Quantifying aphid behavioral responses to environmental change

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Abstract

Aphids are the most common vector of plant viruses, and their feeding behavior is an important determinant of virus transmission. Positive effects of global change on aphid performance have been documented, but effects on aphid behavior are not known. We assessed the plant-mediated behavioral responses of a generalist aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), to increased CO_2 and nitrogen when feeding on each of three host species: *Amaranthus viridis* L. (Amaranthaceae), *Polygonum persicaria* L. (= *Persicaria maculosa* Gray) (Polygonaceae), and *Solanum dulcamara* L. (Solanaceae). Via a family of constrained Markov models, we tested the degree to which aphid movements demonstrate preference among host species or plants grown under varying environmental conditions. Entropy rates of the estimated Markov chains were used to further quantify aphid behavior. Our statistical methods provide a general tool for assessing choice and quantitatively comparing animal behavior under different conditions. Aphids displayed strong preferences for the same host species under all growth conditions, indicating that CO_2- and N-induced changes in plant chemistry have minimal effects on host preference. However, entropy rates increased in the presence of non-preferred hosts, even when preferred hosts were available. We conclude that the presence of a non-preferred host species affected aphid-feeding behavior more than changes in plant leaf chemistry when plants were grown under elevated CO_2 and increased N availability.

Introduction

Aphids are a group of phloem-feeding insects that reduce plant performance, suppressing photosynthesis and growth (Flynn et al., 2006), altering plant architecture (Sudderth et al., 2005), and reducing crop yield (Dixon, 1998). Although aphid feeding directly affects plants, the most damaging effects are caused by the pathogens they transmit as they move among host plants (Radcliffe & Ragsdale, 2002; Ng & Perry, 2004; Hogenhout et al., 2008). Aphids transmit over 50% of the 600 viruses with invertebrate vectors (Brunt et al., 1996; Nault, 1997; Hull, 2002), causing major economic losses in crops world-wide (Hooks & Fereres, 2006). Two persistent viruses, beet yellow virus and beet mosaic virus, can lead to sugar beet yield losses of up to 75% (Godfrey & Mauk, 1993, 1995;

Fernandez-Quintanilla et al., 2002). The precise mechanisms of plant pathogen transmission vary depending on whether the virus is circulative or non-circulative (Ng & Perry, 2004), but requires aphid movement between an infected and an uninfected host plant.

Many aphid species have winged and non-winged adult morphs that can each specialize on different ranges of host species (Powell et al., 2006; Webster, 2012). Aphids identify host species using visual (Doring & Chittka, 2007) and olfactory cues (Pickett & Glinwood, 2008; Webster, 2012), combined with leaf surface exploration and probing of plant tissues (Ngumbi et al., 2007). In addition to the long-distance transfer of viruses by winged aphid morphs (Broadbent & Tinsley, 1951; Boiteau & Parry, 1985), viruses can be transferred by non-winged morphs as they walk (Alyokhin & Sewell, 2003) or are moved to neighboring plants by rain, wind, animals, or farm equipment (Bailey et al., 1995; Narayandas & Alyokhin, 2006). Once a virus is transmitted to a field, the local movement of aphids can account for the majority of virus spread within certain crops (Garrett & McLean, 1983; Webster, 2012).

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Aphids may also disperse locally to locate improved food sources for themselves and their progeny (Castle et al., 1998). Aphids tend to change feeding locations more often when feeding on poor food sources compared with higher quality food sources (Dixon, 1998). Thus, differences in plant quality under changing environmental conditions have the potential to alter aphid-feeding behavior, including movement among host plants.

Direct plant responses to global changes such as increased atmospheric CO_2 and soil nitrogen (N) alters the nutritional quality for insect herbivores (Fajer et al., 1992; Coviella et al., 2002; Goverde et al., 2002). Phloem feeders are the only insect herbivore guild with both increased fecundity and abundance when feeding on plants grown under elevated CO_2 (Robinson et al., 2012). Aphids are particularly sensitive to environmental factors that cause changes in nutrient transport within a plant (Sandstrom & Moran, 2001; Jansson & Ekbom, 2002; Karley et al., 2002). Not all aphid species are affected by elevated CO_2, but the species that have positive responses are generalist, polyphagous feeders, such as *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Awmack et al., 1996, 1997; Bezemer & Jones, 1998; Bezemer et al., 1999; Whittaker, 1999; Lawton, 2000; Hughes & Bazzaz, 2001; Sudderth et al., 2005; Flynn et al., 2006). In addition, *M. persicae* has been shown to have higher fecundity and intrinsic rate of population increase when feeding on fertilized compared with control plants (Jansson & Smilowitz, 1986; Stafford et al., 2012). *Myzus persicae* is a major crop pest with a near world-wide distribution (Stoetzel & Miller, 1998) that directly reduces yield and transmits over 180 persistent and non-persistent plant virus strains (Anderson, 1991). *Myzus persicae* is known to demonstrate significant preferences for higher nutrient plants (van Emden & Bashford, 1971). Therefore, this species is ideal for testing whether changes in plant chemistry under increased CO_2 and N conditions affect local-scale aphid movement among plants, with significant implications for the spread of plant pathogens.

We designed a series of experimental trials to characterize the local-scale movement of *M. persicae* between leaf types over time. We utilized discrete Markov chains to model dynamical aspects of aphid behavior, accounting for initial aphid movements and the temporal dependence of aphid location observations. With this approach, we numerically quantified the rate of aphid movement, as well as the degree to which their movement indicates a preference among plants. We first characterized the behavior of *M. persicae* when given a choice of leaves from two plant species grown under the same environmental conditions. We then tested whether this behavior is related to plant quality by restricting aphids to leaves from the same host species grown under different CO_2 and N treatment combinations. We predicted that elevated CO_2 and increased N would differentially affect the quality of three host plant species—*Amaranthus viridis* L. (Amaranthaceae), *Polygonum persicaria* L. (= *Persicaria maculosa* Gray) (Polygonaceae), and *Solanum dulcamara* L. (Solanaceae)—altering aphid behavior and host preferences.

