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Phenotypic plasticity of corn leaf aphid *Rhopalosiphum maidis* under elevated temperature and CO_2

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PHENOTYPIC PLASTICITY OF CORN LEAF APHID *RHOPALOSIPHUM MAIDIS* UNDER ELEVATED TEMPERATURE AND CO_2

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Résumé

Yu CHEN. (2019). Plasticité phénotypique du puceron du maïs, *Rhopalosiphum maidis* sous conditions élevées de température et CO_2 (Thèse de doctorat en anglais). Gembloux, Belgique, Agro-Bio Tech, Liège University, 100 p., 12 tableaux, 12 fig.

Résumé —Dans le contexte actuel du changement climatique à l'échelle planétaire, la concentration moyenne mondiale de dioxyde de carbone (CO_2) dans l'atmosphère a régulièrement augmenté, passant de 280 ppm dans la période préindustrielle à 401 ppm environ actuellement. Les concentrations devraient doubler d'ici 2100. On prévoit également que les changements climatiques augmenteront non seulement la fréquence mais aussi l'ampleur des épisodes de chaleur extrême, ce qui posera de grands défis à la plupart des organismes ectothermes, comme les pucerons.

En tant qu'insecte nuisible à l'échelle mondiale, le puceron du maïs *Rhopalosiphum maidis* a causé des dommages importants aux cultures céréalières comme l'orge, le maïs et le blé. Le puceron du maïs est également vecteur de phytovirus, dont le virus de la mosaïque de la canne à sucre et le virus de la mosaïque naine du maïs, qui entraîne des pertes économiques. Comme tous les ectothermes, les pucerons ont une capacité pour détecter les variations de température. Leur petite taille et leur taux métabolique élevé les rendent sensibles à l'équilibre énergétique et hydrique lors de l'exposition à des conditions extrêmes.

Dans cette étude, nous avons testé la tolérance thermique du puceron du maïs à des températures extrêmement élevées à différents stades de croissance. La température critique élevée (CHT pour « critical high temperature ») de *R. maidis* se situe entre 39,0 et 40,0 °C en fonction du niveau d'alimentation. Les températures létales supérieures (ULTs pour « upper lethal temperatures ») varient d'un stade de développement à l'autre, les valeurs les plus élevées étant observées au quatrième stade. L'alimentation améliore la tolérance thermique des pucerons.

Le polyphénisme, un phénomène courant dans la nature, est une forme importante d'adaptation face à un environnement diversifié. Le polyphénisme des ailes observé chez le puceron du maïs est lié à la mauvaise qualité de l'habitat. Cinq stades de développement des pucerons (du premier au quatrième stade nymphal et jeunes adultes) avec des densités de population accrues ont été suivi sous deux types de régimes de températures. L'émergence d'individus ailés était directement liée au surpeuplement et à la température. Les nymphes de premier et deuxième stades étaient plus sensibles à la densité de population induisant l'émergence d'individus ailés. De plus, la température a joué un rôle important sur l'émergence des ailés, induisant à la fois une augmentation d'individus ailés et un accroissement du taux de survie.

L'augmentation de concentration de CO_2 affecte non seulement la croissance et le développement des plantes, mais aussi l'émission de composés organiques volatils d'origine végétale. Des changements dans le profil d'odeurs des plantes peuvent affecter les interactions plantes-insectes, particulièrement le comportement des

insectes phytophages. Au cours des essais biologiques à double choix, les pucerons ailés et aptères étaient plus attirés par les COV issus des plants d'orge cultivés sous des conditions de concentrations de CO_2 ambiant (aCO_2 ; 450 ppm) que ceux cultivés sous des concentrations de CO_2 élevées (eCO_2 ; 800 ppm). Les nymphes n'étaient pas attirées par les COV des plants d'orge cultivés sous eCO_2. En outre, 16 COV ont été identifiés à partir de plants d'orge cultivés sous aCO_2, alors que seulement 9 COV ont été identifiés à partir des plants d'orge cultivés sous eCO_2.

Aussi, l'effet d'un taux élevé de CO_2 sur le comportement alimentaire des pucerons de maïs sur les plants d'orge a été évalué en utilisant le système d'électro-pénétrographie (EPG). La teneur en éléments nutritifs de la plante hôte et les indices de développement des pucerons dans des conditions eCO_2 et aCO_2 ont été examinés. Les plants d'orge cultivés sous concentration d'eCO_2 contenaient moins de protéines brutes et d'acides aminés. L'analyse EPG a montré que les plants cultivés sous eCO_2 ont influencé le comportement alimentaire des pucerons, en prolongeant le temps total de la phase d'avant piqûre d'essai et d'ingestion passive de phloème. De plus, le poids frais, la fécondité et le taux de croissance intrinsèque de la population de *R. maidis* ont été significativement réduits dans les conditions d'eCO_2 par rapport à celles de aCO_2.

Notre recherche ouvre une nouvelle perspective sur la compréhension des interactions plantes-insectes phytophages dans le contexte du changement climatique.

Mots-clés: Changements climatiques, *Rhopalosiphum maidis*, puceron de maïs, tolérance thermique, alimentation, comportement alimentaire, composés organiques volatils, électro-pénétrographe (EPG), interactions plantes - pucerons.

Abstract

Yu CHEN. (2019). Phenotypic plasticity of corn leaf aphid *Rhopalosiphum maidis* under elevated temperature and CO_2 (PhD Dissertation in English). Gembloux, Belgium, Agro-Bio Tech, Liège University, 100 p., 12 tables, 12 fig.

Abstract — In the current context of global climate change, the world average temperature and carbon dioxide (CO_2) concentration in the atmosphere has steadily increased. Climate change is also predicted to increase the frequency and magnitude of extreme heat events, which will involve great challenges for most of the ectotherm organisms, such as aphids. As a worldwide pest insect, corn leaf aphid, *Rhopalosiphum maidis* caused significant damage on cereal crops such as barley, corn, and wheat. *R. maidis* is also a vector of plant viruses including sugarcane mosaic virus, maize dwarf mosaic virus, which result in economic losses. Similar to all ectotherms, aphids have acute sensory capability for detecting temperature variations. Their small size and high mass-specific metabolic rates makes them sensitive to energy and water balance during exposure to extremes.

In this study, thermal tolerance of *R. maidis* under extreme high temperatures across differential life stages were tested. The critical high temperature (CHT) of *R. maidis* was between 39.0 to 40.0 °C according kinds of feeding treatments. The upper lethal temperatures (ULTs) of *R. maidis* were significantly different between feeding and no feeding treatments. In addition, the ULTs varied significantly across life stages with highest ULTs values for 4^{th} instars. Feeding significantly increased the thermal tolerance of aphids.

R. maidis exhibit wing polyphenism in response to poor habitat quality. Polyphenism is an important form of adaptation in an adverse environment. Five developmental stages of aphids with increased population densities were investigated under two kinds of temperature patterns. Crowding was found to directly impact winged induction. The 1^{st} and 2^{nd} nymphs were more sensitive for alate morphs induction under high density. In addition, temperature played a significant role in wing production, with the 26/39 °C temperature setting inducing higher alate morphs and survival.

The increase in concentrations of CO_2 not only affects plant growth and development, but also impacts the emission of plant organic volatile compounds. During the dual choice bioassays, the winged and wingless aphids were more attracted by the VOCs of barley seedlings cultivated under ambient CO_2 concentrations (aCO_2; 450ppm) than barley seedlings cultivated under elevated CO_2 concentrations (eCO_2; 800ppm), nymphs were not attracted by the VOCs of eCO_2 barley seedlings. While 16 VOCs were identified from aCO_2 barley seedlings, only 9 VOCs were found from eCO2 barley seedlings.

The effect of elevated CO_2 on feeding behavior of *R. maidis* on barley seedlings was tracked using electrical penetration graph (EPG). The nutrient content of host plant and the developmental indexes of aphids under eCO_2 and aCO_2 conditions were examined. Barley seedlings under eCO_2 concentrations had lower contents of crude

protein and amino acids. EPG analysis showed plants cultivated under eCO_2 influenced the aphid feeding behavior, by prolonging the total pre-probation time and the ingestion of passive phloem sap. Moreover, fresh body weight, fecundity and intrinsic population growth rate of *R. maidis* was significantly decreased in eCO_2 in contrast to aCO_2 condition. Our reasearch provide a new perspective on understanding plant-insect herbivore interactions under climate change.

Keywords: climate change, *Rhopalosiphum maidis*, corn leaf aphid, thermal tolerance, feeding, foraging behavior, volatile organic compounds, electrical penetration graph (EPG), aphid-plant interactions

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General introduction

1 Dominant variables of climate change

Human-induced climate change is potentially the most important factor affecting natural and agricultural ecosystems. Its impact on the performance of herbivores is central to informing ameliorative or adaptive strategies. The dominant variables of climate change constitute: elevated temperature and CO_2. Global mean surface air temperature is predicted to increase about 1.8-6.0 °C by the end of this century, while CO_2 is predicted to continue to increase from the current 400 ppm to between 750 and 1300 ppm in the same period. In addition, climate change will increase in frequency, intensity, and/or amount of extreme temperatures (Stocker et al., 2013). The independent effects of temperature elevation and CO_2 enrichment on the biology and physiology of herbivorous insects are well studied (Rawson, 1992; Griffin and Seemann, 1996; Morison and Lawlor, 1999; Medlyn et al., 1999; Bunce, 2001; Woodward, 2002; Ainsworth & Rogers, 2007; Ge et al., 2010). All in all, elevated temperature will affect herbivorous insects directly (Bale, 2002), it generally accelerates insect growth, shortens the duration between generations, reduces fecundity, expands the distribution of insect populations and also induces some physiological responses (Crozier and Dwyer, 2006; Robinet and Roques, 2010; Zhang et al., 2015). Enhancement of CO_2 may alter larval feeding behavior, extend developmental time, reduce survival, decrease adult weight, and lower fecundity of insect herbivores (Brooks and Whittaker, 1998; Wu et al., 2006). To investigate aphid's phenotypic plasticity under elevated temperature and CO_2 lay the foundation for understanding the adaptation and evolution of insects under extreme climate.

2 Phenotypic plasticity of aphid

Plastic responses are important for species to adapt to environment changes (Gienapp et al., 2008; Chevin et al., 2010). Phenotypic plasticity is the ability of a single genotype to produce more than one alternative phenotype in response to environmental conditions, or in other words the ability of a single genotype to express itself in different ways in different environments (Martel et al., 2011). In short, phenotypic plasticity is equal to genotype plus environmental conditions (Dong et al., 2005). Phenotypic plasticity has important inspiration to study the relationship between ecology and evolution of species. The aphid group includes 4400 species worldwide (Heie, 1995) and is an ideal model for studying the phenotypic plasticity of insects under climate change (Cammell and Knight, 1992). For its global distribution and thus be most likely to experience diverse environmental challenges. Their small size and high mass-specific metabolic rates makes them sensitive to energy and water balance during exposure to extremes (Williams et al., 2014). Secondly, the short generation times and immense capacity for increase of these aphids give them the potential for rapid responses to climate change, further enhancing the pest status of some species (Harrington et al., 1995). Thirdly, aphid feeding represents a continuous carbon sink (Welter, 1989). Sink strength is considered to be one of the most important influences regulating the acclimation response of plants to elevated CO_2 (Rogers et al., 1995).

Aphids exhibit phenotypic plasticity, such that genetically identical individuals can potentially show different phenotypes, as having wings or not at adult stage (Braendle et al., 2006). Winged aphids are specialized for dispersal through flight, as they have a more developed sensory system, are more resistant to starvation and live longer (Tsuji and Kawada, 1987). These features are assumed to be beneficial for locating new habitats and host plants by winged aphids in a complex environment. Each aphid species feeds on a restricted range of host plants and thus the locating, landing and quick initialization of reproduction on suitable host plants is imperative to the fitness of the aphid (Powell et al., 2006). Winged aphids have also been shown to have reduced fecundity and longer developmental times, which is likely due to the increased energy cost of having wings (Mackay and Wellington, 1975; Tsumuki et al., 1990). Wing dimorphism marks key shifts in reproductive mode and host plant colonization. Changes in host-plant quality stimulate induction of winged morphs, because unfavorable conditions should be associated with production of individuals adapted for dispersal. However, many aphids also use tactile or chemical cues to gauge density and crowding as signals for winged morph induction, rather than cues derived from plants themselves (Müller et al., 2001). High density treatment of reproductive aphids can induce the production of winged progeny, suggesting that embryonic fate might be governed by “crowd-signaling substances” that accumulate in aphid mothers exposed to crowding (Ishikawa and Miura, 2013).

3 Effects of elevated temperature on aphid

Temperature significantly affects aphid performance and fitness (Angilletta, 2009). In general, a host of physiological functions (e.g. growth, metabolism, reproduction) occur optimally at an intermediate temperature (thermal optimum) and outside of this range, performance is reduced (Martin and Huey, 2008; Angilletta et al., 2002). At extreme high and low temperatures, performance may become inhibited and sustained exposure to these conditions can result in damage and injury that may eventually lead to death (Chown and Nicolson, 2004). The minimum temperature at which aphid development occurs is generally around 4 °C varying within and between species. For example, a range between 2.3 and 6.3 °C was estimated for *Acyrthosiphon pisum* (Harris) (Kilian and Nielson, 1971; Campbell and Mackauer, 1975; Lamb et al., 1987). Optimal temperatures and upper limits are also variable but usually in the range of 20 to 25 °C and 25 to 30 °C for most aphids (Harrington and Stork, 1995). Then, the rate of development in aphids is directly dependent on temperature. A female aphid requires a certain number of degree-days above the developmental threshold to reach adulthood (Harrington et al., 2001). This is variable with different studies showing it to range from 99 to 147 in *A. pisum* (Honek and Kocourek, 1990). This is a particularly short generation duration among insects.

Global warming favors the development of aphid populations. This effect is likely to be even stronger than for other insects, due to the short generation duration and extremely large reproductive capacity (Yamamura and Kiritani, 1998). Then, an increase in temperature of only 2 °C would allow the number of generations produced per year to increase from 18 to 23 (Howling et al., 1994) with a potentially huge

increase in population size. Other biological functions influenced by temperature include dispersal and reproduction. Aphid species disperse principally through the production of large numbers of winged individuals capable of flying considerable distances, both to move to a new plant when experiencing decreasing nutritional quality and for the transfer from winter to summer host plants in the case of species with seasonal alternation between different host plant species. Both the number of winged individuals produced and their flying capacity depend on temperature, with increasing temperatures favoring mobility. Lower temperature thresholds for flight are generally around 13-16 °C and upper thresholds around 31 °C (Kunert and Weisser, 2003).

4 Effects of elevated CO_2 on aphid

Increases in CO_2 concentration stimulate plant growth, but decrease the nutritional quality of plants for phytophagous insects (Lincoln et al., 1993). CO_2 is the raw material for carbohydrate production during photosynthesis. The carbohydrate play an important role in the life cycle of herbivores for storing and transporting the structural components (Rhodes et al., 1996). Rising environmental CO_2 concentrations will increase plant biomass (Amthor, 1995), including both leaf (Seneweera and Conroy, 2005) and root (Ferris and Taylor, 1994) which provide more chemical energy for longevity, fecundity and mobility of phytophagous insects. An elevation in atmospheric CO_2 also increases the carbon : nitrogen ratio (C:N) (Hartley et al., 2000; Johns and Hughes 2002; Chen et al., 2005; Ainsworth and Rogers, 2007). On one hand, increased of carbon content alters patterns of resource partitioning within the plant (Docherty et al. 1997). Organic solutes moving through the phloem affect the sieve element composition directly (Van Bel, 2003; Pritchard et al., 2007). In addition, extra carbon can change the distribution of some metabolites (Veteli et al., 2002; Goverde and Erhardt, 2003; Bae and Sicher, 2004), which may directly modulate aphid feeding behavior. On the other hand, nitrogen, mostly bound in amino acids and proteins, is a limiting factor for herbivores (Mattson, 1980; Wilkinson and Douglas, 2003). The reduction in nitrogen content would decrease insect growth and development (Mittler, 1967; Chen et al., 2005). The responses of aphids to high concentrations of CO_2 are highly variable. Depending on the aphid species considered, development and fertility rates may increase (Awmack et al., 1997; Mondor et al., 2005; Sudderth et al., 2005), decrease (Hughes and Bazzaz, 2001) or remain unaffected by such atmospheric changes (Percy et al., 2002; Awmack et al., 2004). Among 26 aphid-host plant combinations studies, 6 cases highlighted an increase in aphid fecundity and 5 cases showed a reduced production rate at elevated CO_2 (eCO_2) rate, while 12 combinations did not show any difference between elevated eCO_2 and ambient CO_2 (aCO_2) level (Holopainen, 2002). This matches with variability in plant species response to elevated CO_2, and diversity in herbivore performance may result from a differential change in the composition of various plant (Morgan et al., 2004).

5 Insect-plant interactions

Aphids are important model organisms in the study of insect–plant interactions (Rodriguez-Saona and Stelinski, 2009). Their complex lifecycles consist of several morphologically distinct stage including both apterae and winged adult forms, each of which may be specialized on a different range of host plants (Powell and Hardie, 2001). Some species overwinter on a primary host before migrating to a range of secondary hosts in the spring and establishing colonies. Overcrowding on host plant leads to the production of winged adults that move to and colonize new host plants within the secondary host range (Dixon and Glen, 1971; Leather, 1993). In spring, the eggs hatch and after one or two generations, parthenogenetic winged migrants (virginoparae) are produced. These migrants are able to disperse over long distances to a range of secondary herbaceous plants. Following this migration to new plants, the virginoparae are able to acquire and subsequently transmit non-persistent viruses (Dixon, 1971; Swenson, 1968). After settling on a host plant, they produce apterae aphid. However, when the colony becomes crowded or plant quality declines, the colony produces winged virginoparae that migrate in search for another host plant (Müller et al., 2001). As the summer progresses, aphids may become more common on different host-plant species as the availability of particular host plants changes. Finally, back to autumn, host-alternating aphids migrate back to the primary host to undergo sexual reproduction. Migration between summer and winter hosts and movement between summer hosts are therefore integral parts of the aphid life cycle, and this need to locate new host plants presents a challenge to aphids.

6 The response of aphid to plant volatiles

Plants release a variety of volatile organic compounds (VOCs), ranging from fatty acid derivatives, terpenoids, and sulfur compounds to phenylpropanoids (Qualley and Dudareva, 2009). Aphids use these VOCs to interact with their host plant and react to changes in their environment, especially at short distances as suitable cues for host location (Webster, 2012) or as pheromone for conspecific detection (Mustaparta, 1990). Earliest evidence of this came from attempts to assess the behavioral responses of different morphs of *Aphis fabae* to volatiles from its primary and secondary host plants using an olfactometer (Jones, 1944). Increasing evidence in previous years has shown that aphids can both respond to the odor of host plants and use olfaction to discriminate host from non-host (Pickett et al., 1992; Pickett and Glinwood, 2008). Much of the recent works on this topic focused on how plant volatiles provide information on plant identity to host-seeking aphids. Visser (1986) proposed two hypotheses. Firstly, insects respond to volatile compounds that are specific to insect host plants. Secondly, insects use blends of more commonly distributed volatile compounds, with species-specific ratios of different compounds being used to identify the plant. It has been suggested that the use of such blends is widespread among insect taxa and evidence is gradually accumulating to support this hypothesis (Bruce et al., 2005).

