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Elevated CO_2 Levels Impact Fitness Traits of Vine Mealybug *Planococcus ficus* Signoret, but Not Its Parasitoid *Leptomastix dactylopii* Howard

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Abstract: Carbon dioxide (CO_2) is one of the primary factors driving climate change impacts on plants, pests, and natural enemies. The present study reports the effects of different atmospheric CO_2 concentrations on the vine mealybug *Planococcus ficus* (Signoret) and its parasitoid wasp *Leptomastix dactylopii* (Howard). We investigated the life-history parameters of both species on grapevine *Vitis vinifera* (L.) plants grown under elevated (eCO_2) and ambient (aCO_2) CO_2 levels in a greenhouse and in a vineyard free-air carbon dioxide enrichment (FACE) facility. The greenhouse experiments with an eCO_2 level of around 800 ppm showed a significant increase in survival rates, a strong trend towards declining body size, and an increasing fecundity of female mealybugs, while fertility and development time did not change. However, none of these parameters were altered by different CO_2 concentrations in the VineyardFACE facility (eCO_2 level around 450 ppm). On the other hand, the parasitism success, development time and sex ratio of *L. dactylopii*, reared on *P. ficus* under eCO_2 or aCO_2, varied neither in the greenhouse nor in the FACE facility. These results suggest that future CO_2 levels might cause small-scale changes in vine mealybug fitness; however, this is not necessarily reflected by parasitoid performance.

Keywords: climate change; elevated CO_2; grapevine pest; mealybug; parasitoid; FACE

1. Introduction

Atmospheric CO_2 levels are on the rise, with the latest reports published by the Intergovernmental Panel on Climate change (IPCC) reporting an increase of 20 ppm per decade, resulting in an increase of over 35% since pre-industrial times. Current atmospheric CO_2 levels are close to 400 ppm, and this value is expected to double by the end of this century [1]. Plants react to elevated atmospheric CO_2 levels with a whole range of morphological and physiological adaptations. Most C3 plants increase their photosynthesis rates and primary production [2,3]. This also applies to grapevine plants (*Vitis vinifera* L.). For example, Bindi et al. [4] noted that atmospheric CO_2 enrichment stimulated grapevine growth and enhanced fruit and total biomass. Stimulated growth and yield, as well as enhanced stomatal conductance and transpiration, under elevated CO_2, were also reported from a vineyard free-air carbon dioxide enrichment (FACE) facility in Geisenheim, Germany [5].

Alterations in temperature, precipitation, and other climatic factors are expected to have substantial direct impacts on grape insect pests and pathogens, as well as on the suitability of a grapevine as a host plant for a range of organisms [6]. The increased availability of carbon (C) vs. nitrogen (N) leads to an accumulation of carbohydrates in the foliage and consequently to a higher C:N ratio and a lower nutritional value for herbivores [2,3,7]. This has negative effects, especially on leaf-chewing herbivores,

while phloem-feeders seem to be less affected. Several studies report an improved aphid fitness under elevated CO_2 (eCO_2) [3,8], although the mechanisms behind this are not yet fully understood. While aphids are a primary pest in many agricultural and horticultural systems, mealybugs, such as the vine mealybug *Planococcus ficus* Signoret (Hemiptera: Pseudococcidae), are a much bigger concern in grapevine production.

Planococcus ficus is an invasive phloem-feeding insect from the Mediterranean area, which has become a serious invasive pest in many grape-growing regions worldwide [9,10]. Mealybugs affect grapevines both directly and indirectly. By feeding on the phloem sap of all plant organs, mealybugs weaken the plants' vigour. Furthermore, the excreted honeydew promotes the growth of sooty mould on leaves and fruits, reducing photosynthesis, grape marketability and wine quality [9,11,12]. *Planococcus ficus* is also known to transmit grapevine leafroll-associated virus (GLRaV) and other diseases, which reduce the crop yield and wine quality [13,14]. Mealybug control was based on repeated applications of broad-spectrum insecticides, although with limited success, as mealybugs feed not only on the canopy, but also under the bark and in the root area [15], where they are inaccessible to contact-active pesticides. Additionally, the reiterated use of broad-spectrum insecticides is associated with negative effects on non-target organisms, including biological control agents, and the risk of future pesticide resistance [10,16,17]. Therefore, alternative methods for mealybug control include employing new pesticides, pheromone-based mating disruption, and biological control [18–22]. Models predict that under future climate change scenarios, *P. ficus* will thrive and continue to expand its range [23,24]. The only available study on the effects of eCO_2 on scale insects concludes that temperature, rather than CO_2, alters the performance of the Madeira mealybug, *Phenacoccus madeirensis* (Green) [25].

The biological control of mealybugs with natural enemies forms an important part of sustainable pest management programs. *Planococcus ficus* can be parasitised by several encyrtid species, such as *Anagyrus pseudococci* (Girault), *Leptomastix dactylopii* (Howard), *Leptomastidea abnormis* (Girault), *Coccidoxenoides perminutus* Girault, and *Coccidoxenoides peregrinus* (Timberlake) [10]. *Leptomastix dactylopii* (Hymenoptera: Encyrtidae) is a solitary, arrhenotokous, koinobiont endoparasitoid, probably native to eastern Africa [26], which has been introduced into Europe, the United States, Pakistan, India, and Australia [27]. *Leptomastix dactylopii* has been used in biological control programs against the citrus mealybug, *Planococcus citri* (Risso) [28,29], but it has also been found in vineyards infested with *P. ficus* and other vineyard mealybugs in California (US), Iran, South Africa and Tunisia [9,30–32]. Laboratory trials showed that *P. ficus* is a suitable host for *L. dactylopii* and wasps might even have an innate preference for this host [26,33]. *Leptomastix dactylopii* is susceptible to low temperatures, and its geographical range might expand due to global warming, similar to other encyrtid wasps [24]. There are no studies available on the effects of eCO_2 on *L. dactylopii* or other Encyrtidae. Considering other related pest-natural enemy complexes, some studies investigated the knock-on effects of eCO_2 on aphid parasitoids, but results vary between neutral, beneficial, and detrimental effects [34,35]. If host quality decreases under eCO_2, parasitoids will be adversely affected. If eCO_2 increases host quality, parasitoid performance may be maintained or increased. However, the number of available studies is too low to derive general patterns.