Materials and methods

Plant growth conditions

We selected old-field annual host plants that utilize different photosynthetic pathways because previous studies have demonstrated smaller changes in leaf N under elevated CO_2 for C_4 species, and larger responses for C_3 species (Bazzaz, 1990). In addition, the host species chosen grow rapidly and have similar leaf sizes. We also had evidence from related experiments (Sudderth et al., 2005; Flynn et al., 2006; Sudderth & Bazzaz, 2008) that these particular species are differentially sensitive to elevated CO_2 and N availability. Seeds of *A. viridis* (C_4), *P. persicaria* (C_3), and *S. dulcamara* (C_3) collected from local old-fields (Bedford, MA, USA; 42°30.501′N, 71°17.7′W) were germinated in flats containing a 2:1 mixture of Promix to sand, then transplanted into 2.5-l pots containing the same soil mixture. The plants were grown in four environmentally controlled greenhouse zones maintained at ambient CO_2 (370 µmol mol^{-1}) or elevated CO_2 (750 µmol mol^{-1}), and L16(25 °C):D8(20 °C) h periodic regime. Light levels were supplemented with 1 000-W high-density discharge lights suspended over the benches when daytime light levels dropped below 600 µmol m^{-2} s^{-1}. The nutrient treatments were delivered to each plant via two continuous feed injection systems (Dosatron International, Bordeaux, France) connected to two parallel automatic watering systems (The Drip Store; Escondido, CA, USA), both installed in all four zones used in the experiment. A water-soluble fertilizer (MiracleGro EXCEL Cal-Mag 15-5-15) was mixed to deliver 25 p.p.m. N for both nutrient treatments, while the high-N treatment received an additional 50 p.p.m. ammonium nitrate, for a total treatment level of 75 p.p.m. N. Plants were maintained under the experimental treatments for 60–90 days before the choice experiments.

Aphid choice experiments

The experimental aphid colonies were established from a single aphid collected at Drumlin Farms in Lincoln (MA, USA); therefore, the colony consisted of genetically identical clones. They were reared in growth chambers at 23 °C and ambient CO_2, in large insect cages

(50 × 50 × 30 cm) containing *S. dulcamara*, *A. viridis*, and *P. persicaria*. Fresh plants were provided every 2 weeks. The plants removed from the colonies were closely inspected for signs of aphid-transmitted disease: no symptoms were observed during the study.

The plant-mediated effects of elevated CO_2 and increased N on *M. persicae* feeding behavior were tested in two types of experiments: between and within species. The between-species experiment was designed to test whether aphid behavior indicated significant preference between host plants and whether their preferences changed when plants were grown under elevated CO_2, increased N, or the combination of both. In a series of four trials with 18 replicates in each trial, aphids were given a choice of a *P. persicaria* (C_3) or an *A. viridis* (C_4) leaf grown under the same environmental conditions (AL = ambient CO_2 + low nitrogen, AH = ambient CO_2 + high N, EL = elevated CO_2 + low N, or EH = elevated CO_2 + high N). The within-species experiment was designed to test whether plant growth under different CO_2 and N conditions affected aphid behavior when they were constrained to a single plant species. In a series of 18 trails with 18 aphid replicates for each trial, aphids were given the choice between two leaves of the same species (*A. viridis*, *P. persicaria*, or *S. dulcamara*) grown under different combinations of CO_2 and N conditions (AL, AH, EL, or EH).

Clip cages were used as aphid arenas in both experiments. The cages were constructed of 0.5-cm-thick rings cut from 25-ml centrifuge tubes (2.5 cm diameter) with paint strainer mesh glued over one end. The mesh-covered ring was glued to one side of a 5-cm-long metal hair clip and a neoprene ring was glued to the other side to provide a tight seal when the cage was clipped onto leaves. In each of the 18 replicates in each trial, a leaf from each of two plants of similar size and age were arranged such that each

leaf filled one half of the cage (Figure 1). The leaves remained attached to the whole plants throughout the duration of the experiment to avoid affecting water balance or volatile emissions. To begin a trial, a fine paintbrush was used to place one adult apterous aphid inside the cage on the net. The aphid was required to walk off the cage to access either leaf type, thus making an initial choice between leaf types. The behavior of each aphid over time was monitored by noting its location (cage, leaf 1, or leaf 2), every 30 min for 8 h.

At the end of each trial, the experimental leaves were collected and the fresh mass of each was recorded. The remaining aboveground biomass of each plant was harvested (each plant was only used for one feeding trial) and dried at 65 °C to a constant mass. Total aboveground dry mass was used to determine the growth responses of plants to the CO_2 and N treatments. Water content of the experimental leaves was determined by subtracting the dry mass from the fresh mass of each leaf. The N content of a random sub-sample of eight leaves from each feeding trial was analyzed by the University of Georgia Soil Ecology Lab using a C/H/N analyzer (NA1500; Carlo Erba Strumentazione, Milan, Italy).