7 Aphid feeding behavior

To locate their host plants, aphids use the sensilla in antennae to distinguish the host plant from other environmental volatiles and locate the feeding sites on the leaf surface (Bromley and Anderson, 1982; Visser et al., 1996), such as color, shape, texture and odor perceived from their environment (Bruce et al., 2005). These information acquisition is gathered in a number of consecutive behavioral steps and integrated within the central nervous system (Bruce et al., 2005; Powell et al., 2006; Bruce and Pickett, 2011). After landing, and before stylet penetration, aphids evaluate plant surface characteristics (Powell et al., 2006). The chemical cues in the plant boundary layer, trichomes, epicuticular waxes, substrate topology and color, may influence their behavior (Ibbotson and Kennedy, 1959; Goffreda et al., 1989; Storer et al., 1996; Powell et al., 1999). Stylet penetration starts as brief probes to the epidermal layer during which small amounts of leaf sap are ingested (McLean and Kinsey, 1968; Tjallingii, 1985; Tjallingii and Esch, 1993). During probing the plant tissue, aphids' stylets transiently puncture epidermal, mesophyll, and parenchyma cells (Tjallingii and Esch, 1993). Aphid stylet eventually reaches to the plant sieve elements and keeps drawing phloem sap. Because phloem sap has high sucrose concentration, aphids sometimes suck xylem containing lower amounts of sugars and carbohydrates than the phloem to ensure the water balance (Spiller et al., 1990; Pompon et al., 2010). Winged aphids may also ingest xylem shortly after plant contact has been made (Powell and Hardie, 2002).

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2

Objectives and thesis structure

Current trends and projections of climate change in ecosystems encompasses not only increased mean temperatures and CO_2 concentrations but also increased variability and higher frequencies of extreme heat events, which has posed a great challenge for most of the ectotherm organisms. Aphids are strongly affected by environmental conditions. Elevated temperatures affect aphid directly by accelerating developmental rates and increasing the generation numbers, by reducing fecundity and also inducing some physiological responses. Enhancement CO_2 may alter larval feeding behavior, extend developmental time, reduce survival, decrease adult weight, and lower fecundity of aphid.

The first objective of this dissertation was to explore the degree to which a brief thermal exposure affects thermal tolerance across life stages with feeding and no feeding conditions. To achieve this aim, feeding effect on thermal tolerance of corn leaf aphid, *Rhopalosiphum maidis* was tested (Chapter III). Aphids exhibit polyphenism, which can be winged or wingless in order to adapt poor habitat quality. The second objective was to analyze the differential wing polyphenism adaptation across life stages under extreme high temperatures which provide an insight into understanding the two fundamental ecological processes, survival and dispersal (Chapter IV).

Also, the CO_2 effect was investigated as it not only affects plant growth and development, but also changes the emission of plant organic volatile compounds (VOCs), which may finally influence the aphid behavior. The third objective was to investigate the effects of eCO_2 on foraging behavior of *R. maidis*. The foraging behavior of aphids to chemical cues of host plant was assessed by using Y glass tube olfactometer. VOCs from isolated barley seedlings *Hordeum vulgare* L. reared under ambient CO_2 (aCO_2) and elevated CO_2 (eCO_2) conditions were analyzed by GC-MS (Chapter V).

The fourth objective was to assess eCO_2-mediated changes in the composition of phloem sap in barley and the resulting consequences for *R. maidis*. We quantified eCO_2-mediated changes in leaf chemistry including crude proteins, carbohydrates and free amino acids of barley seedlings. The biological parameters of aphid developed on barley seedlings grown under aCO_2 and eCO_2 conditions were assessed. The effects of eCO_2 on aphid stylet ingestion on host plants by tracking corn leaf aphid feeding behavior with electrical penetration graph (EPG) was determined (Chapter VI).

Finally, a conclusion and future prospects following our result are proposed (Chapter VII).

3

Differential thermal tolerance across life stages under extreme high temperatures crossed with feeding status

From Chen, Y., Quan, Y.D., Verheggen, F.J., Wang, Z.Y., Francis, F., & He, K.L. Differential thermal tolerance across life stages under extreme high temperatures crossed with feeding status in corn leaf aphid. Submitted to *Scientific Reports.*

Abstract: Climate change is predicted to increase the frequency and magnitude of extreme heat events, which will involve great challenges for most of the ectotherm organisms, such as insects. In this study, we address the thermal tolerance of corn leaf aphid, *Rhopalosiphum maidis* (Hemiptera: Aphididae) under extreme high temperatures across differential life stages. Immature instars and adults of *R. maidis* were exposed to brief events of increasing temperatures (0.5 °C min^{-1}), starting at 22.0 °C and reaching one of the 14 maximal temperature selected (from 38.5 to 44.5 °C). In each temperature regime, the mortality of *R. maidis* was compared with and without barley seedlings. Results showed that the critical high temperature of *R. maidis* was between 39.0 to 39.5 °C in no feeding treatments, while it was between 39.5 to 40.0 °C in feeding treatments. The upper lethal temperatures (ULTs) of *R. maidis* were significantly different between feeding and no feeding treatments. In addition, the ULTs varied significantly across life stages with highest ULTs values for 4^{th} instars. Feeding significantly increased the thermal tolerance of phytophagous insect. Variable balances in plant-herbivore interactions will be induced according to the insect feeding diet status and particular instar during the time of extreme temperature as illustration of global warming as part of climate changes. Our findings could be valuable in developing a reliable phenological model for the prediction of population dynamics of *R. maidis*.

Key words: climate change, upper lethal temperatures (ULT), *Rhopalosiphum maidis*, thermal tolerance, feeding

1 Introduction

The report from Intergovernmental Panel on Climate Change (IPCC) pointed out that the anthropogenic climate change has already caused approximately 1.0 °C of global warming and projected that global warming is likely to increase 1.5 °C between 2030 and 2052. Moreover, climate change will increase in frequency, intensity, and/or amount of extreme temperatures. The increase of extreme temperatures on land are speculated to higher than global mean surface temperature, which may increase by 3 °C at global warming of 1.5 °C (IPCC, 2018). The extreme high temperatures posed great challenges for most ectotherm organisms, because their biological functions are mostly driven by ambient temperature (May, 1979). The impact of temperature has been studied extensively on development and growth rates (Forster and Hirst, 2012), reproduction (Geister and Fischer, 2007) and survival (Huey and Berrigan, 2001) of ectotherms. However, experimental data are limited on thermal tolerance and mechanisms of ectotherms under extreme high temperatures.

In general, ectotherms have their own thermal optimum regime. When ambient temperature is out of the optimal ranges, the performance including growth, metabolism, reproduction, etc. will inevitably decrease (Martin and Huey, 2008). However, a number of studies have reported the rapid shifts in distribution, life cycle traits for many species under a global warming scenario (Parmesan and Yohe, 2003; Chown and Nicolson, 2004; Hsieh et al., 2009; Bale and Hayward, 2010; Chen and Ma, 2010; Franke et al., 2014; Xie et al., 2018). Even a brief exposure to extreme temperature could impact organism phenology (Semenov, 2008), species distribution (Overgaard et al., 2014), population dynamics (Welbergen et al., 2008), community structure (Ma et al., 2015), and sustained exposure to extreme high temperatures can result in damages that may eventually lead to death (Chown and Terblanche, 2006; Hoffmann et al., 2013). Therefore, determining the thermal tolerance is essential for understanding the effects of extreme heat events on fitness and dynamics of a given population under climate change scenarios (Terblanche and Chown, 2007).

Under field conditions, the temperatures fluctuate frequently (Kingsolver et al., 2012; Woods, 2013). The daily average temperature usually ranges from 22 to 26 °C during the corn growing season (May to September) in Langfang, Hebei, China, the maximum is 5 to 8 °C higher than average (http://data.cma.cn/). The daily maximum temperature often exceeds 35 °C in July. Therefore, the ectotherms typically experience thermally variable environments, their thermal performance in fluctuating temperatures may differ from the ones at constant temperatures (Hokanson et al., 1977; Martin and Huey, 2008; Colinet et al., 2015). For instance, egg-to-adult development under fluctuating temperatures increased the heat tolerance of Drosophila buzzatii compared with their development under constant temperatures (Sarup and Loeschcke, 2010). Larvae of tobacco hornworm, *Manduca sexta* L., reared in fluctuating temperatures had significantly higher optimal temperatures and maximal growth rates than larvae reared in constant temperatures (Kingsolver et al., 2011).

Although theories and empirical work has provided large evidences for well understanding the thermal biology of ectotherms (Blanckenhorn and Demont, 2004; Davidowitz et al., 2004; Kingsolver et al., 2009), other aspects such as interactions between temperature and other environmental factors have received limited attention. In particular, the physiology of the ectotherms transduces thermal experience into performance, which can be reversed by feeding behaviours (Diamond and Kingsolver, 2010). With the increasing temperature, the metabolism activity of ectotherms was enhanced (Dillon et al., 2010). Therefore, ectotherms need to increase food intake in order to gain enough energy for growth, reproduction or other basic functions (Swindell, 2012; McLeod et al., 2013; Warner et al., 2015). However, studies on thermal tolerance often ignored the influence of feeding which might be co-limiting or improve ectotherm performance (Koussoroplis and Wacker, 2016). Also, the effect of insect instar on the sensitivity related to extreme temperatures is poorly known and should be investigated. Therefore, understanding the relationships between the feeding status, the developmental stage and tolerance to thermal environment are the basis for most efficient predictions about insect responses to climatic variation and climate change.

Corn leaf aphid, *Rhopalosiphum maidis* (Hemiptera: Aphididae), is the most important aphid pest of cereals in tropical and warm temperature climates regions (Blackman and Eastop, 2000). Severe infestation of this aphid can cause serious yield and economic losses (Xie et al., 2014). In this study, feeding effect on thermal tolerance of *R. maidis* was tested. Life-specific (1^{st} to 4^{th} instars, apterous and alate adults) *R. maidis*, which were fed on barley leaves, were exposed to 14 temperature regimes. The aim of our study is to address three major questions with *R. maidis*: (1) How do brief high temperatures affect survival across life-stages? (2) How do temperature and feeding interact to affect survival? (3) Does feeding affect thermal tolerance? We address these questions by examining the upper lethal temperature (ULT) (basal tolerance) and critical high temperature (CHT), which are commonly considered as excellent index and standard for evaluating the thermal requirements and physiology of a given population (Lutterschmidt and Hutchison, 1997; Terblanche and Chown, 2007). CHT is the threshold of upper optimum thermal, which is a turning point. When temperature gets over this point, the mortality is generally considered to be significantly increased. The ULT is usually estimated from the temperature-response (mortality) curve (Beitinger et al., 2000).

2 Materials and methods

2.1 Plant material

Barley, *Hordeum vulgare* L., was sown in a black plastic pot (7.5 cm diameter and 9 cm high), with 25-30 seedlings per pot. These plants were cultivated in a greenhouse at 22.0 ± 0.5 °C with a 16 hour-light photoperiod. When plants grew up to two fully expanded leaves, they were used for aphid rearing.

2.2 Insect stocks

A colony of R. maidis originally collected from a corn field at the Experiment Station of Chinese Academy of Agricultural Sciences (39°30′42″N, 116°36′7′E) in Langfang, Hebei Province, China, was reared on barley seedlings in a cage (L 36×W 27×H 28 cm) at a constant temperature of 22.0 ± 0.5 °C, 65 ± 5% relative humidity (RH) and a photoperiod of 16 : 8 h (L : D). Active individuals used in the experiments had been reared for ten or more generations in the laboratory.

2.3 Developmental duration assay

In order to easily distinguish and get large amount of stages specific aphids for thermal testing experiments, the developmental duration of each instar was observed. The nymphs of aphids typically experience 4 instars and then moult to adults. Instars of the nymphs were systematically distinguished by recognizing every exuviae that were generally nearby the aphids from each moult (El-Ibrashy et al., 1972). Experiments were carried out in environmental chambers (Heraeus Group, HPS 500, Germany) at a constant temperature of 22.0 ± 0.5 °C, 65 ± 5% RH and a 16 hour-light photoperiod. Sixty neonate nymphs were observed in the experiment. By recording the moulting dates, we calculated the developmental duration of each stage under rearing condition. Barley seedlings were individually inserted into Eppendorf tubes (1.5 ml). The root of the seedling was wrapped with wet filter paper. A new nymph (<6 h old) was infested on the barley seedling (about 5 cm in height, 7 days after germination), which was placed into a transparent plastic box (9 cm diameter and 7 cm high) covered with double-deck gauze on the top. In order to clearly observe the aphids two filter paper disks were placed on the bottom of the box. Developmental stage of each aphid was recorded daily until aphids died. In order to get alate adults, alatiform 4^{th} instar aphids were selected from the rearing colonies and transferred into the new pots, about 2 days later, newly alate adults were well developed.

2.4 Upper lethal temperature (ULT) assays

The experimental treatments included six developmental stages of *R. maidis* (1^{st} to 4^{th} instars, apterous and alate adults), feeding and no feeding status (with or without barley seedlings), and 14 temperature regimes.

In the no feeding treatment, 30 same aged aphids were transformed to a transparent plastic box (the same box used in developmental time test). Triplicates were performed here with 90 aphids in total per treatment with feeding and no-feeding treatments across 14 temperatures. In feeding treatments, barley seedling was provided (using the same protocol as described in developmental time test) to feed the aphids in transparent plastic box, all the subsequent processes were similar to no feeding treatments.

Experiments were carried out in environmental chambers (HPS 500, Germany) to achieve the 14 temperature regimes with 65 ± 5% RH (Fig. 3-1). The temperature

was risen at 0.5 °C min^{-1} from rearing temperature of 22.0 °C to each maximal temperature (ranging from 38.5 to 44.5°C) and held at the maximal temperature for 60 s, then returned at 0.5 °C min^{-1} rate to 22.0 °C. Aphids were then transferred into the environmental chambers at a constant temperature of 22.0±0.5 °C, 65±5% RH and a 16 hour-light photoperiod. Mortality was recorded in 24 h.

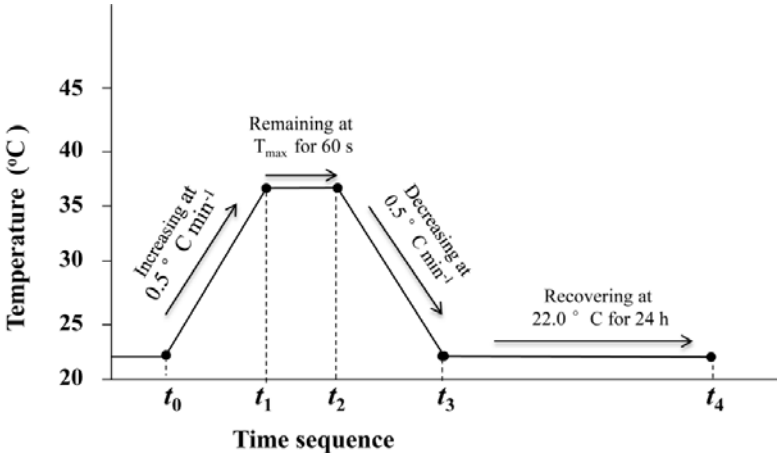

Figure 3-1: Brief extreme high temperatures of ULT assays. Time from t_0 to t_1, the treatment temperature was increased from rearing temperature of 22.0 °C to maximal temperature (T_{max}) at a rate of 0.5 °C min^{-1}; from t_1 to t_2, the temperature was holding at T_{max} for 60 s; from t_2 to t_3, the temperature was decreased from T_{max} to rearing temperature of 22.0 °C at a rate of 0.5 °C min^{-1}; From t_3 to t_4, the aphids were reared at 22.0 °C for 24 h. T_{max} ranged from 38.5 to 44.5 °C with an increased interval of 0.5 °C for 13 temperature regimes.

2.5 Statistical analysis

Mortality data from each temperature, feeding status and life stages treatments were subjected to an analysis of variance by using the general linear model with SAS procedure PROC GLM (SAS V9.2). Treatment means were compared using Fisher-protect least significant difference (LSD) test to determine significant difference at a 95% confidence level. All percentage data were normalized using the transformation y = arsine $(x/100)^{1/2}$ before test. Because all aphids could not survive after exposed to the brief extreme temperature of 44.5 °C, therefor, data from these treatments were excluded in the analysis of variance. Mortality curves for the different life stages as a function of temperatures with either feeding or no feeding status were fitted using logit model by PoLoPlus Program (LeOra Software), which yielded the values of sample size, ULT50 and ULT90 with 95% fiducial limits, slope with standard errors (Slope±SE), chi-square ($\chi 2$) values and heterogeneity. We used the components from PoloPlus logit analyses to calculate 95% confidence intervals (CI) for the ratio of ULT_{50}s and ULT_{90}s. The variations among the slopes were estimated by u test (SAS V9.2).

3 Results

3.1 Developmental duration under laboratory conditions

The developmental duration of *R. maidis* reared in the laboratory conditions was almost synchronized. The developmental duration for 1st and 4th instars reared on barley seedlings at 22 °C was about 2.0 days, whereas 2nd, and 3rd instars required about 1.5 days to complete developmental duration. Therefore, excepting 1st instar was new nymphs (<6 h old), the 2nd, 3rd, 4th instars and adults used for experiments were approximately 2.0, 3.5, 5.0, and 7.0 days after born from apterous parthenogenetic adults.

3.2 Effects of extreme temperature, feeding status and life stage on survival of R. maidis

The brief extreme high temperature played a significant impact on performance of *R. maidis* (Table 3-1). Comparing to the control (22.0 °C), the mortality of *R. maidis* was not significantly different when they experienced to the exposure of brief extreme high temperatures of 38.5 °C. However, as the brief extreme high temperature rose up from 38.5 °C, the mortality was increased significantly (Fig. 3-2). The aphid could not survive after exposed to the brief extreme temperature of 44.5 °C. Feeding could significantly enhance the performance of *R. maidis*. The average mortality declined to 9.7% in the aphids from feeding compared to no feeding. Survivals of the aphid were significantly different across the life stages.

Table 3-1: Summary results of a general linear model (GLM) analysis of the effect of temperature, feeding status and stage on the mortality in *Rhopalosiphum maidis*.

Source	df	*F*	*P*
Temperature	12	717.38	< 0.001
Feeding status	1	254.77	< 0.001
Stage	5	214.79	< 0.001
Temperature * Feeding status	10	19.26	< 0.001
Feeding status * Stage	5	28.38	< 0.001
Temperature * Stage	52	5.52	< 0.001
Temperature * Feeding status * Stage	42	1.49	0.030

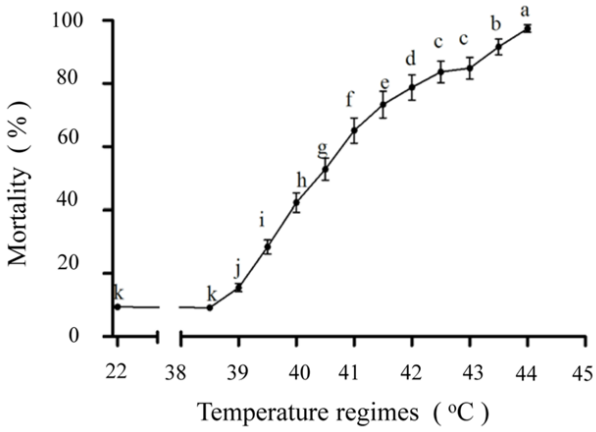

Figure 3-2: Means and standard errors of the mortality of *Rhopalosiphum maidis* under 13 temperature regimes. Means with same letters indicated no significant difference based on Fisher's Protected LSD test ($\alpha \geq 0.05$).

3.3 Critical high temperature (CHT) across life stage

The bi- or tri-interactions among the brief extreme high temperature, feeding status and life stage for the aphid survival were significant (Table 3-1). The mortalities varied significantly when *R. maidis* experienced to different levels of brief extreme temperature events across life stages in no feeding and feeding treatments (Fig. 3-3, Fig. 3-4). Comparing to the control (22.0 °C), the mortality of *R. maidis* was not significantly different when they experienced to the brief extreme temperatures of 39.0 °C in no feeding treatments. The mortality was significantly accelerated when they experienced to >39.0 °C exposure of brief extreme high temperature events. The 1st to 4th instars could not survival when they experienced to 41.5 to 43.5 °C exposure of brief extreme high temperature events, respectively. The CHT of *R. maidis* was between 39.0 to 39.5 °C. In addition, the 3rd and 4th instars were more tolerant to brief extreme high temperature events when they were fed. The CHT was ranging from 39.5 to 40.0 °C.