Besides host quality, parasitism success depends on the parasitoid's ability to locate possible hosts. When attacked by herbivores, plants release attack-specific volatiles which help natural enemies to locate these herbivores [36]. While chewing insects stimulate the jasmonic acid-signalling pathway, phloem-feeding insects trigger the salicylic acid signalling pathway [34]. Considering the reactions of grapevine plants to attacks by *P. ficus*, the transcriptional response of vine plants is rather weak [37]. However, elevated CO_2 stimulates the production of salicylic acid, which might favour parasitoid host location of phloem-feeders and improve parasitism success [38].

Systematic investigations on the consequences of changing temperature, precipitation or CO_2 concentration on grapevine diseases and pests are few in number [6,39] and multi-trophic interactions of grapevine pests and their natural enemies have never been studied under elevated CO_2 levels. The present study aims to investigate the effects of elevated CO_2 on the performance of *L. dactylopii* and its host, the vine mealybug, reared on grapevine plants in an FACE system and in the greenhouse.

We hypothesised that (I) vine mealybugs reared under eCO_2 would react like other phloem-feeders, as aphids, predicting that (II) life history parameters would not be altered or even improve from higher atmospheric CO_2 levels, and (III) the parasitoid *L. dactylopii* would either be unaffected or benefit from eCO_2, depending on the mealybug performance.

2. Materials and Methods

Insects were obtained from the Department of Crop Protection at Geisenheim University, Germany. *Planococcus ficus* was reared on sprouted potatoes at 26 ± 1 °C and 60–70% relative humidity (RH) in darkness in an incubator. *Leptomastix dactylopii* cultures were reared on *P. ficus* on sprouted potatoes in transparent plastic containers (37 × 22 × 25 cm) at 26 °C, 60–70% RH, and a photoperiod of 16:8 (L:D) in an environmental chamber. Containers were also supplied with honey agar as an additional food for wasps.

In order to answer the question of whether life-history parameters of *P. ficus* vary under different CO_2 concentrations, we conducted greenhouse experiments as well as field studies in the VineyardFACE facility (Geisenheim University, Geisenheim, Germany). The greenhouse experiment was performed with potted Riesling grapevine plants exposed to ambient and elevated CO_2 concentrations of 400 CO_2 and 800 ppm CO_2, respectively (Table S1). At the onset of the experiment, plants were 12 weeks old and approximately 40 cm high. Grapevine plants used in the greenhouse experiments in this study were grown under ambient CO_2 conditions for 12 weeks before exposure to eCO_2 started. Each plant was infested with ca. 100 *P. ficus* nymphs using leaf disc transport [40]. Briefly, two to three ovisacs harvested from *P. ficus* females were transferred to 2 × 2 cm paper towel squares and placed on 5 cm vine leaf discs. Leaf disks were placed on water-soaked cotton wool and 5 cm filter papers to avoid leaf edges entangling in the cotton wool. After 48 h, paper towel squares were removed and the number of crawlers on the leaf disks was counted. Then, two to three leaf discs were placed on each experimental plant, applying a total number of approx. 100 crawlers per plant. Plants were covered with a fabric gardening bag (60 × 40 cm, 19 g/m^2, Classic80, HECO Textilverlag, Memmingen, Germany), ensuring oxygen and light supply while preventing mealybugs from escaping and being attacked by natural enemies. A total of 40 plants were placed in a greenhouse chamber with elevated CO_2 concentrations, while another 40 plants served as the control in an ambient CO_2 greenhouse chamber. Half of the 40 plants in each cabin were used to study life history parameters of *P. ficus*, while the other 20 plants were used to raise adult *P. ficus* females for the parasitism experiments with *L. dactylopii*. During the course of the experiments (mid-July to late August 2016), an average temperature of 22 °C was reached in the greenhouse (average minimum temperature 16.0 °C, average maximum temperature 30 °C) (Table S2), and plants were watered twice per week with rainwater.

The VineyardFACE facility enhances open-air CO_2 concentrations using ring-like structures with a diameter of 12 m, which are placed over the rows of an actual vineyard. Three rings are sparged with CO_2 reaching eCO_2 levels of approx. 450 ppm, while the other three rings use air and serve as the aCO_2 (ca. 400 ppm) control. For a detailed description of the VineyardFACE design, see [5,41] and Supplementary Figure S1. Field experiments were conducted between mid-July and mid-September 2016. During the experiments described here, CO_2 concentrations were measured by using two LI-8100 analyser control units (Li-CorLI8100/8150 Multiplexer, Li-Cor Biosciences, Lincoln, NE, USA) installed at two heights (1.7 m and 0.75 m) in the grapevine canopy. Within aCO_2 rings, an average level of 401 ± 1.3 ppm was reached during the experimental periods, while in eCO_2 rings, air was enriched during daylight hours to approximately 12% to 18% above the ambient CO_2 (456 ± 16.1 ppm), which is the concentration predicted for the mid-21st century. Supplementary Figure S2 illustrates CO_2 concentrations in aCO_2 and eCO_2 rings during the first period of the experiments (mid-July to beginning of August 2016) described here. Data on weather conditions during the experimental periods are provided in the Supplementary Table S3. During the course of the experiments, an average temperature of 20.5 °C was reached in the field (average minimum temperature 14.0 °C, average maximum temperature 27 °C). Experiments were conducted on 10 five-year-old Riesling grapevine

plants per ring, resulting in three replicates, with 10 sub-replicates each. Similar to the greenhouse experiment, two canes per plant were inoculated with approximately 100 1st instar mealybug nymphs and covered with gardening fabric. Of the two infested canes per plant, one was used for the study on mealybug life-history parameters, while the other one served to provide adult mealybugs reared under eCO_2 as hosts for *L. dactylopii*.