Statistical analysis of plant performance

The main effects of CO_2 and N on aboveground biomass, leaf N, and leaf water content were tested by three-factor ANOVAs (JMP; SAS Institute, Cary, NC, USA) using a split-plot design with CO_2 and block as the main effects and N as the sub-plot effect, according to a model used in a similar experiment (Sudderth et al., 2005). The block effects were not significant, and thus were removed from the final analysis. All data were transformed if necessary to meet the assumptions of ANOVA analysis using Box–Cox methods.
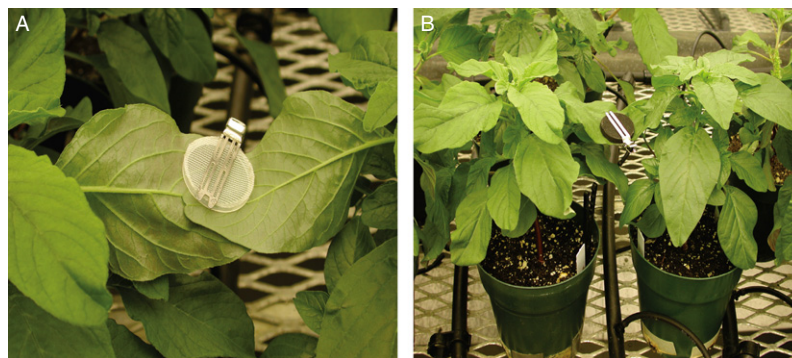

Figure 1 Photographs showing the clip cages and leaf arrangement for one replicate of the *Amaranthus viridis* AL (= ambient CO_2 + low nitrogen) vs. AH (= ambient CO_2 + high N) trial of the within-species aphid choice experiment. (A) Leaves are overlapped, such that each leaf type fills half of the clip cage. (B) One aphid is placed on the clip cage net, exposed to the underside of the leaves. The location of the aphid (on the cage, on leaf type 1, or on leaf type 2) was recorded over the observation period.

Statistical methods for analyzing aphid behavior

Here, we develop statistical methods to test the degree to which caged aphids demonstrate preference among a pair of leaves. There is significant temporal dependence in the locations at which aphids are observed during each trial. In addition, initial observations are influenced by the fact that aphids begin each trial on the cage. We do not want to discard these early observations entirely, however, because initial aphid movements provide important information about their host plant preferences. Furthermore, because each observation falls into one of three discrete categories (leaf 1, leaf 2, or the cage), classical Gaussian methods are inappropriate. To address these issues, we model aphid behavior using discrete Markov chains. This analysis explicitly accounts for dynamical aspects of aphid behavior, rather than just their final positions. It thus allows us to numerically quantify their rate of movement, as well as the degree to which their movement indicates a preference among plants.

Markov models of aphid behavior

Consider a single observed sequence of aphid movements, and let x_t denote the aphid's location for observation number t. This observation can take one of three values, which we denote by $x_t = 1$ for the first leaf, $x_t = 2$ for the second leaf, and $x_t = 3$ for the cage. For all sequences, the aphid begins on the cage, and thus $x_0 = 3$. A first-order Markov chain assumes that, given the aphid's current location, previous observations provide no extra information about its likely future behavior:

$$P[x_{t+1}|x_t, x_{t-1}, \ldots, x_1, x_0] = P[x_{t+1}|x_t].$$

This model is parameterized by a 3×3 state transition matrix, which gives the conditional probability of the three potential future states x_{t+1} given each current state x_t. The spacing between experimental observations (30 min) is much larger than the time needed for an aphid to move between leaves, thus a first-order model is sufficiently expressive to capture aphid preference.

We formalize our experimental analysis as a hypothesis testing problem. The null hypothesis H_0 is that the aphids show no preference, whereas the alternative H_1 is that they do indeed prefer one leaf type over the other. To make these hypotheses mathematically precise, we associate each with a different class of Markov chains. Under H_0, we place constraints on the transition matrix, which ensure that all aspects of aphid behavior treat the leaves symmetrically. First, when aphids show no preference, the probability that they remain on each leaf for two subsequent observations should be identical:

$$P[x_{t+1} = 1|x_t = 1] = P[x_{t+1} = 2|x_t = 2].$$

In addition, they should behave symmetrically when moving between the two leaves:

$$P[x_{t+1} = 1|x_t = 2] = P[x_{t+1} = 2|x_t = 1].$$

Finally, movements to and from the cage should display similar symmetry:

$$P[x_{t+1} = 3|x_t = 1] = P[x_{t+1} = 3|x_t = 2],$$

and

$$P[x_{t+1} = 1|x_t = 3] = P[x_{t+1} = 2|x_t = 3].$$

Our null hypothesis (no preference) is then that the observed sequence of aphid movements was generated by some Markov model satisfying these constraints. Note that the null hypothesis states that aphids show no preference between leaf types, but makes no assumptions about the dynamics of transitions between the leaves and the cage. The alternative, H_1 (preference), is that they follow some more general, unconstrained Markov model, which may violate one or more of these constraints.

Given an estimated Markov model, the amount of movement between states (aphid locations) is nicely captured by the Markov chain's entropy rate (Cover & Thomas, 1991). The entropy of a probability distribution measures the degree to which samples are inherently random, or difficult to predict. The entropy rate then measures the average variability in long observation sequences:

$$H(P) = \sum_{x_t} P_{ss}[x_t] \sum_{x_{t+1}} P[x_{t+1}|x_t]\log_2 \frac{1}{P[x_{t+1}|x_t]}.$$

Here, $P_{ss}[x]$ is the steady-state probability of being in state x, which is easily computed from the state transition matrix. The entropy rate is largest for Markov chains in which the next state is always chosen uniformly at random, and zero for models which eventually reach a 'trapping' state from which they never leave. Thus, small entropy rates indicate that aphids rarely change locations after their initial movements from the cage to a leaf, whereas large entropy rates indicate frequent location changes.