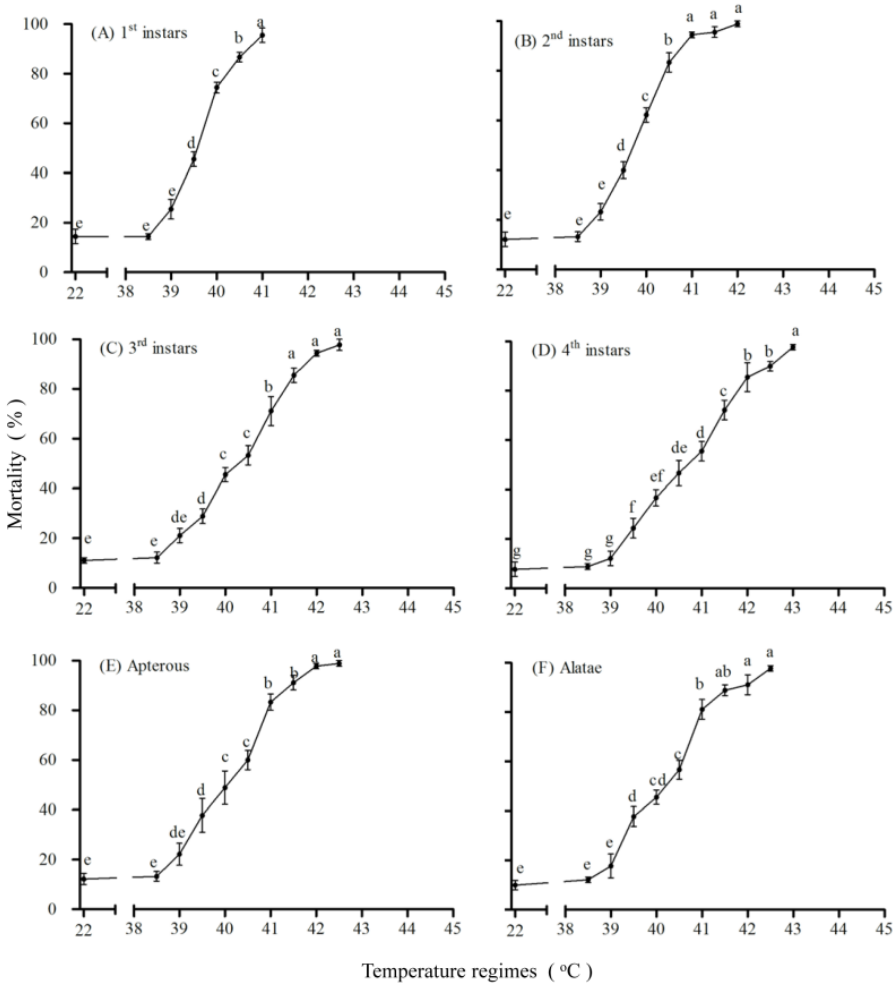

Figure 3-3: Mortality of life specific *Rhopalosiphum maidis* exposed to different levels of brief extreme temperatures in no feeding treatment. (A) to (F) represented the 1st to 4th instar, apterous and alatae adults.

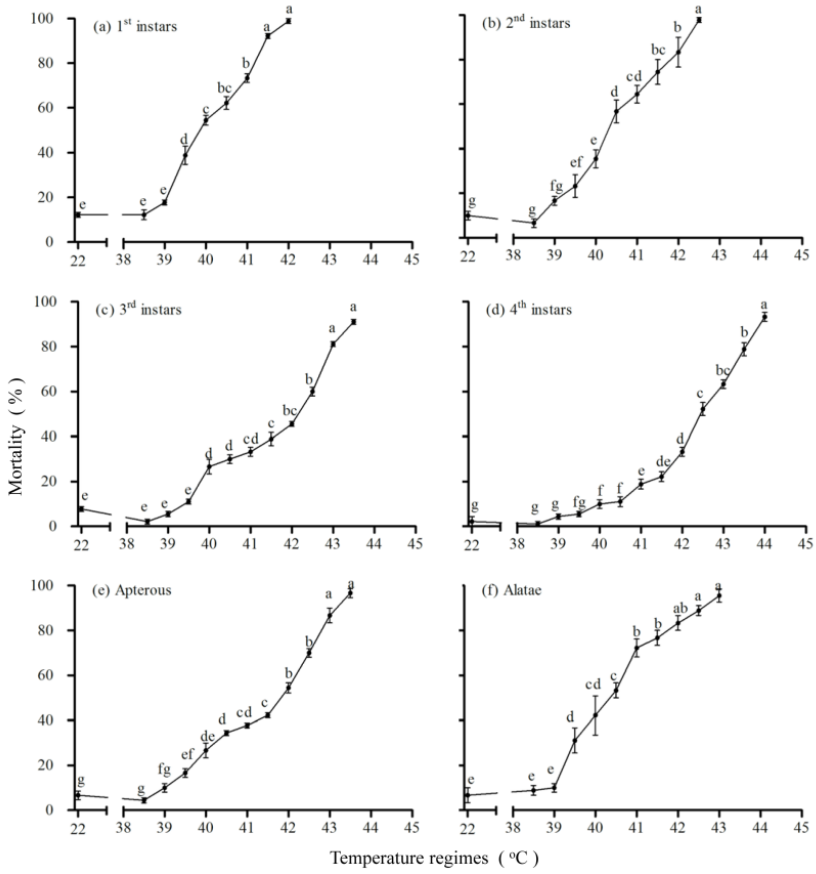

Figure 3-4: Mortality of life specific *Rhopalosiphum maidis* exposed to different levels of brief extreme temperatures in feeding treatment. (a) to (f) represented the 1st to 4th instar, apterous and alatae adults.

3.4 Upper lethal temperature (ULT) of R. maidis

Exposure (of brief extreme high temperature events)-response (mortality) relationship in *R. maidis* was fitted logit model (Table 3-2). The ULT_{50} and ULT_{90} (upper lethal short temperature necessary for 50% and 90% mortalities) values ranged from 39.5 to 40.6 °C and 40.6 to 42.4 °C among four instars, apterous and alatae adults. There were significant differences (P<0.05) in tolerance to short extreme temperature events among developmental stages of *R. maidis*. The 4th instar was the most tolerant. In contrast, the 1st instar was the most susceptible. In addition, the ULTs were higher in feeding *R. maidis* than in no feeding, especially the ULT_{90}. Meanwhile, the slopes of the exposure response regression equations were significantly greater in feeding *R. maidis* than in no feeding ones. These results suggested that feeding could increase the heat tolerance in *R. maidis*.

Table 3-2: Values of ULT_{50}s and ULT_{90}s (95% fiducial limits) of life stage specific *Rhopalosiphum maidis* in no feeding and feeding treatments (Logit model).

Status	Stages	n[a]	ULT_{50}(95% fl) °C	ULT_{90} (95% fl) °C	Slope ± SE	χ^2	df[b]	h[c]
No Feeding	1st instar	630	39.5 (39.4-39.6) c	40.6 (40.5-40.9) c	1.9±0.2 a	5.9	15	0.39
	2nd instar	720	39.7 (39.5-39.8) c	40.9 (40.7-41.1) c	1.7±0.1 a	9.2	19	0.48
	3rd instar	810	40.2 (40.0-40.3) b	41.8 (41.6-42.1) b	1.2±0.1 bc	13.3	23	0.58
	4th instar	900	40.6 (40.4-40.7) a	42.4 (42.2-42.7) a	1.2±0.1 c	17.4	27	0.64
	Apterous	810	39.9 (39.7-40.1) b	41.5 (41.3-41.8) b	1.4±0.1 b	18.5	22	0.84
	Alatae	810	40.1 (39.9-40.2) b	41.7 (41.5-41.9) b	1.3±0.1 bc	17.8	24	0.74
Feeding	1st instar	720	39.9 (39.8-40.1) d	41.6 (41.3-42.0) d	1.3±0.1 a	9.9	19	0.52
	2nd instar	810	40.5 (40.3-40.6) c	42.3 (42.0-42.7) c	1.2±0.1 ab	21.8	23	0.95
	3rd instar	1080	41.7 (41.5-41.9) b	44.0 (43.8-44.5) ab	0.9±0.1 c	26.4	31	0.85
	4th instar	1170	42.3 (42.1-42.5) a	44.3 (44.1-44.7) a	1.1±0.1 b	20.3	34	0.60
	Apterous	1080	41.4 (41.2-41.6) b	43.7 (43.2-44.4) b	0.9±0.1 c	26.3	29	0.91
	Alatae	1080	40.4 (40.2-40.5) c	42.3 (42.1-42.6) c	1.1±0.1 b	25.0	27	0.93

4 Discussion

As the natural stresses are often variable and ubiquitous, establishing a clear definition and standards for the effect of environment on insects has been challenging. Extreme summer temperatures during the growing season in the natural habitats are predicted to increase in both intensity and frequency in geographic region of *R. maidis* in China (Wang et al., 2002). The main goal of our study was to explore the degree to which a brief thermal exposure affects thermal tolerance across life stages with feeding and no feeding.

The majority of studies often emphasized the effect of constant temperature on the life history of aphid. It has been stated that 30 °C is a lethal temperature for nymphs of *Sitobion avenae* (Fabricius), *Metopolophium dirhodum* (Walker) (Asin and Pons, 2001; Dean, 1974), and constant 35 °C was lethal to immature *Aphis gossypii* (Glover) (Satar et al., 2005). A few nymphs of *R. maidis* survived and completed development at 35 °C (Kuo et al., 2006), while 26-28 °C had a higher impact on the mortality rate of *Nasonovia ribisnigri* (Mosley) (Diaz and Fereres, 2005). However, organisms in natural environments experience fluctuations rather than constant temperatures (Colinet et al., 2015), and constant temperatures is limited in prediction about development rates of organisms, such as marsh frog (Niehaus et al., 2012), tobacco hornworm (Kingsolver et al., 2015), aphid (Ma et al., 2015). Moreover, only few studies have focused on the response of corn leaf aphids to brief extreme high temperature events. In this study, we imitated natural fluctuated extreme climates as much as possible, considered most important abiotic (temperature) and biotic (food) factors, combined with the aphid own physiological conditions (life stage) to investigate thermal tolerance of *R. maidis* under brief

extreme high temperatures. Our results indicated that the CHT was 39 ^{o}C for *R. maidis* with a brief extreme high temperature. This information could be valuable in developing a reliable phenological model for the prediction of population dynamics of *R. maidis*.

In our experiments, the highest ULT_{50}s and ULT_{90}s were observed in 3rd and 4th instars compared to 1st and 2nd instar nymphs and adults. These results are consistent with some studies addressing stage-related variation in thermal sensitivity, reporting a greater thermal resistance in young stage compared to adults (Zizzari and Ellers, 2014; Zhang et al., 2015; Zhao et al., 2017). The younger instar nymphs were less thermal tolerant, which was probably because of their low mobility and weak thermal inertia to use behaviour to evade extreme conditions (Zhang et al., 2015). Adult survival might be depressed if there is a trade-off between survival under stress and reproductive output (Cox et al., 2010; Marshall and Sinclair, 2010) or as a result of senescence (Colinet et al., 2013). Once sexual maturity is reached, an individual's energy budget might evolutionarily support an increased fitness by spending more effort on reproduction (Sørensen and Loeschcke, 2002). This might evidently result in a physiological trade-off between protection and reproduction and may account for the observed differences in survival rate between juveniles and adults exposed to heat shock.

While ectotherms do not maintain a constant body temperature, they make behavioural, physiological and cellular adjustments to maintain thermal homeostasis (Bicego et al., 2007). The interaction between energetics and life history traits is evident from studies showing different diet regimens producing a range of outcomes in traits such as reproduction and lifespan. For example, dietary restriction can extend lifespan in many species (Swindell, 2012), whereas increased food availability can result in more and larger offspring (Warner et al., 2015). This response to energy availability can be described as a form of phenotypic plasticity (Wada and Sewall, 2014). In our study, feeding with barley seedlings significantly increased thermal tolerance of the aphids regardless the developmental stages when compared with no feeding treatments. Feeding treatments also increased a constant heat tolerance. One of the possible reasons is as a food source, barley seedlings provide nutrition and water supply which could guarantee the basic life requirements of the aphids and indirectly increased their thermal tolerance. Studies indicated that the metabolism activity of ectotherms enhanced exponentially as the temperature increased (Dillon et al., 2010). Therefore, ectotherms need to increase food intake in order to rapidly balance water loss through mass feeding (Klok and Chown, 2001; Benoit et al., 2007) and gain enough energy for growth, reproduction or other basic functions (McLeod et al., 2013). In addition, barley seedlings could provide suitable micro environmental for aphids to avoid high temperatures. Previous study showed that the 1st instar aphids usually inhabited on the underside of leaves, where temperature was lower during day (Ma et al., 2004).

In conclusion, our results highlighted that feeding can increase thermal tolerance of herbivorous insect under brief high temperatures cross-life stages. The responses to thermal extremes were found to be different with a significant degree of variation

when comparing fed or food stress aphids but also mainly when coupled with the considered developmental stages. Variable balances in plant-herbivore interactions will be induced according to the insect feeding diet status and particular instar during the time of extreme temperature as illustration of global warming as part of climate changes.

Our study is helpful to reveal the thermal tolerance mechanism of small arthropod under extreme climates according to developmental stages. From an evolutionary perspective, extreme events may serve as major selective factors that influence the evolution of physiological capacities and resistances (Gutschick and BassiriRad, 2003; Hoffmann et al., 2003; Denny et al., 2009; Somero, 2010). Further studies will be required to determine the relative importance of costs and benefits of phenotypic plasticity under extreme climates. For instance, further testing is needed to assess thermal resistance and winged aphid induction and how this will affect population persistence. Furthermore, studies on field-acclimatized individuals are required to elucidate how seasonal heterogeneity influences winged-induced genetic variation in physiological sensitivity but also in further spatio-temporal dynamics of insect natural communities as a major challenge.

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4

Differential wing polyphenism adaptation across life stages under extreme high temperatures in corn leaf aphid

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Abstract: Polyphenism, a common phenomenon in nature, is an important form of adaptation in a diverse environment. Corn leaf aphid (CLA), *Rhopalosiphum maidis*, (Hemiptera: Aphididae), exhibit wing polyphenism in response to poor habitat quality. In this study, we focused on the effects of crowding and thermal cues on morph determination of CLA. Five developmental stages of aphids (1^{st} to 4^{th} nymphs and maternal adults) with increased population densities, were tested under two kinds of temperature patterns, i.e., A) a constant temperature of 22 °C with 2 h exposure to high temperature in the range of 35 to 39 °C during mid-photophase and B) different constant temperatures in the range of 22-30 °C with 2 h exposure to high temperature of 39 °C during mid-photophase. Crowding was found to directly impact winged induction. The 1^{st} and 2^{nd} nymphs were more sensitive for alate morphs induction under high density. In addition, temperature played a significant role in wing production, with the temperature setting of 26/39 °C in pattern B inducing higher alate morphs and survival than other temperature settings. So, we hypothesize that warmer climate with brief high temperature is more favorable for survival and alate morphs production, but cool weather and transient extreme high temperature (>39 °C) is detrimental for CLA. Our results provide a new perspective on understanding the interactions between changes in extreme high temperatures and insect densities that differentially affect wing polymorphism for further demographic and distribution rates of species across temporal and spatial scales.

Keywords: Climate change, polyphenism, *Rhopalosiphum maidis*, crowding, temperature

1 Introduction

Polyphenism is a form of developmental plasticity in which organisms respond to environmental cues by producing adaptive, discrete, alternative phenotypes known as morphs (Brisson and Davis, 2016). This phenomenon is common and important as a form of adaptation and a source of variation for natural selection. Particularly, the ambient temperature is the main abiotic factor influencing development and further vital stages in insect life cycles. The impact of extreme high temperature in the context of global warming should than be investigated more precisely considering individual developmental stage for direct effects but also for further adult morph induction. Aphids exhibit polyphenism, as genetically identical individuals can potentially show different phenotypes (Braendle et al., 2006; Grantham and Brisson, 2018). Compared to the apterous phenotype, the winged aphids have a longer nymphal development period, lower offspring production, and higher longevity (Johnson, 1957; Mackay and Wellington, 1975; Tsumuki et al., 1990; Simon and Peccoud, 2018). Moreover, winged forms are also more resistant to starvation (Hazell et al., 2005). The morphological and physiological characteristics of winged aphids enable them to survive in harsh conditions, have the chance to disperse and clone to a new environment (Dixon et al., 1993).

First, a considerable number of studies have addressed the environmental conditions that affect the production of winged individuals in aphids including both biotic (crowding, nutrition, interspecific interactions, natural enemies, alarm pheromone, maternal, etc.) and abiotic (temperature, photoperiod, precipitation, etc.) factors (Kawada, 1965; Mittler and Sutherland, 1969; Kunert and Weisser, 2003; Kunert et al., 2005; Brisson, 2010; Mehrparvar et al., 2013; Grantham et al., 2016). Crowding has been established as a prime factor in the production of winged forms among aphids: higher aphid densities lead to more tactile stimulations between individual aphids, triggering wing induction (Johnson, 1965; Lees, 1967; Purandare et al., 2014; Martínez and Costamagna, 2018;). However, the stage in the life history of the aphid at which crowding has the most influence differs between species(Shaw, 1970). The sensitivity of *Rhopalosiphum insertum* (Walker), *Therioaphis ononidis* (Kalt.), *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulz.) to crowding seems to be confined almost entirely to the first instar (Bonnemaison, 1958; Noda, 1958; Kawada, 1964; Toba et al., 1967). On the other hand, Awram (1968) showed that *M. persicae* may belong to a class of species typified by *Macrosiphum granarium* and *Aphis craccivora* (Koch) (Awram, 1968), in which prenatal crowding of mothers and postnatal crowding of larvae both influence the production of winged forms (Johnson, 1965; Noda, 1960). However, the sensitive stage of Corn leaf aphid (CLA) for winged induction is still unclear.

Second, the increased temperature, which is a major response of global change, has a direct impact on the life activities of ectotherms, such as survival, development, fecundity and migration (Hoffmann et al., 2013; Colinet et al., 2015; Sentis et al., 2017). Similar to all ectotherms, aphids have acute sensory capability for detecting temperature variations (Chen and Ma, 2010). There were different

opinions in the study of temperature affecting winged aphid induction. Most research reported that low temperature would induce *M. persicae*, *L. erysimi*, *B. brassiae*, *A. glycines*, and *Macrosiphoniella sanborni* to become alatae, and high temperature would inhibit wing dimorphism (Johnson, 1965; Lees, 1966; Lv and Chen, 1993). However, Diaz and Fereres found that an increase in temperature led to a significant increase in the proportion of alatae lettuce aphid *Nasonovia ribisnigri* (Diaz and Fereres, 2005). Müller et al. pointed out that higher temperature might be expected to influence aphid morph determination indirectly, as associated with the increase in activities and contacts between aphids, which may result in more winged aphids (Müller et al., 2001). Interactions between temperature increase and insect density are being studied for the cumulative and critical role on the production of winged aphids.

The corn leaf aphid, *Rhopalosiphum maidis*, (Hemiptera: Aphididae), is an important herbivorous insect pest that feed on corn, barley, millet and many other plants. Similar to most Aphidinae species, CLA can have wings or be wingless, but the roles of particular environmental factors on wing polyphenism have not been examined in this species. The aim of this work was to study the wing polyphenic response both to crowding and thermal cues in CLA, depending on the considered developmental stage under selective pressure. We also consider maternal effect to see whether it is true in our CLA colony. Different temperature patterns were used to identify the thermal environment that induces winged aphids, and the interaction effect between crowding and temperature was analyzed. This information will improve the ability to forecast the occurrence and potential changes in temporal and geographical distribution of this kind of insect under global warming.

2 Materials and methods

2.1 Plant material

Barley, *Hordeum vulgare* L., was sown in a black plastic pot (7.5 cm diameter and 9 cm high), with approximately 30 seedlings per pot. These plants were cultivated in a greenhouse at 22.0 ± 0.5 °C with 16:8 h (L:D) cycle. When plants grew up to two leaves fully expanded, they were then used for aphid rearing.