In both the greenhouse and FACE experiments, the development time, body size, fecundity, fertility, and survival of *P. ficus* females were recorded. The developmental stages of mealybugs were determined according to Walton & Pringle [9]. Three weeks after infesting vines with mealybug nymphs, greenhouse and FACE plants were assessed for the presence of females reaching the oviposition stage. Plants were checked every other day and the first ten (greenhouse) or five (VineyardFACE) ovipositing females from every plant were collected and stored in ethanol until further analysis. The infestation and collection dates for each female were used to determine the development time. Body length (from head to anal lobes) of the collected *P. ficus* females was measured under a stereomicroscope. To determine the fecundity and fertility, one additional female at the onset of oviposition was sampled from each treated and untreated plant (Greenhouse: $n = 20$; VineyardFACE: $n = 3$). These females were transferred individually to a small piece of paper towel in a sealed Petri dish and incubated in the respective greenhouse chambers (eCO_2 and aCO_2) for two weeks. Then, Petri dishes were frozen, and the number of crawlers and unhatched eggs was counted under a microscope. The sum of unhatched eggs and hatched crawlers accounted for fecundity, while fertility was determined by the percentage of hatched crawlers. Greenhouse experiments finished after six weeks, while the duration of the VineyardFACE experiments extended over 9 weeks, due to the slower mealybug development under field conditions. At the end of the experiments, the number of surviving adult females was recorded on each plant. In accordance with Ross et al. [42] and Cocco et al. [43], we assumed a baseline survival rate of 60% of females in our *P. ficus* culture. Hence, the survival of females was calculated as

$$\frac{\text{(Number of surviving female adults post-experiment + number of females collected for body size + 1 female collected for the fertility)}}{\text{Number of applied } P.\ ficus \text{ nymphs} \times 0.6} \quad (1)$$

A second experiment aimed to answer the question of whether the parasitism success, development time, or sex ratio of *L. dactylopii* vary under different CO_2 concentrations in the greenhouse or in the VineyardFACE facility. In the greenhouse, 20 potted cv. Riesling grapevine plants were inoculated with approximately 100 1st instar nymphs of *P. ficus* and placed in respective greenhouse chambers with ambient or elevated CO_2. Likewise, in the VineyardFACE facility, one cane of each of the ten vine plants per FACE ring was infested with 100 1st instar *P. ficus* nymphs. When female mealybugs reached the 3rd instar to preoviposition stage, one leaf of each plant was placed in a small, water filled tube; glued to the ground of a round, transparent experimental arena (diameter 15 cm, height 15 cm); and covered with a detachable lid. Subsequently, five mealybugs, reared from the same plant from which the leaf was collected, were transferred to the leaf using a sable brush. Mealybugs were allowed to settle overnight; then, a male and a female individual of *L. dactylopii* were introduced into the arena for 24 h (greenhouse) or 48 h (VineyardFACE) and then removed. Experimental arenas were placed in their respective treatment greenhouse chambers or FACE ring (eCO_2 or aCO_2). Water was refilled in the tubes, when necessary. After two weeks, arenas from the VineyardFACE facility were moved to the greenhouse and placed in the respective aCO_2 and eCO_2 treatments, since temperatures in the field decreased to unfavourable levels for *L. dactylopii* development. Subsequently, arenas were visually checked for parasitised mealybugs in order to determine parasitism success. In the following weeks, arenas were checked for *L. dactylopii* adult emergence every 24 h and wasps were removed. The date of emergence was recorded to determine the development time. Emerged individuals were transferred to glass vials and sexed. The experiment finished after 5 weeks when wasp emergence ended.

To detect the possible effects of eCO_2 on *P. ficus* and *L. dactylopii*, data of each measured parameter were tested for normality, followed by an unpaired t-test. Only *P. ficus* fertility data from the FACE

experiment did not follow a normal distribution and was analysed with a Mann-Whitney test for non-parametric data sets. Contingency tables and Fisher's exact test were used to detect possible differences in categorical variables, i.e., the sex ratio of newly emerged *L. dactylopii*. Pearson's correlation analysis was conducted to investigate the relation of female body size and development time (onset of oviposition) of female *P. ficus* mealybugs reared under elevated or ambient CO_2 conditions. All analyses were performed with Graphpad Prism version 7.00 for Windows (GraphPad Software, La Jolla, CA, USA).

3. Results

3.1. Life History Parameters of P. ficus Under Different CO_2 Levels

3.1.1. Greenhouse Experiments

The mean body size of mealybugs reared on grapevine plants under aCO_2 was larger compared to those reared under eCO_2. However, this difference was not significant, even though there was a tendency ($p = 0.05$) towards smaller sized mealybugs under eCO_2 (Figure 1A; Tables S4 and S5). There was a significant negative correlation between the time of exposure and body size of ovipositing females in the eCO_2 treatment ($R^2 = 0.63$, $p = 0.01$), while such a correlation was not detected with aCO_2 (Figure 2).

The survival of adult females was significantly greater for mealybugs reared on grapevine plants under eCO_2 than for mealybugs under aCO_2 conditions (53.2% and 40.1% survival, respectively) ($p = 0.04$) (Figure 1B; Tables S4 and S6). There was no difference between aCO_2 and eCO_2 in terms of the development time from the 1st instar to oviposition, neither for the mean development time (Figure 1C, Tables S4 and S5) nor considering the first ovipositing female of each plant. Fecundity, on the other hand, resulted in 230.9 (aCO_2) and 290.1 (eCO_2) mealybug eggs. The improved fecundity under eCO_2 was not significant, but might indicate a strong trend ($p = 0.05$); while fertility, calculated as the percentage of 1st instar nymphs hatched from these eggs, was not different between the CO_2 treatments (Figure 1D; Tables S4 and S7).

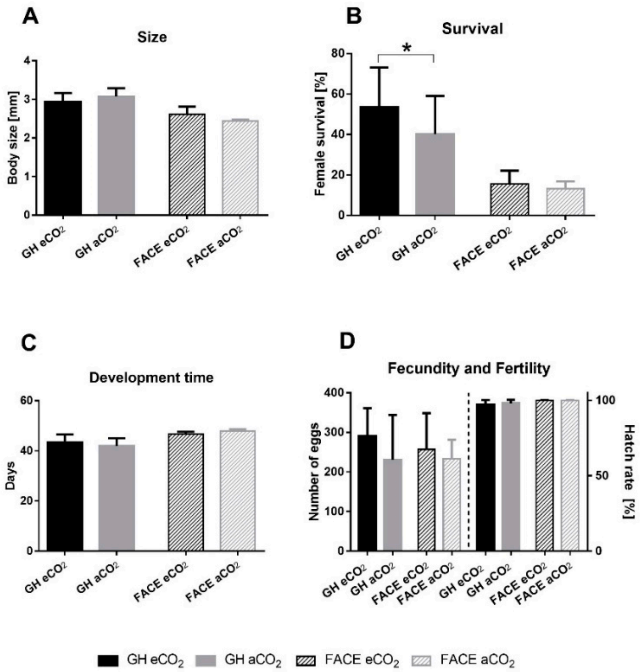

Figure 1. Life history parameters of mealybug females reared under eCO_2 and aCO_2 in the greenhouse and in the VineyardFACE facility. Data displayed as means + SD. Statistically significant differences between CO_2 treatments are marked with *. Solid columns display greenhouse data (GH), and hatched columns display data from the VineyardFACE experiment. (**A**) Body size of females at the onset of oviposition. (**B**) Survival of females. (**C**) Development time of females from 1st instar to oviposition. (**D**) Fecundity, shown as the total number of eggs laid per female, and fertility, shown as the percentage of hatched crawlers from these eggs.

