

*the plant journal***Physiological responses of pepper (*Capsicum annuum*) to combined ozone and pathogen stress**

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3 1 **Physiological responses of pepper (*Capsicum annuum*) to combined ozone and pathogen**  
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5 2 **stress**  
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## 16 Summary

17 Tropospheric ozone [O<sub>3</sub>] is a secondary air pollutant formed from the photochemical oxidation  
18 of volatile organic compounds in the presence of nitrogen oxides, and it is one of the most  
19 damaging air pollutants to crops. O<sub>3</sub> entry into the plant generates reactive oxygen species  
20 leading to cellular damage and oxidative stress, leading to decreased primary production and  
21 yield. Increased O<sub>3</sub> exposure has also been shown to have secondary impacts on plants by  
22 altering the incidence and response to plant pathogens. We used the *Capsicum annum* (pepper)-  
23 *Xanthomonas perforans* pathosystem to investigate the impact of elevated O<sub>3</sub> (eO<sub>3</sub>) on plants  
24 with and without exposure to *Xanthomonas*, using a disease-susceptible and disease-resistant  
25 pepper cultivar. Gas exchange measurements revealed decreases in diurnal photosynthetic rate  
26 ( $A'$ ) and stomatal conductance ( $g_s'$ ), and maximum rate of electron transport ( $J_{max}$ ) in the disease-  
27 resistant cultivar, but no decrease in the disease-susceptible cultivar in eO<sub>3</sub>, regardless of  
28 *Xanthomonas* presence. Maximum rates of carboxylation ( $V_{c,max}$ ), midday  $A$  and  $g_s$  rates at the  
29 middle canopy, and decreases in aboveground biomass were negatively affected by eO<sub>3</sub> in both  
30 cultivars. We also observed a decrease in stomatal sluggishness as measured through the Ball-  
31 Berry-Woodrow model in all treatments in the disease-resistant cultivar. We hypothesize that the  
32 mechanism conferring disease resistance to *Xanthomonas* in pepper also renders the plant less  
33 tolerant to eO<sub>3</sub> stress through changes in stomatal responsiveness. Findings from this study help  
34 expand our understanding of the trade-off of disease resistance with abiotic stresses imposed by  
35 future climate change.

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3 37 **Significance statement**  
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5 38 This paper examines the impact of combined biotic and abiotic stress on plant physiology and  
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7 39 finds that cultivars of pepper resistant to *Xanthomonas* infection have decreased leaf-level carbon  
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9 40 assimilation and stomatal conductance in response to elevated O<sub>3</sub>, regardless of inoculation  
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11 41 status. Our data suggests the mechanism conferring pathogen disease resistance in pepper also  
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13 42 renders the plant less tolerant to elevated O<sub>3</sub> stress through changes in stomatal responsiveness.  
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## 44 **Introduction**

45 Tropospheric O<sub>3</sub> is located 6-20 kilometers above the earth's surface and is a secondary air  
46 pollutant formed from the photochemical oxidation of volatile organic compounds (VOC) in the  
47 presence of sunlight and nitrogen oxides (NO<sub>x</sub>) (Kangasjarvi *et al.*, 2005; The Royal Society,  
48 2008). This is separate from stratospheric O<sub>3</sub> which plays an integral part in blocking harmful  
49 UV radiation from reaching the earth's surface (Ainsworth, 2017). Tropospheric O<sub>3</sub> levels are  
50 predicted to increase by 0.5 - 2.5 % annually due to human activities (IPCC 2022). Tropospheric  
51 O<sub>3</sub> concentration is greatest in areas with moderate to high air pollution, warmer temperatures,  
52 and sunlight, which are also conditions where it is favorable for maximum plant photosynthesis  
53 (Royal Society, 2008). Tropospheric O<sub>3</sub> levels are also dynamic, as they exhibit seasonal  
54 variability with concentrations rising during warmer months and increasing during days with  
55 stronger sunlight intensity (Ainsworth *et al.*, 2020).

56 Elevated O<sub>3</sub> concentrations can have a severe impact on plants in many ways. Upon  
57 entering the plant through the stomata, O<sub>3</sub> rapidly degrades to form reactive oxygen species  
58 (ROS), leading to oxidative stress in the plants (Kangasjärvi *et al.*, 2005). ROS presence disrupts  
59 chloroplast activity by damaging stromal membrane proteins, proteins in photosystem II, and  
60 effecting electron transport activity required in photosystem I and II (Khorobrykh *et al.*, 2020).  
61 This ROS formation can also cause stomatal closure and a subsequent decrease in stomatal  
62 conductance, which can lead to a decrease in plant photosynthesis and productivity (Kangasjärvi  
63 *et al.*, 2005). Plants are able to combat this ROS formation with the help of antioxidants, as they  
64 work to scavenge the ROS present under both stress and normal conditions (Tiwari & Agrawal,  
65 2018).

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3 66 Elevated O<sub>3</sub> has also been shown to impact gas exchange at the leaf level. While the  
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5 67 impact of elevated O<sub>3</sub> on stomatal conductance is species dependent, O<sub>3</sub> has been shown to  
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7 68 impact stomatal closure and lead to stomatal sluggishness, which is characterized by decreases in  
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9 69 stomatal conductance which ultimately limit photosynthesis (Paoletti & Grulke, 2010).  
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12 70 Additionally, analysis using the Ball-Woodrow-Berry (BWB) model (Ball *et al.*, 1987) has  
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14 71 demonstrated that elevated O<sub>3</sub> can proportionally decrease the relationship between  
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16 72 photosynthetic rate and stomatal conductance in certain varieties of C<sub>3</sub> and C<sub>4</sub> plants, as well as  
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18 73 decrease water use efficiency in C<sub>3</sub> plants (Li *et al.*, 2019; Masutomi *et al.*, 2019). These impacts  
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20 74 of O<sub>3</sub> on photosynthesis directly affect overall plant growth and primary metabolism, ultimately  
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22 75 contributing to decreases in net primary productivity (Ainsworth *et al.*, 2012).  
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26 76 Recent reviews of plant responses to O<sub>3</sub> indicate exposure to O<sub>3</sub> can alter the ability of  
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28 77 the plant to respond to biotic pressures (Ainsworth *et al.*, 2017; Leisner *et al.*, 2023). Romero *et*  
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30 78 *al.*, (2020) found that O<sub>3</sub> exposure in tomato (*Solanum lycopersicum*) cultivars that were tolerant  
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32 79 to *Xanthomonas* species exhibited an increase in disease intensity upon O<sub>3</sub> exposure. This was  
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34 80 seen in both their O<sub>3</sub> sensitive and tolerant cultivars, even after the application of a  
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36 81 benzothiadiazole, a disease resistance enhancer. Conversely, when strawberry plants (*Fragaria*  
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38 82 *virginiana*) were subjected to acute O<sub>3</sub> fumigation and inoculation with *Xanthomonas fragariae*,  
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40 83 an inhibition of lesion formation was observed (Laurence & Wood, 1978). Elevated O<sub>3</sub> has also  
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42 84 been shown to change the profile of the leaf surface, as it can lead to an increased leaf  
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44 85 wettability, which is the affinity for water to form and hold to the leaf's surface. This becomes  
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46 86 ideal for pathogenesis, as it in turn increases the ability for bacterial pathogens to attach and  
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48 87 successfully colonize (Karnosky *et al.*, 2002; Grinberg *et al.*, 2019).  
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3 88 At the cellular level the signaling pathways induced or altered by elevated O<sub>3</sub> can mirror  
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5 89 those from pathogen infection. The oxidative ROS burst that plants experience in response to O<sub>3</sub>  
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7 90 entry into the plant mimics the same ROS burst plants experience in response to pathogens  
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9 91 (Langebartels *et al.*, 2002). O<sub>3</sub> can also trigger a hypersensitive defense (HR) response like that  
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11 92 induced by pathogens. An HR is elicited to help increase expression of defense-related genes,  
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13 93 attempting to slow or stop the movement of a biotic or abiotic threat (Zurbriggen *et al.*, 2010).  
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15 94 The oxidative burst caused by O<sub>3</sub> can also impact interactions among major plant hormones, all  
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17 95 of which are important elements of plant defense responses to pathogens (Leisner *et al.*, 2023).  
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19 96 Plant pathogen presence has been shown to cause changes in total antioxidants in infected tissue,  
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21 97 which are an important to quench ROS generated from elevated O<sub>3</sub>, but this response is species-  
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23 98 dependent and dependent on pathogen trophic lifestyles (De Gara *et al.*, 2003). Taken together, it  
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25 99 is important to understand the complex interplay of biotic and abiotic stress responses in plants at  
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27 100 the physiological level.  
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33 101 In this study we used the pepper (*Capsicum annuum*) – *Xanthomonas perforans*  
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35 102 pathosystem to understand how plants will respond against a simultaneous challenge of elevated  
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37 103 O<sub>3</sub> and a foliar pathogen, given their overlapping signaling pathway and impact on plant  
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39 104 productivity. *Xanthomonas* spp. cause bacterial spot symptoms on Solanaceous plants, such as  
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41 105 tomato and pepper (Potnis *et al.*, 2015). Bacterial spot symptoms start with circular lesions that  
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43 106 lead to necrosis on leaves and fruit. Defoliation can also occur, and lead to sun scalding (Potnis  
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45 107 *et al.*, 2015). Similarly, chronic exposure to elevated O<sub>3</sub> concentrations can lead to foliar damage  
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47 108 including symptoms such as bronzing, yellowing, necrosis, and chlorotic spotting (Iriti *et al.*,  
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49 109 2006). In this study physiological and yield responses of pepper plants to growth in these  
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51 110 combined growth environments were evaluated in an experimental near-field setting. We  
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3 111 hypothesized that *Xanthomonas*-susceptible cultivars of *C. annuum* would have limited  
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5 112 photosynthetic performance, yield, and slower stomatal responsiveness compared to the  
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7 113 *Xanthomonas*-resistant cultivars when subject to both elevated O<sub>3</sub> and pathogen stress. We  
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9 114 expect these limitations to be caused primarily in response to elevated O<sub>3</sub> compared to the  
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11 115 pathogen presence. From this study, we aim to test the resiliency of the disease-resistant cultivar  
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13 116 when faced with both an abiotic stress and pathogen by evaluating plant physiological  
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15 117 parameters. Using this novel approach, we can gain further insights into the underlying  
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17 118 mechanisms that promote greater biotic resistance in plants when challenged with combined  
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19 119 stresses.  
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## 25 26 121 **Results**

### 27 28 122 *Diurnal carbon assimilation and stomatal conductance were only impacted by ozone in the* 29 30 123 *disease-resistant cultivar*