2.2 Aphid rearing

A corn leaf aphid, *R. maidis*, colony was reared on barley seedlings that were planted every week in a climate-controlled room at 22 ± 0.5 °C, 65% relative humidity, and a photoperiod of 16 L:8 D h. Aphids were maintained for at least three generations at a low population density (five individuals per plant) prior to being used in the experiments.

Based on the mass rearing of CLA, the development durations of 1st and 4th instars were approximately 2.0 days whereas 2nd and 3rd instars required approximately 1.5 days. Therefore, excepting 1st instars that are new nymphs (<6 h old), the 2nd, 3rd and 4th instars used for experiments were approximately 2.0, 3.5, and 5.0 days,

respectively. Before the start of the experiments, adult CLA were placed into new pots, and 6 h later, the adults were removed, and new born first instars were reared on the barley seedlings. In this way, we could obtain the synchronously developed stage for respective experiments.

2.3 Experimental design

A glass capillary tube (6 mm diameter and 30 mm height) was used as an experimental cell, which was filled in 3 ml of 2% bacto agar mixed with Miracle-Gro (The Scotts Miracle-Gro Company, Marysville, Ohio) as a medium. A piece of barley seedling (5 mm width × 20 mm height), rather than plants, was inserted into the medium. The top of the glass tube was covered with double-deck gauze to avoid aphid escape. Each tube, with a piece of barley seedling, was used as an experimental unit. One to fifty aphids were infested per unit on the barley seedling in the following experiments. All experiments were carried out in the environmental chamber (Heraeus Group, HPS 500, Germany) at 65 ± 5% RH and a photoperiod of 16 L:8 D, and tested aphids were allowed to develop to adults. Finally, the number of survivors and winged morphs was recorded.

2.4 Crowding experiment

The experiments were conducted at 22 °C including four development stages of aphids (1^{st}, 2^{nd}, 3^{rd}, and 4^{th} instars) and five population densities (1, 5, 10, 30, and 50 aphid(s) per experimental unit). We crowded each stage with five population densities. For example, when the population density was 10, 10 nymphs of the same stage were placed into an experimental unit. Twenty-four hours after crowding, these nymphs were individually transferred to their own experimental unit (for a total of 450 nymphs in each treatment). The numbers of survivors and winged morphs was recorded after these nymphs had developed into adults.

2.5 Maternal effects

The newly enclosed adult aphids were carefully collected from rearing condition using a brush and then placed in the experimental unit at densities of 1, 5, 10, 30, and 50 aphid(s). After 24 h of crowding, one adult from each of 5, 10, 30, and 50 density treatments was randomly selected and placed into a new experimental unit to assess its fecundity. The offspring of the adult were individually transferred to a separate unit daily and allowed to develop into an adult. Finally, survival and winged morphs were recorded. There were 10 replicates for each density.

2.6 Temperature experiment

Each experimental unit infested with 10 1^{st} instars (< 6 h) was subjected to two patterns of temperature regimes. Each pattern had four temperature settings. In pattern A, the nymphs were reared at 22 °C with a transient (2 h) high temperature of 35, 37, 39 or 41 °C. The transient (2 h) high temperature exposure was set at mid-photophase. In pattern B, the nymphs were reared at 24, 26, 28, or 30 °C with a transient (2 h) high-temperature of 39 °C. The transient (2 h) high temperature

exposure was also set at mid-photophase. The constant environment temperature at 22 °C is considered as the control. All experiments were maintained at 65 ± 10% RH, with a 16:8 h light: dark photoperiod. There were 45 replicates for each treatment. The number of survivors and alatae was checked after tested nymphs developed into adults.

2.7 Assessment of interaction between crowding and temperature

The experiment was conducted as 2 factors with a completely randomized design including three temperature levels (rearing temperature/2 h high temperature: 24/39 °C, 26/39 °C, 28/39 °C) and two density levels (10 and 50 1st instars per unit). There were 45 replicates for each treatment. The number of survivors and alatae was recorded after the 1st instars developed into adults.

2.8 Statistical analysis

The proportion of survival and winged aphids between different treatments was subjected to an analysis of variance by using the general linear model with SAS procedure PROC GLM (SAS V9.2). Before analysis, all data were checked for normality and homogeneity of variances using the Kolmogorov-Smirnov and Levene's test. The percentage data were normalized using the transformation y=arsine (x/100)1/2. Treatment means were compared using Tukey's multiple range tests to determine significance at a 95% confidence level. The logit model was used to fit the survivorship of the nymphs under five densities. A Chi-square contingency table was used to analyse the crowding effect on alate morphs induction among nymphs. Correlation analysis was used to analyse the relationship between population density and percentage of winged nymphs.

3 Results

3.1 Crowding effect on survival of nymphs

Crowding induced a rather low (<10%) but constant mortality to all instars by comparing different population density treatments across the lowest to the highest. Accordingly, the survivorship of the aphids reared under five densities was well fitted to the logit model (Table 4-1). In addition, the survival rate was approximately 10% lower for a younger stage than an elder stage (Fig. 4-1) under the same population density.

Table 4-1: Values of survivorship at each developmental stage of *Rhopalosiphum maidis* under five densities (Logit Model).

Instar	n	Intercept	Slope	$\chi 2$	df	Heterogeneity
1st	450	-0.442±0.082	0.190±0.071	0.919	3	0.306
2nd	450	-0.928±0.089	0.129±0.076	0.788	3	0.263
3rd	450	-1.512±0.103	0.181±0.088	1.072	3	0.357
4th	450	-2.418±0.140	0.397±0.115	1.160	3	0.387

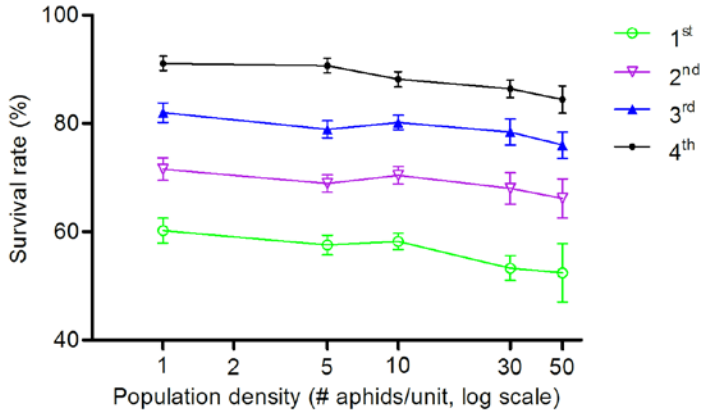

Figure 4-1: Survivorship (mean±SE%) of *Rhopalosiphum maidis* at each developmental stage with different population densities under 22 °C.

3.2 Crowding induction of alatae

The effect of crowding on alate morphs induction varied among the nymphs. The 1st and 2nd instars were significantly affected by crowding in the production of alate morphs, whereas the 3rd and 4th instars were not significantly affected by crowding (Table 4-2). In addition, the 1st instar was 2 times more sensitive than the 2nd instar in response to crowding.

Table 4-2: Values of alatae induction at each developmental stage of *Rhopalosiphum maidis* under five densities (Chi-square Contingency Table Analysis).

Instar	df	$\chi 2$	*P*
1st	4	24.06	< 0.001
2nd	3	9.07	0.028
3rd	3	1.21	0.750
4th	2	0.96	0.619

For the 1st and 2nd instars, the correlation between alate morph proportion (ya and yb) and five empirical population densities was well fitted with second-order polynomial models (Fig. 4-2a-b), respectively: $y_a=0.656+0.448x-0.005x^2$, adjusted $r^2=0.962$; $P<0.05$; $y_b=0.282+0.274x-0.003x^2$, and adjusted $r^2=0.969$; $P<0.05$.

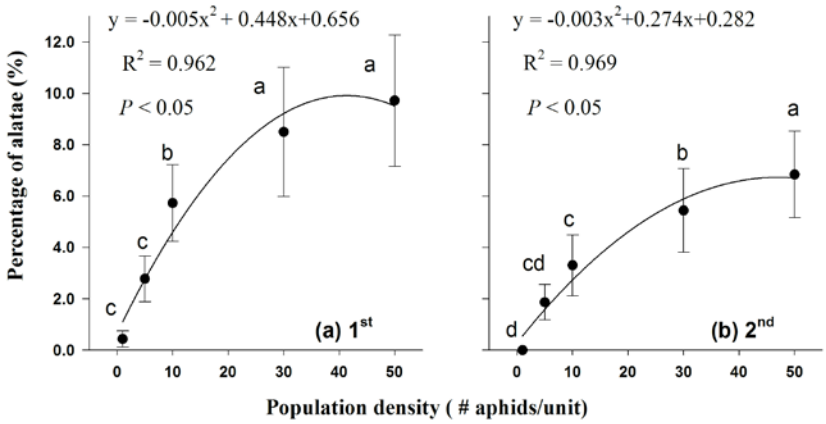

Figure 4-2: Modulation of population density on alate morphs production of *Rhopalosiphum maidis*: (a) Crowding treatment at 1st instar; (b) Crowding treatment at 2nd instar. The curved lines in (a) and (b) were generated based on the regression model using the data shown in the scatterplot in each graph. Different letters indicate significant differences (P<0.05) by using Tukey's multiple range tests.

3.3 Maternal effects

Differences in aphid density in the maternal environment did not influence their survival rates ($F_{4,45}$ = 0.61, P = 0.659), but impacted their offspring polyphenism ($F_{4,45}$ = 6.29, P < 0.001) when aphid adults were crowded with five population densities. A strong wing polyphenic response of the first generation was observed. Under the population density of 50, the alatae rate of first generation was approximately 10.5%, which was significantly higher than population density of 1 and 5, which was 0.9% and 3.7%, respectively (Fig. 4-3).

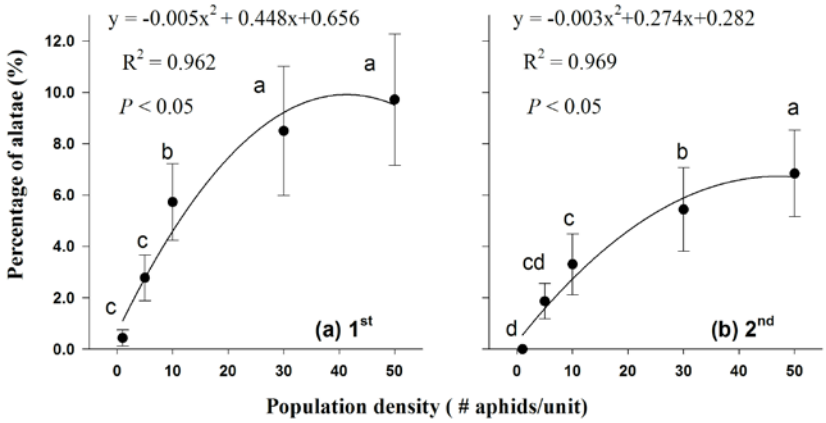

Figure 4-3: Maternal crowding effect on offspring survival and alatae of *Rhopalosiphum maidis*. Different letters indicate significant differences (P<0.05) by using Tukey's multiple range tests.

3.4 Effects of transient high temperatures on survival and alate development

Temperature pattern A ($F_{4, 220}$ = 433.59, P<0.001) and temperature pattern B ($F_{4, 220}$ = 227.28, P<0.001) had a significant effect on the survival rate of CLA. The survival rate was significantly increased when the aphids were reared under temperature pattern A, i.e., constant temperature of 22 °C with a transient (2 h) exposure to a high temperature in the range of 35 to 39 °C during mid-photophase (Fig. 4-4a), whereas 41 °C was detrimental to the aphids, which significantly decreased their survival. In temperature pattern B, the survival rates were significantly high at constant temperatures between 22-26 °C with a transient (2 h) exposure to high temperature of 39°C during mid-photophase (Fig. 4-4b). High temperatures (28-30 °C) significantly affected the survivorship of CLA.

Temperature patterns A ($F_{4, 220}$ = 9.49, P < 0.001) and B ($F_{4, 220}$ = 65.53, P < 0.001) also resulted in significant differences in the induction of alatae. When nymphs were reared under pattern A, the percentage of alatae generated was increased from 7% to 9% as the transient high temperature increased from 35 to 39 °C. Although there were no significant differences in the induction of alatae among three temperature settings, the proportion of alatae at 22/39 °C was significantly higher than control that at 22 °C (Fig. 4-4a). When nymphs were reared under the temperature pattern B, the percentages of alatae were significantly different among these settings. In general, as the temperature increased from 24 to 28 °C, the percentage of alatae was increased from 10% to 27%. However, when temperature was as high as 30 °C, the percentage of alatae declined to 3%, while the suvival was the lowest (Fig. 4-4b).

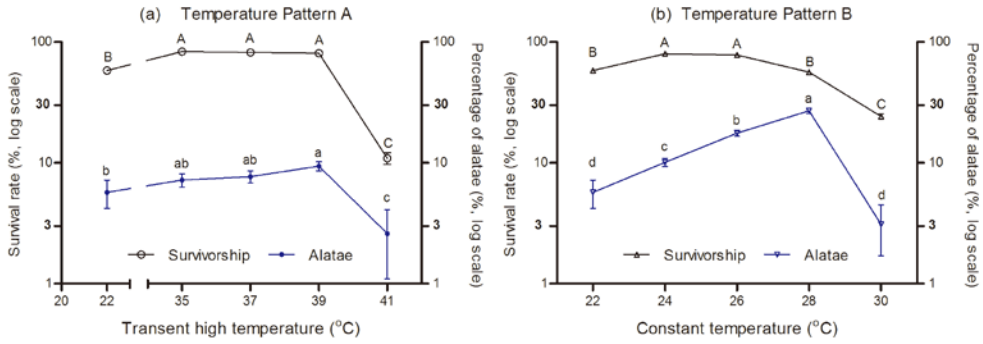

Figure 4-4: Survivorship and alatae induction (mean±SE%) of *Rhopalosiphum maidis* at two temperature patterns: (a) Temperature pattern A; (b) Temperature pattern B. Different capital or lowercase letters represent significant differences (P<0.05) by using Tukey's multiple range tests.

3.5 The interaction effects between crowding and temperature

Crowding and temperature significantly affected the proportion of aphid survival and alatae (Table 4-3). The temperature was found to have a larger influence than density on both aphid survival and alatae emergence. As the density and temperature accelerated, the proportion of survivors was dramatically decreased, while aphid alatae the rate was significantly increased. The proportion of alatae was increased by up to 41% at 28/39 °C when density was 50 nymphs per tube, while the survival rate was as low as 43% (Table 4-4). However, no significant interaction effect was found on survival rates and alatae emergence.

Table 4-3: Summarized models of the effects of density[1] and temperature[2] on the survival and alatae of *Rhopalosiphum maidis* using General Linear Model (GLM).

Variable	Source	df	F	P
Survival (%)	Density	1	10.22	0.002
	Temperature	2	96.27	0.001
	Density * Temperature	2	1.46	0.236
Alatae (%)	Density	1	16.32	< 0.001
	Temperature	2	57.89	< 0.001
	Density * Temperature	2	1.56	0.214

[1]Density: 10 and 50 aphids/unit;

[2]Temperature pattern: 24/39 °C, 26/39 °C and 28/39 °C.

Table 4-4: The percentage of survival and alatae (mean±SE%) of *Rhopalosiphum maidis* under different temperature and density combinations.

Temperature	Survival (%)		Alatae (%)	
	10 nymphs/unit	50 nymphs/unit	10 nymphs/unit	50 nymphs/unit
24/39 °C	79.9±1.5Aa	77.1±4.4 Aa	10.1±0.8 Aa	13.6±0.9 Aa
26/39 °C	78.2 ±1.4 Aa	72.2 ±2.9 Aa	17.6±1.0Ab	21.7±1.4 Ab
28/39 °C	56.4±1.6 Ab	43.8±2.8 Bb	26.9±1.4Ac	41.6±1.2 Bc

Within a row, different lowercase letters indicate significant differences (P<0.05) by using Tukey's multiple range tests;

Within a column, different uppercase letters indicate significant differences (P<0.05) by using Tukey's multiple range tests.

4 Discussion

There are numerous environmental factors that lead to the production of insect alatae forms. Temperature, photoperiod and humidity may all influence the production of winged forms either directly or indirectly through an effect on the host plant (Johnson, 1966a, b; Lees, 1967; Shaw, 1970; Martínez and Costamagna, 2018). These factors were generally considered in continuous conditions, and then, their effects were minimized for shorter duration changes. The host plant is probably another main factor in the development of insect alatae forms (Mittler and Dadd,

1966) but was also kept fixed by using seedlings of the same age, uniform in size and grown under identical conditions. In this way, only some of the direct effects could be studied. Our results confirmed that in addition to fixed changes in the photoperiod, relative humidity rate or host plant, the insect crowding condition is essential for winged induction. Moreover, the temperature had a vital catalytic role in this process. This study contributes to significant insight into understanding the proximate factors that regulate alternative morph production and, lays the foundation for adaptation and evolution of insect polyphenism under extreme climate with short time exposure.

Winged morph production has been considered a driver of density regulation in aphids, and in many species, the production of winged individuals is strongly density dependent (Lees, 1967; Sutherland, 1969; shaw, 1970; Mehrparvar et al., 2013; Purandare et al., 2014;). The production of winged individuals among aphid populations is essential for the aphid life cycle and is possibly the best strategy for dispersal and colonization to more optimal environments (Müller et al., 2001). Our results confirmed earlier studies and clearly showed that aphids are responsive to crowding. While *R. maidis* aphid reared in isolation rarely gave rise to alate forms, the increase in insect density induced larger proportion of alatae. The younger stage appeared to be more sensitive to the density effect. We crowded1st, 2nd, 3rd, and 4th nymphs and wingless adults within parthenogenetic embryos. The first two instars and the progeny under high density were more likely to be alatae. The development of the crowding response for the two latter instars is not obvious at any density compared with other stages. It is also a general phenomenon that aphids produce fewer winged than wingless morphs, as former require more resources (Mehrparvar et al., 2013). Despite the clear effect of crowding for CLA, the effect size was small in comparison with the pea aphid which produces a much higher proportion of winged morphs in response to crowding (Sutherland, 1970).

Climate change will cause rapid modification of environmental conditions and an unpredictable occurrence of extremes (Easterling, 2000; Kingsolver and Buckley, 2015; Ma et al., 2015). Extreme events and the gradual increase in average temperatures affect all biological traits, from physiology to life history, also including polyphenism. Low-temperature (the lowest at 8 °C) adapted *N. ribisnigri* not only performed better in survival and reproduction with the temperature increase and reached its best at 20-24 °C, but also produced more alates (Diaz et al., 2015). Martay et al. (2017) modelled weather variables combined with aphid population abundance of 80 species across 12 sites in Great Britain from 1970-2010 and found that winged aphids increased on average by 0.70% annually, of which 62.7% could be accounted for by climate change (Martay et al., 2017). The empirical study of elevated temperature and CO_2 revealed that higher temperature (22 vs. 26 °C) will lead to shorter generation time and higher rm (the largest intrinsic rate of natural increase) value as well as more alate reduction of CLA under global warming in combination with elevated CO_2, which could result in an increase in population growth and spread, i.e., enhancing risk of serious damage to crops by CLA (Xie et al., 2014). Under constant temperature conditions, the optimum temperature for development, survival and reproduction of CLA is at 30 °C, but it is unfavorable to

adults at 35 °C (El-Ibrashy et al., 1972). In our study, it could favor the survivorship and alate morphs induction of CLA being reared under constant temperature of 22 °C with a transient exposure (2 h) to high temperature (35 to 39 °C) during mid-photophase. In addition, alate morphs induction was increase along with the rearing temperature rising up in the range of 22 to 28 °C, although the optimum temperature for survival was in the range of 22 to 26 °C. Our results demonstrated that not only global warming, but also the frequent extreme heat events would increase the abundance of CLA under the climate change.