Testing for significant preferences

As discussed in the previous section, each of our aphid behavior hypotheses (H_0: no preference; H_1: preference) corresponds to a family of Markov chains with varying parameters. Generalized likelihood ratio tests (GLRTs) provide a mechanism for testing such hypotheses with many desirable properties (Rice, 1995). Suppose that we

have observed S independent sequences of aphid movements (18 for our trials), each containing T observations. Let $x^s = (x^s_1, \ldots, x^s_T)$ denote the observations corresponding to sequence s, and $P[x^s;\theta]$ the joint probability of x^s with respect to a Markov chain with transition parameters θ. The GLRT is then based on the following test statistic:

$$L(x) = \sum_{s=1}^{S} \left[\log P\left[x^s; \hat{\theta}_1\right] - \log P\left[x^s; \hat{\theta}_0\right] \right].$$

Here, $\hat{\theta}_1$ is the transition distribution corresponding to the Markov chain under which the observed data are most likely [the maximum likelihood (ML) parameter estimate], corresponding to H_1. These parameters are easily found by counting the times each transition occurs in the observed data. Similarly, $\hat{\theta}_0$ are the ML parameter estimates satisfying the four constraints under H_0, outlined in the previous section. These parameters provide the best-fitting model, which shows no preference among the two plant leaves.

Because models satisfying the null hypothesis H_0 are more constrained, the GLRT statistic $L(x)$ is always positive. Large values indicate that the unconstrained model (H_1) provides a much better fit to the data, and thus that the aphids significantly prefer one leaf type over the other. In contrast, small values provide little evidence against the null hypothesis. To quantify these differences, we must determine the distribution of $L(x)$ under H_0. Asymptotically, this statistic is known to follow a χ^2 distribution (Rice, 1995). However, because our aphid experiments involve a relatively small number of observations, such asymptotics are unjustified and would overestimate the significance of our results.

To more accurately estimate significance levels, we use bootstrap to construct a non-parametric estimate of the significance of S observation sequences. We begin by drawing S sequences $\tilde{x}_s$ with replacement from the data's empirical distribution. To ensure that these samples conform to the null hypothesis, we also randomly select half of these bootstrap sequences and swap the labels corresponding to plants A and B. This transformation ensures by construction that the resampled sequences show no preference between leaves, but other aspects of the temporal dynamics of aphid movement among feeding locations remain unchanged. Given a bootstrap sample $\{\tilde{x}_1, \ldots, \tilde{x}_s\}$, we determine the best-fitting models under each hypothesis and evaluate the GLRT statistic $L(\tilde{x})$. We repeat this process many times (100 000 in our experiments), and record the proportion of bootstrap datasets, which lead to GLRT statistics $L(\tilde{x})$ that are larger than $L(x)$. This fraction, which estimates the probability of showing similar model differences by random chance, then provides a consistent bootstrap estimate of the trial's significance level, or P-value. A P-value <0.05 in Tables 2 or 3 indicates that the aphids demonstrated a significant preference for one leaf type over the other. Each of the experimental trials presented in these tables was conducted independently on different days with fresh plants and aphids.

Results

Plant growth and leaf traits
Plant biomass and leaf N content were greater under the high-N treatments for all three plant species (Table 1). Elevated CO_2 increased plant biomass (Figure 2A) and reduced leaf N content under low N (Figure 2B) in the C_3 species (*S. dulcamara* and *P. persicaria*), but did not affect *A. viridis*, the C_4 species. A priori contrasts comparing the effect of elevated CO_2 at each N level showed that the biomass of *A. viridis* increased under elevated CO_2 at low N ($P = 0.047$), but not at high N. Leaf water content of *A. viridis* leaves was higher under high N at both CO_2

Table 1 Summary of ANOVA results for effects of elevated CO_2 and increased nitrogen on plant performance traits for the within-species experiment. Plant responses for the plants used in the between-species experiment were nearly identical (data not shown)

| Measurement	Treatment	d.f.	*Amaranthus viridis* (C_4)		*Polygonum persicaria* (C_3)		*Solanum dulcamara* (C_3)	
			F	P	F	P	F	P
Biomass	CO_2	1,1	1.46	0.23	104.32	<0.001	108.84	<0.001
	N	1,8	52.37	<0.001	508.47	<0.001	21.19	<0.001
	CO_2*N	1,8	2.66	0.11	30.58	<0.001	<0.001	0.99
Leaf % N	CO_2	1,1	1.70	0.20	4.55	0.04	68.28	<0.001
	N	1,8	77.80	<0.001	29.38	<0.001	88.6	<0.001
	CO_2*N	1,8	0.35	0.56	7.35	0.01	0.20	0.66
Leaf % water	CO_2	1,1	0.02	0.90	0.09	0.76	0.17	0.68
	N	1,8	70.36	<0.001	2.00	0.16	1.22	0.27
	CO_2*N	1,8	3.36	0.07	4.39	0.04	2.47	0.12
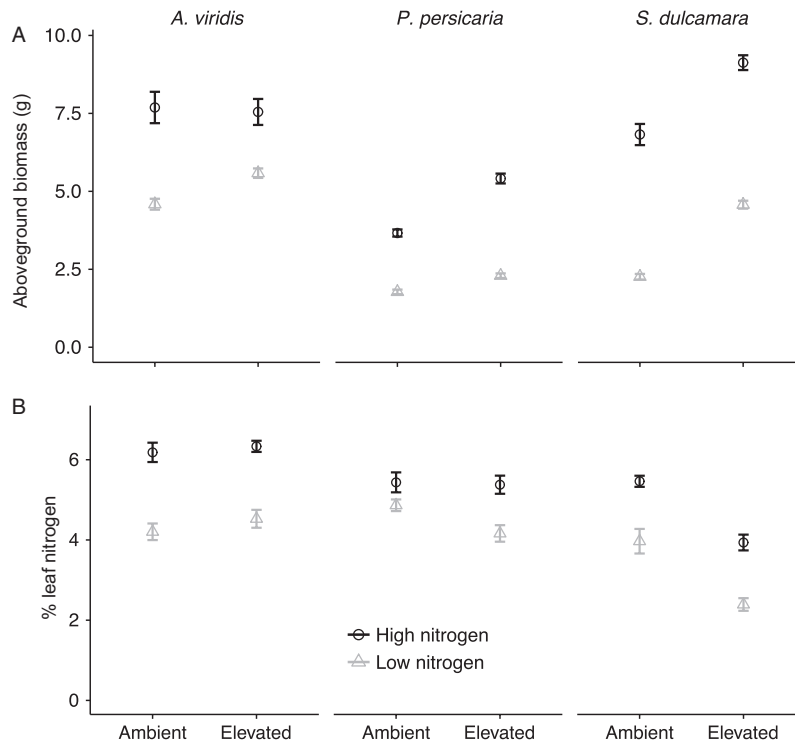
Figure 2 Mean ($\pm$ SE) plant responses to growth under the CO_2 and N treatments. (A) Aboveground biomass increased under the high-N treatments for all species, and under elevated CO_2 in the C_3 (*Polygonum persicaria* and *Solanum dulcamara*), but not the C_4 (*Amaranthus viridis*) species. (B) Leaf N content increased under high N in all species under both CO_2 levels.