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33 124 To determine the overall response of  $A$  and  $g_s$  to our treatment groups, midseason diurnal gas  
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35 125 exchange measurements were taken to obtain integrated daily carbon assimilation ( $A'$ ) and  
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37 126 stomatal conductance ( $g_s'$ ) values at the midseason timepoint. There was a significant main  
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39 127 effect of O<sub>3</sub> (non-O<sub>3</sub> treatments (Control & Inoc) compared to eO<sub>3</sub> treatments (eO<sub>3</sub> & Inoc +  
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41 128 eO<sub>3</sub>) for  $g_s'$  but no significant main effect of inoculation (Inoc and Inoc +eO<sub>3</sub> versus Control and  
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43 129 eO<sub>3</sub>) or interaction of eO<sub>3</sub> x Inoc for the disease-resistant cultivar (Fig. 1A). For the disease-  
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45 130 susceptible cultivar, there was a slightly significant main effect of eO<sub>3</sub> ( $p = 0.063$ ), but no  
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47 131 significant main effect of inoculation or an interaction of eO<sub>3</sub> x Inoc (Fig 1A; Supplemental  
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49 132 Table 1). Overall, the disease-resistant cultivar showed a significant 64% decreased in  $g_s'$  in  
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51 133 elevated O<sub>3</sub> conditions (eO<sub>3</sub> and Inoc + eO<sub>3</sub>) compared to ambient O<sub>3</sub> conditions (Control and  
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3 134 Inoc) (Fig. 1A). Pairwise comparisons of each treatment in the disease-resistant cultivar found  
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5 135 there was a moderately significant decrease in  $g_s$  ' in eO<sub>3</sub> compared to Inoc ( $p = 0.073$ ), eO<sub>3</sub>  
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7 136 compared to the control ( $p = 0.06$ ), and Inoc + eO<sub>3</sub> compared to the control ( $p = 0.085$ ).  
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10 137 Additionally, there was a moderately significant decrease in  $g_s$  ' in Inoc + eO<sub>3</sub> compared to the  
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12 138 control ( $p = 0.085$ ) in the disease-resistant cultivar (Fig. 1A). There was no significant impact on  
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14 139  $g_s$  ' for any treatment for the disease-susceptible cultivar, however (Fig. 1A). Analysis of diurnal  
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16 140 carbon assimilation ( $A$  ' ) found that eO<sub>3</sub> had a significant effect on  $A$  ' in the disease-resistant  
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18 141 cultivar compared to the control and inoculation alone, while neither eO<sub>3</sub> or inoculation  
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20 142 (individually or in combination) had an impact on  $A$  ' for the disease-susceptible cultivar (Fig 1B;  
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22 143 Supplemental Table 2). Elevated O<sub>3</sub> (averaged across eO<sub>3</sub> and Inoc + eO<sub>3</sub> treatments)  
23  
24 144 significantly reduced  $A$  ' in the disease-resistant cultivar by 48%.  
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31 146 ***The impacts of O<sub>3</sub> and inoculation on midday gas exchange measurements were cultivar- and***  
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33 147 ***canopy height-dependent***

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35 148 *Xanthomonas* can affect varying canopy levels, with the middle canopy showing high  
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37 149 susceptibility to infection around the fourth pair of leaves, and younger leaves in the upper  
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39 150 canopy exhibiting high variability in infection (Neves *et al.*, 2014). With this in mind, we also  
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41 151 measured midseason midday photosynthetic measurements at the top and middle canopy height.  
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43 152 Stomatal conductance ( $g_s$ ) at midday in the upper canopy showed significant effects of elevated  
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45 153 O<sub>3</sub> treatments regardless of inoculation for the disease-resistant cultivar, but not on the disease-  
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47 154 susceptible cultivar following a similar trend to the diurnal photosynthesis measurements (Fig.  
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49 155 2A; Supplemental Table 3A). There was a 67% decrease in midday  $g_s$  in elevated O<sub>3</sub> conditions  
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53 156 (eO<sub>3</sub> and Inoc + eO<sub>3</sub>) when compared to the ambient O<sub>3</sub> conditions (Control and Inoc).  
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3 157 Additionally, there was as significant difference in midday  $g_s$  at the upper canopy in the Inoc  
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5 158 versus Inoc + eO<sub>3</sub> treatment, indicating the combined treatment significantly decreased midday  $g_s$   
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7 159 more than inoculation alone (Fig. 2A). Midday photosynthetic rate ( $A$ ) in the upper canopy was  
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9 160 significantly decreased in the disease-resistant cultivar when subject to elevated O<sub>3</sub> (eO<sub>3</sub> and  
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11 161 Inoc + eO<sub>3</sub>) (Fig. 2B). In the disease-resistant cultivar, there was a 55% decrease in midday  $A$  in  
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13 162 elevated O<sub>3</sub> conditions (averaged across eO<sub>3</sub> and Inoc + eO<sub>3</sub>) when compared to the ambient O<sub>3</sub>  
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15 163 conditions (averaged across Control and Inoc). There was a main effect of elevated O<sub>3</sub> (eO<sub>3</sub> and  
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17 164 Inoc + eO<sub>3</sub>) in the disease-susceptible cultivar, resulting in a 36% decrease in midday  $A$  in the  
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19 165 upper canopy compared to ambient O<sub>3</sub> conditions (Control and Inoc). There was no significant  
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21 166 main effect of inoculation or the interaction of eO<sub>3</sub> x inoculation, however (Fig. 2B).  
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26 167         There was stronger impact of eO<sub>3</sub> treatment on midday gas exchange measurements for  
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28 168 both the disease-resistant and disease-susceptible cultivars in the middle canopy. Midday  $g_s$   
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30 169 measurements at the middle canopy showed a significant main effect of elevated O<sub>3</sub>, but not  
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32 170 inoculation, for both the disease-resistant and disease-susceptible cultivar (Fig. 2C; Supplemental  
33  
34 171 Table 3B). Elevated O<sub>3</sub> (including both eO<sub>3</sub> and Inoc + eO<sub>3</sub>) decreased midday  $g_s$  in the middle  
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36 172 canopy by 52% compared to ambient O<sub>3</sub> conditions in both the disease-resistant cultivar and  
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38 173 disease-susceptible cultivar (Fig. 2C). Pairwise comparisons of the treatment found a significant  
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40 174 decrease in midday  $g_s$  at the middle canopy in eO<sub>3</sub> compared to both the control and Inoc in the  
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42 175 disease-resistant cultivar (Fig. 2C), while only eO<sub>3</sub> and Inoc were significantly different in the  
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44 176 disease-susceptible cultivar. Midday  $A$  in the middle canopy was also significantly decreased by  
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46 177 elevated O<sub>3</sub>, regardless of inoculation status in the disease-resistant and disease-susceptible  
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48 178 cultivar (Fig. 2D). Elevated O<sub>3</sub> (eO<sub>3</sub> and Inoc + eO<sub>3</sub>) decreased midday  $A$  in the middle canopy  
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50 179 by 47% in the disease-resistant cultivar and 41% in the disease-susceptible cultivar when  
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3 180 compared to the ambient O<sub>3</sub> treatments (Control and Inoc). There was no significant difference  
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5 181 in midday *A* at the middle canopy between the eO<sub>3</sub> and Inoc + eO<sub>3</sub> treatment for either cultivar,  
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7 182 however, Inoc and Inoc + eO<sub>3</sub> were significantly different in both cultivars (Fig. 2D). This  
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9 183 indicated that the impact of combined *Xanthomonas* and O<sub>3</sub> exposure on gas exchange  
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11 184 parameters is dependent on leaf age in pepper.  
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17 186 ***Maximum rates of carboxylation and electron transport were significantly decreased in***  
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19 187 ***elevated O<sub>3</sub> conditions for the disease-resistant cultivar***

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21 188 The maximum rates of carboxylation ( $V_{c,max}$ ) was significantly lowered in the disease-resistant  
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23 189 cultivar when exposed to elevated O<sub>3</sub> conditions (eO<sub>3</sub> and Inoc + eO<sub>3</sub>) compared to the control  
24  
25 190 and inoculation alone (Fig. 3A; Supplemental Table 4).  $V_{c,max}$  was decreased by 61% in the  
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27 191 disease-resistant cultivar when averaged across elevated O<sub>3</sub> treatments. There was no significant  
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29 192 difference in  $V_{c,max}$  between the Inoc and Inoc + eO<sub>3</sub> treatments in the disease-resistant cultivar.  
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31 193 There was also a significant main effect of elevated O<sub>3</sub> on  $V_{c,max}$  for the disease-susceptible  
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33 194 cultivar (Fig. 3A).  $V_{c,max}$  was decreased by 48% in the disease-susceptible cultivars when  
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35 195 averaged across elevated O<sub>3</sub> treatments (eO<sub>3</sub> and Inoc + eO<sub>3</sub>). Electron transport rate ( $J_{max}$ ) was  
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37 196 significantly decreased in the disease-resistant cultivar when subject to eO<sub>3</sub> compared to the  
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39 197 control and inoculation alone (Fig 3B; Supplemental Table 5). There was no significant  
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41 198 difference in  $J_{max}$  between the Inoc and Inoc + eO<sub>3</sub> treatments in the disease-resistant cultivar  
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43 199 (Fig. 3B). In the disease-resistant cultivar  $J_{max}$  was decreased under elevated O<sub>3</sub> conditions (eO<sub>3</sub>  
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45 200 and Inoc + eO<sub>3</sub>) by approximately 53.8% when compared to ambient O<sub>3</sub> conditions (Control and  
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47 201 Inoc). There was no significant impact of O<sub>3</sub> or inoculation for the disease-susceptible cultivar  
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49 202 (Fig. 3B).  
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5 204 ***Chlorophyll content was significantly impacted by both elevated O<sub>3</sub> and inoculation in both***  
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8 205 ***cultivars in a canopy height-dependent manner***  
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10 206 Chlorophyll content was measured for leaves in both the upper and middle canopy for both  
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12 207 cultivars across all treatments. Chlorophyll content measured in the disease-resistant cultivar was  
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14 208 significantly lowered at both the upper and middle canopies when subject to elevated O<sub>3</sub>  
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16 209 conditions (Fig. 4A,B; Supplemental Table 6). Chlorophyll content was decreased by 36% in the  
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18 210 upper canopy and 32% in the middle canopy when subject to elevated O<sub>3</sub> conditions (eO<sub>3</sub> and  
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20 211 Inoc + eO<sub>3</sub>) when compared to ambient O<sub>3</sub> conditions (Control and Inoc) in the disease-resistant  
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22 212 cultivar. There was also a significant decrease in chlorophyll content between Inoc and Inoc +  
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24 213 eO<sub>3</sub>, but not eO<sub>3</sub> and Inoc + eO<sub>3</sub> for the disease-resistant cultivar at both canopy heights (Fig.  
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26 214 4A,B). Leaf chlorophyll content was only significantly reduced in the disease-susceptible  
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28 215 cultivar between the Inoc and Inoc + eO<sub>3</sub> treatment in both the middle and upper canopy (Fig.  
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30 216 4A,B), with a significant main effect of both O<sub>3</sub> and inoculation in the middle canopy (Fig. 4B).  
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32 217 The disease-susceptible cultivar had a 21% decrease in leaf chlorophyll content in the Inoc +eO<sub>3</sub>  
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34 218 treatment compared to Inoc alone in the upper canopy, and a 29% decrease in the middle canopy.  
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36 219 This indicates that canopy height may play an important role in determining the combined  
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38 220 impacts of *Xanthomonas* presence and elevated O<sub>3</sub> on leaf chlorophyll content.  
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42 222 ***Residual stomatal conductance is significantly impacted by elevated O<sub>3</sub> and inoculation in the***  
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44 223 ***disease-resistant cultivar, whereas the slope constant was not impacted by any treatment in***  
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46 224 ***both cultivars***  
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3 225 Using the BWB model previously described, stomatal conductance was plotted as a linear  
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5 226 relationship to environmental factors such as light, humidity, and CO<sub>2</sub> concentration. From this,  
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7 227 we calculated  $m$ , representing the empirical relationship between stomatal conductance ( $g_s$ ) and  
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9 228 photosynthetic rate ( $A$ ) when subject to these factors, and  $g_0$ , representing the residual stomatal  
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11 229 conductance as photosynthetic rate ( $A$ ) approaches 0 (Ball *et. al.*, 1987; Leuning, 1995). Both  
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13 230 elevated O<sub>3</sub> and the interaction of elevated O<sub>3</sub> x inoculation had significant main effects on  $g_0$  in  
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15 231 the disease-resistant cultivar (Fig. 5A; Supplemental Table 7). Pairwise comparisons of  
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17 232 treatments found that  $g_0$  was decreased by all treatments when compared to the control in the  
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19 233 disease-resistant cultivar (Fig. 5). Elevated O<sub>3</sub> (eO<sub>3</sub> and Inoc + eO<sub>3</sub>) decreased  $g_0$  by 63% (Fig.  
20  
21 234 5A), when compared to ambient O<sub>3</sub> (Control and Inoc). Inoculation (Inoc and Inoc + eO<sub>3</sub>)  
22  
23 235 decreased  $g_0$  in the disease-resistant cultivar by 29% when compared to plants without  
24  
25 236 inoculation with the pathogen (Control and eO<sub>3</sub>). There was a main effect of elevated O<sub>3</sub> on  $g_0$   
26  
27 237 for the disease-susceptible cultivar, with a 72% decrease in  $g_0$  by the Inoc + eO<sub>3</sub> treatment when  
28  
29 238 compared to Inoc (Fig. 5A). The slope constant ( $m$ ) was not significantly impacted by any  
30  
31 239 treatment for the disease susceptible or disease-resistant cultivars (Fig. 5B; Supplemental Table  
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33 240 8).  
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#### 42 242 ***Total antioxidant capacity was not impacted by any treatment across cultivars***

