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**Effects of a heat wave event on the chemical ecology of species interactions in the potato
agroecosystem**

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Abstract

Heat waves, brief periods of unusually high temperatures, are increasing in frequency and intensity globally. Such extreme weather events can alter plant chemistry, disrupting species interactions that contribute to pest suppression or increase their performance. Yet, most heat wave studies focus on pairwise interactions, leaving us with a poor understanding of how complex agroecosystems respond to temperature extremes. We addressed this knowledge gap by simulating an experimental heat wave in the field on potato plants (*Solanum tuberosum*) and the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), in the presence or absence of their mutualistic microbial symbionts and another pest, the potato aphid (*Macrosiphum euphorbiae*). We assessed CPB performance alongside changes in volatile organic compounds (VOCs) and glycoalkaloids from host plants. CPB performance declined in the absence of CPB microbial symbionts and under aphid competition, but this effect was reversed under heat wave conditions. These results corresponded with a downregulation in glycoalkaloids, suggesting that potato prioritizes heat stress response over herbivore attack by divesting resources from the production of defensive compounds. The heat wave strongly affected VOCs composition, reducing emissions of multiple compounds while increasing others, but these changes were not directly linked with CPB performance. Overall, our results demonstrate that heat waves effects on crop-pest dynamics are dependent on the agroecological context and mediated by specialized metabolites. Importantly, under dual herbivore pressure, potato crops appear to prioritize coping with heat over defending against pests, underscoring the urgent need for pest management strategies that account for extreme climate events.

Key words: Colorado potato beetle; potato aphid; *Solanum tuberosum*; symbiotic microbes; thermal stress; volatile organic compounds

Introduction

While most climate change research has focused on the consequences of gradual changes in climate averages, a growing number of studies suggest that extreme weather events can lead to substantial losses in agricultural economic outputs (Thorton et al. 2014, Miller et al. 2021). Among these events, heat waves — brief periods of stressfully high temperatures (Robinson 2001) — can have significant impacts on pest management (Zhang et al. 2015, Skendzic et al. 2021, Carvajal-Acosta et al. 2024). Agroecosystems are composed of multiple interacting species which are largely mediated by plant chemistry (Silva et al. 2018). Nonetheless, most of our understanding of the chemical ecology of heat waves are based on experiments involving a focal species and are often conducted in greenhouses or laboratory conditions. As a result, our knowledge of how heat waves impact the chemical ecology of crop-pest interactions, particularly under field conditions where insect pests interact with multiple other species, remains underdeveloped.

In their agroecological contexts, insect pests interact with multiple other species including crops, other pest competitors, mutualists, among other species that contribute to pest suppression or enhance pest performance. Plant chemistry plays a major role in modulating these interactions (Silva et al. 2018). For instance, plant response to herbivore attack is often highly specific depending on the herbivore feeding guild or whether they are being attacked by a single or multiple herbivores (Dicke and Baldwin 2010, Moreira et al. 2015). Thus, the presence of other insect pest species, by altering plant chemistry, may significantly alter interactions between a crop and a focal pest (Ponzio et al. 2017, Davidson-Lowe and Ali 2021). There is also growing evidence of the importance of microbial mutualistic symbioses in insect–plant interactions. For example, several studies report cases of mutualistic relationships between insect herbivores and

symbiotic microbes that allow them to bypass or confer resistance against plants defenses (Barr et al. 2010, Frago et al. 2012, Chung et al. 2013). In addition to attracting natural enemies of pest (Aartsma et al. 2019, Takabayashi & Shiojiri 2019), some volatile organic compounds (VOCs) have direct toxic or deterrent effects on insect herbivores (Gershenzon and Dudareva 2007, Jander 2022) and are also involved in mediating interactions among herbivores from separate feeding guilds (Davidson-Lowe & Ali, 2021). Although recent work has shown that high temperatures can change the plant chemistry that govern these important interactions (Jan et al. 2021, Chávez-Arias et al. 2022), studies investigating heat wave impacts on the chemical ecology of interacting species are lacking.

The majority of thermal studies suggest that plants upregulate the production of secondary compounds under stressfully high temperatures (Dias et al. 2019, Liu et al. 2021, Laoue et al. 2022, Yeshi et al. 2022), which are thought to act as a protective mechanism against thermal and oxidative stress (Jan et al. 2021, Laoue et al. 2022). Nonetheless, it is unclear whether the accumulation of these chemicals translates into higher plant resistance against herbivores (Havko et al. 2020, Kuczyk et al. 2021, Cope et al. 2023, Lopez-Goldar et al. 2024). Likewise, research shows that elevated temperatures enhance the emission of VOCs (Holopainen and Gershenzon 2010, Copolovici et al. 2012, Liu et al. 2021). For instance, heat stress can induce glucosinolate hydrolysis leading to the release of volatile byproducts such as isothiocyanates (Kask et al. 2016). However, heat stress can also suppress stomatal aperture (Haworth et al. 2018) reducing the emission of certain volatiles compounds. Moreover, research is limited on how these chemical defenses are affected by the simultaneous occurrence of different biotic or abiotic stresses such as multiple-herbivore attack and high temperatures (Huber and Bauerle 2016, Peng et al. 2022).

Plant response to combined or contrasting stressors can be highly complex as they are largely controlled by physiological pathways that may interact synergistically or antagonistically which each other to elicit a wide range of plant responses (Fujita et al. 2006, Suzuki et al. 2014). For example, it has been widely reported that plants response to combined biotic stresses (leaf chewers and phloem feeder) or conflicting stressors such as abiotic and biotic stress in an antagonistic fashion (i.e., negative phytohormone crosstalk) (Anderson et al. 2004, Ku et al. 2018, Aerts 2021). This leads to an unbalanced plant response where one stress is prioritized over another. However, plant response can also be synergistic or additive (no-interaction) depending on the ecological context, stress level or timing (Rejeb et al. 2014, Khan et al. 2020, Cinto Mejía and Wetzel 2023). A synergistic response leads to higher overall resilience than plants exposed to a single stressor (Rejeb et al. 2014) while an additive response results in a balance response to conflicting stresses (Cappetta et al. 2020). The complexity of plant responses further highlights the need for studies that account for the ecological context (i.e., diversity of interacting species) to fully grasp the impacts of heat waves on agricultural systems.