concentrations (data not shown). There were significant CO_2*N interactions affecting aboveground biomass, leaf N, and leaf water content in *P. persicaria*, but not in the other two plant species (Table 1).

Aphid behavior

The plant-mediated effects of elevated CO_2 and soil N on aphid behavior were modeled using a family of constrained Markov models to test the degree to which aphid movements demonstrate preference between plants. Generalized likelihood ratio tests supported significant aphid preference for *A. viridis* over *P. persicaria* hosts, regardless of plant growth conditions (Table 3). For most of the within-species feeding trials, aphids moved off the cages onto each leaf type in roughly equal proportions. When feeding on *A. viridis*, the aphids rarely left the leaf type that they initially chose (Figure 3, top panels: observed data). In the between-species trials, aphids moved from *P. persicaria* to *A. viridis* leaves more often than they moved from *A. viridis* to *P. persicaria* leaves (Figure 4, top panels: observed data).

Entropy rates of the estimated Markov chains were used to quantify aphid motion over time (Table 2). Larger values represent more movement, whereas smaller values

show that the aphids did not often switch between locations. In the within-species experiment, aphids on *A. viridis* rarely moved between leaf types or back to the cages after they initially moved to one of the two leaves in the cage (Figure S1). In four of the six trials, the aphids never left one of the leaf types, resulting in entropy rates of zero. In contrast, aphids constrained to *P. persicaria* frequently moved between leaf types and from the leaves back to the cages, resulting in the largest entropy rates. Aphids on *S. dulcamara* showed moderate amounts of movement between leaf types and from the leaves back to the cages, giving intermediate entropy rates.