43  
44 243 Total antioxidant capacity was measured in leaf tissue collected from the upper canopy of plants  
45  
46 244 from each OTC, collected at the midseason timepoint, 57 days after planting. The concentration  
47  
48 245 of total antioxidants ranged from ~ 185- 226 nmol TE mg FW<sup>-1</sup> in the disease-resistant cultivar,  
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50 246 and from ~195-237 nmol TE mg FW<sup>-1</sup> in the disease-susceptible cultivar (Fig. 6; Supplemental  
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3 247 Table 9). There was no significant impact of any treatment on total antioxidant capacity, in both  
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5 248 the disease-resistant and disease-susceptible cultivars.  
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10 250 ***Total aboveground biomass was lowered in elevated O<sub>3</sub> conditions while fruit weight remained***  
11  
12 251 ***unaffected***  
13

14 252 At the final harvest in August, we collected aboveground biomass, including any plant material  
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16  
17 253 aside from fruits from soil level up. Elevated O<sub>3</sub> was a significant factor in decreasing  
18  
19 254 aboveground biomass in both the disease-resistant and disease-susceptible cultivars (Fig 7A;  
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21 255 Supplemental Table 10). Aboveground biomass collected for the disease-resistant cultivar in  
22  
23 256 elevated O<sub>3</sub> conditions (eO<sub>3</sub> and Inoc + eO<sub>3</sub>) was decreased by 43% when compared to ambient  
24  
25  
26 257 O<sub>3</sub> (Control and Inoc) conditions (Fig. 7A). There was also a significant decrease in aboveground  
27  
28 258 biomass between the Inoc and Inoc + eO<sub>3</sub> treatment, but not the eO<sub>3</sub> and Inoc + eO<sub>3</sub> treatment in  
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30  
31 259 the disease-resistant cultivar (Fig. 7A). In the disease-susceptible cultivar, there was also a  
32  
33 260 significant main effect of inoculation on aboveground biomass, with a significant decrease in the  
34  
35 261 Inoc + eO<sub>3</sub> treatment as compared to the control (Fig. 7A). Aboveground biomass decreased by  
36  
37 262 13% in the elevated O<sub>3</sub> conditions (eO<sub>3</sub> and Inoc + eO<sub>3</sub>) for the disease-susceptible cultivar when  
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39  
40 263 compared to ambient O<sub>3</sub> (Control and Inoc) conditions (Fig. 7A).  
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42 264 Total fruit number and ripened fruit dry weights were measured and then the average fruit  
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44 265 weight per plant was calculated. Fruits that had decayed or fallen prior to collection were not  
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46  
47 266 counted. There was a total of 201 fruits collected for the disease-susceptible pepper plants  
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49 267 collected across all treatments, with a total weight of 2870.1 g. There was a total of 194 fruits  
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51 268 collected for the disease-resistant pepper plants collected across all treatments, with a total  
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54 269 weight of 1990.9 g. There were no significant differences for average weight per pepper across  
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3 270 all treatments for both the disease-resistant and disease-susceptible cultivars (Figure 7B;  
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5 271 Supplemental Table 11).

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10 273 **Discussion**

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12 274 The goal for this study was to investigate the impacts of a combined abiotic and biotic stress  
13  
14 275 environment on the physiology of a major agricultural crop. We utilized the *Xanthomonas* –  
15  
16 276 *Capsicum annuum* pathosystem for this study as it is a crop relevant in the Southeastern United  
17  
18 277 States, and *Xanthomonas perforans* which is an emerging pathogen on *Capsicum annuum* among  
19  
20 278 other Solanaceous species (Newberry *et al.*, 2019; 2023). The resistant cultivar used in this study  
21  
22 279 is considered to possess intermediate level of resistance against all eleven races of bacterial spot  
23  
24 280 *Xanthomonas* (Stall *et al.*, 2009) and contains *bs5* recessive resistance gene (Clause & Monroy  
25  
26 281 2019). In selecting the resistant and susceptible cultivars used for this study we can evaluate if  
27  
28 282 there are any tradeoffs in abiotic stress resistance associated with enhanced biotic stress  
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30 283 resistance.  
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38 285 *The disease-susceptible cultivar demonstrates stronger O<sub>3</sub> tolerance than the disease-resistant*  
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40 286 *cultivar when leaf-level physiological parameters are evaluated*

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42 287 Our study found that O<sub>3</sub> had the largest negative impact on leaf-level physiology compared to  
43  
44 288 inoculation alone. This response was almost exclusively seen in the disease-resistant cultivar  
45  
46 289 (Fig. 1-3). The strongest impact on diurnal and midday gas exchange parameters, as well as  
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48 290  $V_{c,max}$  and  $J_{max}$  was seen with the eO<sub>3</sub> and Inoc + O<sub>3</sub> treatments for the disease-resistant cultivar.  
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50 291 We also observed significant impacts of elevated O<sub>3</sub> alone and in combination with inoculation  
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53 292 on leaf chlorophyll content in the disease-resistant cultivar at the upper and middle canopy (Fig.

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3 293 4). For the disease-susceptible cultivar we only saw significant negative treatment impacts on  
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5 294  $V_{c,max}$ , midday gas exchange and chlorophyll content taken at two canopy heights (Fig. 2 & 4).  
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7  
8 295 Midday  $A$  taken at the upper canopy had a significant main effect of  $O_3$  for the disease-  
9  
10 296 susceptible cultivar (Fig. 2B). Additionally,  $A$  at the middle canopy was significantly negatively  
11  
12 297 impacted by inoculation and inoculation with elevated  $O_3$  compared to the control in the disease-  
13  
14 298 susceptible cultivar (Fig. 2D). There was also a significant main effect of elevated  $O_3$  on  $V_{c,max}$   
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16 299 (Fig. 3A), and leaf chlorophyll content at the upper and middle canopy was also significantly  
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18 300 negatively impacted by inoculation with elevated  $O_3$  for the susceptible cultivar (Fig. 4B). Taken  
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20 301 together, this indicates that the disease-susceptible cultivar has higher  $O_3$  tolerance at the leaf-  
21  
22 302 level than the disease-resistant cultivar, and canopy height is an important factor in evaluating  
23  
24 303 physiological responses to elevated  $O_3$ .  
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28 304 Previous literature has demonstrated that elevated  $O_3$  negatively impacts leaf-level  
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30 305 physiological parameters (Ainsworth *et al.*, 2012). Elevated  $O_3$  has also been shown to decrease  
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32 306 leaf chlorophyll content across multiple depths in the canopy (Tenga & Omrod, 1990), which in  
33  
34 307 turn, can negatively impact photosynthetic efficiency. Work done in lettuce (*Lactuca sativa* L.)  
35  
36 308 also found that chronic  $O_3$  exposure can cause limitations in  $J_{max}$  and  $V_{c,max}$  (Goumenaki *et al.*,  
37  
38 309 2010). In this study they hypothesized that chronic  $O_3$  exposure may lead to a decrease in the  
39  
40 310 abundance of Rubisco and Rubisco-activase and may be associated with developmental stage of  
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42 311 the leaf. In our study, we observed that the disease resistant cultivar showed stomatal and  
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44 312 biochemical limitations (decreases in  $V_{c,max}$  and  $J_{max}$ ) at elevated  $O_3$  conditions, while the disease  
45  
46 313 susceptible cultivar only showed a decrease in rubisco activity ( $V_{c,max}$ ) which could have been  
47  
48 314 caused by decreases in rubisco concentration (Goumenaki *et al.*, 2010). These results indicate  
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50 315 there is cultivar variation in the elevated  $O_3$  response in pepper, as we see increased sensitivity to  
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3 316 O<sub>3</sub> in the cultivar with greater disease resistance. Cultivars of other crops including corn and  
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5 317 soybean have also shown to have this variable sensitivity to O<sub>3</sub>, with some soybean cultivars  
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7 318 exhibiting greater antioxidant capacity in chronic elevated O<sub>3</sub> conditions (Betzberger *et al.*,  
8  
9 319 2010; Ainsworth, 2012).

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12 320 Additional work has shown that leaf age is a factor in elevated O<sub>3</sub> vulnerability. When  
13  
14 321 exposed to elevated O<sub>3</sub> compared to ambient and charcoal filtered conditions, older leaves in  
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16 322 black cherry (*Prunus serotina*) exhibited decreased stomatal conductance and photosynthetic  
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18 323 rate, as well as increased visual leaf damage caused by O<sub>3</sub> (Zhang *et al.*, 2010). Furthermore,  
19  
20 324 previous work has also shown that elevated O<sub>3</sub> can cause decreases in leaf chlorophyll content  
21  
22 325 across multiple depths in the canopy (Tenga & Omrod, 1990), which in turn, can negatively  
23  
24 326 impact photosynthetic efficiency. Burton *et al.*, (2016) found older leaves in the soybean canopy  
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26 327 exhibit greater foliar damage caused by elevated O<sub>3</sub>, suggesting that the genes involved in O<sub>3</sub>  
27  
28 328 response may differ depending on the leaf age, and may be linked to the redox state within the  
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30 329 apoplast. Future work is needed therefore to evaluate O<sub>3</sub> impacts on leaf-level physiology at  
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32 330 multiple developmental ages during the growing season, as this may impact the plant  
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34 331 physiological response.  
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42 333 *Both disease pressure and elevated O<sub>3</sub> affect stomatal responses in pepper as seen through*  
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44 334 *changes in the BWB relationship*

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47 335 The BWB model describes the relationship between net photosynthesis ( $A_n$ ) and stomatal  
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49 336 conductance ( $g_s$ ). Parameterization of the BWB model was done to determine impacts of  
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51 337 pathogen pressure and O<sub>3</sub> on the slope constant ( $m$ ) and residual stomatal conductance ( $g_0$ ) (Fig.  
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53 338 5). Residual stomatal conductance ( $g_0$ ) was significantly decreased by all treatments compared to  
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3 339 the control in the disease-resistant cultivar (Fig. 5A), while neither inoculation nor O<sub>3</sub> had a  
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5 340 significant effect on the slope constant ( $m$ ) in either cultivar (Fig. 5B). The results from our study  
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7 341 report for the first time the relationship between  $A_n$  and  $g_s$  is altered by elevated O<sub>3</sub> alone and in  
8  
9 342 combination with pathogen infection in pepper. Previous studies have already demonstrated the  
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11 343 BWB relationship is impacted by elevated O<sub>3</sub> (Lombardozzi *et al.*, 2012; Hoshika *et al.*, 2015;  
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13 344 Masutomi *et al.*, 2019). For example, in rice and in beech it was observed that residual stomatal  
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15 345 conductance was increased due to O<sub>3</sub> for cultivars most sensitive to O<sub>3</sub> (Hoshika *et al.*, 2015;  
16  
17 346 Masutomi *et al.*, 2019). It was also found in rice that the slope constant was not impacted by O<sub>3</sub>  
18  
19 347 (Masutomi *et al.*, 2019). Similarly in our study we found the slope constant was not impacted by  
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21 348 O<sub>3</sub>, however we found that elevated O<sub>3</sub> in combination with pathogen inoculation led to a  
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23 349 decrease in  $g_0$  in pepper.

