

Order of arrival promotes coexistence via spatial niche preemption by the weak competitor

Inês Fragata¹, Raul Costa-Pereira², Agnieszka Majer³, Oscar Godoy^{4*}, Sara Magalhães^{1*}

1 – Centre for Ecology, Evolution and Environmental Changes; Department of Animal Biology, Faculty of Sciences, University of Lisbon, Portugal

2 – Department of Animal Biology, Institute of Biology, Universidade Estadual de Campinas, Brasil ORCID 0000-0003-2370-5866

3 – Population Ecology Lab, Faculty of Biology, Adam Mickiewicz University, Poland ORCID 0000-0002-2425-7885

4 – Department of Biology, Instituto Universitario de Investigación Marina (INMAR), Universidad de Cádiz, Puerto Real. 11510, Spain. ORCID 0000-0003-4988-6626

*Co-last authorship

Running title: Niche preemption facilitates coexistence

Keywords: Order of arrival, Priority effects, Modern coexistence theory, Spatial segregation, *Tetranychus urticae* and *T. evansi*, Herbivores, Niche modification, Competitive ability

Article type: Letter

Number of words in abstract: 150

26 **Number of words main text:** 5006

27

28 **Number of references:** 66

29

30 **Number of figures:** 3

31

32 **Corresponding Author:** Inês Fragata, irfragata@fc.ul.pt, cE3c, Centre for Ecology, Evolution
33 and Environmental changes, Faculdade de Ciências da Universidade de Lisboa, Edifício C2,
34 Campo Grande 1749-016 Lisbon, Portugal, Phone. +351 217500577, ext. 22311.

35

36 **Authorship statement:** IF, OG and SM designed the experiment, IF, AM and RCP collected
37 data, IF and OG performed modelling work and analysed data. IF and SM wrote the first
38 draft of the manuscript, and all authors contributed substantially to revisions.

39

40 **Data accessibility statement:** Data and scripts for data analyses will be deposited in a public
41 repository upon acceptance.

42

43

Abstract

Historical contingency, such as the order of species arrival, can modify competitive outcomes via niche modification or preemption. However, how these mechanisms ultimately modify stabilising niche and average fitness differences remains largely unknown. By experimentally assembling two congeneric spider mite species feeding on tomato plants during two generations, we show that order of arrival interacts with species' competitive ability to determine competitive outcomes. Contrary to expectations, we did not observe that order of arrival cause priority effects. In fact, coexistence was predicted when the inferior competitor (*Tetranychus urticae*) arrived first. In that case, *T. urticae* colonized the preferred feeding stratum (leaves) of *T. evansi* leading to spatial niche preemption, which equalized fitness but also increased niche differences, driving community assembly to a close-to-neutrality scenario. Our study demonstrates how the spatial context of competitive interactions interact with species competitive ability to influence the effect of order of arrival on species coexistence.

Introduction

Priority effects are broadly defined as the process by which historical contingencies in community assembly (e.g. order and/or timing of arrival) change the outcome of interspecific interactions (Chase 2003; Fukami 2015). Priority effects can be inhibitory if earlier arrival by one species inhibits the growth of the species arriving subsequently (Chase 2003; Fukami 2015; Ke & Letten 2018). Such effects are expected to result in alternative stable states and hamper coexistence (Fukami 2015; Ke & Letten 2018). In turn, facilitative priority effects occur when population growth is higher when individuals arrive after the settlement of a first species. Facilitative effects are expected to foster coexistence (Fukami 2015), but they have been less often identified in natural communities (Queijeiro-Bolaños *et al.* 2017; Clay *et al.* 2019; Halliday *et al.* 2020).

Two major mechanisms are predicted to cause priority effects: niche preemption, in which early colonizers reduce the amount of resource available to late colonizers, and niche modification, in which the species arriving first modifies the environment, thereby inhibiting or facilitating later colonization (Kardol *et al.* 2013; Vannette & Fukami 2014; Fukami 2015; Delory *et al.* 2019, 2021; Grainger *et al.* 2019). For example, niche preemption in plant communities was found to be strong in environments with high nutrient supply, as early arriving plants grew quickly and prevented growth of later colonizers by depleting space and light (Kardol *et al.* 2013). Also, previous colonization by different plant communities modified the soil metabolome and inhibited population growth of forb, but not grass species, via decreased root foraging (Delory *et al.* 2021). Although distinguishing among niche preemption and modification is not always possible (Grainger *et al.* 2018; Boyle *et al.* 2021), recent advances from coexistence theory can serve as a powerful approach to better understand the importance of historical contingencies for species coexistence. Yet the combination of these theoretical tools has seldom been applied in empirical settings.

Modern coexistence theory posits that the long-term persistence of competing species (i.e., species coexistence) can be attained by two non-mutually exclusive mechanisms: (i) equalizing mechanisms that reduce average fitness differences, and therefore, dominance between species and (ii) stabilising mechanisms, which tend to stabilise the interaction between competitors by increasing the strength of intraspecific competition relative to interspecific competition (Chesson 2000). Therefore, species will stably coexist if the stabilising niche differences, leading to negative frequency dependence, are larger than differences in fitness between competitors. Otherwise the species with higher fitness will eventually dominate the community (Chesson 2000; Barabás *et al.* 2018; Spaak & De Laender 2021). Under this framework, priority effects are strictly defined as the case in which the population dynamics of interacting species are governed by positive frequency dependence (i.e., via negative niche differences), which leads to the dominance of the early-arriving species (Ke & Letten 2018; Grainger *et al.* 2019; Spaak & De Laender 2021). Hence, species cannot coexist locally unless there is spatial variability in the order of arrival. However, despite the fact that recent theory offers predictions on the outcome of coexistence in systems with historical contingencies, empirical tests are conspicuously lacking (but see Cardinaux *et al.* 2018; Grainger *et al.* 2019; Song *et al.* 2020). Therefore, there is as yet scarce knowledge of which species traits interact with historical contingencies to determine outcomes of interspecific interactions.

For the herbivore communities, such traits can be the spatial distribution of consumers associated with resource use. Indeed, niche preemption may arise in this system, as herbivores generally have preferred plant strata and the first arriving species may monopolize that resource (Grainger *et al.* 2018; Godinho *et al.* 2020a). Moreover, herbivores often induce defences on the plants they colonize, which is expected to entail niche modification for species arriving later (Erb *et al.* 2011; Moreira *et al.* 2015; Stam *et al.* 2017). Additionally, some other herbivore species are known to down-regulate plant defences, improving the performance of later

colonizers (Sarmiento *et al.* 2011a; Godinho *et al.* 2016), thereby potentially causing facilitative priority effects. Overall, given the spatial heterogeneity of the environment that herbivores experience (e.g., variation in leaf quality within and between plants), effects of the order of arrival on species coexistence are expected to be prevalent in herbivore communities (Utsumi *et al.* 2010; Erb *et al.* 2011; Moreira *et al.* 2015; Stam *et al.* 2017, 2018; Godinho *et al.* 2020a). But what type of competitive outcome we should expect is unclear. Order of arrival has been a traditional link to priority effects, yet the interaction of the chronology of community assembly with the type of impact of species have on the environment (e.g. where they growth and how they modify the habitat) can result in diverse outcomes, from competitive exclusion to species coexistence. Applying the modern coexistence framework to this open question can shed light on the proximate mechanisms that allow for species to coexist under varied historical contingencies.