In this study we used the potato agroecosystem as a model system to investigate the impacts of a heat wave event on the chemical ecology of potato crops (*Solanum tuberosum*) and its main pest, the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), under increasing levels of ecological complexity, specifically represented by interaction diversity. To accomplish this, we used ceramic heaters and open top chambers to generate a single heat wave event in a potato field with plants exposed to CPB alone or CPB in the presence or absence of phloem-feeders (potato aphids; *Macrosiphum euphorbiae*) and CPB microbial symbionts. We then quantified CPB performance and plant secondary chemistry (glycoalkaloids and VOCs). We hypothesized that the effects of heat waves on crop-pest

interactions will depend on the interaction diversity of an agroecosystem. Specifically, we predicted an antagonistic plant response to higher interaction diversity where plants exposed to high biotic stress (i.e., dual pest damage and gut microbes) and high temperatures, will prioritize responses to extreme heat over herbivore attack (Ku et al. 2018) (Fig. 1). Therefore, heat waves should decrease glycoalkaloids and, as consequence, improve CPB performance in plants exposed to high biotic and abiotic stress compared to plants subjected to lower biotic stress (i.e., no herbivores or CPB-only damage) (Fig. 1). Finally, because VOCs emissions have been shown to be highly sensitive to heat stress and known to change with herbivore damage, we hypothesized that volatiles compounds will be strongly affected by heat wave conditions and damage by CPB and aphids. However, it is unclear how VOCs will be affected by simultaneous biotic and abiotic stress.

Materials and Methods

Study organisms

To study heat wave impacts on crop-pest interactions, we used potato plants Atlantic variety and Colorado potato beetle as the main insect pest. In addition, we used potato aphid to assess whether prior damage by phloem feeders affects the interactions between potato and CPB under a heat wave event. Potato plants defend themselves against insect herbivores by producing toxic glycoalkaloids (Hlywka et al. 1994) and leaf volatiles have been shown to act as repellent for herbivores and deter further feeding (Ali and Wright 2020, Munawar et al. 2022).

In northern climates, CPB adults spend the winter underground and emerge in the spring when they disperse to a suitable host plant to feed and reproduce (Boiteau et al., 2003). CPB chewing herbivore that activates and is susceptible to defenses associated with the jasmonic acid

(JA) pathway and it is also engaged in a symbiotic relationship with bacteria in the genera *Stenotrophomonas*, *Pseudomonas*, and *Enterobacter* that manipulate plant defenses in their favor (Chung et al. 2013). The bacteria present in CPB saliva elicit Salicylic acid (SA) regulated defenses interfering with the activation of JA-associated resistance against leaf-chewers. The potato aphid is a phloem feeder, which activates and is susceptible to plant defenses associated with the SA pathway and may also interfere with the activation of JA-associated defenses against leaf chewers.

Experimental design

To assess the effect of a heat wave event on pest performance and plant chemistry on different ecological contexts, we conducted a field experiment during the 2022 growing season at the Michigan State University Kellogg Biological Station (KBS) (Hickory Corners, MI). We generated a 4-day heat wave event on potato plants exposed to damage by CPB larvae with or without microbial symbionts and/or simultaneous damage by potato aphids.

CPB colony

All larvae were obtained from a colony maintained at the KBS greenhouse facilities with adults sourced from the Montcalm Research Station, MI. We obtained CPB without symbiotic bacteria by splitting the colony into separate cages with antibiotic-free and antibiotic-treated potato plants. Antibiotic-treated plants were sprayed thoroughly with an antibiotic solution consisting of 5% Tetracycline, 5% Neomycin, and 2.5% Streptomycin in DI water (modified from Chung et al. 2013). To ensure that symbiotic bacteria was removed from CPB digestive system, we collected fecal samples from antibiotic-treated and non-treated CPB in Petri dishes containing agar and observed bacterial colony growth for a week (Supp.1 Fig. S1).

Field experiment

We planted seed potatoes individually within 1 m² plots in mid-May. When plants began sprouting and before naturally occurring CPB populations arrived, we allowed a subset of plants to be colonized by natural populations of potato aphids (Supp. 2) and covered the rest with 1 m² by 80 cm tall mesh cages (Lumite, Inc., GA). When plants were about six weeks old, they were assigned to one of the following herbivory treatment combinations: 1) “control” plants with no herbivores; 2) “CPB” alone; 3) CPB treated with antibiotics “CPB(ab)”; 4) CPB with aphids “CPB+aphid”; and 5) antibiotic-treated CPB with aphids “CPB(ab)+aphid”. Plants in the “CPB” and “CPB(ab)” groups received 10 CPB neonate larvae from the antibiotic-free and the antibiotic-treated colony, respectively. Due to high neonate mortality, two days later, we added 10 additional 1st instar stage larvae ensuring each plant had at least 10 CPB larvae. For the “CPB+aphid” and “CPB(ab)+aphid” treatment groups, we added CPB larvae from their corresponding colonies as described above to the aphid colonized plants and covered with the protective cages. Experimental plants assigned to the antibiotic treatment groups were also sprayed once prior to the heat wave event to ensure CPB larvae were free of the symbiotic bacteria.

After one week of initiating bioassays, half of these plants were assigned randomly to either an ambient or heat wave group. Each herbivore x heat wave treatment combination had 15 replicates, totaling 120 plants. We simulated a single 4-day heat wave event in mid-July using an electric 300-Watt ceramic heater (Tempco, Inc., IL) hung in the opening of a pyramidal open-top chamber with a wood frame and anti-condensate greenhouse plastic sides (6 mil, 91% light transmittance, Poly-Ag Corp., CA) (Carvajal-Acosta et al. 2024). This system increased daily temperatures by an average of 10°C and 4°C at night, achieving average temperatures of 41.84°C

± 9.86 (diurnal) and 25.16°C ± 4.2 (nocturnal) in the heat wave plots compared to 31.8°C ± 9.26 (diurnal) and 21.91°C ± 3.43 in ambient plots (Supp.1 Fig. S2). To prevent heat shock in plants and insects, we initiated the heat wave during the night when temperatures are cooler. At the end of the heat treatment, we removed the heaters and chambers but left the cages in place.