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28 350 Previous work suggests the increase in  $g_0$  of the BWB relationship may be caused by  
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30 351 stomatal sluggishness, which is impairment or delay in stomatal response due to physiological or  
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32 352 structural damage caused by O<sub>3</sub> due to unknown mechanisms (Reich & Lassoie, 1984; Reiling &  
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34 353 Davison, 1995; Paoletti, 2005; Grulke *et al.*, 2007; Paoletti & Grulke, 2010; Hoshika *et al.*,  
35  
36 354 2012). An increase in  $g_0$  caused by greater stomatal sluggishness and increased stomatal opening  
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38 355 in response to elevated O<sub>3</sub> would increase O<sub>3</sub> uptake in leaves, which would lead to additional O<sub>3</sub>  
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40 356 damage (Masutomi *et al.*, 2019). Additionally, an increase in  $g_0$  in response to elevated O<sub>3</sub> could  
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42 357 lead to decreased water-use efficiency, as seen in work done in rice (Masutomi *et al.*, 2019),  
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44 358 further exacerbating the negative impacts of O<sub>3</sub> on plant physiology and yield.

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49 359 In our study however, we found a decrease in  $g_0$ , or lack of stomatal sluggishness which  
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51 360 may reflect the complex relationship between O<sub>3</sub> and pathogen infection on the BWB  
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53 361 relationship. In our study we found elevated O<sub>3</sub> in combination with inoculation decreased  $g_0$  by

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3 362 71% when compared to the control in the disease-resistant cultivar. The lack of stomatal  
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5 363 sluggishness (decrease in stomatal opening) observed in the disease-resistant cultivar in response  
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7 364 to elevated O<sub>3</sub> and pathogen infection may also explain the negative impacts we observed on  
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9 365 leaf-level carbon assimilation (Fig. 1 & 2) and biomass accumulation (Fig. 7A), as previous  
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11 366 work has demonstrated that *Xanthomonas* infection alone does negatively impact photosynthesis  
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13 367 in other species (Nogués *et al.*, 2003; Zhou *et al.*, 2004; Debona *et al.*, 2014). We did not see an  
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15 368 impact of any treatment on fruit pepper weight (Fig. 7B), however. This aligns with findings  
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17 369 from Thwe *et al.*, (2015), which suggest that there may be a conservatory mechanism when  
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19 370 indeterminate plants are exposed to O<sub>3</sub> that may help protect seed count and reproductive  
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21 371 structures. This decrease in stomatal sluggishness may also explain why no changes in  
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23 372 antioxidant capacity were observed across treatments for either cultivar (Fig. 6). If O<sub>3</sub> uptake is  
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25 373 limited by changes in  $g_s$  and  $g_0$  the need to quench additional ROS may be decreased.  
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31 374 The disease-resistant cultivar used in this study contains the recessive resistance gene  
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33 375 *bs5*, which conveys a non-HR response to *Xanthomonas* (Clause & Monroy, 2019). We  
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35 376 hypothesize that one mechanism by which this non-HR response provides intermediate level of  
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37 377 resistance to *Xanthomonas* is by triggering quicker stomatal closure. While the mechanism by  
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39 378 which this would occur in pepper is unknown, previous work in *Arabidopsis thaliana* gives some  
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41 379 possible insights. The dominant *Bs5* gene is a susceptibility locus and encodes a protein with a  
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43 380 cystine-rich transmembrane module (CYSTM) domain (Szabó *et al.*, 2023). CYSTM proteins are  
44  
45 381 involved in both biotic and abiotic stress responses (Mir *et al.*, 2013) and are hypothesized to aid  
46  
47 382 in the delivery of virulence factors, specifically, type III effectors. The *bs5* recessive gene in the  
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49 383 disease-resistant cultivar contains a deletion variant of this CYSTM protein aiding in the non-HR  
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51 384 response. Work done in *Arabidopsis thaliana* found the *Pathogen and Circadian Controlled 1*  
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3 385 (*PCCI*) gene, which encodes for a protein with a CYSTM domain, regulates abscisic acid  
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5 386 (ABA)-mediated developmental transitions. This study showed RNA interference constructs for  
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7 387 the *PCCI* gene (*iPCCI*) were hypersensitive to ABA, and stomata of *iPCCI* leaves remained  
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9 388 more closed than wild type plants under non-stressed conditions (Mir *et al.*, 2013).

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12 389 This phenotype of more closed stomata with a silenced CYSTM domain containing  
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14 390 protein in *Arabidopsis* is like what we observed with the resistant cultivar, showing reduced  
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16 391 stomatal conductance (both  $g_0$  and  $g_s$ ) under elevated O<sub>3</sub> conditions, regardless of pathogen  
17  
18 392 infection. Our observations suggest that the resistant cultivar containing the CYSTM variant,  
19  
20 393 *bs5*, shows an altered physiological response when exposed to elevated O<sub>3</sub>, regardless of  
21  
22 394 pathogen infection. This altered response, as measured by reduced photosynthetic capacity and  
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24 395 stomatal conductance, warrants further experiments with near-isogenic lines to test the role of the  
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26 396 *bs5* variant in simultaneously mediating biotic and abiotic stress tolerance pathways.

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29 397 It is of note that while the overall disease severity for the disease-resistant cultivar was  
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31 398 lower (0.37%) compared to the disease-susceptible cultivar (53.01%) at mid-season, elevated O<sub>3</sub>  
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33 399 increased the disease severity only in the disease-resistant cultivar (Bhandari *et al.*, 2023). This  
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35 400 was also accompanied by high variability in disease severity values, suggestive of a plastic  
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37 401 response of the plant (Bhandari *et al.*, 2023). We hypothesize that the variable disease severity  
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39 402 observed under elevated O<sub>3</sub> may be caused by variation in the extent to which *Xanthomonas* cells  
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41 403 were able to gain entry through stomata on different leaves and reaching the plant apoplastic  
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43 404 space, taking advantage of the higher humidity and water availability caused by the closed  
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45 405 stomata, and allowing them to proliferate and increase disease severity (Lajeunesse *et al.*, 2023).

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## 52 53 54 407 **Conclusions**

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3 408 Taken together, findings from this work demonstrate that the combination of elevated O<sub>3</sub> and  
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5 409 pathogen pressure can significantly impact plant photosynthetic parameters more than pathogen  
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7 410 pressure alone. Our work finds that cultivars of pepper resistant to *Xanthomonas* infection have  
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9 411 decreased leaf-level carbon assimilation and stomatal conductance in response to elevated O<sub>3</sub>,  
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11 412 regardless of inoculation status. We believe this is possibly due to changes in the BWB  
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13 413 relationship, as we observed a decrease in  $g_0$  in response to all treatments compared to the  
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15 414 control in the disease resistant cultivar. We hypothesize that the *bs5* recessive gene that aids in  
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17 415 *Xanthomonas* resistance also may be triggering early stomatal closure, which resulted in negative  
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19 416 impacts on leaf-level gas exchange and subsequent biomass accumulation. Future work is needed  
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21 417 to understand the *Xanthomonas* resistance that exists in *Capsicum annuum*, and to uncover the  
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23 418 breakdowns seen when faced with additional abiotic stressors.  
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## 420 **Experimental Procedures**

### 421 ***Plant growth information and field experimental design***

422 This work was conducted over a single growing season (May-August) in 2021 at the  
423 Atmospheric Deposition site (AtDep) located at Auburn University in Auburn, AL. Pepper  
424 seedlings were germinated starting in late March and grown at the Plant Science Research Center  
425 at Auburn University for ~ 5 to 6 weeks before transplanting to pots. Two cultivars of pepper  
426 were used in the experiment: a *Xanthomonas*-susceptible cultivar, Early Calwonder, and a  
427 resistant cultivar PS 09979325. The resistant cultivar contains several traits that have been  
428 proven resilient to common biotic stressors of Solanaceous species such as bacterial spot disease  
429 and tobacco mosaic virus (Bhandari *et al.*, 2023). Seventy-two seedlings of each cultivar were  
430 grown at the AtDep site totaling 144 samples for the experiment.

431 Following transplanting, half of the plants were inoculated with *X. perforans* and then  
432 relocated to the field for the duration of the experiment according to Bhandari *et al.* (2023).  
433 Thirteen-liter pots were filled with ProMix potting soil. The soil was amended before  
434 transplanting with 114g of Osmocote slow-release fertilizer following recommendations of  
435 Auburn University Extension practices for pepper. Plants were irrigated twice a day, once  
436 starting at 8:45AM and once starting at 4:45PM each for 30 minutes with a single 7.5 LPH (liter  
437 per hour) dripper, amounting to a total of 3.8 liters of water/day.