Here shed new light on the drivers of competitive outcomes by combining theoretical and empirical tools to experimentally investigate the mechanisms through which order of arrival affects species coexistence. We use as a model system, the two closely-related competing herbivorous species, the spider mites *Tetranychus urticae* and *T. evansi* (Alzate *et al.* 2020). *Tetranychus evansi* generally outcompetes *T. urticae* on tomato plants (Sarmiento *et al.* 2011b; Orsucci *et al.* 2017; Alzate *et al.* 2020), although both species are also commonly observed to co-occur on the same location (Ferragut *et al.* 2013). Niche modification is expected to be at play in this system, because the two species interact with plant defences, albeit differently: *T. urticae* induces tomato defences, whereas *T. evansi* suppresses them (Sarmiento *et al.* 2011a; Alba *et al.* 2015). This asymmetrical niche modification is predicted to facilitate coexistence by hampering growth of the stronger competitor and favouring growth of the inferior one, when they arrive on plants colonized by the other species. Moreover, niche preemption may occur, as both *T. evansi* and *T. urticae* prefer the upper, more nutritious leaves of tomato plants, where

their performance is higher (Godinho *et al.* 2020a). Thus, early-arriving species could occupy the preferred niche and displace the other species to lower, less optimal, plant strata. We tested this, by performing a series of multi-generational experiments where we varied order of arrival and measured space use by the two competing species. We then applied modern coexistence theory framework to unravel the conditions favouring coexistence or potentially leading to priority effects.

Material and Methods

Model system, species characteristics, and maintenance of experimental populations

Tetranychus urticae is a generalist herbivore that feeds on many economically important crops (Helle & Sabelis 1985; Grbić *et al.* 2011; Sousa *et al.* 2019), whereas *T. evansi* is a solanaceous specialist that has recently invaded Europe from South America (Boubou *et al.* 2012). Both species colonize tomato plants, although *T. urticae* may shift to other hosts if *T. evansi* is present (Ferragut *et al.* 2013).

All experiments were performed with outbred populations of *T. urticae* and *T. evansi* spider mites, formed via controlled crosses among four *T. evansi* and three *T. urticae* populations collected in different locations in Portugal (Godinho *et al.* 2020b). Before the experiment, spider mite populations were maintained in boxes containing leaves detached from five-week-old tomato plants (*Solanum lycopersicum*, var MoneyMaker), with their petiole in a small pot containing water, under controlled conditions (25 °C, 68% of humidity, photoperiod of 16 hours light/8 hours dark). Twice a week, overexploited leaves were removed, and water and new tomato leaves were added. Before infestation, tomato plants were kept in a climatic chamber at 25°C, with a photoperiod of 16 hours light/8 hours dark with 75% humidity and watered three times per week.

To ensure that females used in the experiments were of similar age, we created cohorts of mated *T. urticae* and *T. evansi* females for each block. To this aim, females were placed during 48h in petri dishes (14.5 cm diameter) with a layer of wet cotton and two freshly cut tomato leaves. One week later, another tomato leaf was added. Petri dishes were watered twice per week. In the experiment, we used females with 13-15 days of age.

Theoretical approach for predicting competitive outcomes: quantifying niche and fitness differences

Data collected in the experiments were used to parameterize a mathematical model from which niche and average fitness differences can be quantified as well as the predictions of competitive outcomes. We assume that the population dynamics in our experiment can be described by a Beverton-Holt function (Hart *et al.* 2018):

$$(1) N_{i,t+1} = \frac{\lambda_i * N_{i,t}}{(1 + \alpha_{ii} * N_{i,t} + \alpha_{ij} * N_{j,t})}$$

Where $N_{i,t+1}$ is the number of individuals of species i in the next generation, λ_i the growth rate of species i in absence of competitors, α_{ii} the intraspecific competitive ability, α_{ij} the interspecific competitive ability and $N_{i,t}$, $N_{j,t}$ the number of individuals of species i and j in the current generation, respectively. In this model, we assume that spider mites do not have a dormant stage. This means that λ_i represents only the fraction of eggs that hatch and become female individuals that grow and reproduce in the next generation.

From this model, niche overlap (ρ) is defined as follows (see details in Chesson 2012; Godoy & Levine 2014).

$$(2) \rho = \sqrt{\frac{\alpha_{ij} \alpha_{ji}}{\alpha_{jj} \alpha_{ii}}}$$

This formula reflects the average degree to which species limit individuals of their own species relative to heterospecific competitors. If species limit individuals of their own species more strongly than competitors (α_{jj} , α_{ii} , are much greater than α_{ij} , α_{ji}), then niche overlap will be low, favouring coexistence. Alternatively, if species limit individuals of their own species and those of their competitor equally, niche overlap equals one, hampering stable coexistence. With ρ defining niche overlap between a pair of species, stabilising niche differences are expressed as $1-\rho$.

Average fitness differences ($\frac{\kappa_j}{\kappa_i}$) (Chesson 2012; Godoy & Levine 2014) are defined as:

$$(3) \quad \frac{\kappa_j}{\kappa_i} = \frac{\lambda_j - 1}{\lambda_i - 1} * \frac{\sqrt{\alpha_{ij} * \alpha_{ii}}}{\sqrt{\alpha_{ji} * \alpha_{jj}}}$$

The greater the ratio, ($\frac{\kappa_j}{\kappa_i}$), the greater the fitness advantage of species j over i . If this ratio is one, species are equivalent competitors. With niche overlap and average fitness defined in eqn. 2 and 3 respectively, we can determine the coexistence condition. Specifically, coexistence requires both species to invade when rare (Chesson 2012), which is satisfied when (Godoy & Levine 2014):

$$(4) \quad \rho < \frac{\kappa_j}{\kappa_i} < \frac{1}{\rho}$$

Stable coexistence is possible whenever species have either large niche differences (corresponding to small niche overlap) that overcome large average fitness differences, or at the other extreme, via an a close-to-neutral scenario (Scheffer *et al.* 2018), where, even with weak niche differences, small fitness differences stabilise the interaction between competitors. If competitors are not predicted to coexist, we can pinpoint if this is due to competitive exclusion (when fitness differences are larger than niche differences) or due to priority effects, leading to alternative states when niche differences are negative (Fukami & Nakajima 2011;

Ke & Letten 2018). We used data from the fully parameterized models (see below) to generate these predictions.

Experiments

We performed a series of experiments in which we either manipulated the order of arrival and relative frequency (i.e., relative initial abundance with a constant density of 20 individuals), or the initial density of each of the two species.

In the first experiment, both species were introduced simultaneously using the following proportions of *T. evansi* / *T. urticae*: 1:19; 10:10 and 19:1, along with the single-species controls (20:0 and 0:20). To manipulate the order of arrival, we introduced (i) 10 *T. evansi* females 48h before 10 *T. urticae* females and vice versa and (ii) 19 *T. evansi* females 48h before 1 *T. urticae* female and vice versa. The experiment was done in two blocks, one week apart. Each block contained five boxes of each experimental treatment (nine treatments, with ten boxes per treatment), each with a pot filled with water and two freshly cut tomato leaves from five-week-old tomato plants. Leaf pairs consisted of leaves 2 and 4 or 3 and 5 (leaf number indicates leaf age: leaves 2 and 5 correspond to the oldest and youngest leaves, respectively). This was done to ensure that each box contained a younger and an older leaf, since both species have a preference for younger (upper) leaves (Godinho *et al.* 2020a). Adult females were distributed by the two leaves, following the treatments described above. After one generation (circa 14 days), two more leaves were added to ensure enough resources for the second mite generation. Boxes that initially received the leaf pair 2-4, received leaves 3-5 and vice versa. After two generations, we counted the number of adult females of each species per leaflet and leaf.

In the second experiment we also estimated the growth rate of each species by counting the number of adult females obtained from the progeny of a single *T. urticae* or *T. evansi* female

ovipositing for 48h in two overlapping 18mm leaf disks, replicated 18 times. These disks were placed in square petri dishes with a layer of wet cotton and were watered every two days. The number of adult females produced was assessed after one generation.