Data collection

Insect performance

We censused CPB larvae twice, once before the heat wave and once after to quantify performance (searching for 5 minutes per plant). Adult beetles were collected in mid-August prior to removing experimental cages, frozen immediately after collection, oven-dried and sexed. We then weighted them and measured pronotum length to assess adult performance.

Plant chemistry

We analyzed potato's four main glycoalkaloids (solanine, chaconine, dehydrosolanine, and dehydrochaconine), and leaf volatiles to assess whether heat stress changes plant secondary chemistry under different types of biotic stresses. Glycoalkaloids were extracted from plant tissue and analyzed using LC/MS/MS at the Michigan State University's Mass Spectrometry and Metabolomics Core. Samples were collected at the end of the experiment to avoid plant induction which may alter biotic interactions or interfere with the accuracy of volatile measurements.

Approximately 100 mg of plant tissue from each plant were collected in pre-weighted 2 mL tubes containing three 3mm stainless steel beads (VXB.com; Kit 12064). Frozen tissue was ground in a pre-frozen bead beater (Retsch, MM400) at 30/s until fully ground. Samples were extracted with 1 mL extraction buffer (80:20 v/v methanol:water, 0.1% formic acid, and 100 nM

of digitoxin as internal standards). After incubating at 4°C on a rocking platform for 16 hours, samples were centrifuged at 4°C for 10 minutes at 14,000 rpm (Eppendorf, 5810 R). Supernatants (10 µl) were diluted into a 990 µL cold extraction buffer and stored at -20°C. Samples were analyzed using a Waters Xevo G2-XS Quadrupole-Time-of-flight LC/MS/MS system with a Waters Acquity BEH-C18 UPLC column (2.1 x 100mm) in positive ion mode. Compounds were eluted using a binary gradient of solvent A (0.1% formic acid in water) and solvent B (acetonitrile) at a flow rate of 0.3 mL/ minute at 40°C following a stepwise gradient: 98.0% A, 2.0% B; 0.50 min, 85.0% A, 15.0% B; 5.00 min, 40.0% A, 60.0% B; 7.00 min, 1.0% A, 99.0% B; 8.00 min, 1.0% A, 99.0% B; 8.01 min, 98.0% A, 2.0% B; 10.00 min, 98.0% A, 2.0% B. We identified compounds in the Waters MassLynx software based on mass spectrometry, confirmed with digitoxin as internal standard, and quantified using the Waters Quanlynx MS software. Prior to statistical analysis data was normalized to internal standards and tissue sample mass.

Leaf volatiles were collected immediately after the heat wave event over a period of three days from 118 plants including at least 10 replicates per each treatment combination. Sampling occurred between 11 AM and 2 PM during which time temperatures ranged from 23-43°C following dynamic headspace sampling procedures modified from (Campbell et al. 2019). Briefly, we selected one vegetative stem per plant of similar biomass and enclosed them in a 35 x 35 cm nylon oven bags (Reynolds, USA) unsealed on one side. The bags were tied with twisters and volatiles were allowed to equilibrate for 30 minutes, after which, samples were pumped for 30 minutes through a scent trap using an air sampling pump (Ointik; Push-Pull Active Air Sampling Vacuum Pump) set to a pre-trap flow rate of 350 mL/min. Stems were saved, dried, and weighted. Ambient controls (n=10) were taken from the area adjacent to the experimental

plots where air was collected using an empty oven bag and sampled following the same methodology as the experimental samples. These ambient samples were used to identify contaminants or background compounds from surrounding vegetation.

Leaf and ambient samples were analyzed at the Rocky Mountain Biological Laboratory, CO using thermal desorption Gas Chromatography-Mass Spectrometry (GC-MS), together with one blank/unused scent traps to detect potential contaminants in the trap system. Settings for thermal desorption and GC temperature ramp followed those in Mullins et al. (2020). Peak deconvolution, integration, and tentative compound identification were performed in the Automated Mass Spectral Deconvolution and Identification System (Davies 1998) using the 2020 NIST mass spectral library. Data filtering was performed in the bouquet package (Eisen et al. 2022). Peaks were included if they had mass spectral match scores greater than 80%, a maximum peak area of at least 20,000 counts, and if they were present in more than 20% of the samples. Additionally, we only included compounds with peak areas four times higher than the mean area of the ambient and blank controls. Caprolactam (compounds present in oven bags) and compounds with high retention times (above 15 minutes) were excluded as contaminants. Stems used for volatile collection were saved, dried, and weighted. Volatile emissions were quantified based on peak values and were standardized by dry weight of the sampled stem.

Statistical Analysis

We estimated the effect of the heat wave event on CPB performance and leaf chemistry using Bayesian linear models in R v4.4.0 (R Core Team 2024) in the brms package (Bürkner 2021, Stan Development Team 2024) which uses a Hamiltonian Montecarlo sampling algorithm. Models were fitted using six Markov chain Monte Carlo (MCMC) chains with 40,000 iterations and weakly informed priors (Supp. 1 Table S1). We assessed convergence by ensuring all “Rhat”

values were < 1.03 , and visually checked fits via posterior predictive checks (Gabry et al. 2019). Glycoalkaloid were analyzed using linear models with a normal error distribution. Leaf chemistry data was log-transformed as needed and priors were adjusted accordingly. All models, CPB performance and glycoalkaloids, included heat wave and herbivore treatments and their interaction as fixed effects. CPB performance (mass, survival, pronotum length), was modeled using linear mixed models with plant as a random effect to account for correlations among individuals on the same plant. For CPB larval and adult survival, we used a binomial error distribution and the number of CPB recorded in a pre-heat wave census as the initial number. For CPB adult mass and pronotum length, we used a linear mixed models with a normal error distribution. Initial aphid count (pre-heat wave census) was included as a predictor in models assessing CPB performance while CPB and aphid initial count was included in the leaf chemistry analyses.