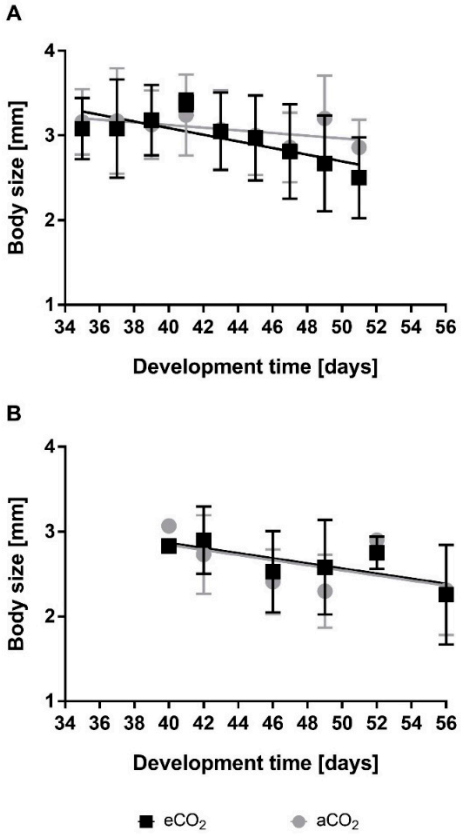

Figure 2. Pearson's correlation analysis of female body size and sampling date of female *P. ficus* mealybugs at the onset of oviposition reared under elevated (eCO_2, black squares) or ambient (aCO_2 grey circles) CO_2 conditions. Data displayed as means + SD. (**A**) Greenhouse data: eCO_2 $R^2 = 0.63$, $p = 0.01$; aCO_2 $R^2 = 0.37$, $p = 0.08$. (**B**) VineyardFACE data: eCO_2 $R^2 = 0.60$, $p = 0.07$; aCO_2 $R^2 = 0.31$, $p = 0.25$.

3.1.2. VineyardFACE Experiments

Field data on mealybug survival, body size, development time from 1st instar to oviposition stage, fecundity, and fertility were compared between aCO_2 and eCO_2 VineyardFACE rings ($n = 3$), but none of these parameters showed significant differences (Figure 1; Tables S8–S11). Generally, the field results followed similar trends to the results obtained in greenhouse experiments, but did not reach statistical significance. The relationship between body size and the onset of oviposition was not significant in the FACE experiment. (Figure 2B).

3.2. Parasitism by L. dactylopii

The parasitism experiment with *L. dactylopii* did not reveal any differences between parasitism success, development time, or sex ratio under eCO_2 and aCO_2 treatments, neither in the greenhouse nor in the VineyardFACE facility (Table 1). In the greenhouse, the aCO_2 treatment resulted in a total of 15 parasitized *P. ficus* females, from which 14 wasps emerged: 10 females (69%) and 3 males, while 1 escaped before being sexed. Under eCO_2 greenhouse, 16 mealybug females were parasitized, all of which emerged: 12 females (80%) and 3 males, while 1 escaped before being sexed (Table 1, Table S12). In the field and under FACE eCO_2, 40 mealybugs were parasitised and 36 wasps hatched (58% females) (Table 1, Table S13). In the FACE aCO_2 treatment, a total of 52 mealybugs were parasitized and 44 wasps hatched, 61% of which were female. The development time of *L. dactylopii* from egg to adult was approximately 24 days in both eCO_2 and aCO_2 greenhouse treatments, while wasps from the VineyardFACE facility needed 25 days (eCO_2) and 26 days (aCO_2) to complete their development.

Parasitism success was 15% in the aCO_2 treatment and 16% under eCO_2 conditions in the greenhouse, while in the field, more mealybugs were parasitised (35% under aCO_2, and 29% under eCO_2).

Table 1. Results from the parasitism experiment with *L. dactylopii* and *P. ficus* in a greenhouse and field (VineyardFACE) under ambient (aCO_2) and elevated (eCO_2) conditions. Values are expressed as means ± SD.

Site	Treatment	Parasitism Success (%)	Development Time (days)	Sex Ratio (% Females; % Males)
Greenhouse	aCO_2	15 ± 17, n = 20	23.86 ± 1.35, n = 14	69.23; 30.77, n = 13
	eCO_2	16 ± 22.1, n = 20	23.69 ± 0.79, n = 16	80; 20, n = 15
	p	0.87	0.67	0.67
VineyardFACE	aCO_2	34.67 ± 34.01, n = 30	25.67 ± 3.34, n = 44	61.36; 38.63, n = 44
	eCO_2	29.33 ± 27.66, n = 30	25.13 ± 2.54, n = 36	58.33; 41.67, n = 36
	p	0.51	0.41	0.82

4. Discussion

Here, we report for the first time on the possible effects of eCO_2 in a grapevine-based pest-parasitoid system using the vine mealybug *P. ficus* and its parasitoid *L. dactylopii* as model organisms. Our greenhouse experiments with eCO_2 showed a significant increase in survival rates, and strong trends for a decreased body size and increased fecundity of *P. ficus* females, while fertility did not change under eCO_2 concentrations. Body size was negatively correlated with sampling date, as females that started oviposition later were of a smaller size. Body size is usually positively related to fecundity in mealybugs [42,43], but we did not find evidence for this in the present study. It is possible that the number of eggs suffered a decline over time, similar to mealybug size, but this was not evaluated. On the contrary, fecundity did increase under eCO_2, although this trend was not significant.