Data Analyses

Effect of order of arrival and initial frequency on species abundance

To test the impact of order of arrival and frequency and their interaction on the proportion of adult females of each species after two generations, we performed the following general linear mixed model (lme4 package, Bates et al. 2015), using the binomial family:

$$(5) Y = \text{Frequency} + \text{Order} + \text{Frequency} \times \text{Order} + \text{Block} + \varepsilon$$

Where Y corresponds to the combination of two vectors with the number of *T. evansi* and *T. urticae* females after two generations, Frequency (fixed factor) to the initial ratio between the two species, Order (fixed factor) to the order of arrival, Block (random factor) to whether the experiment was performed on week one or two, and ε to the residual error. Additionally, we ran the same model as above, but merging Frequency, Order and their interaction in a single factor (Treatment, 7 levels). This allowed performing *a priori* contrasts to compare between different orders of arrival and frequencies for each species, since our experimental design was not orthogonal. To compare the effect of different orders of arrival, we performed contrasts between the treatments with same initial frequency but different orders of arrival. To compare the effect of frequency, we performed contrasts between treatments with same order of arrival but different initial frequencies. Contrasts were done using testInteractions fromphia package (Rosario-Martinez 2015) and were then corrected for multiple comparisons using FDR correction (Benjamini & Yekutieli 2001).

Effects of order of arrival on coexistence

We used a maximum likelihood approach to estimate the growth rate in absence of competitors (λ) and the intra and interspecific competitive interactions (the α 's) for each species. To ensure model convergence, we took a nested perspective in which the results of the first model were used as priors for the subsequent models which increased in complexity (Matías et al. 2018). That is, estimates obtained from model 6A were used as priors for model 6B, and those obtained from model 6B were used as priors for model 6C.

$$6A) N_{t+1} = \lambda * N_t$$

$$6B) N_{i,t+1} = \frac{\lambda * N_t}{(1 + \alpha * N_t)}$$

$$6C) N_{i,t+1} = \frac{\lambda_i * N_{i,t}}{(1 + \alpha_{ii} * N_{i,t} + \alpha_{ij} * N_{j,t})}$$

The initial model (6A) considers only the intrinsic growth rate in the absence of interactions (λ). This model was parameterized using estimates from the experiment with single *T. urticae* or *T. evansi* female. Model 6B adds an α parameter, which accounts for the overall effect of competition, and finally, model 6C separates this overall competitive effect into intra and interspecific components.

Effect of order or arrival and initial frequency on leaf occupancy and aggregation

To test if coexistence vs. exclusion outcomes could be explained by niche preemption due to changes in leaf occupancies, we compared occupancy patterns of each species across the four leaves. For the single species treatment, we tested if the number of females differed across leaves (model 7). For the double species treatment, we tested if the order of arrival or initial frequency, or their interaction changed mite distribution across leaves (model 8). For that, we compared the number of females in each leaf to the distribution of the single species treatment.

We applied the following binomial models, with Leaf and/or Treatment and their interaction as fixed factors, for the control (model 7) and experimental (model 8) treatments:

$$(7) Y = \text{Leaf} + \varepsilon$$

$$(8) Y = \text{Leaf} + \text{Treatment} + \text{Leaf} \times \text{Treatment} + \varepsilon$$

where Y corresponds to the combination of two vectors with the number of *T. evansi* (or *T. urticae*) females on each leaf per box and the total number of individuals on each box that were not on that leaf. For the double treatment, a posteriori contrasts were done between each treatment and the corresponding single species treatment. The initial fitting with Block as a random factor, indicated no variance in this factor, thus we fitted only fixed factors.

Since *T. evansi* suppresses defences locally, it is expected that *T. urticae* aggregates with it (Sato *et al.* 2016). To test if aggregation changed with order of arrival or initial frequency, we calculated the Checkerboard score (C-score) (Gotelli & Rhode 2002) per replicate. The C-score quantifies species co-occurrence, measuring the extent to which they segregate or aggregate across environments (Gotelli & Rhode 2002). The bipartite package (Dormann *et al.* 2008) normalizes the C-score between 0 (no aggregation) and 1 (aggregation), allowing comparisons between treatments. To calculate the C-score per leaf, we created a presence-absence matrix per leaflet and leaf for each box. We then applied the following general linear mixed model to test for differences in aggregation between treatments:

$$(9) Y = \text{Treatment} + \text{Block} + \varepsilon$$

Where Y is the computed C-score and Treatment is a fixed factor. Contrasts were performed between initial frequency and order of arrival, applying FDR correction for multiple comparisons, as described above.

All analyses were done using R (R Core Team 2021) using package “cxr” (García-Callejas *et al.* 2020), and plots were done using ggplot2 (Wickham 2016) and cowplot (Wilke 2020) packages. Data and scripts are available in the github repository: https://github.com/irfragata/priority_effects.

Results

Effect of order of arrival and initial frequency on species abundance

The number of individuals of each species on tomato plants were affected by the order of arrival ($\chi^2 = 298.93$, $df = 2$, $p\text{-value} < 0.0001$), their initial frequency ($\chi^2 = 568.12$, $df = 2$, $p\text{-value} < 0.0001$), and the interaction among these factors ($\chi^2 = 188.61$, $df = 2$, $p\text{-value} < 0.0001$). Specifically, the abundance of *T. evansi* females after two generations was higher when this species arrived first or simultaneously with *T. urticae*, independently of initial frequencies. However, the additional advantage provided by arriving first was much larger in the equal frequency treatment, as contrasts comparing order of arrival were highly significant for equal frequencies (10:10), but not for the high *T. evansi* frequency (19:1) (Table S1, Fig.1). The abundance of *T. urticae* after two generations was also affected by initial frequency and order of arrival. Indeed, the final number of *T. urticae* females was higher when this species arrived first and was at high initial frequency, than in the equal frequency treatment (Table S1, Fig. 1). Overall, these results confirm that *T. evansi* is a superior competitor as expected from previous field and laboratory observations (Sarmiento *et al.* 2011b; Ferragut *et al.* 2013; Alzate *et al.* 2020).

Effect of order of arrival on coexistence

The order of arrival modified the outcome of competition between the two species. *Tetranychus evansi* (the superior competitor) excluded *T. urticae* when it arrived first or at the same time. Under this exclusion scenario, the rate of competitive exclusion is expected to be faster when *T. evansi* arrived first due to an increase in fitness differences and due to a decrease in niche differences (Fig 2). Coexistence was only possible when *T. urticae* arrived first (Fig. 2). This outcome was due to small fitness and niche differences among competitors, leading to a quasi-neutral scenario. Specifically, when *T. urticae* arrived first, we observe almost similar

strengths of intra- and interspecific interactions among species (Fig S1A), combined with an increase in the intrinsic growth rate of *T. urticae* (in comparison to other orders of arrival, Fig S1B). Contrary to expectations and previous studies, order of arrival was not associated with priority effects.

Effect of order of arrival and initial frequency on leaf occupancy and aggregation

To explore whether changes in coexistence outcomes were driven by a shift in leaf occupancy, we tested how initial frequency and order of arrival affected the proportion of females of the two species on each leaf (Fig 3 A, C, Fig S2) vs. the occupancy in the single species treatments (Fig 3B, D). When *T. evansi* was alone, it reached a consistently higher abundances on leaves 3 and 4 (Table S2A, Fig 3B), whereas *T. urticae* was less abundant on leaf 2 (the oldest leaf) in comparison to all others (Table S2A, Fig 3D). In treatments where *T. urticae* arrived first, significantly fewer *T. evansi* females were found on leaf 4 (Fig 3, Fig S2A, Table S2B). This pattern was observed for leaf 3 when *T. urticae* started with higher frequency and both species arrived at the same time. When *T. evansi* arrived first or started at higher frequency, we observed fewer changes on its own leaf occupancy (Fig S2A). The distribution of *T. urticae* showed a slight shift when it arrived first, with a reduction on the prevalence of leaves 2 and 5 and slightly higher occupation of leaves 3 and 4 (Fig. S2B, Table S2B). When *T. evansi* started at high frequency, there was also a shift in *T. urticae* distribution, with a lower occupancy of leaves 2 and 5 (Fig S2B).