We evaluated our hypotheses using the Region of Practical Equivalence (ROPE) and probability of direction (PD) of the posterior distribution (Kruschke and Liddell 2018). We used ± 0.1 SD of zero for our ROPE range. If less than 5% of the posterior distribution fell within the ROPE range, this was considered strong evidence against the null hypothesis, 5-20% moderate evidence, while 20% or more of the posterior within ROPE was considered weak evidence against the null (Liao et al. 2021). Finally, for PD, 50% probability indicates poor evidence against the null and 95% or above strong evidence against the null.

We analyzed plant volatile using a constrained ordination approach which allows to partition the variance attributed to the explanatory variables and thus, more directly tests the effect of each treatment on volatile composition. Plant volatiles emissions were square-root transformed and analyzed using a distance-based redundancy analysis (db-RDA) or CAPSCALE

on a dissimilarity matrix with Euclidean distances. Multivariate analysis was performed in R v4.4.0 (R Core Team 2024) in the vegan package (Oksanen et al. 2007) and all models included herbivore inclusion and heat wave treatments, and their interaction as explanatory variables.

Results

Herbivore performance

In the ambient temperature group, CPB that experienced both competition with aphids and the antibiotic treatment had 28% lower larval survival (ROPE=0%, PD=99%), 38% lower adult survival (ROPE=6%, PD=95%), and 9% smaller adult body sizes (ROPE=3%, PD=94%) compared to antibiotic-untreated CPB feeding alone (Fig. 2 a, b, c). We also found moderate evidence for an interactive effect of the heat wave treatment on CPB performance in the presence of both aphid competition and the antibiotic treatment. This indicates that heat wave effects on CPB performance is determined by an interaction with aphid competition and their microbial symbionts. In antibiotic-treated CPB under aphid competition the heat wave event increased the odds of survival for larvae by 100% (ROPE=7%, PD=93%; Fig. 2a) adults by 50% (ROPE=9%, PD=89%; Fig. 2b) and pronotum length by 10% (ROPE=4%, PD=90%; Fig. 2d) compared to the “CPB” control group. This suggests that beetles suppressed by competition with aphids and reductions in symbiotic bacteria benefited from the heat wave event.

Plant chemistry

Glycoalkaloids

Glycoalkaloids levels in host plants decreased in plants assigned to the “CPB” group and increased in the “CPB(ab)” group, suggesting that symbiotic microbes suppressed production of glycoalkaloids. Chaconine (ROPE=5%, PD=95%), dehydrochaconine (ROPE=0%, PD=99%),

and dehydrosolanine (ROPE=2%, PD=97%) all decreased by ~30-40% on average in all compounds when damaged by microbial-hosting CPB compared to control undamaged plants (Fig. 3a, c, d). In contrast, feeding by antibiotic-treated CPB larvae had the opposite effect on two glycoalkaloids, chaconine (ROPE=5%, PD=94%) and solanine (ROPE=6%, PD=94%) more than doubling concentrations of both compounds (Fig. 3a and b). Plants assigned to both groups with aphids had similar glycoalkaloids concentrations compared to control plants (ROPE>18%, PD<55% across models).

Similar to CPB performance, we detected moderate evidence for an interaction between the heat wave and the “CPB(ab)+aphid” group. Plants subject to the heat wave and simultaneous feeding by antibiotic-treated CPB larvae and aphids led to increases in most glycoalkaloids, chaconine (ROPE=6%, PD=92%) by 21%, dehydrosolanine (ROPE=3%, PD=96%) by 37%, and dehydrochaconine (ROPE=6%, PD=93%) by 34% (Fig. 3 a, c, d). This in contrast to undamaged plants where we observed minor increases in glycoalkaloids under the heat wave.

Leaf volatiles

Among the 57 VOCs identified, 35 contributed to the overall differences between the heat wave and ambient groups (Fig. 4, $P=0.007$). We detected no significant differences in volatiles emissions among the different herbivore inclusion treatment groups ($P=0.28$) and no interactive effect between these terms ($P=0.13$). Constrained inertia was 0.14 indicating that the model explained 14% of the variance in VOCs composition. We visualized differences in emissions between the heat wave and ambient groups with a CAPSCALE ordination from a reduced model including only the heat wave treatment as the explanatory variable (Fig. 4 a). Specifically, β -Sesquiphellandrene, 2,6-Dimethylnonane, β -Longipinene, (β -cis) Farnese, and Sesquisabinene were downregulated under heat wave conditions. On the other hand, emission

rates of Hexamethylcyclotrisiloxane, (α -trans) Bergamotene, (+)-Sativin, 1-Hexadecanol, and Guaia-1(10),11-diene, were higher in plants subjected to the heat wave treatment (Supp. 1 Fig. S3).

Discussion

Extreme climatic events such as heat waves, are currently emerging as major threats for agricultural sustainability and pest management (Zhang et al. 2015, Skendzic et al. 2021, Carvajal Acosta et al. 2024).. By simulating heat wave conditions in a potato field, we showed that heat wave impacts on pest performance depend on the diversity of species interactions and that such impacts are mediated by secondary chemistry. Plants exposed to a heat wave and simultaneous feeding by aphids and microbe-free CPB larvae had lower levels of glycoalkaloids which translated into higher CPB performance. Although VOCs were highly sensitive to higher temperatures these changes were uncorrelated with pest performance. Altogether, these results show that heat wave impacts on crop-pest interactions depend on their agroecological context. Therefore, the successful management of insect pests under increasingly common extreme weather events will require a holistic approach accounting for the various pests present in the field, their feeding modes, as well as their microbiota.