Several patterns of aphid behavior are represented in the time series observation and model data. In the *P. persicaria* within-species experiments, aphids were slow to leave the cages and many aphids remained on the cages throughout the observation period (Figure 3). The aphids left the cages most quickly in the trial in which they showed a slight preference for the AH leaf type compared with the EL leaf type. In contrast, aphids in the within-species trials on *A. viridis* very quickly left the nets and initially chose each leaf type in roughly equal proportions. There was minimal switching between leaf types during the observation period. The aphids rarely moved back to the cages
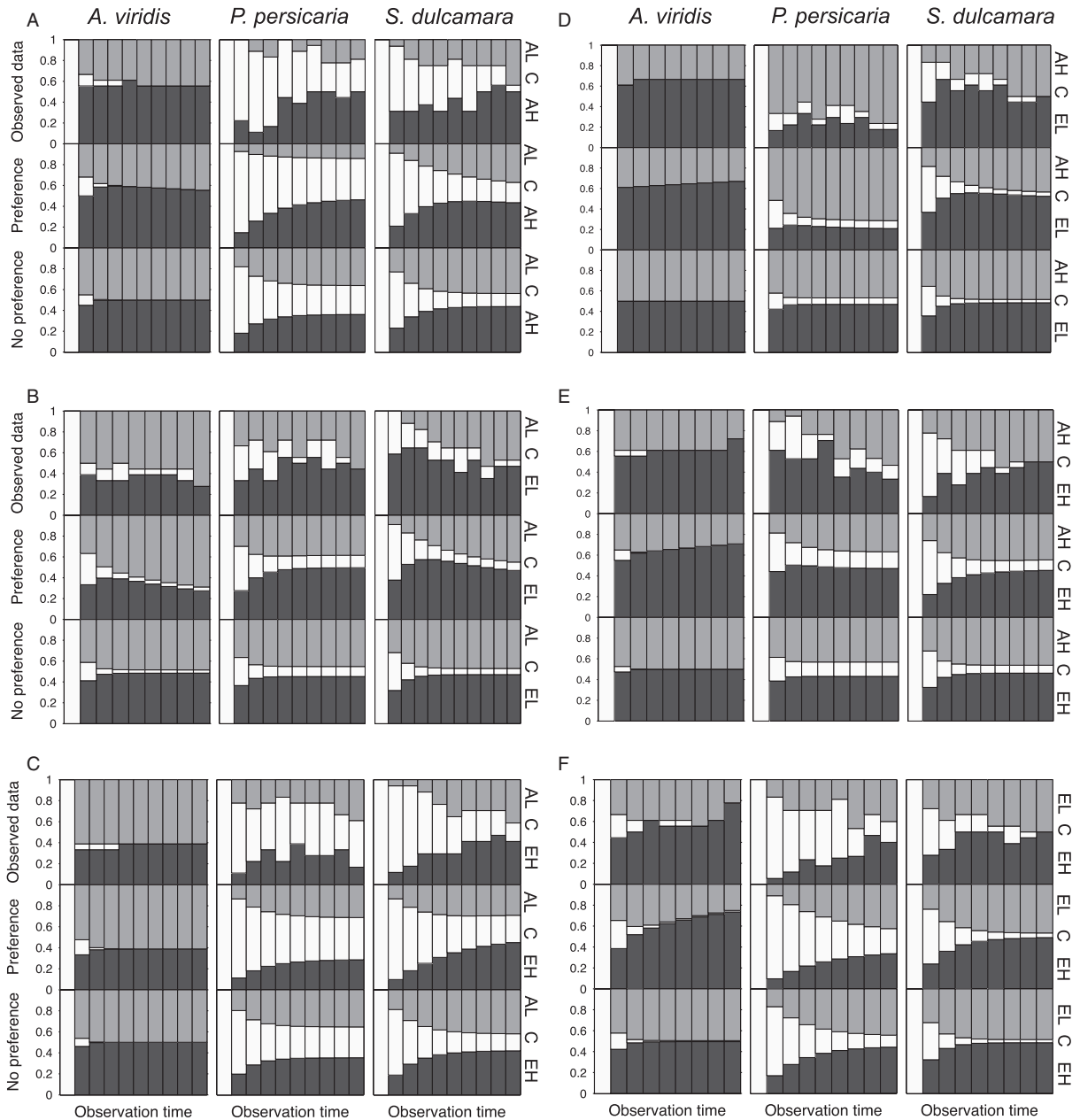
Figure 3 Observed and modeled data for the within-species experiments indicating the movement of *Myzus persicae* aphids between feeding locations over time. For each trial (A–F), the top panels show the observed data, the middle panels show the Markov model allowing aphid preference (H_1), and the bottom panels show the restricted Markov model, which does not allow aphid preferences (H_0). At each observation point, the proportion of replicates in which aphids were observed at each of the three possible feeding locations is indicated [white = cage (C), light gray = leaf type 1, dark gray = leaf type 2]. The leaf types for each feeding trial are shown along the right hand side of each graph (AL = ambient CO_2 + low nitrogen, AH = ambient CO_2 + high N, EL = elevated CO_2 + low N, and EH = elevated CO_2 + high N). Visually, larger differences between the unrestricted and restricted Markov chain models indicate a stronger aphid preference for the feeding location with the greatest area in the unrestricted model (middle). For all feeding trials in the within-species experiment, aphids spent the most time on the cages when given a choice between two *Polygonum persicaria* leaves, less time when feeding on *Solanum dulcamara*, and almost no time on the cages when given a choice between two *Amaranthus viridis* leaves.
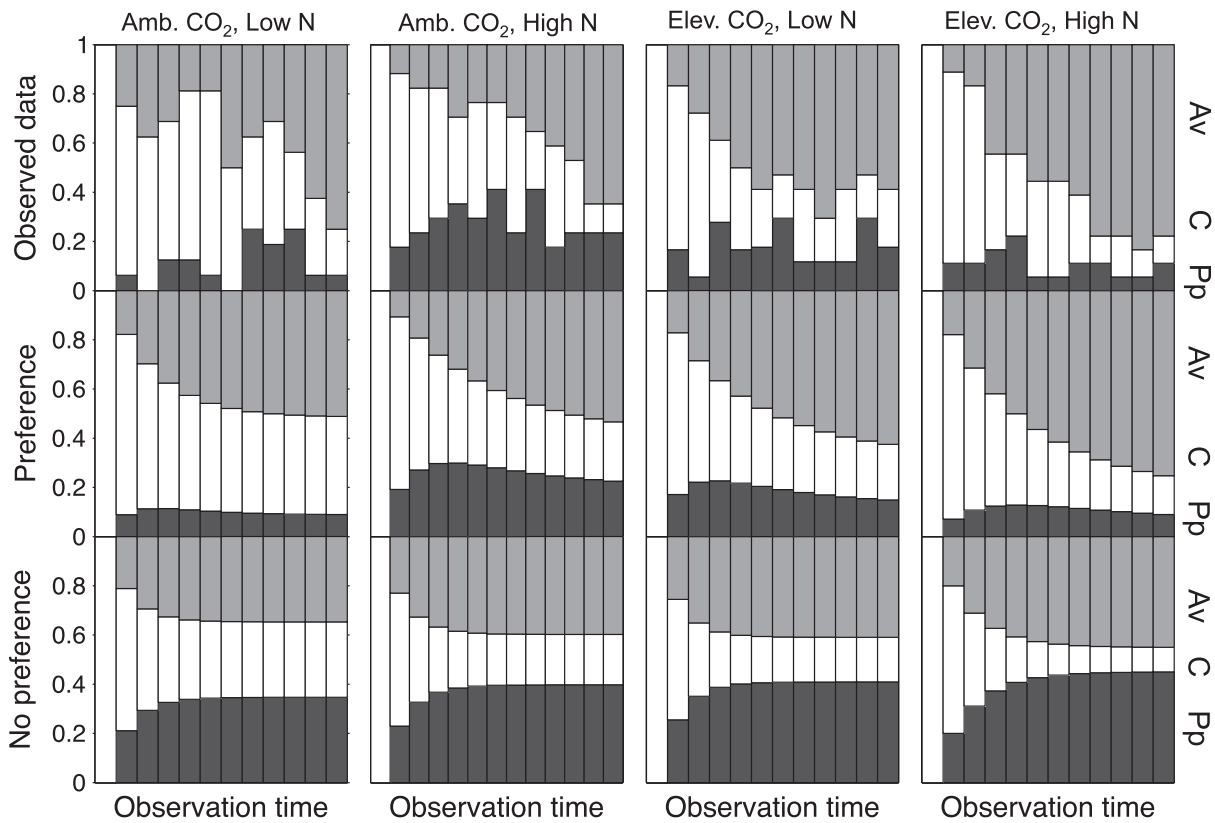
Figure 4 Observed and modeled data for the between-species experiments indicating the movement of *Myzus persicae* aphids between feeding locations over time. For each trial, the top panels show the observed data, the middle panels show the Markov model allowing aphid preference, and the bottom panels show the restricted Markov model, which does not allow aphid preferences. At each observation point, the proportion of replicates in which aphids were observed at each of the three possible feeding locations is indicated [white = cage (C), light gray = *Amaranthus viridis* (Av), dark gray = *Polygonum persicaria* (Pp)]. Aphids were slow to leave the cages and moved frequently throughout the observation period, but spent the most time on *A. viridis* leaves, less time on the cages, and the least time on *P. persicaria* leaves.