438 The AtDep site was used to expose plants to elevated and ambient levels of O<sub>3</sub> for the  
439 duration of their life cycle using the OTC system. Each chamber was equipped with a fanbox  
440 that supplied elevated or ambient O<sub>3</sub> air through a double lined plastic wall, with perforations to  
441 create a ring of air blown around the chamber. For the elevated O<sub>3</sub> chambers, there were four  
442 ozone generators (HVAC-1100 Ozone generator, Ozone Technologies, Hull, IA, USA) that

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3 443 controlled two ultraviolet lightbulbs each (Model GPH380T5VH/HO/4 P, Ozone Technologies,  
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5 444 Hull, IA, USA). The intensity of these lightbulbs was set using an analog module, set from 0-  
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7 445 10V, and adjusted depending on factors that may increase or decrease natural O<sub>3</sub> levels such as  
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9 446 strong UV index or heavy cloud coverage, respectively. O<sub>3</sub> concentration for all 12 chambers  
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11 447 were measured every 24 minutes, sampling each chamber for 2 minutes at a time. Plastic tubing  
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13 448 directed air from the chambers to a gas manifold, which redirected air to its coinciding solenoid  
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15 449 valve to be read by the ozone monitor (Model 205 Dual Beam Ozone Monitor, 2B Technologies,  
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17 450 Boulder, CO, USA). Plants were fumigated with elevated O<sub>3</sub> or ambient air for 8 hours a day,  
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19 451 between the hours of 10am and 6pm. Plants were placed inside the OTCs directly following  
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21 452 inoculation using a randomized complete block design. Six plants of each cultivar (resistant and  
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23 453 susceptible) were placed in each chamber, with 36 plants per treatment. Utilizing 12 OTCs, we  
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25 454 exposed plants to four different treatments: ambient O<sub>3</sub> with no inoculation (Control), ambient  
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27 455 O<sub>3</sub> with *X. perforans* inoculation (Inoc), elevated O<sub>3</sub> with no inoculation (eO<sub>3</sub>), and elevated O<sub>3</sub>  
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29 456 with *X. perforans* inoculation (Inoc + eO<sub>3</sub>) (Supplemental Figure 1). OTCs containing inoculated  
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31 457 plants were placed inside the first 6 chambers, to decrease the risk of cross contamination  
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33 458 amongst OTCs not containing the pathogen. Across the field season, ambient O<sub>3</sub> chambers  
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35 459 averaged 30.6 ppb of O<sub>3</sub>, and elevated O<sub>3</sub> chambers averaged 90.3 ppb of O<sub>3</sub> (for additional  
36  
37 460 information see Bhandari *et al.* 2023). O<sub>3</sub> levels in elevated OTCs were set to represent double  
38  
39 461 the concentration of ambient O<sub>3</sub>, to reflect regions in the United States where ambient O<sub>3</sub>  
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41 462 concentrations regularly exceed levels that are damaging to major crops (Dentener *et al.*, 2006,  
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43 463 Heagle, 1989).  
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#### 54 465 ***Midday Gas Exchange Measurements***

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3 466 Gas exchange parameters were measured using three LI-6800 Portable Photosynthesis System  
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5 467 (LICOR biosciences. Lincoln, Nebraska, USA). The chamber light, temperature, and humidity  
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7 468 were set to match the conditions outside of the chamber at the start of the measurements.  
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10 469 Measurements were taken on a newly developed, fully expanded leaf, often found at the 3rd  
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12 470 node from the top. Midday photosynthesis measurements were taken starting at 11am and were  
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14 471 taken for 2 plants of each cultivar per chamber totaling 48 measured plants. These measurements  
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16 472 were taken in June (57 days post-inoculation), to compare photosynthetic impacts of *X*.  
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18 473 *perforans* in the upper canopy compared to the middle canopy.  
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#### 23 24 475 ***Diurnal Gas Exchange Measurements***

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26 476 Diurnal gas exchange measurements were taken every 3 hours at the following times: 8am,  
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28 477 11am, 2pm, and 5pm. Measurements were taken with the LI-6800 on the most recently fully  
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30 478 expanded leaf. Prior to each measurement, environmental temperature, humidity, and light  
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32 479 intensity were all evaluated, and the settings for each parameter were set on the LI-6800 to match  
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34 480 that of the environment. Carbon dioxide (CO<sub>2</sub>) concentrations were set at 410 ppm. These  
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36 481 diurnal measurements were only taken at the midseason in June (57 days post-inoculation). Total  
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38 482 diurnal photosynthetic rate ( $A'$ ) and stomatal conductance ( $g_s'$ ) were estimated by calculating the  
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40 483 area under the curve created by plotting the measured values over the times during the day these  
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42 484 measurements were taken. These integrations were calculated according to methods described in  
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44 485 Soba *et al.*, (2020).  
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#### 51 487 ***Photosynthetic Response Curves***

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3 488 Curves representing the photosynthetic ( $A$ ) response to internal  $\text{CO}_2$  concentration ( $C_i$ ) were  
4  
5 489 generated using the LI-6800 in June (57 days post-inoculation). This was done using an  
6  
7 490 automatic program that adjusts  $\text{CO}_2$  levels (410, 310, 260, 160, 110, 50, 410, 610, 810, 1110,  
8  
9 491 1310, 1510  $\mu\text{mol mol}^{-1} \text{CO}_2$ ) while maintaining light intensity ( $1750 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), temperature  
10  
11 492 ( $28.0^\circ\text{C}$ ) and VPD (1.1 kPa) at constant conditions. Measurements were taken on a newly  
12  
13 493 developed, fully expanded leaf, often found at the 3rd node from the top. With the  $A/C_i$  curves,  
14  
15 494 we calculated the maximum rates of electron transport ( $J_{max}$ ) and carboxylation ( $V_{c,max}$ ) with the  
16  
17 495 method developed by Sharkey *et al.* (2007).  $J_{max}$  and  $V_{c,max}$  were then normalized at  $25^\circ\text{C}$  as  
18  
19 496 Khan *et al.* (2021) demonstrated that leaf temperatures between  $20\text{--}35^\circ\text{C}$  did not alter the  
20  
21 497 measurement of these photosynthetic parameters.  
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### 29 ***Ball-Woodrow-Berry Model Parameterization***

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31 500 To understand stomatal sensitivity to each treatment, a Ball-Woodrow-Berry Model (BWB)  
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33 501 parameterization was conducted. This was completed by measuring the response of  $A$  to various  
34  
35 502  $\text{CO}_2$  concentrations, vapor pressure deficit (VPD) and light levels using the LI-6800 (LICOR  
36  
37 503 biosciences, Lincoln, Nebraska, USA) in June (57 days post-inoculation). The  $A$  and  $\text{CO}_2$  curves  
38  
39 504 were measured as described above while maintaining VPD and light constant at about 1.1  
40  
41 505 kilopascals (kPa) and  $1750 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. Levels of VPD used were 1, 1.5, 2, 2.5,  
42  
43 506 3, 3.5 kPa while maintaining  $\text{CO}_2$  at  $410 \mu\text{mol mol}^{-1}$  and light intensity at  $1750 \mu\text{mol s}^{-1} \text{m}^{-2}$   
44  
45 507 PAR. Levels of light intensity used were 1750, 1500, 1000, 700, 400, 200, 100, 75,  $50 \mu\text{mol s}^{-1}$   
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47 508  $\text{m}^{-2}$  PAR while maintaining the  $\text{CO}_2$  level at  $410 \mu\text{mol mol}^{-1} \text{CO}_2$ , VPD at 1.1 kPa, and  
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49 509 temperature at  $28^\circ\text{C}$ . Measurements were taken on a newly developed, fully expanded leaf, often  
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3 510 found at the 3rd node from the top. Data points used from these experiments were plotted to a  
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5 511 linear equation described in Ball *et al.* (1987). The linear equation is as follows:

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8 512 
$$g_s = g_0 + mA \frac{h_s}{[CO_2]}$$
  
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10  
11 513 where  $g_s$  is stomatal conductance,  $g_0$  represents residual stomatal conductance,  $A$  is  
12  
13 514 photosynthetic rate,  $h_s$  is humidity on the leaf surface,  $[CO_2]$  is the concentration of  $CO_2$ , and  $m$   
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15 515 represents the empirical slope constant.  
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20 517 ***Measurement of Chlorophyll Content***  
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22 518 Chlorophyll content was measured using a portable chlorophyll meter (SPAD-502 Plus, Konica  
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24 519 Minolta Sensing Inc, Osaka, Japan). These measurements took place on the same day of the  
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27 520 midseason diurnal measurements in June (57 days post-inoculation). The data collected from  
28  
29 521 each plant is a representation of 5 chlorophyll measurements averaged for one leaf from the  
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31 522 upper canopy and 5 chlorophyll measurements averaged for one leaf from the middle canopy.  
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33 523 Two plants from each cultivar were measured per OTC.  
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38 525 ***Measurement of Total Antioxidant Capacity***  
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40  
41 526 Measurement of total antioxidant capacity in leaves was done using the oxygen radical  
42  
43 527 absorbance capacity (ORAC) assay as described in Gillespie *et al.* (2007). Approximately 6  
44  
45 528 leaves per cultivar in each chamber were collected in June (57 days post-inoculation) from the  
46  
47 529 upper canopy, and immediately frozen in liquid nitrogen. Approximately 20 mg of ground frozen  
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49 530 leaf tissue per sample was submerged in a 50% acetone solution, and centrifuged at 4,500g for  
50  
51 531 15 minutes at 4 °C. Following centrifugation, the supernatant was removed and diluted 1:40 to fit  
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54 532 within our standard curve. The standard used in this assay was Trolox ( $C_{14}H_{18}O_4$ ), which is a  
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3 533 water-soluble form of the antioxidant Vitamin E. Fluorescein was used as the fluorescent tracer  
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5 534 to detect the quenching of antioxidants and monopotassium phosphate ( $\text{KH}_2\text{PO}_4$ ) buffer was used  
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7  
8 535 as a blank. The assay was conducted within a Cytation 3 imaging reader (Biotek, Winooski,  
9  
10 536 Vermont, USA) absorbance microplate reader using the excitation filter of 485 nm, and the  
11  
12 537 emission filter to 520 nm. The 96-well plate was incubated first at 37 °C for 10 minutes.  
13  
14 538 Immediately following this incubation, 2,2'-azobis-2-methyl-propanimidamide, dihydrochloride  
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16 539 (AAPH) was added to initialize the oxygen radical formation. Following addition of AAPH  
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18 540 quenching of the oxygen radicals was measured using the microplate reader. Total antioxidant  
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20 541 capacity was measured as nmol Trolox Equivalents (TE) per mg fresh weight.  
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### 25 26 543 ***Biomass and Fruit Harvest***

27  
28 544 Ripened fruits were collected starting at the midseason timepoint in June, and collected each  
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30 545 month through the final harvest at the end of the growing season for all plants (6 plants of each  
31  
32 546 cultivar from every OTC). Aboveground biomass, including all stems and leaves, were collected  
33  
34 547 at the final harvest in August, 15 weeks after inoculation. Both ripened fruits and aboveground  
35  
36 548 biomass were dried out at 60°C for approximately 10 days to obtain dry weight data. Fruit and  
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38 549 aboveground biomass dry weights were represented as total grams (g) individually by chamber,  
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40 550 and then data were pooled to represent the four treatment groups. Images of visible foliar damage  
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42 551 at the same time of gas exchange measurements have been included in Supplemental Figure 2.  
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### 48 49 553 ***Statistical Analysis of Physiology***

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51 554 Data collected from all physiological measurements are represented by  $n=3$ , with each  
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53 555 biological replicate represented by an average of two measurements per chamber. Overall  
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3 556 significance of treatment conditions was calculated using two-way interaction Analysis of  
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5 557 Variance (ANOVA) tests. These ANOVA tests were run separately for each cultivar and used O<sub>3</sub>  
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7 558 level and inoculation status as the fixed effects with chamber (block) as a random effect.  
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10 559 Pairwise comparisons across treatment groups were done using Tukey Honest Significant  
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12 560 Differences tests, for each cultivar separately. Levene's Test was used to check homogeneity of  
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14 561 variance and the Shapiro-Wilk normality test was used to test the for any violations in normality  
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16  
17 562 of the data. All statistics were carried out in RStudio (R Version 4.1.1 – “Kick Things”).  
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3 564 **Acknowledgements**  
4