The field experiment at the VineyardFACE site did not detect any differences between aCO_2 and eCO_2 treatment groups. There are several possible explanations for this. Firstly, field experiments take place in a more complex environment, including a whole series of uncontrolled variables, e.g., humidity, wind, and putatively other natural enemies. While this more realistic scenario is important to put laboratory results into context, it also makes it difficult to detect small-scale effects, as they might occur in the case of *P. ficus* under eCO_2. Secondly, VineyardFACE eCO_2 concentrations were much lower (ca. 450 ppm) than in the greenhouse (ca. 800 ppm), due to the open-air nature of the facility. The differences detected under greenhouse eCO_2 were rather small; hence it is not surprising that the lower VineyardFACE eCO_2 levels did not achieve comparable results. Thirdly, despite its large scale and technical sophistication, the Geisenheim VineyardFACE facility only allows a limited number of replicates. There are only three independent test rings for each CO_2 treatment, which complicates the detection of subtle differences *per se*. To gain statistical power, we also analysed the ten biological replicates (subreplicates) per ring, see Supplement Tables S9–S11, S13). These subreplicate-based results are limited in their implications and no general conclusions should be drawn from them. Even so, none of the analysed life history parameters of *P. ficus* showed significant differences between aCO_2 and eCO_2. This combined evidence suggests that VineyardFACE eCO_2 levels did not affect *P. ficus* and it appears unlikely that a higher number of independent treatments (i.e., FACE rings) would change this result.

Since the experimental conditions were substantially different, no statistical testing was done to compare field and greenhouse data. There are some differences which might be attributed to other environmental stress factors, such as temperature and precipitation amounts. Our field experiment yielded low survival rates, but they are comparable to those measured in a screenhouse experiment with *P. ficus* [43]. Female mealybugs in the field needed more time to reach the oviposition state and were slightly smaller than those from the greenhouse, especially in the aCO_2 treatment. Mealybug fecundity and fertility were similar at both sites. Interestingly, a negative correlation between size and development time was detected under eCO_2 at both experimental set-ups, although this relation was not significant (p = 0.07) in the VineyardFACE facility. It would be interesting to study these findings

in more detail. The overall difference between the obtained field (FACE) and greenhouse data might also be attributed to an overall plus of an average temperature of 2–3 °C in the greenhouse, which might have been more favourable for mealybug development.

Higher atmospheric levels of CO_2 have been shown to result in a higher biomass, increased yield, and lower nutritional values in grapevine and other plants [2,5,44]. However, plant tissues and fluids vary in their response to elevated CO_2 [2,3,45] and influence different feeding guilds of herbivores in different ways [3,7]. Leaf-chewing herbivores generally perform worse and phloem-feeders have been shown to be less affected by rising CO_2 concentrations [3,46]. Most literature on the effects of CO_2 and climate change on Hemiptera focuses on aphids. In the only study on mealybugs, Chong et al. [25] found that temperature rather than elevated CO_2 influenced the survival, development time and fecundity of *P. madeirensis*. Aphids can benefit from elevated CO_2, showing increases in fecundity and survival, and decreases in development time [3,46]. However, several studies showed that the direction and size of the effect of eCO_2 depend on the specific combination of host plant and insect species [47–50]. Hughes & Bazzaz [47] tested five aphid species and their host plants under eCO_2; one species was negatively affected, another positively affected, and no significant effects were found for the remaining three.

Cocco et al. [43] investigated the performance of female mealybugs reared on a grapevine with increasing levels of nitrogen (N) fertilisation. There are parallels between the results of the study by Cocco et al. [43] on *P. ficus* under elevated N and the results of our study on *P. ficus* under eCO_2. In both studies, survival rates increased, and fecundity showed an increasing tendency, although this tendency was not significant in the present study. Fertility was not affected in both studies; body size augmented with increasing N levels, but decreased under eCO_2. Cocco et al. [43] attributed their findings to a higher nutritional value of the phloem sap caused by fertilisation. It is known that the effects of eCO_2 can be mitigated by elevated temperatures, drought [25,46] or fertilisation [3]. Clearly, these three factors and eCO_2 interact in future agriculture, especially in perennial systems such as vineyards. A study by Sudderth et al. [8] investigated the effects of the interaction of fertilisation and eCO_2 on aphids by manipulating soil N and atmospheric CO_2 levels. At ambient CO_2 levels, high soil N increased the aphid population size, similar to the mealybugs in the study by Cocco et al. [43]. However, the aphid population size also increased in response to elevated CO_2 on plants grown under low soil N [8], which might relate to the higher survival rates of mealybugs in the present study. Apparently, elevated CO_2 and increased N fertilisation affect phloem-feeders in a similar manner, but this does not correspond to changes in the C:N ratios of the leaf tissue [8]. Studies on the nutritional quality of phloem sap suggest that nitrogen composition (predominantly free amino acids in phloem) is probably the most important determinant of aphid performance [45,51].

While the present study observed a decreasing mealybug body size with the duration of the eCO_2 treatment, we also found that elevated CO_2 levels were positively related to survival, but development time, fertility and fecundity were not significantly affected. Hence, we detected indicators for reduced fitness (body size), as well as factors that support the assumption that fitness was unaffected (development time, fertility and fecundity) or even improved (survival). These mixed results might also be caused by the experimental design. Grapevine plants used in the greenhouse experiments in this study were grown under ambient CO_2 conditions for 12 weeks before exposure to eCO_2 started. Elevated CO_2 levels affect sap-feeding insects mainly through changes in the host plant [3]. These changes do not happen instantly, hence more clear-cut results on their influence on life history parameters of *P. ficus* might take longer than the duration of the present experiment. It would be interesting to study the long-term effects of eCO_2 on *P. ficus* and *L. dactylopii* on grapevines grown under eCO_2 for a longer period than in the present study. Long-term studies could make changes of life-history of both species become more evident.

Host size is known to influence the sex ratio of parasitoid wasps, with females being more likely to emerge from bigger hosts according to de Jong & Alphen [28]. Despite the subsequent size decrease of *P. ficus* females at the onset of oviposition in the greenhouse experiments in our study, no

significant differences in the sex allocation of wasps were observed between eCO_2 and aCO_2 treatments. This might also be attributed to the fact that host size differences were rather small. In fact, none of the evaluated variables of *L. dactylopii* (parasitism success, development time, sex ratio) were altered by CO_2 levels, neither in the greenhouse nor in the VineyardFACE. Parasitism success was greater in the field than in the greenhouse, because wasps remained for 48 h in the VineyardFACE host arenas, compared to 24 h in the greenhouse. To the authors' best knowledge, there have been no studies investigating the performance of mealybug parasitoids under elevated CO_2 conditions. Studies on natural enemies of aphids showed mixed results [34,35]. Hymenopteran aphid parasitoids display the whole range of possible reactions: *Myzus persicae* (Sulzer) and *Brevicoryne brassicae* (L.) showed unaltered and improved life history parameters without changing the performance of the parasitoid wasp *Diaeretiella rapae* (MacInstosh). In a study by Klaiber et al. [52], however, the same parasitoid suffered a decrease in longevity and rates of parasitism on *B. brassicae,* which were lower quality hosts when reared under eCO_2. On the other hand, the biocontrol efficiency of *Aphidius picipes* (Nees) against *Sitobion avenae* (Fabricius) was enhanced under elevated CO_2, although elevated CO_2 had adverse effects on the growth and development of *A. picipes* [53].