Heat wave impacts on CPB performance

We hypothesized that heat wave impacts on pest performance will be dependent on the diversity of interactions among CPB and their antagonistic and mutualistic species. Overall, our results support this hypothesis as the heat wave had limited effects on the performance of CPB when feeding alone. Under ambient conditions, CPB larvae that experienced aphid competition and lacked their microbial mutualists exhibited the lowest performance, but these trends were

reversed under heat wave conditions. As discussed later, the increased CPB performance in this herbivore inclusion treatment group corresponded with a decrease in glycoalkaloids concentrations. These results were surprising as we expected plants subject to a heat wave and simultaneous damage by aphids and microbial-hosting CPB would be the most stressed, leading to lower defenses and higher CPB performance. It is possible that, because plant respond to damage by phloem feeders and microbial pathogens by activating similar molecular pathways (Lazebnik et al. 2014), plants are unable to perceive the presence of both microbes and aphids as a greater threat. Nonetheless, our results show that heat wave events have stronger effects on pest performance when more than a single herbivorous pest was present.

It is worth noting that in the absence of aphid competition, microbial symbionts did not play a significant role in how CPB performed under the heat wave. In addition to manipulating plant defenses in favor of their herbivorous hosts (Clay and Fox 2014), some symbiotic bacteria are known to confer their hosts with heat resistance (Renoz et al. 2019). As such, we expected that larvae with microbial symbionts will perform better under heat stress compared to antibiotic-treated CPB. Consistent with past studies (Chung et al. 2018), plants damaged by CPB hosting microbes had significantly lower glycoalkaloids concentrations confirming their mutualistic role in CPB, but they appear to play no significant role in thermal tolerance. It is also possible that at stressfully high temperatures, this mutualistic relationship is broken down. Some symbiotic bacteria are known to be especially vulnerable to thermal stress (Wernegreen 2012). Thus, the loss of some symbiotic partners under heat wave conditions and the antibiotic-treatment larvae might have comparable effects on CPB performance.

Effect of heat stress and herbivore inclusion on plant chemistry

Glycoalkaloids

Previous research suggests that heat stress can lead to significant and long-lasting increases in glycoalkaloid concentrations in potato plants (Lafta and Lorenzen 2010). However, it is still unclear how plants respond to elevated temperatures when faced with simultaneous biotic stress. We hypothesized that plant response to high biotic stress (i.e., dual pests and microbes) and high temperatures would be antagonistic, channeling resources into mitigating the effects of abiotic stresses over herbivore defense (Ku et al. 2018). Our results provide partial support for this hypothesis as plants exposed to the heat wave and dual damage by aphids and CPB larvae had significantly lower glycoalkaloids concentrations compared to undamaged plants. However, this effect was only observed in plants damaged by aphids and larvae lacking microbial symbionts. This result suggests that plants exposed to dual pest damage and microbes were not necessarily experiencing higher cumulative stress. A likely explanation, as discussed earlier, is that plants may not perceive the co-occurrence of aphids and microbes as additive threats, since both trigger similar defense pathways (Lazebnik et al. 2014). This perceptual overlap may limit the plant's ability to escalate defense responses beyond a baseline level.

While an antagonistic response to conflicting stressors appears to be common, plant responses can also be synergistic or additive. A synergistic response can enhance plant resilience, leading to stronger defenses under concurrent biotic and abiotic stress (Rejeb et al. 2014), whereas an additive response may result in cross-tolerance, where plants balance their responses to both stress types (Cappetta et al. 2020). For example, research in potato has shown a synergistic response to simultaneous drought stress and herbivory by green peach aphid (*Myzus persicae*) resulting in elevated secondary metabolite production (Quandahor et al. 2020). This suggests that the outcome of plant secondary chemistry under various stresses may be dependent on the specific combination of abiotic stress and biotic stressors imposed on the plant.

390 Plant volatiles have long been recognized to be sensitive to high-temperature stress across
391 a wide range of plant taxa (Holopainen and Gersherzon, 2010). Most previous studies report an
392 increase in VOC emissions under elevated temperatures (Loreto et al. 2006, Duhl et al. 2008,
393 Pazouki et al. 2016), including in *Solanum tuberosum* (Munawar et al. 2022). In this study,
394 however, we observed both increases and decreases in the emission of several compounds—
395 primarily mono- and sesquiterpenes—suggesting a more nuanced response to heat stress.

396 Some compounds, such as terpenes, are known to mitigate abiotic stress (Vickers et al.
397 2009, Copolovici et al., 2012), which may explain the increased emission rates of this compound
398 class. This upregulation is likely linked to enzymatic activity as elevated temperatures can
399 enhance enzyme-substrate interactions and increase reaction rates, resulting in higher volatile
400 production (Xu and Barringer 2009, Yilmaz 2001). Conversely, reductions in VOC emissions
401 under high temperatures may result from reduced stomatal conductance (Haworth et al. 2018),
402 which limits the release of some volatile compounds. Another plausible explanation is that
403 extreme temperatures push plants into a negative carbon balance due to elevated respiration and
404 reduced photosynthesis, leaving fewer carbon resources for VOC biosynthesis (Werner et al.
405 2020).

406 Interestingly, unlike previous findings on VOCs responses to herbivore or pathogen
407 attack in potato plants (Laothawornkitkul et al. 2010, Rajabaskar et al. 2013, Davidson-Lowe
408 and Ali 2021), we did not detect significant VOCs changes in response to these biotic stressors.
409 Furthermore, contrary to studies showing additive effects of combined biotic and abiotic stress
410 (Gouinguene and Turlings 2002, Vuorinen et al. 2004), we found no additive effect between the
411 heat wave and any of the herbivore inclusion groups. This supports the hypothesis of an

antagonistic response to contrasting stresses in which resource limitations prevent plants from mounting simultaneous responses to both biotic and abiotic challenges. Instead, plants may prioritize responses against stresses deemed most severe, those representing the greatest threats to survival, or those for which the most resources are available for mitigation.

In summary, while the heat wave strongly influenced leaf volatile emissions, neither CPB damage alone nor its interaction with aphids or microbes significantly affected VOC profiles. Notably, despite the pronounced shifts in volatile composition between heat-stressed and non-heat-stressed plants, these chemical changes had no apparent effect on how pests responded to the heat wave.

Conclusions and implications

Our study demonstrates that the impacts of heat waves on crop-pest interactions are context-dependent, shaped by the ecological interactions in which insect pests are engaging. Traditionally, pest management targets a single focal insect at a time, often the most damaging species. However, we found that some pests perform better under heat wave conditions in the presence of herbivore competitors. This suggests that with the growing frequency of heat waves, effective pest management will require integrated strategies that reflect the diversity of pest species and their distinct feeding modes.