Wing polyphenism is essential for the aphid life cycle and, by allowing for migration to fresh food resources, it may contribute to determining the overall fitness of an aphid population. The effects of external environmental conditions on the wing polyphenism of aphids are likely to be independent and may also be mediated by interacting with multiple factors at the same time. It is still unclear how and when these factors start to work. To understand the costs and benefits, it is important to evaluate the intensity of wing polyphenism associated with different habitat quality indicators. We compared the wing polyphenic response of CLA to crowding and high temperature for a short duration. Our results provide an insight into understanding the two fundamental ecological processes, survival and dispersal. First, the aphids must survive, and then, they should be able to move and disperse. In the future, multiple approaches are necessary to analyze the mechanisms that trigger alternative morph determination by integrating functional “omics” to investigate the biochemical pathways. A better physiological understanding of the wing development of aphids will provide novel targets for studying the evolution of polyphenism in relation to environmental conditions such as extreme climate change and population density from species to community levels. Studying higher levels of organization will allow for a better understanding of ecological and evolutionary responses to forecast how climate change could impact biological diversity.

Author contributions

K.L. He and Y. Chen contributed to the experimental protocol design. Y. Chen performed the experiments. D.D. Sun reared the insects and plants. Wang provided insect materials and experimental instruments. Y. Chen, F. J. Verheggen, F. Francis and K.L. He contributed to the preparation of the manuscript. F. Francis and K.L. He supervised the research.

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5

Effects of host plants reared under elevated CO_2 concentrations on the foraging behavior of different stages of corn leaf aphid *Rhopalosiphum maidis*

From Chen, Y., Martin, C., Fingu Mabola, J.C., Verheggen, F.J., Wang, Z.Y., He, K.L., & Francis, F.. Effects of host plants reared under elevated CO_2 concentrations on the foraging behavior of different stages of corn leaf aphid *Rhopalosiphum maidis*. *Insects*, https://doi.org/10.3390/insects10060182 (2019).

Abstract: Climate change is a major environmental concern and is directly related to the increasing concentrations of greenhouse gases. The increase in concentrations of atmospheric carbon dioxide (CO_2), not only affects plant growth and development, but also affects the emission of plant organic volatile compounds (VOCs). Changes in the plant odor profile may affect the plant-insect interactions, especially the behavior of herbivorous insects. In this study, we compared the foraging behavior of corn leaf aphid (*Rhopalosiphum maidis*) on barley (*Hordeum vulgare* L.) seedlings grown under contrasted CO_2 concentrations during the dual choice bioassays, the winged and wingless aphids were more attracted by the VOCs of barley seedlings cultivated under ambient CO_2 concentrations (aCO_2; 450 ppm) than barley seedlings cultivated under elevated CO_2 concentrations (eCO_2; 800 ppm), nymphs were not attracted by the VOCs of eCO_2 barley seedlings. Then, volatile compositions from 14-d-old aCO_2 and eCO_2 barley seedlings were investigated by GC-MS. While 16 VOCs were identified from aCO_2 barley seedlings, only 9 VOCs were found from eCO_2 barley seedlings. At last, we discussed the potential role of these chemicals observed during choice bioassays. Our findings lay foundation for functional response of corn leaf aphid under climate change through host plant modifications.

Keywords: Climate change, corn leaf aphid, foraging behavior, volatile organic compounds (VOCs).

1 Introduction

Since the industrial revolution, the atmospheric concentration of carbon dioxide (CO_2), have been steadily rising from approximately 280 ppm to 401 ppm (Mauna Loa Observatory: NOAA-ESRL). Forecasts suggest that the concentrations could double by the year 2100 (Stocker et al., 2013). As CO_2 is a substrate for plant photosynthesis, an increase in its concentration in the atmosphere directly impact plant growth and composition (Hartley et al., 2000; Bae and Sicher, 2004; Seneweera and Conroy, 2005). Hence, the carbon:nitrogen (C:N) ratios of plant increase with the concentration of CO_2 (Johns and Hughes, 2002; Chen et al., 2005; Ainsworth and Rogers, 2007; Sun et al., 2018; Zhang et al., 2018), which enhanced the photosynthetic rate of C3 plants, such as wheat and barley (Ziska et al., 1997; Drake et al., 1999; Kim et al., 2003; Ainsworth and Long, 2005). In addition, alteration of secondary plant chemistry by CO_2 rise was documented (Karowe et al., 1997; Coviella et al., 2002; Stiling and Cornelissen, 2007; Karowe and Grubb, 2011). Indeed, plant grown under eCO_2 condition usually elicits the production of phenolic compounds, tannins, and flavonoids and suppresses the production of terpenoids (Robinson et al., 2002; Stiling and Cornelissen, 2007). However, no consistent trend has been found in the response of insects to these allelochemicals adaptation (Bidart-Bouzat and Imeh-Nathaniel, 2008).

Most of herbivorous insect species rely on olfactory signals from their environment to find mating and to locate a host plant (Hansson and Wicher, 2016). The VOCs from plant range from fatty acid derivatives, terpenoids, and sulfur compounds to phenylpropanoids (Qualley and Dudareva, 2009). The composition and amounts of plant VOCs can vary depending on several parameters: plant taxon (Knudsen et al., 1993), stage of development (Holopainen et al., 2010), physiological status (Jiménez-Martínez et al., 2004) and environmental stresses (Maffei, 2010). However, the effects of increasing concentration of CO_2 on the emissions of plant VOCs are not well-defined (Boullis et al., 2016).

Aphids (Hemiptera: Aphididae) are the most important pest insects under temperate climate (Minks and Harrewijn, 1987). They are responsible for the transmission of more than 50% of insect-transmitted plant viruses (Nault, 1997). Many aphid species produced two types of morphs: a winged morph that is mainly responsible for dispersal and the colonization of new plants, and an unwinged morph that mostly stays on the plant on which it was born (Braendle et al., 2006). Aphids use olfaction to recognize plant host from non-plant host which allow them to determine the suitability of different plants (Pickett et al., 1992; Pettersson et al., 2008). Moreover, variation in behavioral responses to volatiles can also be found in winged and wingless morphs (Webster, 2012). Volatile blends based on headspace collections from wheat and oat plants elicited similar behavioral responses from both morphs of *R. padi* in olfactometer studies. When compounds were tested individually, the two morphs responded differently (Quiroz and Niemeyer, 1998). Winged aphids were only attracted/arrested by four of the compounds, whereas wingless morphs responded to 11. However, studies dealing with the behavior of

aphids expose to VOCs of host plant grown under ambient and elevated atmospheric CO_2 are not widespread (Boullis et al., 2018; Blanchard et al. 2019).

As a worldwide pest insect, corn leaf aphid, *Rhopalosiphum maidis* caused significant damage on cereal crops such as barley, *Hordeum vulgare* L., corn, Zea mays L., wheat, *Triticum aestivum* L., and broad bean, *Vicia faba* L (El-Ibrashy et al., 1972). Corn leaf aphid is also a vector of plant viruses including sugarcane mosaic virus (SCMV), maize dwarf mosaic virus (MDMV), which result in serious damage (Bing et al., 1991; Everly, 1960; Foott and Timmins, 1973). This work aims to investigate the effects of elevated CO_2 concentrations on foraging behavior of corn leaf aphids. The foraging behavior of aphids to chemical cues of host plant was assessed by using Y glass tube olfactometer. Then, volatile organic compounds from isolated barley seedlings Hordeum vulgare L. reared under ambient CO_2 (aCO_2) and elevated CO_2 (eCO_2) condition were analyzed by GC-MS.

2 Materials and methods

2.1 CO_2 Condition Chambers

Six chambers (60 cm in length, 50 cm in width and 50 cm in height, PLEXIGLAS® GS, clear 0F00 GT, 8 mm thick; Evonik Industries, Essen, Germany) were used for rearing plants and insects under two different concentration of CO_2. In each chamber, a constant airflow (30 $Lmin^{-1}$) was pushed through an air pump (Koi flow 30; Superfish, Netherlands). Two levels of CO_2 concentrations were applied: ambient level (aCO_2, 450 ± 50 ppm) and elevated level (eCO_2 , aCO_2 + 350 ppm) by using a CO_2 gas tank (>99% purity; Airliquide®, Paris, France). Three chambers were used for each CO_2 treatment. These chambers were maintained at 23 ± 1 °C and 65 ± 10% relative humidity (RH), with a 16 hour light photoperiod under cool white light-emitting diode (LED) lights (77 lmol/sqm/s). Carbon dioxide concentrations, temperature, and RH were continuously monitored in each chamber with MCH-383 SD data loggers (Lutron, Taipei, Taiwan).

2.2 Plant material

Barley, *Hordeum vulgare* L., was sown in single plastic plots (7.5 cm diameter, 9.0 cm high), with 25 to 30 seedlings per pot. After sowing, these pots were introduced in aCO_2 and eCO_2 chambers separately. These seedlings were growing up during two weeks to be used for host finding behavior tests and volatile analysis.

2.3 Aphid rearing

A colony of the aphid, Corn leaf aphid, *Rhopalosiphum maidis*, were originally collected from a corn field in the experiment station of Chinese Academy of Agricultural Sciences in Hebei Province, China, was maintained under ambient CO_2 concentration at a constant temperature of 23±1 °C, 65±10% RH and a 16 hour light photoperiod. The colony was reared on barley seedlings in a cage (36 cm in length,

27 cm in width and 28 cm in height) for about 30 generations before tests. Barley seedlings were replaced once a week.

To ensure uniform experiments, numerous apterous reproductive adults were transferred to new pots. After 24 h, the adults were removed from the plants, and their offspring were kept rearing on the barley seedlings. Three days' nymphs and eight days' winged and wingless adults were used for the host finding behavior tests.

2.4 Foraging behavior bioassay

A two-arms glass olfactometer (Y tube olfactometer) was used to investigate the behavioral response of nymphs, winged and wingless adults to different olfactory stimuli from barley grown under different concentration of CO_2 (eCO_2 : 800 ± 50 ppm; aCO_2 : 450 ± 50 ppm). The aphids were offered one of the three following odor source combinations: control (clean air) vs. aCO_2 barley seedling, control vs. eCO_2 barley seedling, or eCO_2 barley seedling vs. aCO_2 barley seedling.

All trials were conducted at 23 ± 1 °C in an observation chamber (60 cm in length, 50 cm in width and 40 cm in height) lightened with three 16-W cool white fluorescent lights which provided uniform lightening. The main arm of Y-olfactometer (15 cm long and 1.5 cm I.D.) and the two arms (20 cm long and 1.5 cm I.D.) were made of glass. Three black lines (two centimeters from the bottom of the stem or two arms) were drawn on the stem and two arms of Y tube olfactometer separately in order to observe the position of aphids. Plants grown under elevated or ambient concentration of CO_2 in glass pot were placed into sealed glass chamber (4L, 20 cm I.D.) (Analytical Research Systems, Gainesville, FL) and randomly connected to each arm of the Y-olfactometer with Teflon® pipes. A push pump system (PVAS11; Volatile Assay Systems®, Rensselaer, New York) was connected to each chamber to carry volatile organic compounds (VOCs) released by barley to the Y-olfactometer. The air was, first, purifying through a charcoal filter to avoid any outdoor contamination. The pushed air flow was kept at 0.7 L min^{-1}.

Aphids were individually placed at the entrance of the stem part, alternating with nymphs, wingless and winged adult aphids. Each insect was allowing to spend five minutes in the Y tube olfactometer. Totally 180 aphids were tested for each morph. The host finding behaviors of aphids were visually observed and simultaneously encoded using The Observer 5.0 software (Noldus®). The following behaviors were recorded during the experiment.

No response: when aphids stayed at the entrance, didn't cross the black line marked on the stem part;

Only searching: when aphids crossed the black line marked on the stem, but didn't cross the black line marked on the chosen arm;

Selection: when aphids made a choice and crossed the black line marked on the arm of the Y tube olfactometer.

Between each experiment, new plants were introduced to the chambers. The Y-olfactometer were cleaned with pure n-hexane (>99.7%; VWR®, Radnor,

Pennsylvania) and dried at room temperature for about five minutes after testing 15 aphids. Moreover, the chambers and all of the Teflon pipes were washed with an n-hexane (>99.7%; VWR®, Radnor, Pennsylvania).

2.5 Headspace analysis of volatiles from plants by GC-MS

The upper seedling parts (about 12 cm in length) of aCO_2 and eCO_2 barley were carefully sealed in the bell-like glass collection chamber (2 L) separately. To avoid volatile contamination, the base root parts were wrapped with aluminum foil and placed in a cleaned glass bottle. Headspace volatiles from aCO_2 and eCO_2 barley seedlings were collected using a dynamic 'push–pull' pump system. The pushed airflow was set at 0.7 $Lmin^{-1}$ and the pulled air flow was set at 0.3 $Lmin^{-1}$. The air entering into the chamber was cleaned by an activated charcoal filter. A 60 mg Tenax TA® thermodesorption tube (Gerstel, Germany), which is made of a microporous polymer of 2,6-diphenylen oxide, was placed at the exit of the glass chamber to trap the volatile compounds carried by the air pulling from the chamber. The tubes were previously cleaned by a thermal conditioner (TC2, Gerstel, Mülheim an der Ruhr, Deutschland), for a period of 11 hours at 300 °C. Volatile collection took place over a 24-h period at rearing conditions. Straight after volatile collection, the entire aerial portion of the plants was removed to determine dry weight. It allowed to calculate the amount of VOCs in nanogram per gram of aboveground dry plant. Six replicates were conducted for each condition of CO_2 concentration of growing, along with the same number of controls (only soil and glass pots wrapped with aluminum).

The volatiles were analyzed by Gas Chromatography coupled with a Mass Spectrometer (GC-MS) (model 7890A; Agilent Technologies Inc., Santa Clara, CA, USA). In this system, the Tenax TA cartridge was thermally desorbed (Thermal Desorption Unit, Gerstel, Mülheim an der Ruhr, Deutschland) at 250 °C for 10 minutes prior to the injection. In each sample, one microliter of butylbenzene (2.15 ng/µl) was injecting as an internal standard.

The entire sample was injected in a HP-5 capillary column (5% Phenyl Methyl, 30.0 m, internal diameter: 0.25 mm, thickness: 0.25 µm, Agilent Technologies®, Clara, USA). The carrier gas used was Helium (Initial flow: 1.5 ml/min, Post flow: 0.4 ml/min), The temperature program started at 40 °C for 2 min, and was increased at 4 °C min^{-1} to 95 °C, and then increased at 6 °C min^{-1} to 155 °C for 10 minutes, and was finally increased at 25 °C min^{-1} to 280 °C hold for 5 minutes. The detected peaks were identified based on their mass spectrum by using spectral libraries, Pal 600k and Wiley 275 (the MS spectra match factor was minimum 70 %).

2.6 Statistical analyses

Binomial proportion tests (equal distribution hypothesized) were used to compare the foraging behavior of nymphs, wingless and winged aphids in Y tube. The residence time of each choice was subjected to an analysis of variance by using a

general linear model (GLM). Treatment means were compared using the Tukey's multiple range tests to determine significant difference at a 95% confidence level. Plant VOCs between two CO_2 levels were tested with independent samples t-test. All analyzes were performed using SAS version 9.2 (SAS Institute, Cary NC).

3 Results

3.1 Foraging behaviors of aphid

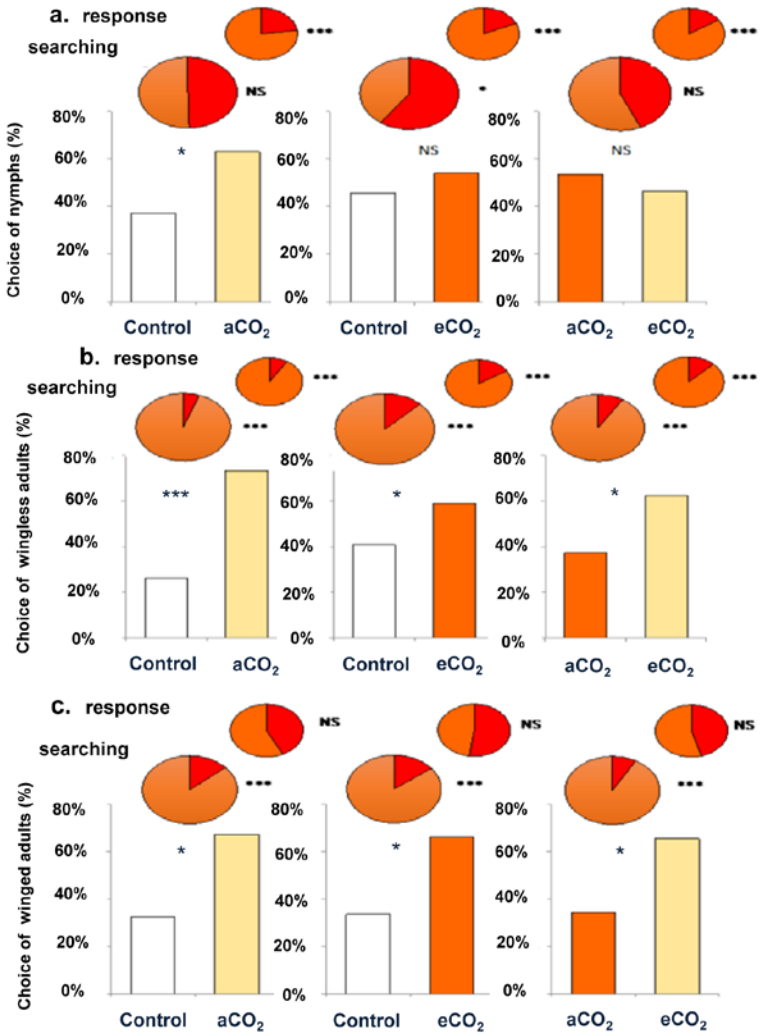

Figure 5-1: Foraging behavior (in %) of nymph (a), wingless (b) and winged (c) corn leaf aphid during three dual choices including control, aCO_2 and eCO_2 barley seedling

combinations. Response and searching status assessment corresponded to mobility in the first 2 cm and before the split of the olfactometer 2 arms respectively. Red color in pies was negative behaviors. *, *** and NS for $P \leq 0.05$, $P \leq 0.001$ and not significant at $\alpha = 0.05$

We tested the foraging behavior of corn leaf aphid for three developmental stages (Fig. 5-1) according to different dual choice, namely control vs. aCO_2 barley seedling, control vs. eCO_2 barley seedling and eCO_2 vs. aCO_2 barley seedlings. The winged and wingless aphids were more attracted by odors of aCO_2 barley seedlings when tested in combination with control air or in combination with eCO_2 barley seedlings. However, nymphs were only attracted by aCO_2 barley seedlings when it was tested in combination with control, otherwise no significant difference was observed when eCO_2 was tested in combination with aCO_2 or with control.

3.2 Residence duration for searching and selection behaviors

The typical behavior of nymphs in Y tube olfactometer consisted mainly of searching activities with more than 38% of experimental time. Also, more than 33% of experimental duration of winged aphids corresponded to no response to odor sources, being stationary at the entrance of the olfactometer stem. When aphids made a choice, the residence duration on each arm was affected by kind of morphs (Table 5-1 and Fig. 5-2). The wingless and winged aphids spent significantly more time in the arms of aCO_2 barley seedlings when tested against control or eCO_2 barley seedlings. However, the residence duration of three morphs did not show any significant difference when toward control vs. eCO_2 barley seedlings.

Table 5-1: Summary of a general linear model (GLM) analysis of the effect of morphs and choices on residence duration of *Rhopalosiphon maidis* in Y tube olfactometer during three dual choices including control, aCO_2 and eCO_2 barley seedling combinations.