Spatial aggregation significantly differed among treatments ($\chi^2 = 18.186$, $df = 6$, $p\text{-value} = 0.01279$), being higher in treatments with similar initial densities (cf. Fig S3 with Fig 1, Table S3). We observed a significant difference in C-score (the degree of spatial aggregation) with higher aggregation when both species arrived at the same time and had equal frequency, and a

lower aggregation when both species arrived at the same time and *T. evansi* started at higher frequency (Table S3). Order of arrival did not change the C-score (Fig. S3, Table S3).

Discussion

This study shows that order of arrival interacts with the competitive ability to determine the probability of coexistence between congeneric species that share common resources such as food and/or space. When both species arrive at the same time or the superior competitor (*Tetranychus evansi*) arrived first, *T. urticae* was predicted to be excluded. Coexistence was only predicted when the inferior competitor (*T. urticae*) was the first species colonizing the habitat. Analyses of leaf occupancy show that these competition outcomes are linked to a spatial niche preemption process in which *T. evansi* was displaced from its preferred food stratum when *T. urticae* arrived first. As a result of this complex interaction between order of arrival, species competitive ability, and spatial occupancy, we observed a particular configuration that allows species coexistence: both species increased niche differences and reduced fitness differences to the extent that they can coexist despite small niche differences. These multiple lines of evidence challenge current paradigms on the direct connection between order of arrival and priority effects.

We found that *T. evansi* had higher competitive ability and growth rate, and often excluded *T. urticae* (Fig 1, 2). This is in line with laboratory observations showing that *T. evansi* outcompeted *T. urticae* on tomato plants (Sarmiento *et al.* 2011b; Alzate *et al.* 2020, but see Orsucci *et al.* 2017) and with field observations showing a reduction in the prevalence and a shift in host use in *T. urticae* upon invasion by *T. evansi* (Ferragut *et al.* (2013). Still, these two species can co-occur in the field in the same plant species (Ferragut *et al.* 2013; Orsucci *et al.* 2017; Zélé *et al.* 2018). The advantage created by the earlier arrival of *T. urticae*, and associated reduction in interspecific competition by *T. evansi*, could be one of the possible mechanisms

fostering coexistence of the two species in nature. Indeed, *T. urticae* can withstand colder temperatures than *T. evansi* (Gotoh *et al.* 2010; Khodayari *et al.* 2013; Riahi *et al.* 2013; White *et al.* 2018), hence it is expected to arrive first in the season. Field surveys that sample both species in the same location across seasons are needed to further explore this hypothesis.

Historical contingencies emerging from order of arrival can happen through two main mechanisms: niche modification or niche preemption (Fukami 2015). In our system, niche modification may arise via interactions between spider mites and plant defences. As *T. evansi* suppresses plant defences, *T. urticae* could benefit from an early colonization from its competitor (Alba *et al.* 2015; de Oliveira *et al.* 2016, 2017; Godinho *et al.* 2020a). However, we observe competitive exclusion when *T. evansi* arrives first, suggesting that if *T. urticae* gained this benefit, it was not enough to outcompete *T. evansi*. In turn, niche preemption can occur through monopolization of nutrients or space, which can be particularly important in intraguild competitive interactions (Grainger *et al.* 2018; Holditch & Smith 2020). In this study, resource depletion could not explain the differences we observe in competitive outcomes, since order of arrival did not have a large impact on the growth rate of both species. However, we observed a shift in the leaf occupancy pattern of *T. evansi* females when *T. urticae* arrived first. This displacement of *T. evansi* from the preferred food stratum (i.e., younger, more nutritious leaves) by early-arriving *T. urticae* can explain the decreased competitive ability of the superior competitor. Thus, our results indicate that variation in species performance driven by habitat quality heterogeneity (Orians *et al.* 2000; Orians & Jones 2001) combines with order of arrival to generate niche preemption, providing a mechanism for the two herbivores to coexist.

Order of arrival is a major determinant of community assembly across diverse taxa, from microbes to plants (Chase 2003; Erb *et al.* 2011; Kardol *et al.* 2013; Stam *et al.* 2017; Grainger *et al.* 2018, 2019; Clay *et al.* 2019, 2020; Halliday *et al.* 2020). Most of these studies show that early colonizers inhibit growth and decrease performance of late arriving species, especially in

those that occupy very similar niches (Fargione *et al.* 2003; Vannette & Fukami 2014; Delory *et al.* 2019, 2021; Grainger *et al.* 2019), although very few concern herbivorous species competing for the same niche (e.g. Grainger *et al.* 2018; Holditch & Smith 2020). Nevertheless, other studies show that order of arrival does not always affect community assembly (e.g. Delory *et al.* 2021) or that initial colonizers may facilitate later colonization of other species (e.g. Queijeiro-Bolaños *et al.* 2017; Delory *et al.* 2019). Here, we show that coexistence is promoted by early colonization by the inferior competitor species, which reduces the competitive ability of the superior competitor and increases the fitness of the inferior competitor. Through fully parameterized models from modern coexistence theory, we found that niche preemption increases niche differences and strongly reduces fitness differences, leading to an equalising effect that allows species to coexist. Our study adds a novel perspective to the growing body of evidence that historical contingencies shape ecological communities, by showing that the probability of coexistence of two competing herbivores changes due to an interaction between order of arrival and species competitive ability.

Priority effects were recently incorporated into modern coexistence theory (Ke & Letten 2018; Spaak & De Laender 2021), but to our knowledge, this is one of the very few studies that empirically tested the impact of changes in order of arrival on species coexistence so far, using this theoretical framework. Grainger *et al.* (2019) observed that positive frequency dependence, due to strong priority effects, arose from changes in order or arrival in yeast species feeding on floral nectars. In contrast, our results show that order of arrival did not lead to priority effects caused by positive frequency dependent alternative states. Instead, when *T. evansi* arrived first, it excluded *T. urticae* due to a large increase in fitness differences and a reduction in stabilising niche differences. However, when *T. urticae*, arrived first, coexistence was predicted, via an increase in niche differences and a decrease in fitness differences, making the competition between the two species more neutral. These results suggests that, in the event

of a small environmental perturbation that produces changes in niche or fitness differences, the two studied species might not be able to coexist, compared to a scenario in which species coexist via strong niche differences. Framing priority effects in the modern coexistence theory (Ke & Letten 2018) is undoubtedly an important step to mechanistically understand how order of arrival affects community assembly processes. However, here we show that order of arrival does not always affect competitive outcomes via priority effects; instead, it can lead to coexistence via niche preemption by the inferior competitor. Thus, our results show that order of arrival can produce a wide range of competitive outcomes from coexistence to competitive exclusion due to positive and negative frequency dependence. Therefore, it is urgent that ecologists widen the scope of the multiple outcomes that historical contingency can produce on species coexistence.

Most empirical and theoretical studies emphasize the inhibitory nature of niche preemption (Fargione *et al.* 2003; Fukami 2015; Vieira *et al.* 2018; Delory *et al.* 2019), with the early arriving species outcompeting the other. However, recent theory suggests that, in a resource competition model of two species, niche preemption by the inferior competitor could facilitate coexistence under a trade-off between order of arrival and the resource levels of zero net growth (R^*) (Qi *et al.* 2021). Our study is, to the best of our knowledge, the first empirical study showing that niche preemption by the weaker competitor facilitates coexistence. This striking change in the outcome of competitive interactions emerge mostly due to a decrease in fitness differences coupled with the decrease in niche overlap. This suggests that even small differences in order of arrival can be sufficient for the monopolization of a resources in plant-herbivore interactions, which may suffice to allow coexistence between competitor species. Therefore, our results demonstrate how small temporal differences percolate into small spatial heterogeneities, fostering coexistence and the maintenance of diversity.