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6  
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14  
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3 **572 Legend for Supporting Information**  
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5 **573 Supplemental Figure 1:** Atmospheric Deposition Site located in Auburn, AL. Open top  
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7  
8 **574** chambers (OTCs) with elevated O<sub>3</sub> are indicated by blue circles, and ambient O<sub>3</sub> OTCs are  
9  
10 **575** indicated by white circles. OTCs 1-6 were inoculated with *Xanthomonas perforans*, and  
11  
12 **576** chambers 7-12 were not inoculated. The inset image is of OTCs used in the study.  
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14 **577**  
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17 **578 Supplemental Figure 2:** Image of plants grown in each treatment for the disease-resistant and  
18  
19 **579** disease-susceptible cultivar.  
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21 **580**  
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24 **581 Supplemental Table 1.** ANOVA tests for fixed effects diurnal stomatal conductance ( $g_s$ )  
25  
26 **582** presented in this study.  
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28 **583**  
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31 **584 Supplemental Table 2.** ANOVA tests for fixed effects for diurnal photosynthetic rate ( $A$ )  
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33 **585** presented in this study.  
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38 **587 Supplemental Table 3A.** ANOVA tests for fixed effects for upper canopy midday stomatal  
39  
40 **588** conductance ( $g_s$ ) and photosynthetic rate ( $A$ ) presented in this study.  
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42 **589**  
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44  
45 **590 Supplemental Table 3B.** ANOVA tests for fixed effects for middle canopy midday stomatal  
46  
47 **591** conductance ( $g_s$ ) and photosynthetic rate ( $A$ ) presented in this study.  
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49 **592**  
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52 **593 Supplemental Table 4.** ANOVA tests for fixed effects for maximum rate of carboxylation  
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54 **594** ( $V_{c,max}$ ) presented in this study.  
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3 5954  
5 596 **Supplemental Table 5.** ANOVA tests for fixed effects for maximum rate of electron transport6  
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8 597 ( $J_{max}$ ) presented in this study.9  
10 59811  
12 599 **Supplemental Table 6.** ANOVA tests for fixed effects for midday SPAD measurements13  
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15 600 presented in this study.16  
17 60118  
19 602 **Supplemental Table 8.** ANOVA tests for fixed effects for Ball-Woodrow-Berry slope constant20  
21 603 ( $m$ ) presented in this study.22  
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24 60425  
26 605 **Supplemental Table 9.** ANOVA tests for fixed effects for oxygen radical absorbance capacity27  
28 606 (ORAC) presented in this study.29  
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31 60732  
33 608 **Supplemental Table 10.** ANOVA tests for fixed effects for aboveground biomass presented in34  
35 609 this study.36  
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40 611 **Supplemental Table 11.** ANOVA tests for fixed effects for pepper fruit dry weight presented in41  
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## 837 **Figure Legends**

838 **Figure 1:** Midseason integrated diurnal [A] stomatal conductance rate ( $g'$ ) and [B]  
839 photosynthetic rate ( $A'$ ) measured in the disease-resistant and the disease-susceptible cultivars.  
840 The box plot represents the average of measured plants across three OTCs ( $n = 3$ ). Significance  
841 denoted is between treatments within a given cultivar (not across cultivars), where different  
842 letters represent significant differences (Tukey post-hoc test  $p < 0.05$ ). Results of main effect  
843 statistics per cultivar are placed in the upper corners (Two-way ANOVA,  $p < 0.05$ ). Brackets  
844 represent significance ( $p < 0.05$ ) of main effects across treatment groups from the two-way  
845 ANOVA and are provided for clarity. NS represents no significance.

847 **Figure 2:** Midseason midday stomatal conductance ( $g_s$ ) [A] and photosynthetic rate ( $A$ ) [B] in  
848 the upper canopy, and midday stomatal conductance ( $g_s$ ) [C] and photosynthetic rate ( $A$ ) [D] in  
849 the middle canopy. The box plot represents the average of measured plants across three OTCs ( $n$   
850  $= 3$ ). Significance denoted is between treatments within a given cultivar (not across cultivars),  
851 where different letters represent significant differences (Tukey post-hoc test  $p < 0.05$ ). Results of  
852 main effect statistics per cultivar are placed in the upper corners (Two-way ANOVA,  $p < 0.05$ ).  
853 Brackets represent significance ( $p < 0.05$ ) of main effects across treatment groups from the two-  
854 way ANOVA and are provided for clarity. NS represents no significance.

856 **Figure 3:** Maximum rate of carboxylation ( $V_{c,max}$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ ) [A] and electron transport  
857 ( $J_{max}$ ,  $\mu\text{mol electrons m}^{-2}\text{s}^{-1}$ ) [B] measured in the disease-resistant and the disease-susceptible  
858 cultivars in June (57 days post-inoculation). The box plot represents the average of measured  
859 plants across three OTCs ( $n = 3$ ). Significance denoted is between treatments within a given

