic Variation of an Aphid Host on the Endosymbiont Associations in Natural Host Populations. Insects 12, 217. https://doi.org/10.3390/insects12030217

Zhang B, Leonard SP, Li Y, Moran NA (2019) Obligate bacterial endosymbionts limit thermal tolerance of insect host species. PNAS 116:24712–24718. https://doi.org/10.1073/pnas.1915307116

Zytynska SE, Weisser WW (2016) The natural occurrence of secondary bacterial symbionts in aphids. Ecol Entomol 41:13–26. https://doi.org/10.1111/een.12281