454

455 **Acknowledgments:** This work was financed by an ERC (European Research Council)
456 consolidator grant COMPCON, GA 725419 attributed to SM and by FCT (Fundação para
457 Ciência e Tecnologia) with the Junior researcher contract (CEECIND/02616/2018) attributed
458 to IF. RC-P is supported by grant #2020/11953-2 São Paulo Research Foundation (FAPESP)
459 and grant R-2011-37572 Instituto Serrapilheira. OG acknowledges financial support provided
460 by the Spanish Ministry of Economy and Competitiveness (MINECO) and by the European
461 Social Fund through the Ramón y Cajal Program (RYC-2017-23666). AM was funded by
462 National Science Centre, Poland (grant no. 2018/28/T/NZ8/00060) and Excellence Initiative -
463 Research University programme (support for the internationalization of the Adam Mickiewicz
464 University PhD students, no. 003/13/UAM/0018).

465

466 **Competing interests:** Authors declare no competing interests.

467

468

469

Bibliography

- Alba, J.M., Schimmel, B.C.J., Glas, J.J., Ataide, L.M.S., Pappas, M.L., Villarroel, C.A., *et al.* (2015). Spider mites suppress tomato defenses downstream of jasmonate and salicylate independently of hormonal crosstalk. *New Phytol.*, 205, 828–840.
- Alzate, A., Onstein, R.E., Etienne, R.S. & Bonte, D. (2020). The role of preadaptation, propagule pressure and competition in the colonization of new habitats. *Oikos*, 129, 820–829.
- Barabás, G., D’Andrea, R. & Stump, S.M. (2018). Chesson’s coexistence theory. *Ecol. Monogr.*, 88, 277–303.
- Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.*, 67, 1–48.
- Benjamini, Y. & Yekutieli, D. (2001). The control of the false discovery rate in multiple testing under dependency. *Ann. Stat.*, 29, 1165–1188.
- Boubou, A., Migeon, A., Roderick, G.K., Auger, P., Cornuet, J.M., Magalhães, S., *et al.* (2012). Test of colonisation scenarios reveals complex invasion history of the red tomato spider mite *tetranychus evansi*. *PLoS One*, 7.
- Boyle, J.A., Simonsen, A.K., Frederickson, M.E. & Stinchcombe, J.R. (2021). Priority effects alter interaction outcomes in a legume-rhizobium mutualism. *Proc. R. Soc. B Biol. Sci.*, 288, 1–8.
- Cardinaux, A., Hart, S.P. & Alexander, J.M. (2018). Do soil biota influence the outcome of novel interactions between plant competitors? *J. Ecol.*, 106, 1853–1863.
- Chase, J.M. (2003). Community assembly: When should history matter? *Oecologia*, 136, 489–498.
- Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annu. Rev. Ecol. Syst.*, 31, 343–366.

495 Chesson, P. (2012). Species Competition and Predation. In: *Encyclopedia of Sustainability*
 496 *Science and Technology* (ed. Meyers, R.A.). Springer, New York, pp. 10 061–10 085.
 497 Clay, P.A., Dhir, K., Rudolf, V.H.W. & Duffy, M.A. (2019). Within-host priority effects
 498 systematically alter pathogen coexistence. *Am. Nat.*, 193, 187–199.
 499 Clay, P.A., Duffy, M.A. & Rudolf, V.H.W. (2020). Within-host priority effects and epidemic
 500 timing determine outbreak severity in co-infected populations. *Proc. R. Soc. B Biol. Sci.*,
 501 287.
 502 Delory, B.M., Schempp, H., Spachmann, S.M., Störzer, L., van Dam, N.M., Temperton,
 503 V.M., *et al.* (2021). Soil chemical legacies trigger species-specific and context-
 504 dependent root responses in later arriving plants. *Plant Cell Environ.*, 44, 1215–1230.
 505 Delory, B.M., Weidlich, E.W.A., von Gillhaussen, P. & Temperton, V.M. (2019). When
 506 history matters: The overlooked role of priority effects in grassland overyielding. *Funct.*
 507 *Ecol.*, 33, 2369–2380.
 508 Dormann, C., Gruber, B. & Fruend, J. (2008). Introducing the bipartite Package: Analysing
 509 Ecological Networks. *R news*, 8, 8–11.
 510 Erb, M., Robert, C.A.M., Hibbard, B.E. & Turlings, T.C.J. (2011). Sequence of arrival
 511 determines plant-mediated interactions between herbivores. *J. Ecol.*, 99, 7–15.
 512 Fargione, J., Brown, C.S. & Tilman, D. (2003). Community Assembly and Invasion: An
 513 Experimental Test of Neutral versus Niche Processes. *Proc. Natl. Acad. Sci. U. S. A.*,
 514 100, 8916–8920.
 515 Ferragut, F., Garzón-Luque, E. & Pekas, A. (2013). The invasive spider mite *Tetranychus*
 516 *evansi* (Acari: Tetranychidae) alters community composition and host-plant use of
 517 native relatives. *Exp. Appl. Acarol.*, 60, 321–341.
 518 Fukami, T. (2015). Historical Contingency in Community Assembly: Integrating Niches,
 519 Species Pools, and Priority Effects. *Annu. Rev. Ecol. Evol. Syst.*, 46, 1–23.

520 Fukami, T. & Nakajima, M. (2011). Community assembly: Alternative stable states or
521 alternative transient states? *Ecol. Lett.*, 14, 973–984.

522 García-Callejas, D., Godoy, O. & Bartomeus, I. (2020). cxr: A toolbox for modelling species
523 coexistence in R. *Methods Ecol. Evol.*, 11, 1221–1226.

524 Godinho, D.P., Cruz, M.A., Charlery de la Masselière, M., Teodoro-Paulo, J., Eira, C.,
525 Fragata, I., *et al.* (2020a). Creating outbred and inbred populations in haplodiploids to
526 measure adaptive responses in the laboratory. *Ecol. Evol.*, 10, 7291–7305.

527 Godinho, D.P., Janssen, A., Dias, T., Cruz, C. & Magalhães, S. (2016). Down - regulation of
528 plant defence in a resident spider mite species and its effect upon con - and
529 heterospecifics. *Oecologia*, 180, 161–167.

530 Godinho, D.P., Janssen, A., Li, D., Cruz, C. & Magalhães, S. (2020b). The distribution of
531 herbivores between leaves matches their performance only in the absence of
532 competitors. *Ecol. Evol.*, 10, 8405–8415.

533 Godoy, O. & Levine, J.M. (2014). Phenology effects on invasion success: Insights from
534 coupling field experiments to coexistence theory. *Ecology*, 95, 726–736.

535 Gotelli, N.J. & Rhode, K. (2002). Co-occurrence of ectoparasites of marine fishes a null
536 model analysis.pdf. *Ecol. Lett.*, 5, 86–94.

537 Gotoh, T., Sugimoto, N., Pallini, A., Knapp, M., Hernandez-Suarez, E., Ferragut, F., *et al.*
538 (2010). Reproductive performance of seven strains of the tomato red spider mite
539 *Tetranychus evansi* (Acari: Tetranychidae) at five temperatures. *Exp. Appl. Acarol.*, 52,
540 239–259.

541 Grainger, T.N., Letten, A.D., Gilbert, B. & Fukami, T. (2019). Applying modern coexistence
542 theory to priority effects. *Proc. Natl. Acad. Sci. U. S. A.*, 116, 6205–6210.

543 Grainger, T.N., Rego, A.I. & Gilbert, B. (2018). Temperature-dependent species interactions
544 shape priority effects and the persistence of unequal competitors. *Am. Nat.*, 191, 197–

209.

Grbić, M., Van Leeuwen, T., Clark, R.M., Rombauts, S., Rouzé, P., Grbić, V., *et al.* (2011). The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature*, 479, 487–492.