Source	Model	DF	$\chi 2$	*P*
Control vs aCO_2	Life stages	2	60.03	< 0.001
	Choices	1	124.81	< 0.001
	Life stages * Choices	2	22.83	< 0.001
Control vs eCO_2	Life stages	2	48.43	< 0.001
	Choices	1	2.62	0.106
	Life stages * Choices	2	0.79	0.456
eCO_2 vs aCO_2	Life stages	2	50.60	< 0.001
	Choices	1	130.45	< 0.001
	Life stages * Choices	2	13.37	< 0.001

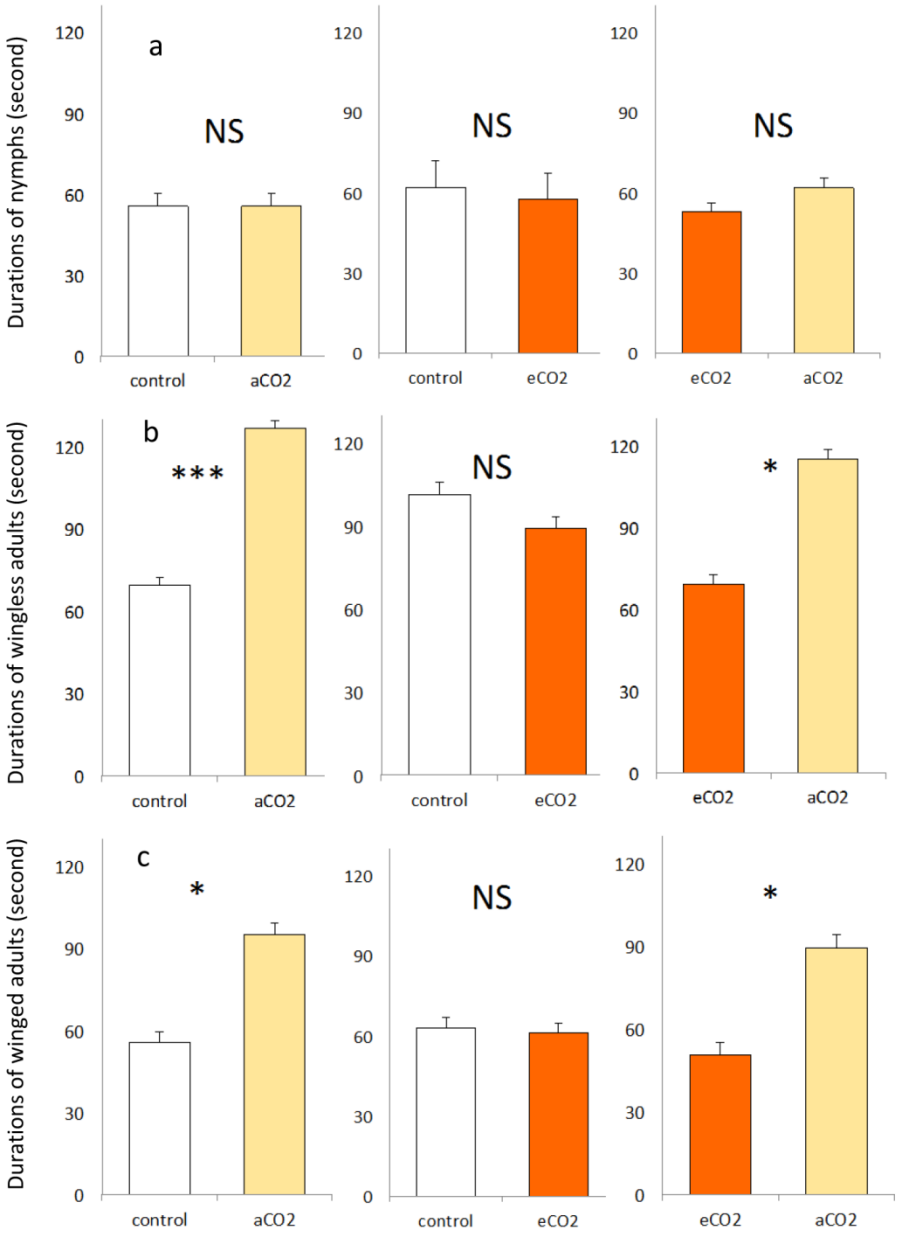

Figure 5-2: Durations (mean ± standard deviation) of nymph (a), wingless (b) and winged (c) corn leaf aphid during three dual choices including control, aCO_2 and eCO_2 barley seedling combinations. *, *** and NS for $P \leq 0.05$, $P \leq 0.001$ and not significant at $\alpha = 0.05$.

3.3 Volatiles Analysis

According to the GC-MS analysis, 16 and 9 VOCs were identified in aCO_2 and eCO_2 barley seedlings respectively (Fig. 5-3). 1,3-butanediol was the main volatile compound emitted by aCO_2 barley seedling whereas linalool was the most abundant volatile compound emitted by eCO_2 barley seedling. Six volatiles were found in both aCO_2 and eCO_2 barley seedlings, including heptanal, 1,3-butanediol, $_2$,3-butanediol, $_2$-methyl-propanoic acid, heptadecane and pentadecane. However, the relative abundances decreased in eCO_2 barley seedlings. The volatile pattern from aCO_2 barley seedlings was more diversified, including seven supplementary volatiles, namely 2-hexenal, cyclohexane decyl, 3-methyl-hexadecane, 3-methyl-pentadecane, 4-methyl-pentadecane, 1-methyl-2-propyl-benzene, propyl-benzene, 1,2,4-trimethyl- benzene, 1,3,5-trimethyl-benzene and indane.

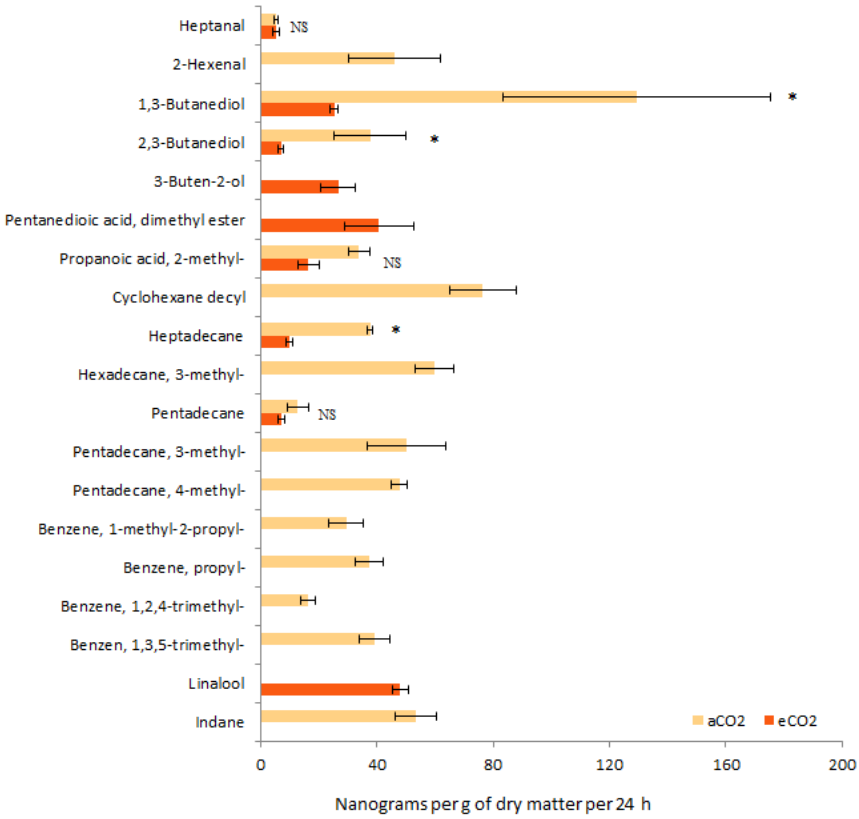

Figure 5-3: Diversity and abundance of volatile emission (mean ± se in ng per g of dry matter per 24 h) from aCO_2 and eCO_2 barley seedling (n = 6 replicates). * and NS for $P \leq 0.05$ and not significant at $\alpha = 0.05$ respectively

4 Discussion

In the study, aphid foraging behaviors were found to be influenced by host plant reared in different CO_2 concentrations. The diversity and abundance of plant VOCs were also differently induced by elevated CO_2 when compared with aCO_2.

In our experiment, the wingless and winged aphids were more attracted by the odors of aCO_2 barley seedlings when tested against eCO_2 barley seedlings or control. They spent more time during dual choice on aCO_2 barley seedlings. However, nymphs were only attracted by aCO_2 when it was tested in combination with control. There was some evidence that aphids can detect a variety of individual plant odor components using the hairs on the tips of antennae (Powell et al., 1995) or that a sensilla at the tibia-tarsus junction may respond to non-volatile chemicals (Pettersson et al., 2007). In aphids, the semiochemicals are perceived by sensory structures called rhinaria, that are classified in two main groups: primary and secondary rhinaria. For example, distal (DPR) and proximal (PPR) primary rhinaria allow all morphs and lifestages of *Aphis fabae Scopoli* to detect 2-hexenal, a common volatile of their host-plants (Park and Hardie, 2004) that is not detected by secondary rhinaria. PPR are usually associated with the perception of host and non-host volatile chemicals, and DPR are probably involved in the perception of the alarm pheromone (Wohlers and Tjallingii, 1983; Pickett et al., 1992).

GC-MS analysis showed that volatiles from barley seedlings including aldehydes, alcohol compounds, acid compounds, alkanes, phenyl compounds and others. We found 2-hexenal in the volatile blends of aCO_2 barley seedlings. Previous study has proved that it is attractive to pea aphid, *A. pisum* Harris (Babikova et al., 2014). In our experiment, an amount of linalool was emitted by eCO_2 barley seedling which had showed a repellent effect on green peach aphid, *M. persicae* (Sulzer) (Harmel et al., 2007), corn leaf aphid, *R. maidis* and bird cherry-oat aphid, *R. padi* (Halbert et al., 2009). Therefore, the presence of those compounds in the odor blends of barley, could explain the preference of aphids towards odors of aCO_2 barley.

The volatiles emitted by barley aerial parts differ both qualitatively and quantitatively probably because of experiment treatments or plant stage. Bukovinszky et al. (2005) analyzed headspace volatiles of 3-4 week old barley, they detected 15 compounds and pointed the volatile profile of barley had the greatest dissimilarity. Wenda-Piesik et al. (2010) collected 11 different volatiles from third leaf stage uninfested barley (Wenda-Piesik et al., 2010). Piesik et al. (2010) tested 6-week barley, identified about 19 volatiles, and mentioned that mechanical injury and insect feeding caused barley to quantitatively release the highest total VOC concentrations after injury (Piesik et al., 2010). In our research reported here in, we used the intact barley seedling after 14 days growth, identified 16 VOCs in aCO_2 barley seedlings and 9 VOCs in eCO_2 barley seedlings, the barley seedlings were so young and not infested by insects or any fungus, that probably be the reason why we collected less volatiles compared with other researches.

The common feature of wingless and winged aphids in the foraging behaviors in the study was that once they had a response to the volatiles of the plant, their search time was short and they make a choice quickly. The average time of response at the

entrance of Y tube for winged aphid was longer than wingless. The sensitivity to the plant volatiles and the variation in behavioral response were partly as a result of differences in morphs. Walking is the main way of wingless aphids expanding to nearby plants in the field. When induced by host-plant odors, the wingless aphid will actively walk towards the odor source in the absence of other host cues (Visser and Taanman, 1987; Nottingham et al., 1991). Winged morphs are capable of making targeted landings on plants under low wind conditions (Storer et al., 1999; Vargas et al., 2005; Goldansaz and McNeil, 2006). However, winged aphids will not attempt to fly unless a certain speed of wind (Hardie et al., 1996; Kennedy, 1990). Which indicate that wind probably is an important precondition for the movement of winged aphid. Behavioral responses of aphid to volatiles were also different in developmental stage within a single morph. Results showed that the nymphs spent more time searching around in the central tube of olfactometer compared to adults, perhaps they spend more energy to move than adults because of their slower walking speed (Sørensen et al., 2003; Gish and Inbar, 2006; Zhang et al., 2015).

The aphid behavioral response to plant VOCs is complex. Presented as a blend, these volatile compounds may be integrated as host cues, leading to aphid attraction/arrestment toward the odor source (Webster et al., 2010). Every compound of the constitutive blend is going to present individually in olfactometric bioassays to test which one have effect on aphid responses. Future work may focus on how aphids gain information on the identity and quality of a plant from the composition of its volatile blend and how interactions between volatiles can affect aphid behavior in changing climatic conditions.

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6

Reduction of plant suitability for corn leaf aphid under elevated carbon dioxide concentrations

From Chen, Y., Serteyn, L., Wang, Z.Y., He, K.L., & Francis, F. Reduction of plant suitability for corn leaf aphid under elevated carbon dioxide condition. *Environmental Entomology*, https://doi.org/10.1093/ee/nvz045 (2019).

Abstract: In the current context of global climate change, atmospheric carbon dioxide (CO_2) concentrations are continuously rising with potential influence on plant-herbivore interactions. The effect of elevated CO_2 (eCO_2) on feeding behavior of corn leaf aphid, *Rhopalosiphum maidis* (Hemiptera: Aphididae) on barley seedlings *Hordeum vulgare* L was tracked using electrical penetration graph (EPG). The nutrient content of host plant and the developmental indexes of aphids under eCO_2 and ambient CO_2 (aCO_2) conditions were also investigated. Barley seedlings under eCO_2 concentration had lower contents of crude protein and amino acids. EPG analysis showed the plants cultivated under eCO_2 influenced the aphid feeding behavior, by prolonging the total pre-probation time of the aphids (wandering and locating the feeding site) and the ingestion of passive phloem sap. Moreover, fresh body weight, fecundity and intrinsic population growth rate of *R. maidis* was significantly decreased in eCO_2 in contrast to aCO_2 condition. Our findings suggested that changes in plant nutrition caused by eCO_2, mediated via the herbivore host could affect insect feeding behavior and population dynamics.

Keywords: climate change, carbon dioxide, corn leaf aphid, feeding behavior, electrical penetration graph (EPG).

1 Introduction

The world average carbon dioxide (CO_2) concentration in the atmosphere has steadily increased from pre-industrial values of approximately 280 ppm to 401 ppm (Mauna Loa Observatory: NOAA-ESRL). Concentrations are projected to increase to 550 ppm by 2050 and may surpass to 700 ppm by 2100 (Stocker et al., 2013). Elevated atmospheric CO_2 (eCO_2) has marked impact on plant growth and individual composition (Hartley et al., 2000; Bae et al., 2004; Seneweera et al., 2005), which indirectly affects the performance of herbivorous insect pests (Bale, 2002; Zavala et al., 2013). Consequently, eCO_2 will in turn influence agro-ecosystem processes and crop productivity (Norby et al., 2005; Zvereva et al., 2006; Lindroth, 2010). CO_2 is the raw material for carbohydrate production for plants during photosynthesis. In general, the most significant changes in foliar chemistry are due to increase of the carbon:nitrogen (C:N) ratio in phloem sap while plants grow under eCO_2 (Hartley et al., 2000; Johns et al., 2002; Chen et al., 2005; Elizabeth A Ainsworth et al., 2007), where nitrogen, mostly bound in amino acids and proteins, is a limiting factor for phloem sap feeding herbivorous insects (Mattson, 1980). Subsequently, this will lead to less fitness of host plants and adverse effects on insects due to nutrition deficiency (Chen et al., 2005), which will result in heavier damage on the host plants (Marks et al., 1996; Bezemer et al., 1998; Sun et al., 2009).

The phloem sap is sugar-rich with dominance of sucrose (Avigad et al., 1997; Jensen et al., 2013) that is the most effective phagostimulant for insect herbivores (Srivastava et al., 1971). Aphids overcome the sugar barrier to phloem sap utilization through their possession in the gut of sucrose-transglucosidase activity (Ashford et al., 2000; Cristofoletti et al., 2003; Douglas, 2006). Overall, a positive relationship between aphid performance and increasing sucrose concentration are mostly invariable (Srivastava and Auclair, 1971; Kriegler, 1972; Pescod et al., 2007; Puterka et al., 2017). However, the adapted range of sucrose concentrations by aphid is species-specific, which may link with its host range (Puterka et al., 2017). Oehme at al. (2013) reported that eCO_2 enhanced significant fructose and glucose levels in spring wheat foliage, which positively affected the relative growth rate of *R. padi*. However, the concentration of sucrose tends to increase at leaf development stage and to decrease at stem elongation stage due to eCO_2, but CO_2-induced these changes were not statistically significant.

Nitrogen is a limiting nutrient source for many herbivores and phloem sap feeding aphid in terms of quality (nutritional components) and quantity (concentration of individual nutritional component) (Mattson, 1980). Although aphid can overcome the nitrogen barrier in terms of essential amino acids in phloem sap by relying on their symbiotic bacteria (endosymbionts) including Buchnera aphidicola (Srivastava et al., 1985; Ohtaka et al., 1991; Douglas, 1998; Spiteller et al., 2000; Nardi et al., 2002; Davis et al., 2006; Schloss et al., 2006; Crotti et al., 2010; Defossez et al., 2011). Histidine, isoleucine, and methionine are required dietary amino acids for *Myzus persicae* in the aphid-bacteria symbiosis (Mittler, 1971).

Rising atmospheric CO_2 directly impacts the plant nitrogen concentration, is

further transformed and affects the amino acid concentration (Docherty et al., 1997; Bertrand et al., 2006; Stiling et al., 2007; Sicher, 2008; Sun et al., 2009). Due to feeding solely on the phloem sap, aphid is one of the most sensitive insect responding to changes in quality and/or quantity of plants exposed to eCO_2 (Pritchard et al., 2007). Previous research suggests that the response of aphid to eCO_2-mediated alternation of foliar quality and/or quantity is species specific (Hughes et al., 2001; Himanen et al., 2008; Oehme et al., 2013), i.e. being positively, negatively, or not significantly affected at both individual and population in terms of growth, development, fecundity, and abundance (Sandström et al., 1994; Awmack et al., 1997; Docherty et al., 1997; Jonas Sandström, 2000; Hughes and Bazzaz, 2001; Holopainen, 2002; Mondor et al., 2005; Oehme et al., 2013; Jiang et al., 2016). Previous reports indicated the changes in individual amino acid levels of phloem sap possibly alter the aphid behavior. Glutamine concentration from high to low levels for aphids M. persicae and Macrosiphum euphorbiae on potato plants could alter the fitness of host plant to aphids (Karley et al., 2002; Sandström, 2000). This highlighted the importance of assessing the entire chemical profile rather than only total concentrations as commonly reported (Weibull, 1987; Sandström and Pettersson, 1994; Docherty et al., 1997; Sandström, 2000).

Although some studies reported eCO_2-induced less fitness of host plant by altering foliar nutrient availability (Lincoln et al., 1993; Schädler et al., 2007; Cornelissen, 2011), a recent research demonstrated that eCO_2-mediated increasing in foliar soluble sugars, free amino acids and fatty acids could be favorable for ingesting cotton sap by *Aphis gossypii* (Glover), and consequently leading to increases in body weight, fecundity, and population abundance (Jiang et al., 2016). Srivastava et al. (1983) proved that eleven nonessential amino acids and amides play various roles in phagostimulation, growth and survival in *Acyrthosiphon pisum* (Harris). We suspected that changes in foliar compositions would alter the feeding behavior and therefore to change in suitability of corn leaf aphid under eCO_2.

Corn leaf aphid, *Rhopalosiphum maidis*, is a worldwide pest which cause significant damage on cereal crops such as barley, corn, pearl millet, wheat and sorghum (El-Ibrashy et al., 1972). *R. maidis* also a vector of plant viruses including sugarcane mosaic virus (SCMV), maize dwarf mosaic virus (MDMV), which often result in serious yield loss (Everly, 1960; Foott et al., 1973; Bing et al., 1991; Blackman et al., 2000). Prior research reported that eCO_2 positively affected the fecundity of corn leaf aphid (Xie et al., 2014). The objective of this study was to assess eCO_2-mediated changes in the composition of phloem sap in barley and the resulting consequences for corn leaf aphid. Thus, we quantified eCO_2-mediated changes in leaf chemistry including crude protein, carbohydrates like glucose, fructose, sucrose and free amino acids in barley seedlings; examined the biological parameters of aphid developed on barley grown under aCO_2 and eCO_2 conditions; and analyzed the effects of eCO_2 on aphid stylet ingestion on host plants by tracking corn leaf aphid feeding behavior with electrical penetration graph (EPG).