Another key finding is that potato plants appear to react to thermal stress and dual herbivore attack in an antagonistic fashion, prioritizing protection against stressfully high temperatures over the production of plant defenses. Although multiple studies report an increase in secondary chemicals under heat stress conditions (Dias et al. 2019, Liu et al. 2021, Laoue et al. 2022, Yeshe et al. 2022), our results showed a reduction in defensive compounds when plants

faced simultaneous attack by herbivores from distinct feeding guilds. This reduction corresponded with increased pest performance, implying that crops may be especially vulnerable during heat waves when exposed to multispecies herbivory. Because under field conditions plants are often attacked by more than one insect pests, our results indicate that crops would be more at risk under heat wave events. These findings underscore the critical need for pest management approaches that account for interaction diversity within agroecosystems, particularly under climate-driven stress events.

Our study also contributes to existing evidence that plant volatile emissions are highly sensitive to thermal stress (Holopainen and Gershenzon 2010, Copolovici et al. 2012, Liu et al. 2021), but these changes were uncorrelated with heat wave effects on pest performance. Future studies should focus on the role of specialized defensive metabolites in mediating heat wave effects on pests. However, given that plant volatiles are an integral component of plant's indirect defenses (i.e., attracting natural enemies) it would be worth to evaluate whether heat-induced changes in plant volatile composition compromise the effectiveness of biological control strategies. Ultimately, to understand and mitigate climate-related threats to agriculture, we must explore the chemical ecology of crops and pests in ecologically realistic scenarios that reflect the multispecies interactions occurring in the field.

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Authors contributions:

ANCA: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Supervision; Validation; Visualization; Writing—original draft; Writing—review & editing. WCW: Conceptualization; Funding acquisition; Resources; Methodology; Writing—review & editing; JS: Methodology; Writing—review & editing. ZS: Funding acquisition; Resources; Writing—review & editing.

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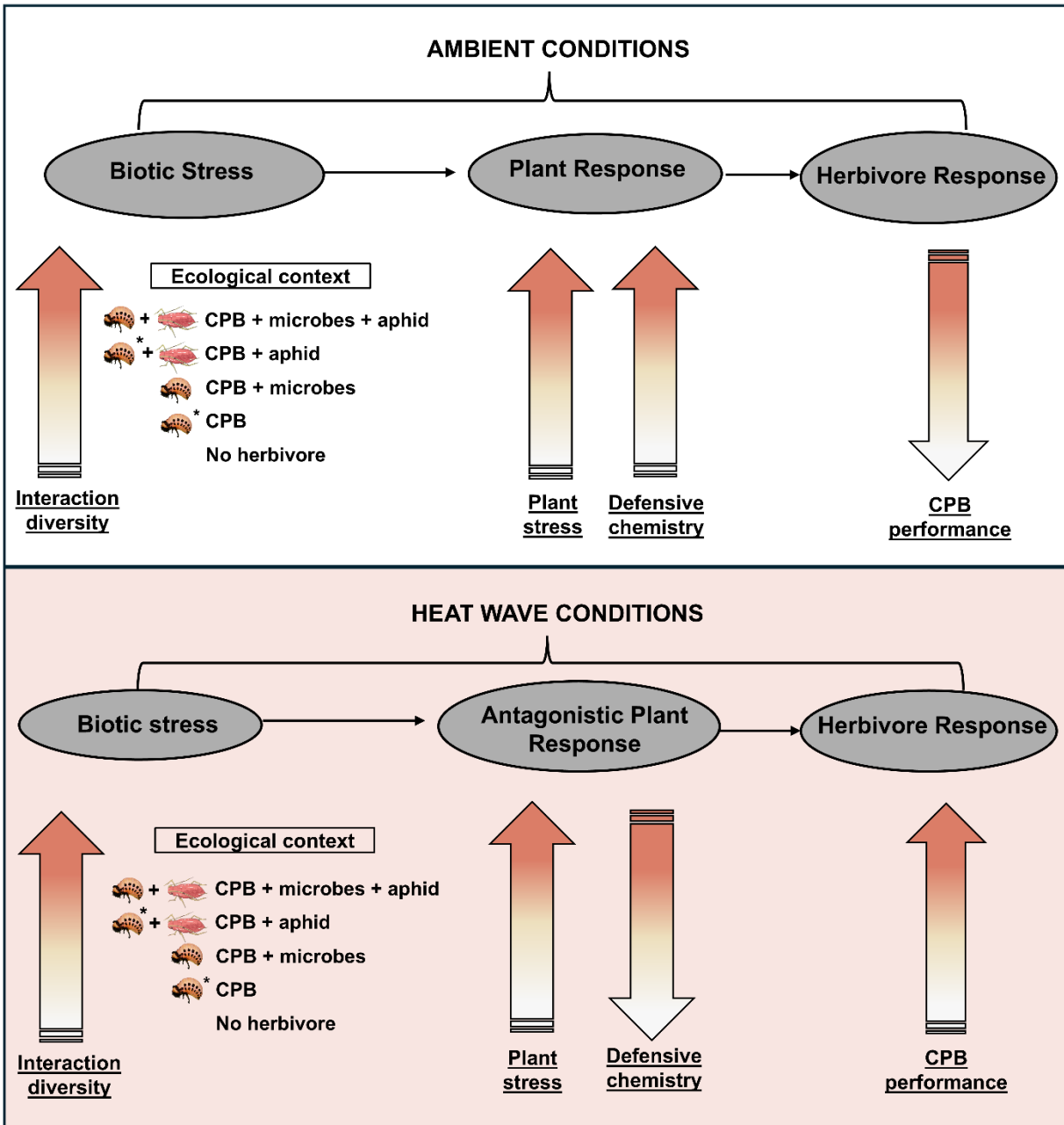


Fig. 1. Conceptual framework showing predicted plant and herbivore response to a heat wave under different levels of species diversity from high to low. CPB marked by asterisk indicates antibiotic treatment to remove microbial symbionts.