Halliday, F.W., Penczykowski, R.M., Barrès, B., Eck, J.L., Numminen, E. & Laine, A.L. (2020). Facilitative priority effects drive parasite assembly under coinfection. *Nat. Ecol. Evol.*, 4, 1510–1521.

Hart, S.P., Freckleton, R.P. & Levine, J.M. (2018). How to quantify competitive ability. *J. Ecol.*, 106, 1902–1909.

Helle, W. & Sabelis, M.W. (1985). *Spider mites their biology, natural enemies and control. World Crop Pests*. Elsevier Science Publishers, Amsterdam.

Holditch, Z. & Smith, A.D. (2020). Priority determines *Tribolium* competitive outcome in a food-limited environment. *PLoS One*, 15, 1–14.

Kardol, P., Souza, L. & Classen, A.T. (2013). Resource availability mediates the importance of priority effects in plant community assembly and ecosystem function. *Oikos*, 122, 84–94.

Ke, P.J. & Letten, A.D. (2018). Coexistence theory and the frequency-dependence of priority effects. *Nat. Ecol. Evol.*, 2, 1691–1695.

Khodayari, S., Colinet, H., Moharramipour, S. & Renault, D. (2013). Seasonal changes in the cold hardiness of the two-spotted spider mite females (Acari: Tetranychidae). *Environ. Entomol.*, 42, 1415–1421.

Matías, L., Godoy, O., Gómez-Aparicio, L. & Pérez-Ramos, I.M. (2018). An experimental extreme drought reduces the likelihood of species to coexist despite increasing intransitivity in competitive networks. *J. Ecol.*, 106, 826–837.

Moreira, X., Abdala-Roberts, L., Hernández-Cumplido, J., Cuny, M.A.C., Glauser, G. &

570 Benrey, B. (2015). Specificity of induced defenses, growth, and reproduction in lima
 571 bean (*Phaseolus lunatus*) in response to multispecies herbivory . *Am. J. Bot.*, 102,
 572 1300–1308.

573 de Oliveira, E.F., Pallini, A. & Janssen, A. (2016). Herbivores with similar feeding modes
 574 interact through the induction of different plant responses. *Oecologia*, 180, 1–10.

575 de Oliveira, E.F., Pallini, A. & Janssen, A. (2017). Herbivore performance and plant defense
 576 after sequential attacks by inducing and suppressing herbivores. *Insect Sci.*, 26, 108–
 577 118.

578 Orians, C.M. & Jones, C.G. (2001). Plants as resource mosaics: A functional model for
 579 predicting patterns of within-plant resource heterogeneity to consumers based on
 580 vascular architecture and local environmental variability. *Oikos*, 94, 493–504.

581 Orians, C.M., Pomerleau, J. & Ricco, R. (2000). Vascular architecture generates fine scale
 582 variation in systemic induction of proteinase inhibitors in tomato. *J. Chem. Ecol.*, 26,
 583 471–485.

584 Orsucci, M., Navajas, M. & Fellous, S. (2017). Genotype-specific interactions between
 585 parasitic arthropods. *Heredity (Edinb.)*, 118, 260–265.

586 Qi, M., DeMalach, N., Sun, T. & Zhang, H. (2021). Coexistence under hierarchical resource
 587 exploitation: the role of R*-preemption tradeoff. *bioRxiv*, 1–23.

588 Queijeiro-Bolaños, M.E., González, E.J., Martorell, C. & Cano-Santana, Z. (2017).
 589 Competition and facilitation determine dwarf mistletoe infection dynamics. *J. Ecol.*,
 590 105, 775–785.

591 R Core Team. (2021). R: A language and environment for statistical computing.

592 Riahi, E., Shishehbor, P., Nemati, A.R. & Saeidi, Z. (2013). Temperature effects on
 593 development and life table parameters of *Tetranychus urticae* (Acari: Tetranychidae). *J.*
 594 *Agric. Sci. Technol.*, 15, 661–672.

595 Rosario-Martinez, H. De. (2015). phia: Post-Hoc Interaction Analysis.

596 Sarmento, R.A., Lemos, F., Bleeker, P.M., Schuurink, R.C., Pallini, A., Oliveira, M.G.A., *et*
597 *al.* (2011a). A herbivore that manipulates plant defence. *Ecol. Lett.*, 14, 229–236.

598 Sarmento, R.A., Lemos, F., Dias, C.R., Kikuchi, W.T., Rodrigues, J.C.P., Pallini, A., *et al.*
599 (2011b). A herbivorous mite down-regulates plant defence and produces web to exclude
600 competitors. *PLoS One*, 6, 8–14.

601 Sato, Y., Alba, J.M., Egas, M. & Sabelis, M.W. (2016). The role of web sharing, species
602 recognition and host-plant defence in interspecific competition between two herbivorous
603 mite species. *Exp. Appl. Acarol.*, 70, 261–274.

604 Scheffer, M., Van Nes, E.H. & Vergnon, R. (2018). Toward a unifying theory of biodiversity.
605 *Proc. Natl. Acad. Sci. U. S. A.*, 115, 639–641.

606 Song, C., Rohr, R.P., Vasseur, D. & Saavedra, S. (2020). Disentangling the effects of
607 external perturbations on coexistence and priority effects. *J. Ecol.*, 108, 1677–1689.

608 Sousa, V.C., Zélé, F., Rodrigues, L.R., Godinho, D.P., Charlery de la Masselière, M. &
609 Magalhães, S. (2019). Rapid host-plant adaptation in the herbivorous spider mite
610 *Tetranychus urticae* occurs at low cost. *Curr. Opin. Insect Sci.*, 36, 82–89.

611 Spaak, J.W. & De Laender, F. (2021). Intuitive and broadly applicable definitions of niche
612 and fitness differences. *Ecol. Lett.*, 23, 1117–1128.

613 Stam, J.M., Chrétien, L., Dicke, M. & Poelman, E.H. (2017). Response of *Brassica oleracea*
614 to temporal variation in attack by two herbivores affects preference and performance of
615 a third herbivore. *Ecol. Entomol.*, 42, 803–815.

616 Stam, J.M., Dicke, M. & Poelman, E.H. (2018). Order of herbivore arrival on wild cabbage
617 populations influences subsequent arthropod community development. *Oikos*, 127,
618 1482–1493.

619 Utsumi, S., Ando, Y. & Miki, T. (2010). Linkages among trait-mediated indirect effects: A

- new framework for the indirect interaction web. *Popul. Ecol.*, 52, 485–497.
- Vannette, R.L. & Fukami, T. (2014). Historical contingency in species interactions: Towards niche-based predictions. *Ecol. Lett.*, 17, 115–124.
- Vieira, E.A., Flores, A.A.V. & Dias, G.M. (2018). Persistence and space preemption explain species-specific founder effects on the organization of marine sessile communities. *Ecol. Evol.*, 8, 3430–3442.
- White, N., Bale, J.S. & Hayward, S.A.L. (2018). Life-history changes in the cold tolerance of the two-spot spider mite *Tetranychus urticae*: applications in pest control and establishment risk assessment. *Physiol. Entomol.*, 43, 334–345.
- Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*.
- Wilke, C. (2020). *cowplot: Streamlined Plot Theme and Plot Annotations for “ggplot2.”*
- Zélé, F., Santos, I., Olivieri, I., Weill, M., Duron, O. & Magalhães, S. (2018). Endosymbiont diversity and prevalence in herbivorous spider mite populations in South-Western Europe. *FEMS Microbiol. Ecol.*, 94, 1–11.

Figure 1 – Proportion of spider mites *Tetranychus evansi* females (y-axis) depending on initial frequency (number of initial females *T. evansi*: *T. urticae*, x-axis) and order of arrival (same time vs. *T. evansi* or *T. urticae* arriving 48h before its competitor) after two generations. *Tetranychus evansi* is the better competitor overall (ratio above 0.5), unless *T. urticae* arrives first or is at higher initial frequency. A posteriori contrasts show a strong effect of order of arrival in the proportion of females of the two species (Suppl. Table 1B). Initial frequency also impacts the final ratio, with a stronger effect when *T. urticae* arrives first or at the same time than *T. evansi* (Suppl. Table 1B). Boxplots represent median and quartiles of the 10 boxes within treatment.