Elevated atmospheric CO_2 levels affect parasitoids mainly through plant-mediated changes in host quality which cascade upwards [35]. Our results indicate a tendency towards a non-altered or even improved mealybug fitness under eCO_2, which is, as hypothesised, in accordance with reports on other phloem-feeders reared in similar conditions under eCO_2. The results of the present study also support the hypothesis that parasitoid performance seemed to be related to mealybug performance.

Climate change influences grapevine plants, their pests, and natural enemies today and in the future [6]. Rising temperatures affect grapevine pests as *P. ficus* directly, speeding up development and voltinism [10]. Gutierrez et al. [24] modelled the future distributions of *P. ficus* and its parasitoids in California based on weather data. Without taking into account elevated CO_2 concentrations, the model explains how mealybugs will boom at elevated temperatures. *Planococcus ficus* will seek cooler sites under the bark and in the root zone, to compensate for its rather narrow optimal temperature range. These refuges also function as a shelter from natural enemies and certain pesticides. Elevated CO_2 levels possibly benefit *P. ficus* and also increase the biomass in vine plants, which might offer more refuge sites and complicate host findings for parasitoids. It has been suggested that, in general, measured responses of manipulated systems to global change decrease over greater spatial and temporal experimental scales, as well as with the number of climate change drivers studied [35].

5. Conclusions

Rising carbon dioxide levels affect agricultural systems worldwide. Crop growers are especially interested in the possible effects of climate change on pest species and their respective antagonists used in biological control programs. This study is the first to test the effects of elevated CO_2 concentrations on a grapevine pest under field conditions and in the greenhouse. It is also the only study to investigate a mealybug-based pest-parasitoid complex under eCO_2 in a multitrophic approach. Our results suggest a trend towards a non-altered or improved fitness of *P. ficus* in the greenhouse with eCO_2 levels around 800 ppm, but not in the field, where eCO_2 concentrations reached approximately 450 ppm. Meanwhile, its parasitoid *L. dactylopii* did not seem to be affected in either of the scenarios tested. Further research should include prolonged eCO_2 exposure of greenhouse plants and investigate the response of grapevine plants to mealybug attack under different CO_2 levels. Additionally, climate change is characterised by a combination of multiple abiotic factors, including rising temperatures and CO_2 levels, as well as varying precipitation patterns. None of these factors act by themselves upon plants, pests and natural enemies and future studies should take this complexity into account. Future research should aim to integrate several trophic levels and environmental stress factors.

Supplementary Materials: The following can be found at http://www.mdpi.com/2073-4395/9/6/326/s1. Figure S1: (A) Aerial view of the FACE facility; (B) Schematic illustration of one FACE ring; Figure S2: FACE CO_2; Table S1: Greenhouse CO_2; Table S2: Greenhouse Temperature; Table S3: FACE climate data; Table S4: GreenhouseResults

(analysed); Table S5: Greenhouse size and develop; Table S6: Greenhouse Survival; Table S7: Greenhouse fertility fecund; Table S8 FACE results (analysed); Table S9: FACE size and development; Table S10: FACE Survival; Table S11: FACE Fertility Fecundity; Table S12: Greenhouse Leptomastix; Table S13: FACE Leptomastix.

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References

1. Pachauri, R.K.; Allen, M.R.; Barros, V.R.; Broome, J.; Cramer, W.; Christ, R.; Church, J.A.; Clarke, L.; Dahe, Q.; Dasgupta, P.; et al. *IPCC Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; IPCC: Geneva, Switzerland, 2014; Available online: https://epic.awi.de/id/eprint/37530/ (accessed on 31 January 2019).
2. Long, S.P.; Ainsworth, E.A.; Rogers, A.; Ort, D.R. Rising atmospheric carbon dioxide: Plants FACE the future. *Annu. Rev. Plant Biol.* **2004**, *55*, 591–628. [CrossRef] [PubMed]
3. Robinson, E.A.; Ryan, G.D.; Newman, J.A. A meta-analytical review of the effects of elevated CO_2 on plant-arthropod interactions highlights the importance of interacting environmental and biological variables. *New Phytol.* **2012**, *194*, 321–336. [CrossRef] [PubMed]
4. Bindi, M.; Fibbi, L.; Miglietta, F. Free air CO_2 enrichment (FACE) of grapevine (*Vitis vinifera* L.): II. Growth and quality of grape and wine in response to elevated CO_2 concentrations. *Eur. J. Agron.* **2001**, *14*, 145–155. [CrossRef]
5. Wohlfahrt, Y.; Smith, J.P.; Tittmann, S.; Honermeier, B.; Stoll, M. Primary productivity and physiological responses of *Vitis vinifera* L. cvs. under Free Air Carbon dioxide Enrichment (FACE). *Eur. J. Agron.* **2018**, *101*, 149–162. [CrossRef]
6. Reineke, A.; Thiéry, D. Grapevine insect pests and their natural enemies in the age of global warming. *J. Pest Sci. (2004)* **2016**, *89*, 313–328. [CrossRef]
7. Bezemer, T.M.; Jones, T.H. Plant-insect herbivore interactions in elevated atmospheric CO_2: Quantitative analyses and guild effects. *Oikos* **1998**, *82*, 212–222. [CrossRef]
8. Sudderth, E.A.; Stinson, K.A.; Bazzaz, F.A. Host-specific aphid population responses to elevated CO_2 and increased N availability. *Glob. Chang. Biol.* **2005**, *11*, 1997–2008. [CrossRef]
9. Walton, V.M.; Pringle, K.L. Vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), a key pest in South African vineyards: A Review. *S. Afr. J. Enol. Vitic.* **2004**, *25*, 54–62. [CrossRef]
10. Daane, K.M.; Almeida, R.P.P.; Bell, V.A.; Walker, J.T.S.; Botton, M.; Fallahzadeh, M.; Mani, M.; Miano, J.L.; Sforza, R.; Walton, V.M. Biology and management of mealybugs in vineyards. In *Arthropod Management in Vineyards*; Bostanian, N.J., Vincent, C., Isaacs, R., Eds.; Springer: New York, NY, USA, 2012; pp. 271–307.
11. Chiotta, M.L.; Ponsone, M.L.; Torres, A.M.; Combina, M.; Chulze, S.N. Influence of *Planococcus ficus* on *Aspergillus* section *Nigri* and ochratoxin A incidence in vineyards from Argentina. *Lett. Appl. Microbiol.* **2010**, *51*, 212–218. [CrossRef]
12. Bordeu, E.; Troncoso, D.O.; Zaviezo, T. Influence of mealybug (*Pseudococcus* spp.)-infested bunches on wine quality in Carmenere and Chardonnay grapes. *Int. J. Food Sci. Technol.* **2012**, *47*, 232–239. [CrossRef]
13. Engelbrecht, D.J.; Kasdorf, G.G.F. Field spreas of corky bark, fleck, leafroll and Shiraz decline diseases and associated viruses in South African grapevines. *Phytophylactica* **1990**, *22*, 347–354.
14. Almeida, R.P.P.; Daane, K.M.; Bell, V.A.; Blaisdell, G.K.; Cooper, M.L.; Herrbach, E.; Pietersen, G. Ecology and management of grapevine leafroll disease. *Front. Microbiol.* **2013**, *4*, 94. [CrossRef] [PubMed]
15. Becerra, V.; Gonzalez, M.; Herrera, M.E.; Miano, J.L. Population dynamics of vine mealybug *Planococcus ficus* Sign. in vineyards. Mendoza. *Rev. Fac. Ciencias Agrar. Univ. Nac. Cuyo* **2006**, *1*, 1–6.
16. Prabhaker, N.; Gispert, C.; Castle, S.J. Baseline susceptibility of *Planococcus ficus* (Hemiptera: Pseudococcidae) from California to select insecticides. *J. Econ. Entomol.* **2012**, *105*, 1392–1400. [CrossRef] [PubMed]