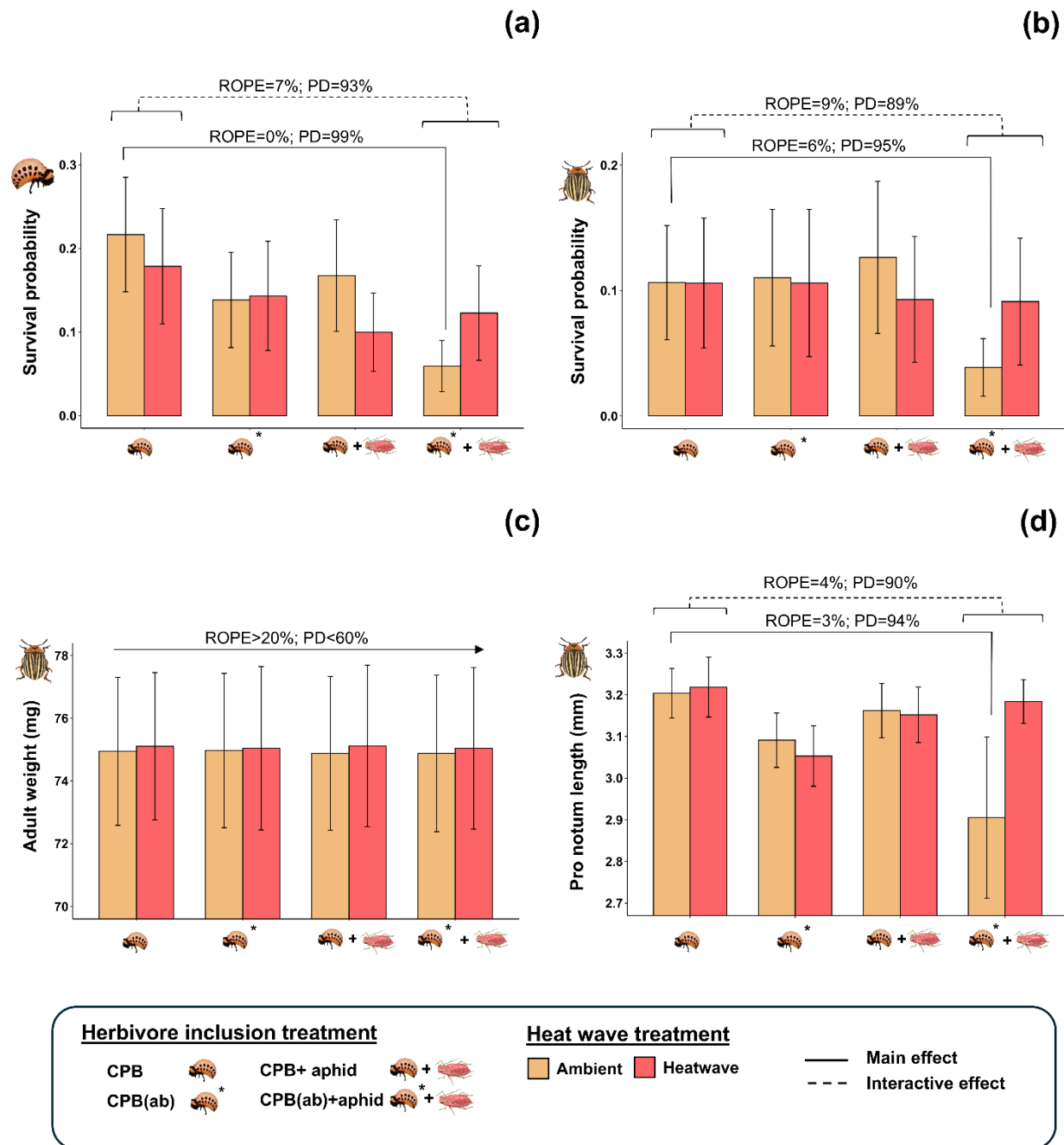


Fig. 2. Effects of a heat wave event on Colorado potato beetle (CPB) performance when interacting with aphids and/or microbial symbionts. Points represent model estimates of average performance and error bars represent standard error. Evidence in support of the alternative

706 hypotheses of an “Herbivore inclusion” effect is indicated by solid lines and its interactive effect
707 with the heat wave treatment is indicated by dashed lines.