Figure 2 – Relationship between average fitness differences ($\frac{\kappa_j}{\kappa_i}$, y-axis) and stabilising niche differences ($1 - \rho$, x-axis) for different orders of arrival (*Tetranychus evansi* first – red, same time – blue, *T. urticae* first – yellow). Plotting average fitness differences against niche differences allows mapping different competitive outcomes predicted by modern coexistence theory (Chesson 2000; Ke & Letten 2018; Spaak & De Laender 2021). The coexistence condition (eq. 4) and its inverse, represented by the two solid black lines, allow defining the space in which species can coexist due to negative frequency dependence or enter alternative stable states due to positive frequency dependence, whenever niche differences are greater or smaller than zero respectively. Otherwise, the species with higher fitness will exclude the other. In our case, the only scenario in which species are predicted to coexist is when *T. urticae* arrives first (yellow). Error bars for each outcome indicate the 95% confidence interval from the maximum likelihood estimates. For the other two cases, it is predicted that the superior competitor *T. evansi* will exclude *T. urticae*.

Figure 3 – Differences between expected and observed leaf occupancy for *Tetranychus evansi* (A) and *T. urticae* (C) for a subset of the experimental treatments (when *T. urticae* arrived first or at the same time as *T. evansi*, note that Figure S2 includes all treatments); leaf occupancy for *T. evansi* (B) and *T. urticae* (D) in the control, single species, treatments. Leaf 2 corresponds to the oldest leaf and leaf 5 to the youngest. For each box, we calculated the ratio of females occupying each leaf in relation to the total number of females present. For the experimental treatments we calculated the difference between this ratio and the average ratio for the control treatments. Thus, positive values indicate that there are more females on that leaf than expected based on the single-species treatment and negative values indicate the reverse pattern. Overall, we see that *T. evansi* reduces occupancy on leaf 4 when *T. urticae* arrives first and on leaf 3 when the two species arrive at the same time. In contrasts, *T. urticae* shows a slight increase in occupancy of leaf 4 when it arrives first and a slight decrease in occupancy of leaves 2 and 5.

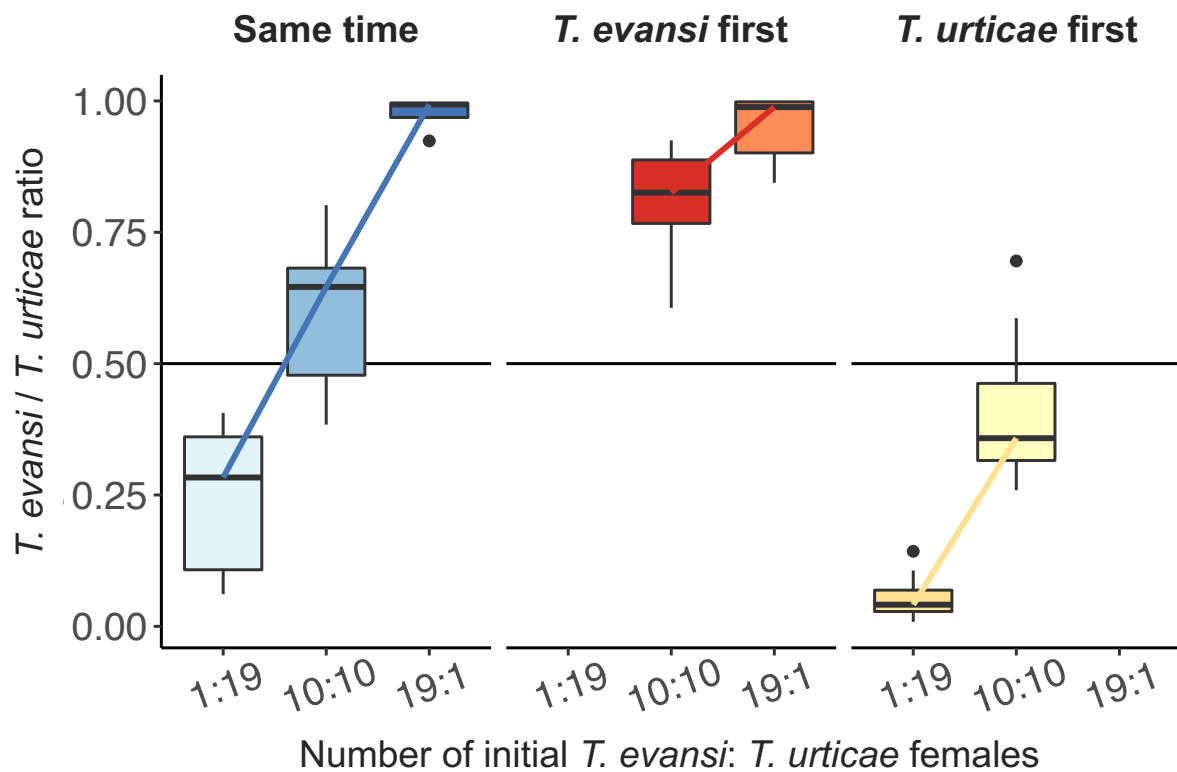
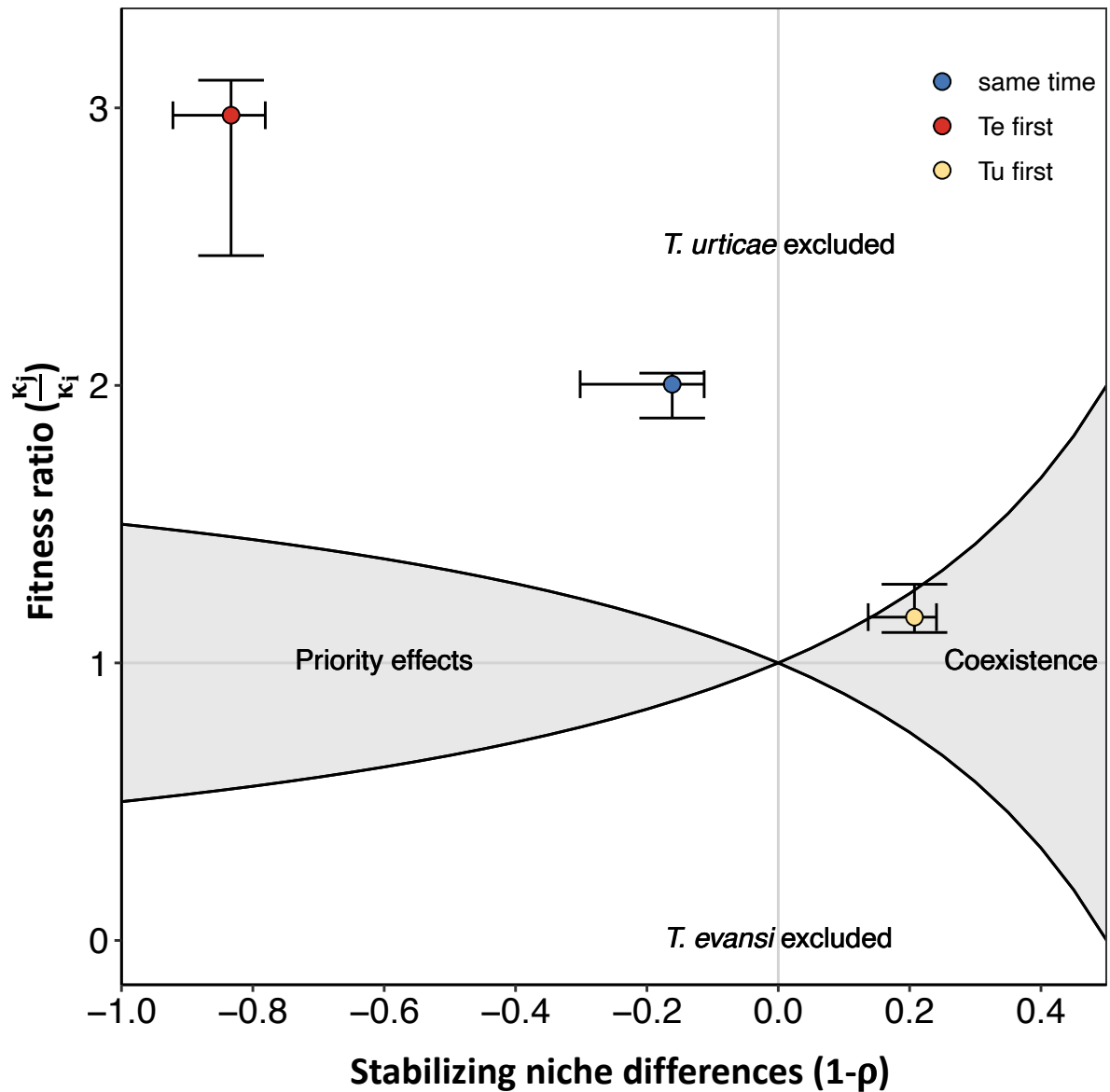