2 Materials and methods

2.1 CO_2 condition Chambers

Six transparent conditioned chambers (L 60×W 50× H 50 cm, PLEXIGLAS® GS, clear 0F00 GT, 8 mm thick; Evonik Industries, Essen, Germany) were used for rearing plants and insects. In each chamber, a constant airflow (30 $L.min^{-1}$) was pushed using an air pump (Koi flow 30; Superfish, the Netherlands). Two levels of atmospheric CO_2 concentrations were applied, i.e. ambient level (aCO_2: 450 ± 50 ppm) and elevated level (eCO_2 = aCO_2 + 350 ppm). The eCO_2 was achieved by using a CO_2 gas tank (> 99% purity; Airliquide, Paris, France). Three chambers were used for each CO_2 level. These chambers were maintained at 23.0 ± 1.0 °C and 60.0 ± 10.0 % RH, with a 16 h light period under cool white light-emitting diode (LED) lights (77 lmol/sqm/s). Carbon dioxide concentrations, temperature, and RH were continuously monitored in each chamber with MCH-383 SD data loggers (Lutron, Taipei, Taiwan).

2.2 Plant material

Barley, Hordeum vulgare L., was sown in black plastic pots (7.5 cm diameter, 9 cm high), with 30 seeds per pot, no chemical fertilizers or insecticides were used. After sowing, 25 pots/chamber were randomly transferred to aCO_2 and eCO_2 chambers. Two weeks old (14 day old), healthy barley seedlings were used for the experiment.

2.3 Aphid rearing

A colony of corn leaf aphid, R. maidis, was originated collected from a corn field in Langfang City, Hebei Province, North China. Virus free aphid and barley seedlings were reared under aCO_2 and/or eCO_2 chambers more than ten generations.

2.4 Crude protein analysis

For the Dumas method (Dumas, 1831), fresh barley seedlings (0.2 g) were weighed in four replicates per CO_2 condition. Samples are wrapped in a tin foil, placed into an automated sample loader, and dropped into the induction furnace of an N analyzer. TruSpec CHNS Macro (Model CNS-2000, LECO, Inc.,St. Joseph, MI, USA) instrument was used for the crude protein determination (Beljkaš et al., 2010). Crude protein content was calculated from the nitrogen content of the material, using a nitrogen conversion factor of 6.25 according to ISO/TS 16634-2 (2016).

2.5 Carbohydrate contents assay

Total soluble sugars were determined in four replicates per CO_2 condition based on the method of phenolsulfuric acid (Dubois et al., 1956). 0.2 g fresh barley seedlings was homogenized with deionized water and centrifuged at 4500rpm for 10 min. Then, 1 ml of extract was treated with 0.125 ml 5% phenol and 2.5 ml 98% sulfuric acid. The mixture remained in the water bath at 30 °C for 20 minutes. Absorbance was read at 490 nm using ultraviolet spectrophotometer (S 2100, Biochrom Ltd, England).

Glucose/Fructose/Sucrose contents of barley seedlings grown under both CO_2 treatments were determined by a high-performance anion-exchange chromatography

with pulsed amperometric detection (HPAEC-PAD). The chromatographic system was a Dionex ICS-5000+ model (Sunnyvale, CA, USA) equipped with an electrochemical detector, and a SP gradient pump. The column was a Dionex CarboPac PA-100 (250 mm × 4 mm i.d.) coupled to a CarboPac guard column (40 mm × 4 mm i.d.). The mobile phase consisted of 500 mM sodium hydroxide (A) and water (eluent B). The flow-rate was 1 mL/min and the injection volume was 25 μL. Four replicates were conducted for each treatment.

2.6 Amino acid assay

In order to define the composition of amino acids, 0.2 g flours of barley seedlings was prepared after hydrolysis under nitrogen with 6M HCl at 110 °C during 24 h. High performance liquid chromatography (HPLC) analysis (Biochrom 20 Plus amino acid analyser, Pharmacia, Cambridge, UK) was performed (Moore et al., 1954) using norleucine as an internal standard. The amino acids were separated by elution with suitable buffers of increasing pH and were detected with ninhydrin in a continuous flow photometric analytical system. Amino acid standard solutions (AA-S-18 from Sigma Aldrich, Germany) (500 nM) containing norleucine were separately injected to calibrate the analyzer and to calculate the amount of amino acid in the samples (Dakia et al., 2007). Four replicates were maintained for each CO_2 level.

2.7 Feeding behavior test by EPG

Feeding behavior of corn leaf aphid on aCO_2 and eCO_2 barley seedlings was monitored by using the Giga-8 DC-EPG system (EPG systems, Wageningen, the Netherlands) at constant temperature 23.0 ± 1.0 °C. Wingless adults reared under aCO_2 conditions were carefully collected by using a brush and then starved for about 30 min. A gold wire (diameter 20 μm, 3 cm length) was connected to the EPG amplifier with a copper wire attached to a copper nail. The other end of the gold wire was attached to the dorsum of the aphid with conductive water-based silver glue. Once a set of eight aphids was wired, the plant electrode was inserted into the soil pot. The recordings started in the morning at 10:00 am and lasted for six hours. Each aphid and each plant was used only once and 21 successful replicates for each treatment (aCO_2 and eCO_2) were obtained. Data were recorded with the Stylet+d software and analyzed with Stylet+a (EPG sytems, Wageningen, The Netherlands) and the feeding activity phases were distinguished based on waveforms resulting from voltage variations (Fig. 6-1). EPG parameters, such as number or duration of waveform events, were automatically calculated using Excel workbook (Sarria et al., 2009).

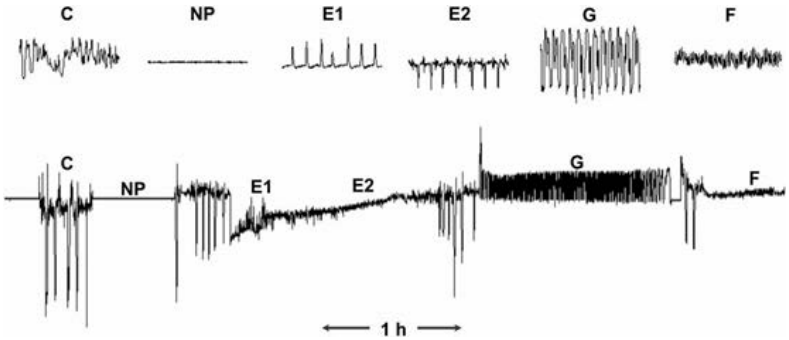

Figure 6-1: Typical waveforms recorded by the electrical penetration graph (EPG) (Tjallingii and Hogen-Esch 1993). C: penetration of stylets into the plant between epidermis and mesophyll cells; np: wandering or stationary on the surface of the plant; E1: saliva transferred into phloem sieve elements; E2: passive sap ingestion from the phloem; G: active sap ingestion from the xylem; F: derailed stylet mechanics.

2.8 Intrinsic rate of aphid under different CO_2 conditions

In both aCO_2 and eCO_2 chambers, one newborn aphid (< 6 h) was placed into a ventilated transparent plastic clip cages (2.7 cm diameter, 2.7 cm high) and restrained on one fresh leaf of a barley seedling. The edge of the clip cage was covered with a sponge to avoid mechanical wounds to the leaf. Development and survival of nymphs and adults were checked daily. New offspring and dead adults were removed after daily counting. The intrinsic rate of increase (r_m) for each system was calculated from the equation rm = 0.738 × (ln Md) / d where d is the period from the aphid birth to its first reproduction and Md is the number of progenies in a reproductive period equal to d (Wyatt et al., 1977; Zhang et al., 2017). A total of 15 replicates per CO_2 condition were maintained.

2.9 Mean relative growth rate of aphid

Twenty newborn aphid nymphs were collected from either aCO_2 or eCO_2 chambers, then placed on 9 cm diameter filter paper and weighed. All aphids were transferred back to aCO_2 or eCO_2 chambers on barley seedlings in clip cages as described above. After 7 days, all 20 aphids were collected and weighed again. A total of 10 replicates were performed for each CO_2 treatment. The mean relative growth rate (MRGR) of *R. maidis* was calculated with the equation: MRGR = (ln 7 days weight − ln birth weight) / 7 (Bruce et al., 2003).

2.10 Statistical analysis

The nutrient contents of barley seedling (including crude protein, carbohydrates and amino acid concentrations) and the biological parameters of aphid (including the development time, fecundity, rm, body weight and MRGR) were examined by using t test. EPG parameters were analyzed by a Mann-Whitney U test. Since EPG waveforms data was non-Gaussian random variables, Spearson's correlation analysis

was conducted to investigate the relationships among EPG waveforms, aphid biological parameters, and nutrient contents of barley seedlings. Pearson's correlation was used analyze the relationship between aphid biological parameters and nutrient contents of barley seedlings. All analyzes were performed with IBM SPSS Statistics 20.0 (IBM Corp, New York, USA, 2011).

3 Results

3.1 Nutrient contents in aCO_2 and eCO_2 barley seedlings

Crude protein and total amino acid were significantly decreased in barley seedlings grown at eCO_2 when compared with aCO_2 condition. Though total soluble sugar was decreased at eCO_2 treatment, there was no statistically significant difference between aCO_2 and eCO_2 barley seedlings (Table 6-1). This indicated that eCO_2 resulted in a relative nutrient deficiency of barley seedlings.

Table 6-1: The concentration of crude protein, carbohydrates and amino acids in barley seedlings under aCO_2 and eCO_2 conditions.

Nutrient contents	aCO_2	eCO_2	P
Crude protein (%)	4.0 ± 0.1	3.5 ± 0.0	0.016*
Total soluble sugar (mg/100 ml)	23.5 ± 1.9	22.2 ± 1.4	0.625
Total amino acid (in g amino acid/100 g of dry material, g/100 D.F.)	30.6 ± 2.0	25.7 ± 0.6	0.043*
Carbohydrates (mg/100 ml)			
Glucose	7.6 ± 0.4	6.7 ± 0.1	0.165
Fructose	0.5 ± 0.0	0.6 ± 0.0	0.101
Sucrose	1.5 ± 0.2	1.2 ± 0.2	0.354
Amino acid concentrations (g/100 D.F.)			
Aspartic acid (Asp)	3.6 ± 0.4	3.1 ± 0.1	0.047*
Threonine (Thr)	1.7 ± 0.3	1.3 ± 0.0	0.016*
Serine (Ser)	1.5 ± 0.3	1.1 ± 0.1	0.045*
Glutamic acid (Glu)	4.0 ± 0.6	3.2 ± 0.1	0.013*
Proline (Pro)	1.6 ± 0.4	1.3 ± 0.0	0.036*
Glycine (Gly)	1.8 ± 0.3	1.5 ± 0.0	0.034*
Alanine (Ala)	2.4 ± 0.3	2.2 ± 0.1	0.028*
Cystine (Cys)	0.2 ± 0.1	0.1 ± 0.0	0.042*
Valine (Val)	1.7 ± 0.2	1.5 ± 0.1	0.203
Isoleucine (Ile)	1.5 ± 0.3	1.2 ± 0.0	0.036*
Leucine (Leu)	2.6 ± 0.4	2.2 ± 0.1	0.046*
Tyrosine (Tyr)	1.3 ± 0.3	1.0 ± 0.0	0.033*
Phenylalanine (Phe)	1.8 ± 0.4	1.4 ± 0.0	0.037*
Histidine (His)	0.7 ± 0.1	0.8 ± 0.0	0.068
Lysine (Lys)	2.2 ± 0.3	1.8 ± 0.1	0.306
Arginine (Arg)	2.0 ± 0.4	1.6 ± 0.0	0.042*

Each value is the mean ± SE of 4 replicates for both aCO_2 and eCO_2 barley seedlings. Asterisk indicates significant differences between treatments ($P < 0.05$).

CO_2 levels also have a major impact on the composition of individual amino acids in barley seedlings (Table 6-1). In the study, Glu and Asp were the predominant free amino acids in barley seedlings. Significant decrease was found for 13 basic amino acids including Asp (- 13.9%), Thr (- 23.5%), Ser (- 26.7%), Glu (- 20.0%), Pro (- 18.8%), Gly (- 16.7%), Ala (- 8.3%), Cys (- 50.0%), Iso (- 20.0%), Leu (- 15.4%), Tyr (- 23.1%), Phe (- 22.2%), Arg (- 20.0%) in eCO_2 barley seedlings ($P<0.05$). However, there is no significant difference in glucose, fructose and sucrose levels.

3.2 Aphid feeding behavior on aCO_2 and eCO_2 barley seedlings

The plant CO_2 treatment had a significant impact on aphid feeding behavior. In the study, the total probing time of aphid was significant longer on eCO_2 barley seedlings.

Table 6-2: EPG parameters of *Rhopalosiphum maidis* on aCO_2 and eCO_2 barley seedlings.

EPG parameters[a]	aCO_2	eCO_2	*P*
General probing behavior			
Number of probes	12.6 ± 1.5	6.9 ± 1.1	0.004*
Total probing time (h)	4.1 ± 0.3	5.4 ± 0.1	<0.001*
Time to 1st probe from start of EPG (min)	2.1 ± 0.5	7.1 ± 1.3	0.002*
Number of short probes (C<3 minutes)	6.0 ± 0.9	2.6 ± 0.6	0.004*
Number of np	12.7 ± 1.5	6.9 ± 1.1	0.003*
Total duration of np (h)	1.8 ± 0.3	0.6 ± 0.1	<0.001*
Pathway phase			
Number of C	16.0 ± 1.7	10.1 ± 1.4	0.007*
Total duration of C (h)	2.1 ± 0.3	1.5 ± 0.2	0.061
Mean duration of C (h)	0.1 ± 0.0	0.2 ± 0.0	0.753
Number of pd	65.0 ± 11.4	67.7 ± 10.7	0.696
Total duration of pd (s)	296.9 ± 53.8	283.0 ± 43.7	0.811
mean duration of pd (s)	4.5 ± 0.2	4.3 ± 0.1	0.385
Phloem phase			
Number of E1	3.8 ± 0.7	3.6 ± 0.6	0.919
Total duration of E1 (min)	20.1 ± 4.8	9.8 ± 3.2	0.068
Mean duration of E1 (min)	5.6 ± 1.3	3.4 ± 1.4	0.092
Number of single E1	2.2 ± 0.5	0.9 ± 0.3	0.054
Total duration of single E1 (min)	5.2 ± 1.5	2.1 ± 0.9	0.052
Number of probes to the 1st E1	4.9 ± 1.1	3.8 ± 0.6	0.970
Number of E2	1.3 ± 0.2	2.5 ± 0.5	0.019*
Total duration of E2 (min)	80.1 ± 18.6	187.9 ± 21.3	0.001*
Mean duration of E2 (min)	56.4 ± 13.9	103.8 ± 18.6	0.044*
Time from start of EPG to 1st sustained E2 (10 minutes) (h)	3.5 ± 0.4	2.3 ± 0.3	0.054
Duration of the longest E2 (min)	75.5 ± 18.7	168.4 ± 21.5	0.002*
Number of sustained E2 (>10 min)	0.9 ± 0.1	1.4 ± 0.2	0.029*
Other phases			
Number of G	0.3 ± 0.1	0.6 ± 0.2	0.223
Duration of G (min)	9.5 ± 3.6	21.3 ± 8.5	0.315
Number of F	0.2 ± 0.1	0.3 ± 0.1	0.461
Total duration of F (min)	7.0 ± 4.0	16.9 ± 10.2	0.628

[a]All parameters were calculated for the whole 6-h recording. Each value is the mean ± SE of 21 replicates for both aCO_2 and eCO_2 barley seedlings.

Asterisk indicates significant differences between treatments ($P < 0.05$).

Aphid had a longer latency to start the first probe on eCO_2 barley seedling than on aCO_2 barley seedlings. The number and duration of np and of short probes were greater on aCO_2 barley seedlings than on eCO_2 barley seedlings. No significant difference was observed in total duration of C wave and pd between the two treatments. However, the passive sap ingestion (E2 waves) was significantly longer and more frequent in eCO_2 than in aCO_2. Despite the absence of significant difference in xylem ingestion phases between treatments, total duration of G waves in eCO_2 barley seedlings was about twice longer than on aCO_2 barley seedlings (Table 6-2).

3.3 Biological parameters of aphid

The fecundity, rm and fresh body weight of 7 days *R. maidis* were significantly less under eCO_2 condition than under aCO_2 condition (Table 6-3). However, no significant differences were found in development time, weight of newborn nymphs, MRGR between aCO_2 and eCO_2 treatments.

Table 6-3: Biological parameters (Mean±SE) of *Rhopalosiphum maidis* on aCO_2 and eCO_2 barley seedlings.

Biological parameters	aCO_2	eCO_2	*t*	*P*
Development time (d)	7.0 ± 0.2	7.5 ± 0.2	-1.916	0.067
Fecundity	4.7 ± 0.2	3.3 ± 0.1	6.866	<0.001*
Intrinsic rate of increase (rm) [a]	0.4 ± 0.0	0.3 ± 0.0	5.514	<0.001*
Wnymph [b]	0.0 ± 0.0	0.0 ± 0.0	1.948	0.067
Wadult [c]	0.5 ± 0.0	0.4 ± 0.0	4.944	<0.001*
Mean relative growth rate (MRGR) d	0.4 ± 0.0	0.4 ± 0.0	1.043	0.311

$r_m = 0.738 \times (\ln Md) / d$, where d is the period from the aphid birth to its first reproduction and Md is the number of progenies in a reproductive period equal to d;

[b] Wnymph means fresh body weight of newborn nymphs (< 6h) (mg);

[c] Wadult means fresh body weight of 7 day adults (mg);

[d] MRGR = (ln 7 days weight - ln birth weight) / 7;

Asterisks indicates significant differences between treatments ($P < 0.05$).

3.4 Relationships among EPG waveforms, nutrient contents of barley seedlings and biological parameters of aphid

The total probing time and duration of E2 were negatively correlated with the nutrient contents of barley seedlings and biological parameters of aphid (Table 6-4). Under aCO_2 and eCO_2 conditions, the total probing time was significantly correlated with the concentration of Ser, Ala and Glu. The total duration of E2 was significantly correlated with the concentration of sucrose, Glu, Phe in both treatments. In addition, glucose was also significantly correlated under aCO_2 treatment.

The biological parameters we tested were positively correlated with the nutrient contents of barley seedling under both aCO_2 and eCO_2 treatments (Table 6-5). Furthermore, His, Lys and Arg were significantly correlated with fecundity under aCO_2 treatment. *Rm* was significantly correlated with crude protein, Arg under aCO_2 treatment, while Gly, Ala, Ile and Phe were significantly correlated under eCO_2 treatment. Fresh body weight of 7 day old aphid was significantly correlated with the concentration of total soluble sugar, glucose, fructose, sucrose, and some individual amino acids like Ile, Lys, Arg under aCO_2 treatment. Also, fructose, sucrose, Gly, Val, Phe, His, Lys and Arg were significantly correlated under eCO_2 treatment.

4 Discussion

Elevated CO_2 was found to affect the feeding behavior via the change of the plant nutrient contents and exerting an influence on the herbivore performance. Rearing in eCO_2 condition, barley seedlings significantly decreased the concentration of crude protein, total amino acids and 13 individual amino acids. The corn leaf aphid prolonged the total probing time and sustained ingestion on eCO_2 barley seedlings but was lower in fecundity, rm and fresh body weight which negatively impacted population abundance of aphid.

Rising atmospheric CO_2 is likely to decrease the protein concentration of many crops, including wheat (Jablonski et al., 2002; Loladze, 2002), potato (Fangmeier et al., 2002) and soybean (Ainsworth et al., 2002). The reduced concentration of plant protein under eCO_2 condition mainly due to accumulation of non-structural carbohydrates dilutes the concentration of proteins (Kimball et al., 1994; Gifford et al., 2000). In this study, eCO_2 level significantly decreased the concentration of 13 basic amino acids of barley seedlings. In accordance, Wang and Nobel (1995) reported doubled CO_2 concentrations led to 17% less amino acids in phloem of *Opuntia ficus-indica* (L.). The amino acid concentrations were lower in needles of black spruce (Bertrand and Bigras, 2006) and in phloem of cotton (Sun et al., 2009) at eCO_2 condition. Decreased amino acid concentration in plant under eCO_2 condition may as a result of “nitrogen dilution” (Docherty et al. 1997).