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7 862 corners (Two-way ANOVA,  $p < 0.05$ ). Brackets represent significance ( $p < 0.05$ ) of main effects  
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17 866 **Figure 4:** Chlorophyll content (SPAD units) in the upper canopy [A] and middle canopy [B].  
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19 867 The box plot represents the average of measured plants across three OTCs ( $n = 3$ ). Significance  
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21 868 denoted is between treatments within a given cultivar (not across cultivars), where different  
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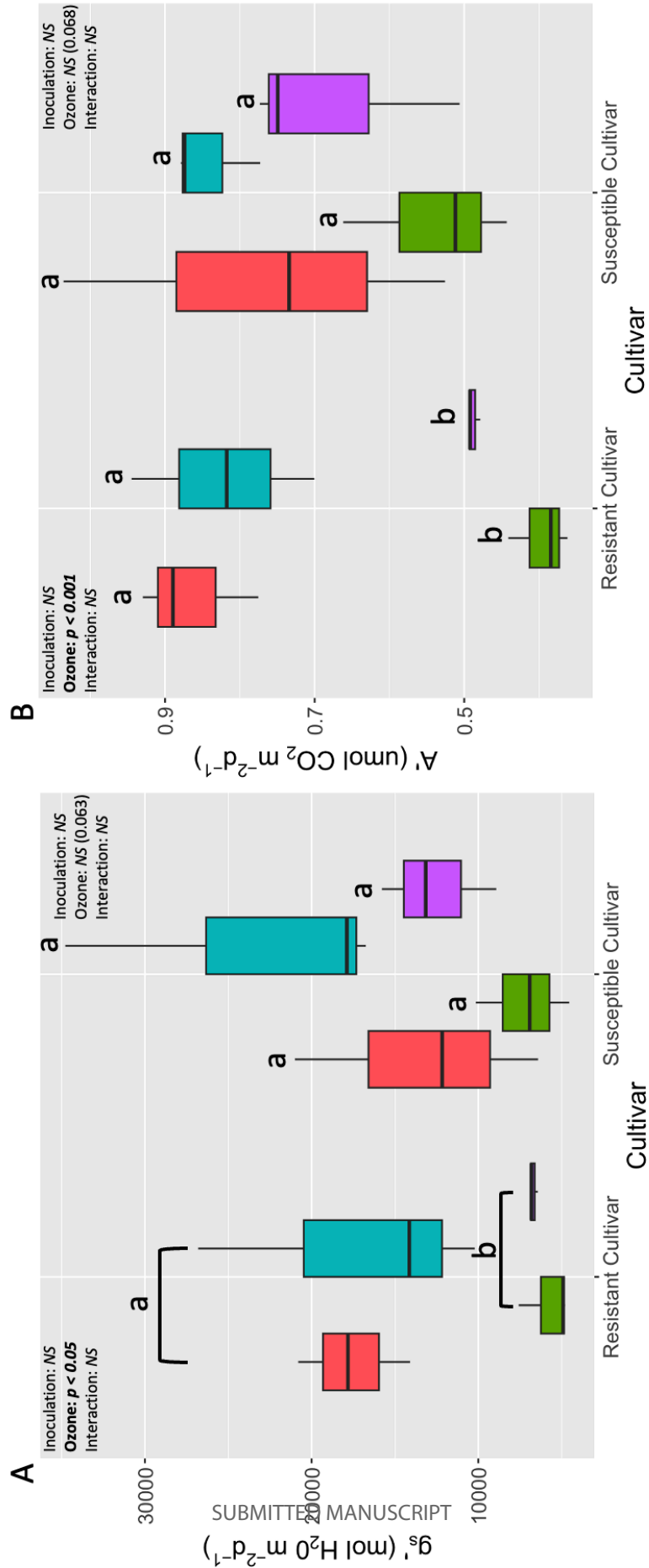
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33 873 **Figure 5:** Ball-Woodrow-Berry Parameterization for the disease-resistant and the disease-  
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35 874 susceptible cultivars at the midseason timepoint, plotting [A] the residual stomatal conductance  
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37 875 ( $g_0$ ) and [B] the slope constant ( $m$ ). The box plot represents the average of measured plants  
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39 876 across three OTCs ( $n = 3$ ). Significance denoted is between treatments within a given cultivar  
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45 879 way ANOVA,  $p < 0.05$ ). NS represents no significance.  
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51 881 **Figure 6:** Leaf oxygen radical antioxidant capacity (ORAC) measurements, representing total  
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53 882 antioxidant capacity ( $\text{nmol TE mg FW}^{-1}$ ) in the disease-resistant and the disease-susceptible  
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3 883 cultivars. The box plot represents the average of measured plants across three OTCs ( $n = 3$ ).  
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12 887 represents no significance.  
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17 889 **Figure 7:** [A] Aboveground biomass ( $\text{g Plant}^{-1}$ ) and [B] average fruit weight per pepper (g), for  
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19 890 the disease-resistant and the disease-susceptible cultivars. All values represent dry weight. The  
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21 891 box plot represents the average of measured plants across three OTCs ( $n = 3$ ). Significance  
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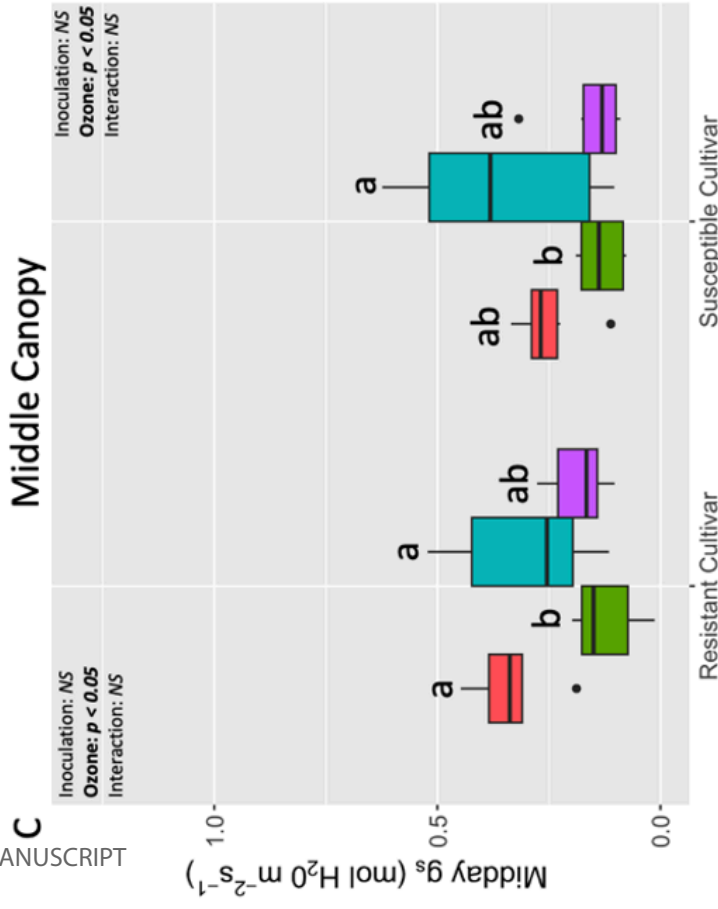
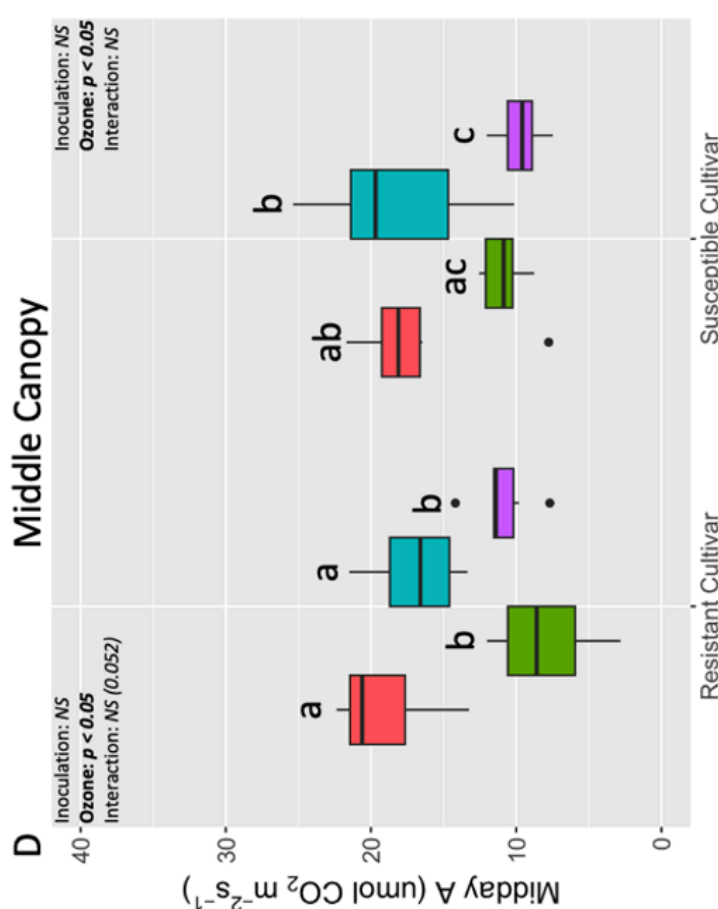
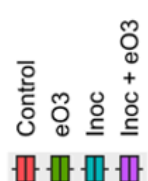
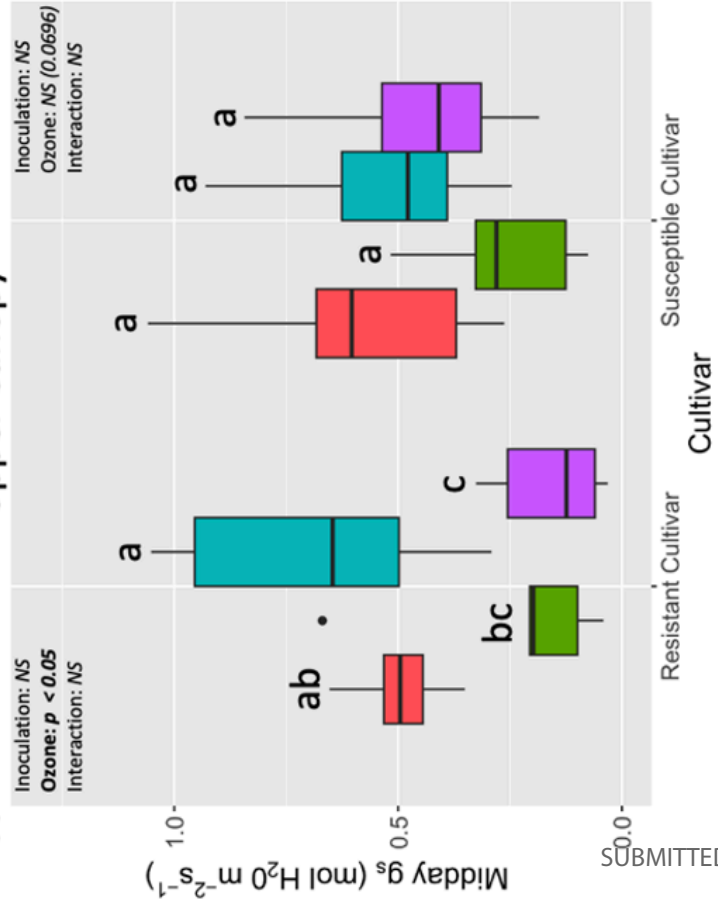
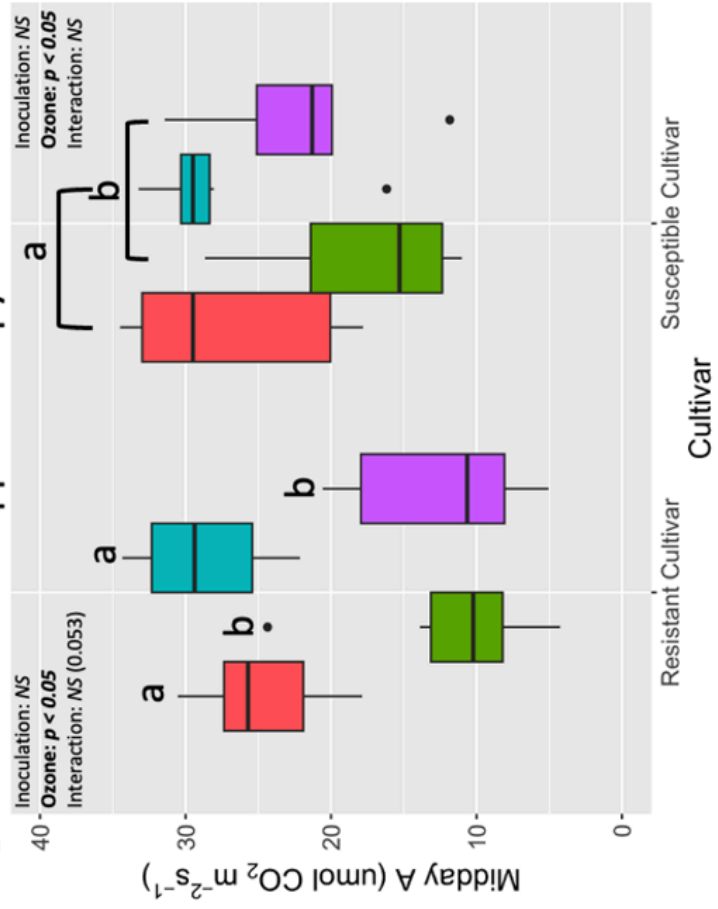
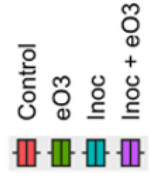
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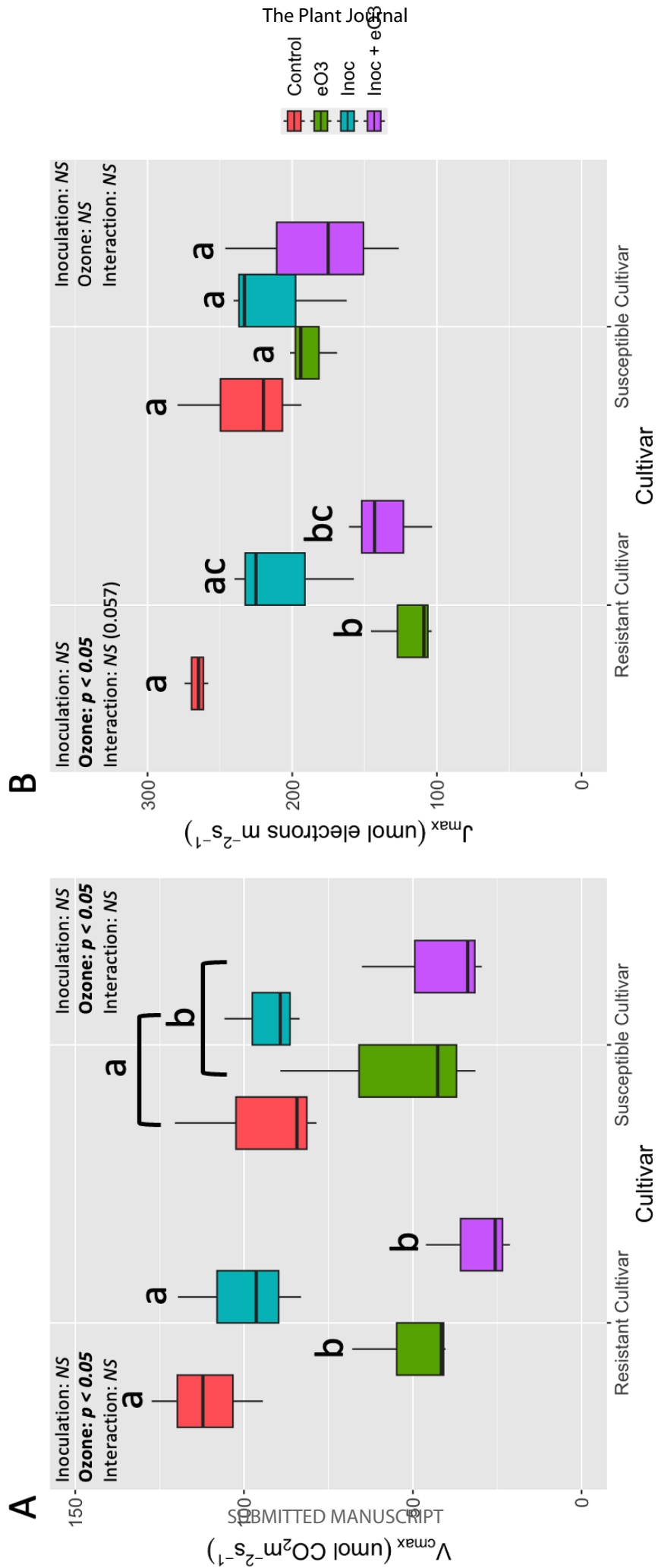