Table 2 Generalized likelihood ratio tests and entropy values for the within-host feeding trials

Choice	*Solanum dulcamara*		*Polygonum persicaria*		*Amaranthus viridis*	
	P	Entropy	P	Entropy	P	Entropy
AL vs. AH	0.44	0.47	0.11	0.69	0.42	0.00
AL vs. EL	0.04 (EL)	0.45	0.71	0.75	0.12	0.18
AL vs. EH	0.20	0.52	0.83	0.69	0.48	0.00
AH vs. EL	0.55	0.43	0.01 (AH)	0.47	0.24	0.00
AH vs. EH	0.89	0.45	0.24	0.80	0.09 (EH)	0.00

Each choice result represents an independent trial where new plants and aphids were utilized from the stock colonies. $P<0.05$ indicates significant *Myzus persicae* preference for the leaf type shown in parentheses (AL = ambient CO_2 + low nitrogen, AH = ambient CO_2 + high N, EL = elevated CO_2 + low N, and EH = elevated CO_2 + high N).

from the leaves, and no aphids remained on the cages by the end of the observation period in any of the *A. viridis* within-species trials. In the *S. dulcamara* trials, the rate at which the aphids moved off the cage varied, and was slow

in some trials and fast in others. The aphids generally did not show preferences between *S. dulcamara* leaf types.

In the between-species experiment, the aphids spent the most time on the *A. viridis* leaves, less time on the cages,

Table 3 Generalized likelihood ratio test results and entropy values for the between-host feeding trials (*Amaranthus viridis* vs. *Polygonum persicaria*) under each growth treatment combination

Host growth condition	P	Entropy
Ambient CO_2 + low N	0.01 (Av)	0.60
Ambient CO_2 + high N	0.03 (Av)	0.46
Elevated CO_2 + low N	0.01 (Av)	0.41
Elevated CO_2 + high N	0.01 (Av)	0.23

Each choice result represents an independent trial where new plants and aphids were utilized from the stock colonies. $P<0.05$ indicates significant *Myzus persicae* preference for the leaf type shown in parentheses (Av = *A. viridis*).

and the least time on *P. persicaria* leaves (Figure 4). The aphids were slow to leave the cages and moved frequently throughout the observation period, but did show significant preferences for the *A. viridis* leaves in all of the trials (Table 3). When the movement of the aphids off the cages (Figure S2) is compared with the *A. viridis* within-species trials, the presence of the *P. persicaria* leaves in the clip cages seems to delay aphid movement to either leaf type (Figure 4). However, once the aphids moved to the *A. viridis* leaves, they did not often move away. They showed the least movement and the strongest preference for the *A. viridis* leaves in the EH trial.

Discussion

We utilized a novel combination of Markov chain models, entropy rates, and generalized likelihood ratio tests to characterize the behavior of a phloem-feeding insect herbivore. We first determined whether the generalist aphid species, *M. persicae*, showed significant preferences between leaves from two plant species, then whether these preferences were modified by plant growth under different environmental conditions. We found that the aphids displayed a strong preference for *A. viridis* over *P. persicaria*, regardless of the environmental conditions that the plants were grown under. As predicted, elevated CO_2 and increased N differentially affected the quality (leaf N content and C:N ratio) of three host plant species (*A. viridis*, *P. persicaria*, and *S. dulcamara*). However, these changes in leaf chemistry did not alter movement patterns or leaf type preferences of *M. persicae*. Rather, aphid behavior, quantified using entropy rates, differed dramatically among plant host species. The presence of a non-preferred host species affected aphid-feeding behavior more than changes in plant leaf chemistry.