The eCO_2 significantly increased the sucrose concentration of soybean (Ainsworth et al., 2007) and broccoli (Krumbein et al., 2010). However, there was no significant effect on the concentration of sucrose in leaves of spring wheat under CO_2 enrichment (Högy et al., 2008) and the concentrations of sucrose, fructose and glucose in maize leaves remained unchanged under eCO_2 (Leakey et al., 2006). In our study, we also did not find any significant difference in total soluble sugar, glucose, fructose, sucrose between aCO_2 and eCO_2 barley seedlings. The carbohydrates synthesis of young cereals may be less affected by eCO_2 (Havelka et al., 1984; Cure et al., 1986; Ryle et al., 1992), probably because they do not convert increased photosynthetic capacity in an increased level of extractable carbohydrates (Ingvardsen et al., 1994).

The analysis of EPG recordings revealed that the cultivation of barley in different concentration of CO_2 greatly affected the feeding behavior of *R. maidis*. The total probing time of *R. maidis* was significant longer on eCO_2 barley seedlings. In the study, the total duration of E2 was the main component of total probing time and

which was also significant longer in eCO_2 condition. The total duration of E2 were negatively correlated with nutrient contents and biological parameters of aphid which indicates that the decrease in plant nutrient contents in eCO_2 barley seedlings may increase the time spending in passive phloem feeding which were unfavorable for its reproductive and population abundance.

In contrast with eCO_2 barley seedlings, aphid feeding on aCO_2 barley seedlings had a higher frequency of probes, which suggested the absence of negative factors in epidermis that might have caused the withdrawal of stylets (Dancewicz et al., 2016). Long np phase observed of aphid on aCO_2 barley seedlings would have indicated the presence of barriers during stylets insertion in plant tissues (Alvarez et al., 2006), and vice versa, aphid feeding on eCO_2 barley seedlings may have more obstacles on the surface of epidermis and less barriers during stylets insertion in plant tissues. *R. maidis* had a longer latency to begin its first probe also implied the epidermal obstacles on eCO_2 barley seedlings, in addition, the result analysis pointed that the time to 1st probe was less affected by carbohydrate or amino acids content of plant.

The number of C wave refers to the ease at which aphid moved the stylets from the mesophyll to the phloem (Benatto et al., 2018). Feeding on eCO_2 barley seedlings, aphid had a lower frequency of C phase suggested that aphid may reach easier to deep tissues of eCO_2 barley seedlings by penetrating the meristematic tissues, which also supported the view that the aphid has less barriers during stylets insertion into the tissues of eCO_2 barley seedlings. EPG results showed that, aphid feeding on eCO_2 barley seedlings may have obstacles in initial probing on the surface of the epidermis, but they were successfully penetrated into the deeper tissues of plant and sustained ingestion was significant longer when compared to the aCO_2 barley seedlings.

The biological parameters were positively correlated with nutrient contents of barley seedlings, especially fecundity, rm, weight of 7 day old adults. Barley seedlings cultivation in eCO_2 condition had a significant lower concentration in crude protein, total amino acids and 13 individual amino acids. Aphid performance on plants could not only be affected by the overall amino acid concentration, but also by the relative concentration of different amino acids (Mittler, 1967). According to Dadd (1985), essential amino acids for aphid are His, Thr, Trp, Met, Val, Phe, Ile and Lys. However, the demand of individual amino acids differs with the aphid species (Sandström et al., 2001). While M. persicae needs Met and g-amino butyric acid, the amino acids Thr, His and Ala are important for *R. padi* (Kazemi et al., 1992).

Herbivore insects respond to the poor nutritional quality of the host plant by increasing their food consumption, prolonging development time, and reducing growth rates (Hale et al., 2003; Lincoln et al., 1993; Roth et al., 1995; Tuchman et al., 2002; Williams et al., 2000). Our results support most of these predictions. *R. maidis* feeding on eCO_2 barley seedlings showed significantly decreased body weight, fecundity and rm, which may result in decreased of population abundance under elevated atmospheric CO_2 environment. Further studies will be required to determine the defense mechanisms including epidermal integrity, defense proteins, and secondary metabolites of plant which might also hinder the penetration of stylets.

Table 6-4: Coefficients of Spearman's correlation between EPG waveforms, nutrient contents of barley seedlings, biological parameters of *Rhopalosiphum maidis* under aCO_2 and eCO_2 conditions.

Components	aCO_2								eCO_2							
	Total probing time (min)	Time to 1st probe from start of EPG (min)	Total duration of E1 (min)	Total duration of E2 (min)	Number of probes	Number of np	Number of C	Number of E2	Total probing time (min)	Time to 1st probe from start of EPG (min)	Total duration of E1 (min)	Total duration of E2 (min)	Number of probes	Number of np	Number of C	Number of E2
	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r
Crude protein	-0.400	0.400	0.400	-0.800	0..200	0.400	0.400	0.400	-0.800	0.800	0.400	-0.800	0.200	0.632	0.400	0.211
Total soluble sugar	-0.800	0.200	0.200	-0.800	0.400	0.800	0.200	0.200	-0.800	0.800	0.400	-0.200	0.800	0.949	0.600	0.949
Total amino acid	-0.800	0.200	0.200	-0.400	0.400	0.400	0.200	0.200	-0.400	0.400	0.800	-0.600	0.400	0.316	0.200	0.105
Carbohydrates																
Glucose	-0.800	0.400	0.400	-0.960*	0.600	0.200	0.400	0.400	-0.800	0.800	0.400	-0.800	0.200	0.632	0.400	0.211
Fructose	-0.800	0.200	0.400	-0.800	0.200	0.400	0.600	0.200	-0.800	0.600	0.400	-0.800	0.400	0.632	0.400	0.056
Sucrose	-0.600	0.400	0.600	-0.990*	0.200	0.200	0.400	0.600	-0.800	0.800	0.400	-0.965*	0.400	0.632	0.400	0.211
Amino acid concentrations																
Asp	-0.800	0.400	0.400	-0.800	0.200	0.400	0.400	0.400	-0.800	0.800	0.400	-0.800	0.200	0.632	0.400	0.778
Thr	-0.800	0.400	0.200	-0.800	0.400	0.400	0.400	0.600	-0.800	0.800	0.400	-0.800	0.400	0.389	0.400	0.211
Ser	-0.970*	0.600	0.200	-0.800	0.200	0.200	0.400	0.400	-0.800	0.800	0.400	-0.800	0.200	0.632	0.400	0.211
Glu	-0.800	0.600	0.400	-0.985*	0.600	0.200	0.800	0.600	-0.990*	0.800	0.400	-0.990*	0.400	0.389	0.400	0.056
Pro	-0.400	0.200	0.400	-0.800	0.200	0.200	0.400	0.400	-0.800	0.632	0.632	-0.738	0.200	0.632	0.400	0.211
Gly	-0.600	0.400	0.600	-0.800	0.400	0.200	0.600	0.400	-0.632	0.800	0.400	-0.800	0.316	0.632	0.105	0.211
Ala	-0.955*	0.600	0.600	-0.800	0.200	0.400	0.400	0.600	-0.800	0.800	0.400	-0.949	0.200	0.389	0.400	0.056
Cys	-0.400	0.400	0.200	-0.800	0.400	0.400	0.600	0.400	-0.800	0.632	0.211	-0.800	0.200	0.632	0.400	0.211

Table 6-4 continued:

Components	aCO_2								eCO_2							
	Total probing time (min)	Time to 1st probe from start of EPG (min)	Total duration of E1 (min)	Total duration of E2 (min)	Number of probes	Number of np	Number of C	Number of E2	Total probing time (min)	Time to 1st probe from start of EPG (min)	Total duration of E1 (min)	Total duration of E2 (min)	Number of probes	Number of np	Number of C	Number of E2
	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r
Val	-0.400	0.400	0.400	-0.800	0.200	0.200	0.400	0.400	-0.800	0.800	0.400	-0.800	0.400	0.632	0.316	0.211
Ile	-0.600	0.200	0.200	-0.800	0.200	0.600	0.400	0.600	-0.800	0.600	0.400	-0.800	0.400	0.632	0.400	0.056
Leu	-0.800	0.400	0.400	-0.800	0.400	0.400	0.200	0.400	-0.800	0.800	0.400	-0.975*	0.200	0.576	0.400	0.211
Tyr	-0.400	0.200	0.200	-0.800	0.200	0.400	0.600	0.400	0.949	0.949	0.316	-0.800	0.200	0.833	0.632	0.778
Phe	-0.800	0.600	0.400	-0.955*	0.400	0.600	0.400	0.600	0.800	0.800	0.400	-0.965*	0.316	0.632	0.400	0.056
His	-0.400	0.400	0.200	-0.800	0.200	0.400	0.400	0.400	-0.632	0.632	0.632	-0.738	0.200	0.500	0.105	0.211
Lys	-0.600	0.200	0.600	-0.800	0.600	0.600	0.200	0.200	-0.800	0.800	0.400	-0.800	0.316	0.632	0.400	0.211
Arg	-0.800	0.200	0.400	-0.960*	0.200	0.400	0.600	0.400	-0.800	0.800	0.400	-0.800	0.200	0.632	0.400	0.211
Biological parameters																
Development time	-0.832	0.211	0.211	-0.738	0.105	0.316	0.211	0.211	-0.949	0.800	0.105	-0.316	0.211	0.889	0.949	0.778
Fecundity	-0.800	0.400	0.400	-0.800	0.200	0.600	0.400	0.400	-0.775	0.775	0.258	-0.775	0.258	0.544	0.775	0.272
rm	-0.832	0.211	0.211	-0.738	0.105	0.211	0.211	0.211	-0.632	0.632	0.211	-0.949	0.105	0.389	0.316	0.056
Wnympha	-0.816	0.316	0318	-0.632	0.316	0.316	0.316	0.316	-0.800	0.800	0.400	-0.800	0.200	0.632	0.400	0.211
Wadultb	-0.800	0.400	0.400	-0.800	0.200	0.400	0.400	0.400	-0.800	0.800	0.400	-0.800	0.200	0.632	0.400	0.211
MRGR	-0.800	0.400	0.400	-0.800	0.200	0.400	0.600	0.400	-0.400	0.400	0.400	-0.800	0.400	0.105	0.200	0.316

[a] W_{nymph} means fresh body weight of newborn nymph (< 6h) (mg);
[b] W_{adult} means fresh body weight of 7 day adult (mg);
r = correlation coefficient;
Asterisks indicate level of significance: $P < 0.05$.

Table 6-5: Coefficients of Pearson's correlation between nutrient contents of barley seedlings and biological parameters of *Rhopalosiphum maidis* under aCO_2 and eCO_2 conditions.

Components	aCO_2						eCO_2					
	Development time	Fecundity	rm	Wnympha	Wadultb	MRGR	Development time	Fecundity	rm	Wnymph	Wadults	MRGR
	r	r	r	r	r	r	r	r	r	r	r	r
Crude protein	0.904	0.979*	0.954*	0.921	0.890	0.904	0.884	0.989*	0.896	0.746	0.825	0.808
Total soluble sugar	0.946	0.892	0.936	0.898	0.970*	0.911	0.597	0.932	0.892	0.877	0.938	0.836
Total amino acid	0.917	0.855	0.902	0.925	0.928	0.932	0.803	0.876	0.939	0.892	0.863	0.824
Carbohydrates												
Glucose	0.924	0.937	0.893	0.904	0.972*	0.946	0.85	0.872	0.934	0.811	0.879	0.862
Fructose	0.944	0.892	0.946	0.949	0.998*	0.914	0.759	0.915	0.880	0.904	0.953*	0.939
Sucrose	0.937	0.949	0.945	0.795	0.966*	0.873	0.585	0.725	0.941	0.877	0.99*	0.945
Amino acid concentrations												
Asp	0.742	0.794	0.945	0.942	0.762	0.867	0.804	0.920	0.859	0.841	0.909	0.898
Thr	0.879	0.866	0.781	0.929	0.837	0.928	0.649	0.552	0.725	0.877	0.815	0.735
Ser	0.903	0.744	0.891	0.85	0.804	0.848	0.76	0.702	0.812	0.896	0.865	0.795
Glu	0.852	0.917	0.909	0.841	0.092	0.90	0.703	0.839	0.875	0.873	0.781	0.938
Pro	0.909	0.795	0.898	0.901	0.842	0.895	0.472	0.503	0.799	0.938	0.897	0.845
Gly	0.913	0.751	0.902	0.846	0.812	0.849	0.673	0.801	0.966*	0.925	0.985*	0.678
Ala	0.919	0.763	0.908	0.855	0.823	0.858	0.451	0.706	0.971*	0.874	0.912	0.937
Cys	0.932	0.833	0.922	0.918	0.876	0.919	0.636	0.87	0.921	0.92	0.875	0.929

Table 6-5 continued:

Components	aCO_2						eCO_2					
	Development time	Fecundity	rm	Wnympha	Wadultb	MRGR	Development time	Fecundity	rm	Wnymph	Wadults	MRGR
	r	r	r	r	r	r	r	r	r	r	r	r
Val	0.943	0.879	0.936	0.946	0.914	0.941	0.362	0.605	0.929	0.786	0.976*	0.897
Ile	0.926	0.93	0.936	0.759	0.955*	0.843	0.61	0.769	0.971*	0.917	0.932	0.921
Leu	0.892	0.890	0.788	0.887	0.938	0.921	0.783	0.737	0.831	0.896	0.875	0.808
Tyr	0.783	0.737	0.831	0.896	0.875	0.808	0.802	0.728	0.800	0.896	0.844	0.772
Phe	0.909	0.905	0.92	0.715	0.927	0.806	0.724	0.882	0.985*	0.934	0.972*	0.926
His	0.904	0.992*	0.909	0.931	0.922	0.87	0..455	0.522	0.83	0.922	0.975*	0.878
Lys	0.901	0.996*	0.908	0.894	0.988*	0.945	0.634	0.675	0.881	0.942	0.971*	0.893
Arg	0.946	0.986*	0.952*	0.882	0.995*	0.95	0.6	0.743	0.928	0.932	0.954*	0.928

[a] W_{nymph} means fresh body weight of newborn nymphs (< 6h) (mg);

[b] W_{aduit} means fresh body weight of 7 day old adults (mg);

r = correlation coefficient;

Asterisks indicate level of significance: $P < 0.05$.

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7

Conclusions and future prospects

The corn leaf aphid *Rhopalosiphum maidis* has a worldwide distribution and then exposed to diverse environmental challenges to be faced with exceptional physiological tolerances. Their small size makes them vulnerable to acute shifts in temperature, but also gives them ready access to sheltered retreats. Small size and high mass-specific metabolic rates makes them sensitive to energy and water balance during exposure to extremes (Williams et al., 2014). To investigate the differential thermal tolerance across life stages under extreme high temperatures crossed with feeding status, we imitated natural fluctuated extreme climates as much as possible, considered most important abiotic (temperature and CO_2) and biotic (food) factors, combined with the aphid own physiological conditions (life stage) to investigate thermal tolerance of *R. maidis* under brief extreme high temperatures.

Our results indicated that the critical high temperature was 39 °C for *R. maidis* with a brief extreme high temperature. This information could be valuable in developing a reliable phenological model for the prediction of population dynamics of *R. maidis*. The highest ULT50s and ULT90s were observed in 3rd and 4th instars compared to 1st and 2nd instar nymphs and adults. The younger instar nymphs were less thermal tolerant, which was probably because of their low mobility and weak thermal inertia to use behaviour to evade extreme conditions (Zhang et al., 2015). Adult survival might be depressed if there is a trade-off between survival under stress and reproductive output (Cox et al., 2010; Marshall and Sinclair, 2010) or as a result of senescence (Colinet et al., 2013). Once sexual maturity is reached, an individual's energy budget might evolutionarily support an increased fitness by spending more effort on reproduction (Sørensen and Loeschcke, 2002).

Then, we analyzed wing polyphenism adaptation across life stages under extreme high temperatures of *R. maidis.* This aphid exhibited wing polyphenism, which is an important form to adapt to adverse environment. The production of winged individuals among aphid populations is essential for the aphid life cycle and is possibly the best strategy for dispersal and colonization to more optimal environments (Müller et al., 2001). Our results confirmed earlier studies and clearly showed that aphids are responsive to crowding. While *R. maidis* aphid reared in isolation rarely gave rise to alate forms, the increase in insect density induced larger proportion of alatae. In addition, temperature played a significant role in wing production, with the 26/39 °C temperature setting inducing higher alate morphs and survival. Our results demonstrated that not only global warming, but also the frequent extreme heat events would increase the abundance of CLA under the climate change.

Aphid foraging behaviors were found to be influenced by host plant reared in different CO_2 concentrations. The diversity and abundance of plant VOCs were also differently induced by elevated CO_2 when compared with aCO_2. The wingless and winged aphids were more attracted by the odors of aCO_2 barley seedlings when tested against eCO_2 barley seedlings or control. GC-MS analysis showed amounts of linalool emitted by eCO_2 barley seedling, which had showed a repellent effect on green peach aphid, *Myzus persicae* (Sulzer) (Harmel et al. 2007), corn leaf aphid, *R. maidis* and bird cherry-oat aphid, *R. padi* (Halbert et al. 2009).

Elevated CO_2 was found to affect the feeding behavior via the change of the plant nutrient contents and exerting an influence on the herbivore performance. Rearing in eCO_2 condition, barley seedlings significantly decreased the concentration of crude protein, total amino acids and 13 individual amino acids. The corn leaf aphid prolonged the total probing time and sustained ingestion on eCO_2 barley seedlings but was lower in fecundity, rm and fresh body weight which negatively impacted population abundance of aphid. Our results support most of these predictions. *R. maidis* feeding on eCO_2 barley seedlings showed significantly decreased body weight, fecundity and rm, which may result in decreased of population abundance under elevated atmospheric CO_2 environment.

The aphid behavioral response to plant VOCs is complex. Presented as a blend, these volatile compounds may be integrated as host cues, leading to aphid attraction/arrestment toward the odor source. Future works may focus on how aphids gain information on the identity and quality of a plant from the composition of its volatile blend and how interactions between volatiles can affect aphid behavior in changing climatic. Furthermore, studies on field-acclimatized individuals are required to elucidate how seasonal heterogeneity influences winged-induced genetic variation in physiological sensitivity but also in further spatio-temporal dynamics of insect natural communities as a major challenge.

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Appendix – Publications

1. Publications included in the thesis

1). Chen, Y., Serteyn, L., Wang, Z.Y., He, K.L., & Francis, F. Reduction of plant suitability for corn leaf aphid under elevated carbon dioxide condition, *Environmental Entomology*, https://doi.org/10.1093/ee/nvz045 (2019).

2). Chen, Y., Verheggen, F.J., Sun, D.D., Wang, Z.Y., Francis, F., & He, K.L. Differential wing polyphenism adaptation across life stages under extreme high temperatures in corn leaf aphid. *Scientific Reports*, https://doi.org/10.1038/s41598-019-45045-x (2019) .

3). Chen, Y., Martin, C., Fingu Mabola, J.C., Verheggen, F.J., Wang, Z.Y., He, K.L., & Francis, F.. Effects of host plants reared under elevated CO_2 concentrations on the foraging behavior of different stages of corn leaf aphid *Rhopalosiphum maidis. Insects*, https://doi.org/10.3390/insects10060182 (2019).

4). Chen, Y., Quan, Y.D., Verheggen, F.J., Wang, Z.Y., Francis, F. & He, K.L. Differential thermal tolerance across life stages under extreme high temperatures crossed with feeding status in corn leaf aphid. Submitted to *Scientific Reports*.

2. International conferences related to the subject

1). Chen, Y., Quan, Y.D. et al. Feeding can increase thermal adaptation of herbivorous insect: a case study with corn leaf aphid under acclimation temperature. The 26th International Working Group on Ostrinia and other Maize Pests, Beijing, P.R. China, in 2017.04.10-2017.04.12.

2). Chen, Y., Martin, C. et al. Effects of elevated CO_2 concentrations on foraging behavior of different stages of corn leaf aphids *Rhopalosiphum maidis*. The 70th International Symposium on Crop Protection, Ghent. Belgium, in 2018.05.22.