17. Venkatesan, T.; Jalali, S.K.; Ramya, S.L.; Prathibha, M. Insecticide resistance and its management in mealybugs. In *Mealybugs and Their Management in Agricultural and Horticultural crops*; Springer: New Delhi, India, 2016; pp. 223–229.
18. Muscas, E.; Cocco, A.; Mercenaro, L.; Cabras, M.; Lentini, A.; Porqueddu, C.; Nieddu, G. Effects of vineyard floor cover crops on grapevine vigor, yield, and fruit quality, and the development of the vine mealybug under a Mediterranean climate. *Agric. Ecosyst. Environ.* **2017**, *237*, 203–212. [CrossRef]
19. Cocco, A.; Muscas, E.; Mura, A.; Iodice, A.; Savino, F.; Lentini, A. Influence of mating disruption on the reproductive biology of the vine mealybug, *Planococcus ficus* (Hemiptera: Pseudococcidae), under field conditions. *Pest Manag. Sci.* **2018**, *74*, 2806–2816. [CrossRef] [PubMed]
20. Walton, V.M.; Daane, K.M.; Bentley, W.J.; Millar, J.G.; Larsen, T.E.; Malakar-Kuenen, R. Pheromone-based mating disruption of *Planococcus ficus* (Hemiptera: Pseudococcidae) in California vineyards. *J. Econ. Entomol.* **2006**, *99*, 1280–1290. [CrossRef]
21. Mansour, R.; Grissa Lebdi, K.; Rezgui, S. Assessment of the performance of some new insecticides for the control of the vine mealybug *Planococcus ficus* in a Tunisian vineyard. *Entomol. Hell.* **2017**, *19*, 21–33. [CrossRef]
22. Daane, K.M.; Bentley, W.J.; Walton, V.M.; Malakar-Kuenen, R.; Millar, J.G.; Ingels, C.A.; Weber, E.A.; Gispert, C. New controls investigated for vine mealybug. *Calif. Agric.* **2006**, *60*, 31–38. [CrossRef]
23. Gutierrez, A.P.; Ponti, L.; D'Oultremont, T.; Ellis, C.K. Climate change effects on poikilotherm tritrophic interactions. *Clim. Chang.* **2008**, *87*, 167–192. [CrossRef]
24. Paul Gutierrez, A.; Daane, K.M.; Ponti, L.; Walton, V.M.; Ellis, C.K. Prospective evaluation of the biological control of vine mealybug: Refuge effects and climate. *J. Appl. Ecol.* **2008**, *45*, 524–536. [CrossRef]
25. Chong, J.-H.; van lersel, M.W.; Oetting, R.D. Effects of elevated carbon dioxide levels and temperature on the life history of the Madeira mealybug (Hemiptera: Pseudococcidae). *J. Entomol. Sci.* **2004**, *39*, 387–397. [CrossRef]
26. Kol-Maimon, H.; Ghanim, M.; Franco, J.C.; Mendel, Z. Evidence for gene flow between two sympatric mealybug species (Insecta; Coccoidea; Pseudococcidae). *PLoS ONE* **2014**, *9*, 1–13. [CrossRef] [PubMed]
27. Noyes, J.S.; Hayat, M. *Oriental mealybug parasitoids of the Anagyrini (Hymenoptera: Encyrtidae)*; CAB International: Wallingford, UK, 1994.
28. Jong, P.W.; Alphen, J.J.M. Host size selection and sex allocation in *Leptomastix dactylopii*, a parasitoid of *Planococcus citri*. *Entomol. Exp. Appl.* **1989**, *50*, 161–169. [CrossRef]
29. Krishnamoorthy, A.; Singh, S.P. Biological control of citrus mealybug, *Planococcus citri* with an introduced parasite, *Leptomastix dactylopii* in India. *Entomophaga* **1987**, *32*, 143–148. [CrossRef]
30. Fallahzadeh, M.; Japoshvili, G.; Saghaei, N.; Daane, K.M. Natural enemies of *Planococcus ficus* (Hemiptera: Pseudococcidae) in Fars Province vineyards, Iran. *Biocontrol Sci. Technol.* **2011**, *21*, 427–433. [CrossRef]
31. Mahfoudhi, N.; Dhouibi, M.H. Survey of Mealybugs (Hemiptera: Pseudococcidae) and their Natural Enemies in Tunisian Vineyards. *Afr. Entomol.* **2009**, *17*, 154–160. [CrossRef]
32. Daane, K.M.; Cooper, M.L.; Triapitsyn, S.V.; Andrews, J.W.; Ripa, R. Parasitoids of obscure mealybug, *Pseudococcus viburni* (Hem.: Pseudococcidae) in California: Establishment of *Pseudaphycus flavidulus* (Hym.: Encyrtidae) and discussion of related parasitoid species. *Biocontrol Sci. Technol.* **2008**, *18*, 43–57. [CrossRef]
33. Marras, P.M.; Cocco, A.; Muscas, E.; Lentini, A. Laboratory evaluation of the suitability of vine mealybug, *Planococcus ficus*, as a host for *Leptomastix dactylopii*. *Biol. Control* **2016**, *95*, 57–65. [CrossRef]
34. Hentley, W.T.; Wade, R.N. Global change, herbivores and their natural enemies. In *Global Climate Change and Terrestrial Invertebrates*; John Wiley & Sons, Ltd.: Chichester, UK, 2016; pp. 177–200.
35. Facey, S.L.; Ellsworth, D.S.; Staley, J.T.; Wright, D.J.; Johnson, S.N. Upsetting the order: How climate and atmospheric change affects herbivore–enemy interactions. *Curr. Opin. Insect Sci.* **2014**, *5*, 66–74. [CrossRef]
36. Mumm, R.; Dicke, M. Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. *Can. J. Zool.* **2010**, *88*, 628–667. [CrossRef]
37. Timm, A.E.; Reineke, A. First insights into grapevine transcriptional responses as a result of vine mealybug Planococcus ficus feeding. *Arthropod. Plant. Interact.* **2014**, *8*, 495–505. [CrossRef]
38. DeLucia, E.H.; Nabity, P.D.; Zavala, J.A.; Berenbaum, M.R. Climate change: Resetting plant-insect interactions. *Plant Physiol.* **2012**, *160*, 1677–1685. [CrossRef] [PubMed]