Entropy measures have been shown to effectively quantify time-dependent behavior of animals including bluefin tuna feeding (Kadota et al., 2011), rat locomotion (Paulus

et al., 1990), and the response of parasitic wasps to plant volatiles (Doyle, 2009). We found that aphid behavior was well represented by entropy rates, indicating the degree of variability in observed aphid locations (cage, leaf 1, or leaf 2) over the 8-h trial period. In the within-species experiment, movement (quantified as entropy rates) was rare when the aphids were on *A. viridis* leaves, frequent when they were on *P. persicaria*, and intermediate on *S. dulcamara*. In the between-species experiment, aphids strongly preferred to feed on *A. viridis* over *P. persicaria* leaves under all combinations of plant growth conditions. However, the presence of the non-preferred plant species (*P. persicaria*) along with the preferred plant species (*A. viridis*) resulted in much higher variability in aphid-feeding location (higher entropy rates) compared with the within-species experiment using *A. viridis* only. The same result may not be observed when aphids are feeding on whole plants rather than restricted to clip cages; however, our results suggest that the presence of non-preferred plant species increases aphid movement among plants, even when feeding on preferred species. Previous work has shown that on preferred host plants, aphids have short walking times and prolonged probing periods, whereas the opposite behavior is observed on non-preferred host plants (Traicevski & Ward, 2002). Although aphids are unlikely to feed for extended time periods on non-preferred species such as *P. persicaria*, probing of the leaf surface followed by movement to other plants could result in increased local-scale transfer of non-circulatory viruses among plants. Our findings suggest that changes in the relative abundance of non-preferred and preferred host species under climate change may affect aphid movement among host plant leaves. However, the effects of climate change on the performance of agricultural weeds, and their impact on crop yields, depend on characteristics (e.g., C_3 vs. C_4 and annual vs. perennial) of the weed and crop species (Ziska, 2000; Hatfield et al., 2011). Thus, the effects of changing environmental conditions on virus transmission by aphid vectors will likely vary among agricultural systems.

Aphid probing and feeding was not measured directly in our study (e.g., utilizing electrical penetration graphs) to allow comparisons of aphid behavior when restricted to many combinations of plant leaf types grown under different environmental conditions. Instead, a lack of movement over several time steps was taken as evidence that the aphids were probing leaf surfaces (Kennedy & Booth, 1963). In the within-species experiment, aphids remained on *A. viridis* leaves for long periods of time, moving between locations rarely after they initially left the cage. These observations and a previous study demonstrating rapid growth of *M. persicae* populations on *A. viridis*

(Sudderth et al., 2005) suggest that this weedy plant species may support large aphid populations in agricultural settings. Weedy species surrounding agricultural fields can act as virus reservoirs by allowing the buildup of aphid populations (Duffus, 1971; Fernandez-Quintanilla et al., 2002). The presence of weedy species can also reduce the increase in crop yield expected under elevated CO_2; therefore, weed control will be increasingly critical under future climate conditions (Ziska, 2000). Aphid-feeding trials can be utilized to assess which weedy plant species should be targeted in control efforts to reduce virus reservoirs.

We tested the prediction that plant responses to elevated CO_2 and increased N would affect host quality and modify aphid-feeding behavior. Although plant leaf chemistry (N content and C:N ratios) was significantly altered by growth under different CO_2 and N conditions, these changes were associated with only minor changes in aphid-feeding behavior. However, phloem composition presumably has more direct effects than total leaf N on aphid-feeding behavior, and the two measures of leaf quality may not be directly linked. Numerous studies have shown plant-mediated effects of elevated CO_2 and other environmental factors on the population level performance of various aphid species (Awmack et al., 1997; Bezemer et al., 1998, 1999; Newman et al., 1999; Awmack & Harrington, 2000; Hughes & Bazzaz, 2001; Sudderth et al., 2005; Flynn et al., 2006). Yet, these studies and others have not found predictable responses, and the mechanisms controlling the effects of environmental change on aphids remain unknown (Newman et al., 2003; Oehme et al., 2011). We did not find evidence that change in aphid-feeding behavior in response to altered leaf chemistry was a primary mechanism affecting aphid population responses to environmental change.

Conclusions

Markov chain models of aphid movement capture temporal dependencies in the locations at which they feed and could be incorporated into models that aim to predict virus spread by aphids through plant populations (Sisterson, 2008). Entropy rates effectively quantified aphid behavior, providing a general tool for quantitatively comparing animal behavior under different conditions. Despite significant changes in plant host chemistry under increased CO_2 and N, we did not observe plant-mediated effects on aphid behavior. Rather, we observed significant host preference among plant species and large differences in aphid behavior on different hosts. Aphid movement increased in the presence of non-preferred hosts, even when preferred hosts were present. Thus, changes in the relative abundance of preferred and non-preferred plant species may alter aphid movement among host plants, affecting the transfer of plant viruses by aphid vectors. Feeding trials similar to those presented here could indicate which weedy plant species should be controlled to reduce virus reservoirs and spread among crops.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Transition matrices for the within-species experiments. Light gray indicates the proportion of time steps over which the 18 replicate aphids that began at the location shown on the x-axis (Cage, Leaf 1, Leaf 2) moved to or stayed on the cage by the next observation time. Light gray shows aphid movement from the starting location indicated on the x-axis to leaf type 1, whereas dark gray shows aphid movement to leaf type 2. The two leaf types used in each trial are indicated along the y-axis. The first treatment listed represents leaf type 1, the second leaf type 2. For all figures, AL = ambient CO_2 + low N, AH = ambient CO_2 + high N, EL = elevated CO_2 + low N, and EH = elevated CO_2 + high N.

Figure S2. Transition matrices representing the observed movement of *Myzus persicae* aphids between the cage, *Amaranthus viridis* (Av), and *Polygonum persicaria* (Pp) for the four between-species experiments (AL = ambient CO_2 + low N, AH = ambient CO_2 + high N, EL = elevated CO_2 + low N, and EH = elevated CO_2 + high N). White indicates the proportion of time steps over which aphids that began at the location shown on the x-axis moved to the cage by the next observation time. Light gray shows aphid movement from the starting location to *A. viridis* leaves, whereas dark gray shows aphid movement to *P. persicaria* leaves.