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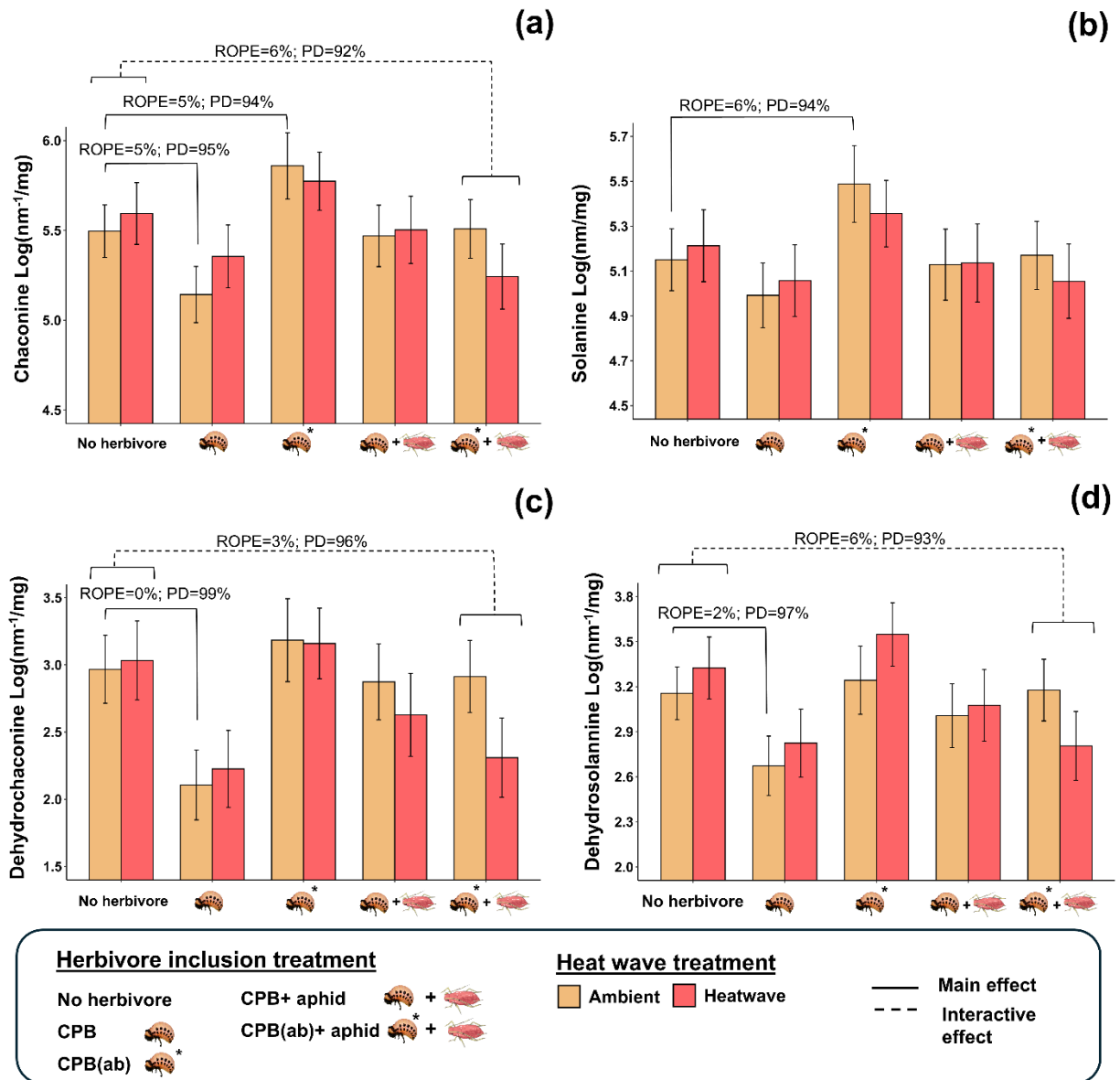


Fig. 3. Glycoalkaloids concentrations in potato host plants under different Colorado potato beetle (CPB) interactions with aphids and/or microbial symbionts and heat wave and ambient conditions. Bars represent model estimates for average glycoalkaloids concentrations and error bars represent standard error. Evidence in support of the alternative hypotheses of an “Herbivore inclusion” effect is indicated by solid lines and its interactive effect with the heat wave treatment is indicated by dashed lines.

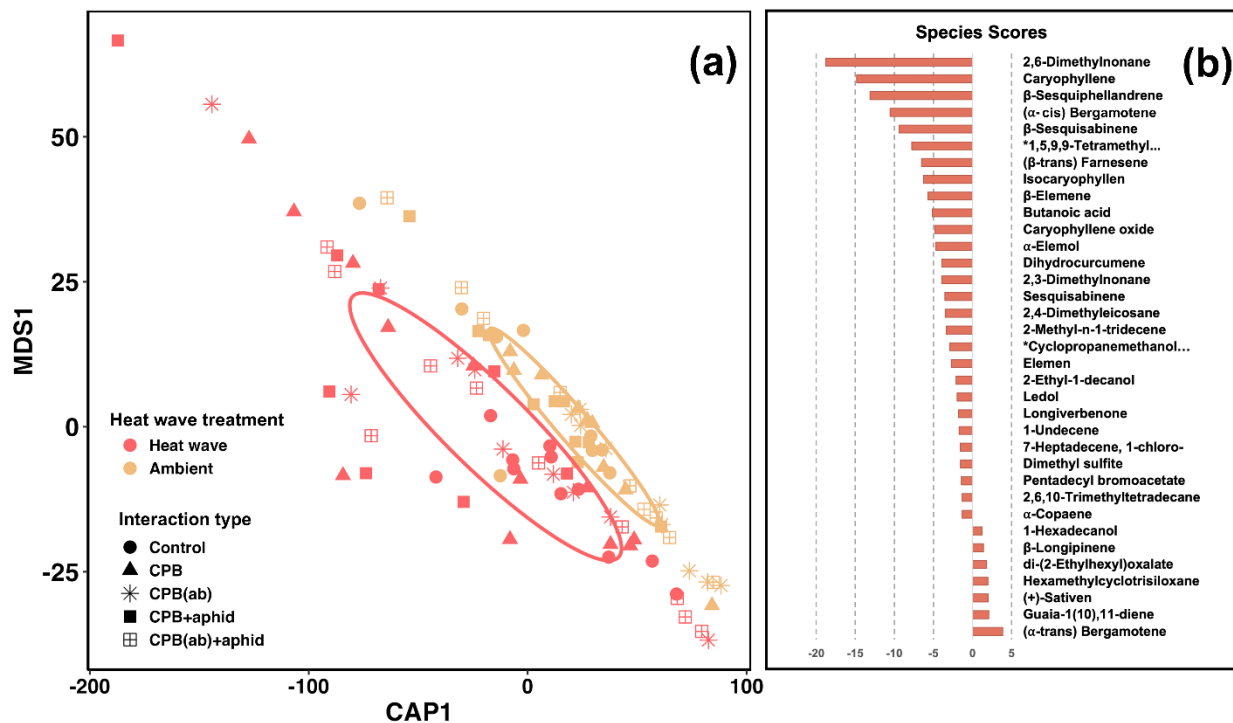


Fig. 4. Effect of a heat wave event on overall volatile composition (a) and specific compounds (b) in potato plants. Panel (a) represents a db-RDA ordination plot showing dissimilarities in plant volatiles between the heat wave and ambient groups. CAP 1 represents the variance explained by the heat wave treatment and MDS1 the total unexplained variance. Panel (b) shows species scores in CAP1 for 35 compounds affected by the heat wave treatment from negative scores to positive. Full name of compounds marked with “*” are 1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene, and Cyclopropanemethanol, 2-methyl-2-(4-methyl-3-pentenyl)-, respectively.