Figure 1 – Proportion of spider mites *Tetranychus evansi* females (y-axis) depending on initial frequency (number of initial females *T. evansi*: *T. urticae*, x-axis) and order of arrival (same time vs. *T. evansi* or *T. urticae* arriving 48h before its competitor) after two generations. *Tetranychus evansi* is the better competitor overall (ratio above 0.5), unless *T. urticae* arrives first or is at higher initial frequency. A posteriori contrasts show a strong effect of order of arrival in the proportion of females of the two species (Suppl. Table 1B). Initial frequency also impacts the final ratio, with a stronger effect when *T. urticae* arrives first or at the same time than *T. evansi* (Suppl. Table 1B). Boxplots represent median and quartiles of the 10 boxes within treatment.



688

689 Figure 2 – Relationship between average fitness differences ($\frac{\kappa_j}{\kappa_i}$, y-axis) and stabilising niche
690 differences ($1 - \rho$, x-axis) for different orders of arrival (*Tetranychus evansi* first – red, same
691 time – blue, *T. urticae* first – yellow). Plotting average fitness differences against niche
692 differences allows mapping different competitive outcomes predicted by modern coexistence
693 theory (Chesson 2000; Ke & Letten 2018; Spaak & De Laender 2021). The coexistence
694 condition (eq. 4) and its inverse, represented by the two solid black lines, allow defining the
695 space in which species can coexist due to negative frequency dependence or enter alternative
696 stable states due to positive frequency dependence, whenever niche differences are greater or

smaller than zero respectively. Otherwise, the species with higher fitness will exclude the other. In our case, the only scenario in which species are predicted to coexist is when *T. urticae* arrives first (yellow). Error bars for each outcome indicate the 95% confidence interval from the maximum likelihood estimates. For the other two cases, it is predicted that the superior competitor *T. evansi* will exclude *T. urticae*.

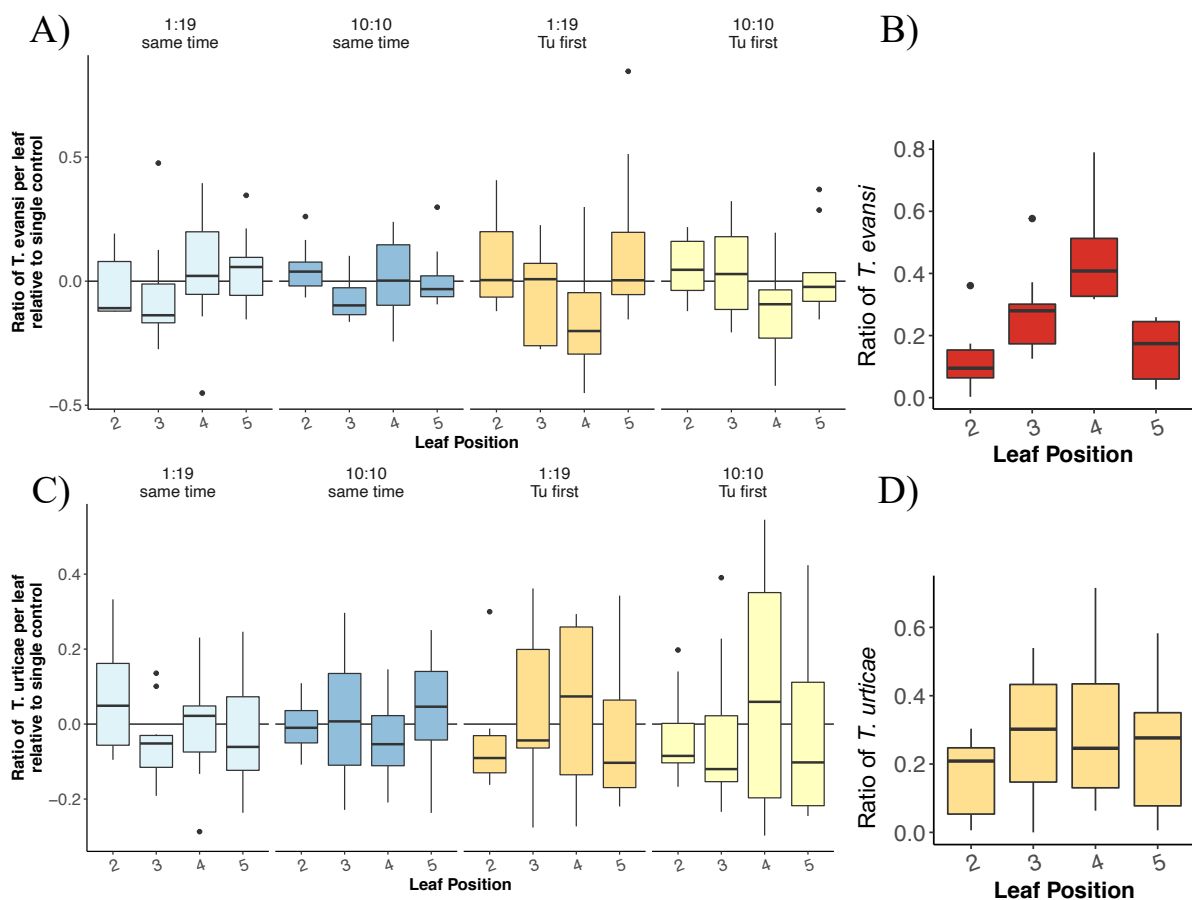


Figure 3 – Differences between expected and observed leaf occupancy for *Tetranychus evansi* (A) and *T. urticae* (C) for a subset of the experimental treatments (when *T. urticae* arrived first or at the same time as *T. evansi*, note that Figure S2 includes all treatments); leaf occupancy for *T. evansi* (B) and *T. urticae* (D) in the control, single species, treatments. Leaf 2 corresponds to the oldest leaf and leaf 5 to the youngest. For each box, we calculated the ratio of females occupying each leaf in relation to the total number of females present. For the experimental

710 treatments we calculated the difference between this ratio and the average ratio for the control
711 treatments. Thus, positive values indicate that there are more females on that leaf than expected
712 based on the single-species treatment and negative values indicate the reverse pattern. Overall,
713 we see that. *T. evansi* reduces occupancy on leaf 4 when *T. urticae* arrives first and on leaf 3
714 when the two species arrive at the same time. In contrasts, *T. urticae* shows a slight increase in
715 occupancy of leaf 4 when it arrives first and a slight decrease in occupancy of leaves 2 and 5.