39. Boudon-Padieu, É.; Maixner, M. Potential effects of climate change on distribution and activity of insect vectors of grapevine pathogens. In Proceedings of the International and multi-disciplinary Colloquim-Global warming, which potential impacts on the vineyards? Beaune, France, 28–30 March 2007; p. 23. [CrossRef]
40. Hogendorp, B.K.; Cloyd, R.A.; Swiader, J.M. Effect of nitrogen fertility on reproduction and development of Citrus mealybug, *Planococcus citri* Risso (Homoptera: Pseudococcidae), feeding on two colors of coleus, *Solenostemon scutellarioides* L. Codd. *Environ. Entomol.* **2006**, *35*, 201–211. [CrossRef]
41. Reineke, A.; Selim, M. Elevated atmospheric CO_2 concentrations alter grapevine (*Vitis vinifera*) systemic transcriptional response to European grapevine moth (*Lobesia botrana*) herbivory. *Sci. Rep.* **2019**, *9*, 2995. [CrossRef] [PubMed]
42. Ross, L.; Dealey, E.J.; Beukeboom, L.W.; Shuker, D.M. Temperature, age of mating and starvation determine the role of maternal effects on sex allocation in the mealybug *Planococcus citri*. *Behav. Ecol. Sociobiol.* **2011**, *65*, 909–919. [CrossRef] [PubMed]
43. Cocco, A.; Marras, P.M.; Muscas, E.; Mura, A.; Lentini, A. Variation of life-history parameters of *Planococcus ficus* (Hemiptera: Pseudococcidae) in response to grapevine nitrogen fertilization. *J. Appl. Entomol.* **2015**, *139*, 519–528. [CrossRef]
44. Bindi, M.; Fibbi, L.; Lanini, M.; Miglietta, F. Free air CO_2 enrichment (FACE) of grapevine (*Vitis vinifera* L.): I. Development and testing of the system for CO_2 enrichment. *Eur. J. Agron.* **2001**, *14*, 135–143. [CrossRef]
45. Ryan, G.D.; Shukla, K.; Rasmussen, S.; Shelp, B.J.; Newman, J.A. Phloem phytochemistry and aphid responses to elevated CO_2, nitrogen fertilization and endophyte infection. *Agric. For. Entomol.* **2014**, *16*, 273–283. [CrossRef]
46. Zvereva, E.L.; Kozlov, M.V. Consequences of simultaneous elevation of carbon dioxide and temperature for plant-herbivore interactions: A metaanalysis. *Glob. Chang. Biol.* **2006**, *12*, 27–41. [CrossRef]
47. Hughes, L.; Bazzaz, F.A. Effects of elevated CO_2 on five plant-aphid interactions. *Entomol. Exp. Appl.* **2001**, *99*, 87–96. [CrossRef]
48. Awmack, C.; Harrington, R.; Leather, S. Host plant effects on the performance of the aphid *Aulacorthum solani* (Kalt.) (Homoptera: Aphididae) at ambient and elevated CO_2. *Glob. Chang. Biol.* **1997**, *3*, 545–549. [CrossRef]
49. Poorter, H. Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. In *CO_2 and Biosphere*; Springer: Dordrecht, The Netherlands, 1993; pp. 77–98.
50. Bezemer, T.M.; Knight, K.J.; Newington, J.E.; Jones, T.H. How general are aphid responses to elevated atmospheric CO_2? *Ann. Entomol. Soc. Am.* **1999**, *92*, 724–730. [CrossRef]
51. Ryan, G.D.; Sylvester, E.V.A.; Shelp, B.J.; Newman, J.A. Towards an understanding of how phloem amino acid composition shapes elevated CO_2-induced changes in aphid population dynamics. *Ecol. Entomol.* **2015**, *40*, 247–257. [CrossRef]
52. Klaiber, J.; Najar-Rodriguez, A.J.; Dialer, E.; Dorn, S. Elevated carbon dioxide impairs the performance of a specialized parasitoid of an aphid host feeding on *Brassica* plants. *Biol. Control* **2013**, *66*, 49–55. [CrossRef]
53. Jun Chen, F.; Wu, G.; Parajulee, M.N.; Ge, F. Impact of elevated CO_2 on the third trophic level: A predator *Harmonia axyridis* and a parasitoid *Aphidius picipes*. *Biocontrol Sci. Technol.* **2007**, *17*, 313–324. [CrossRef]