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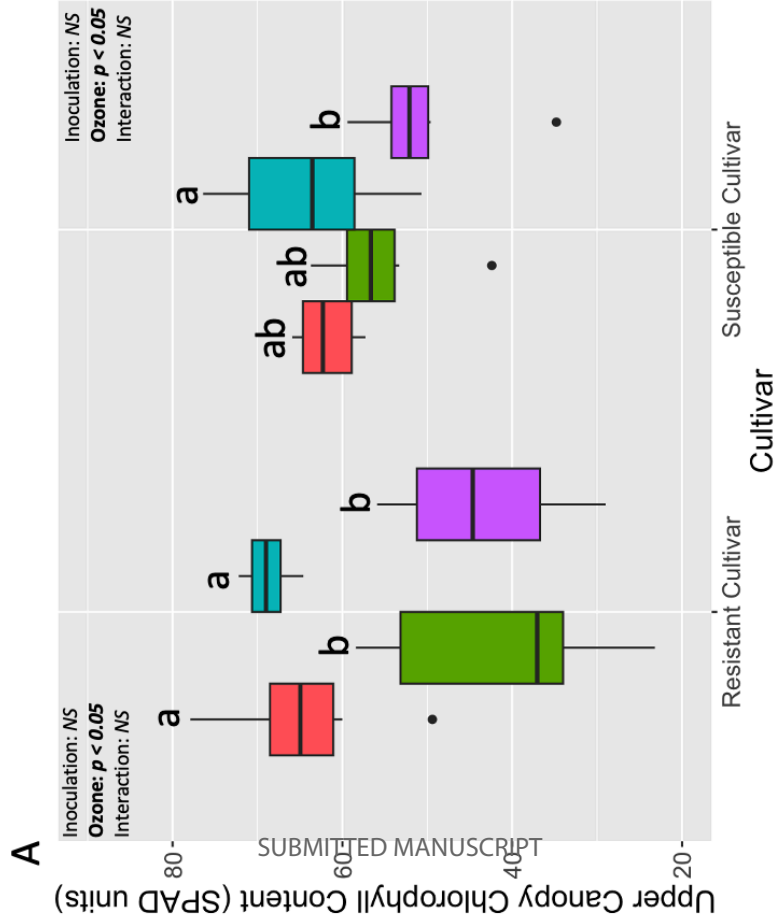
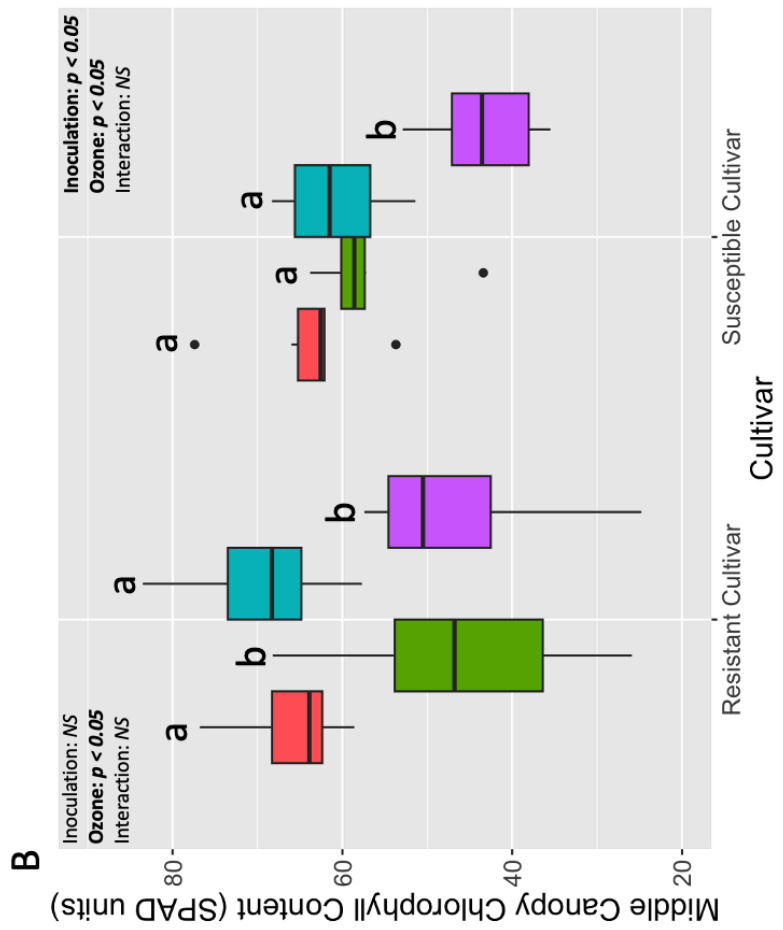
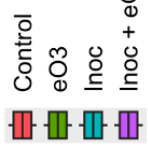
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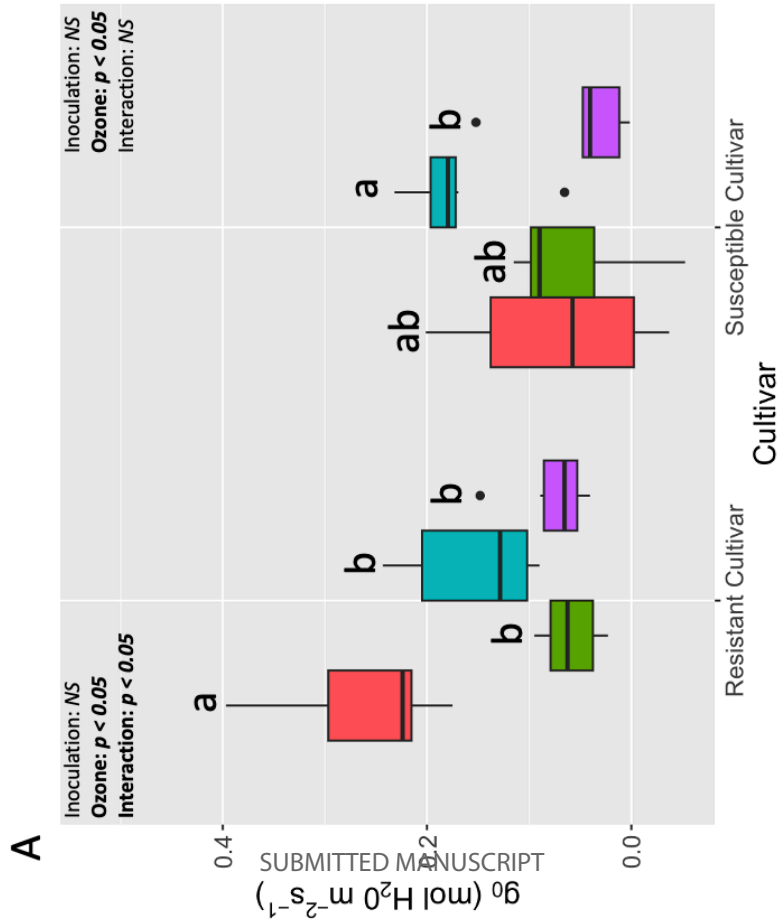
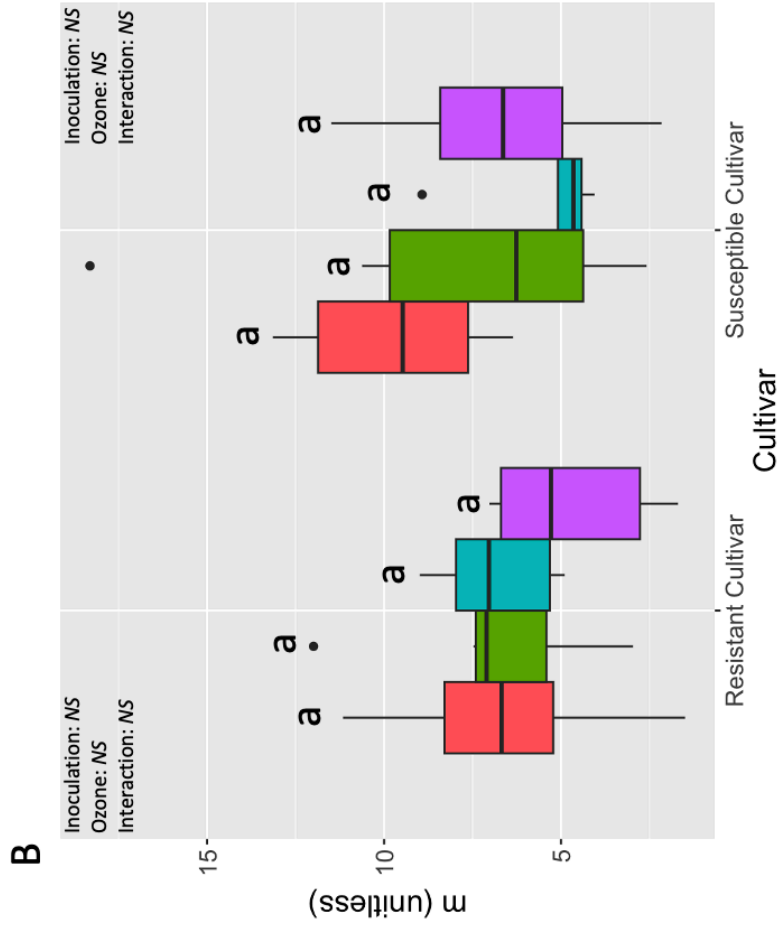


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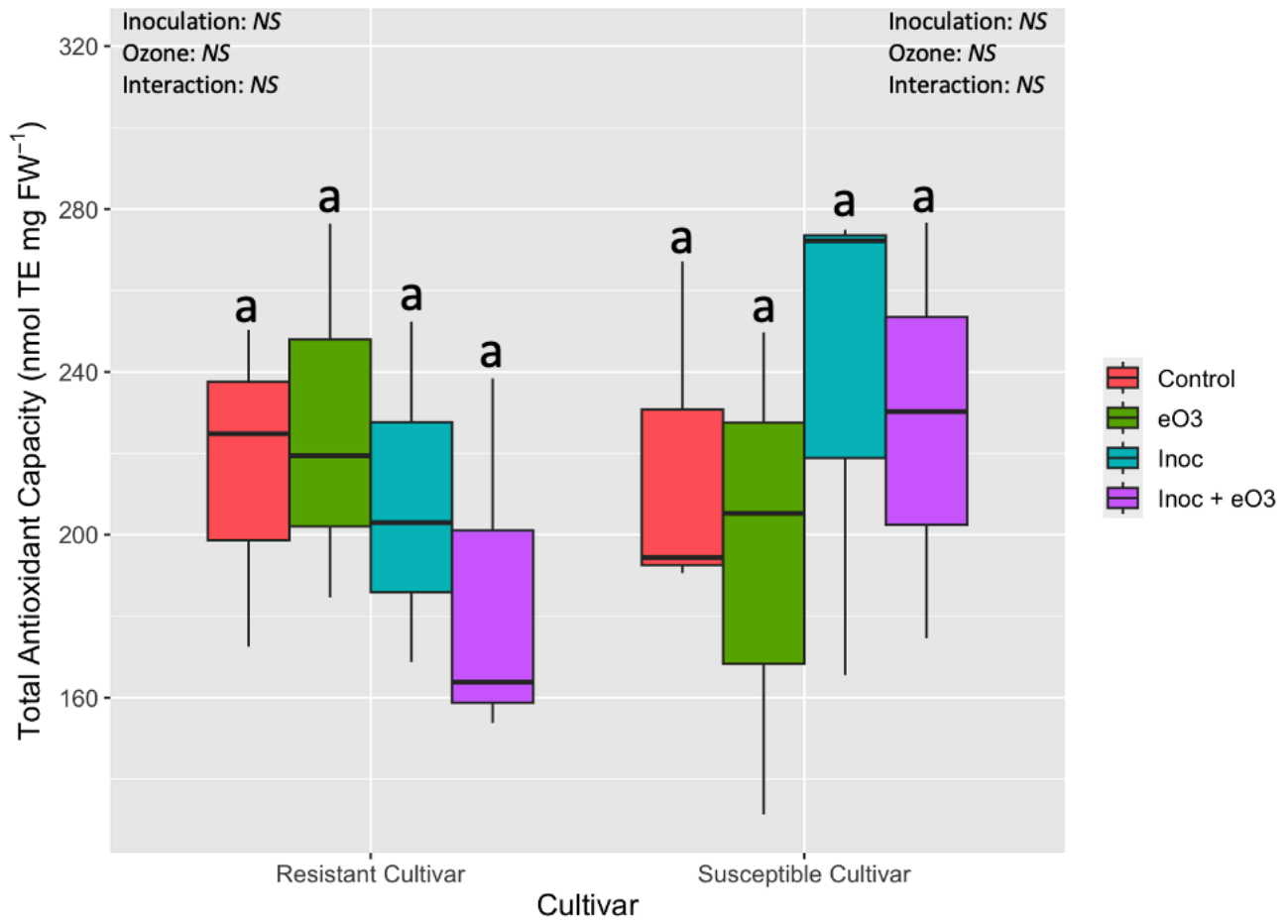
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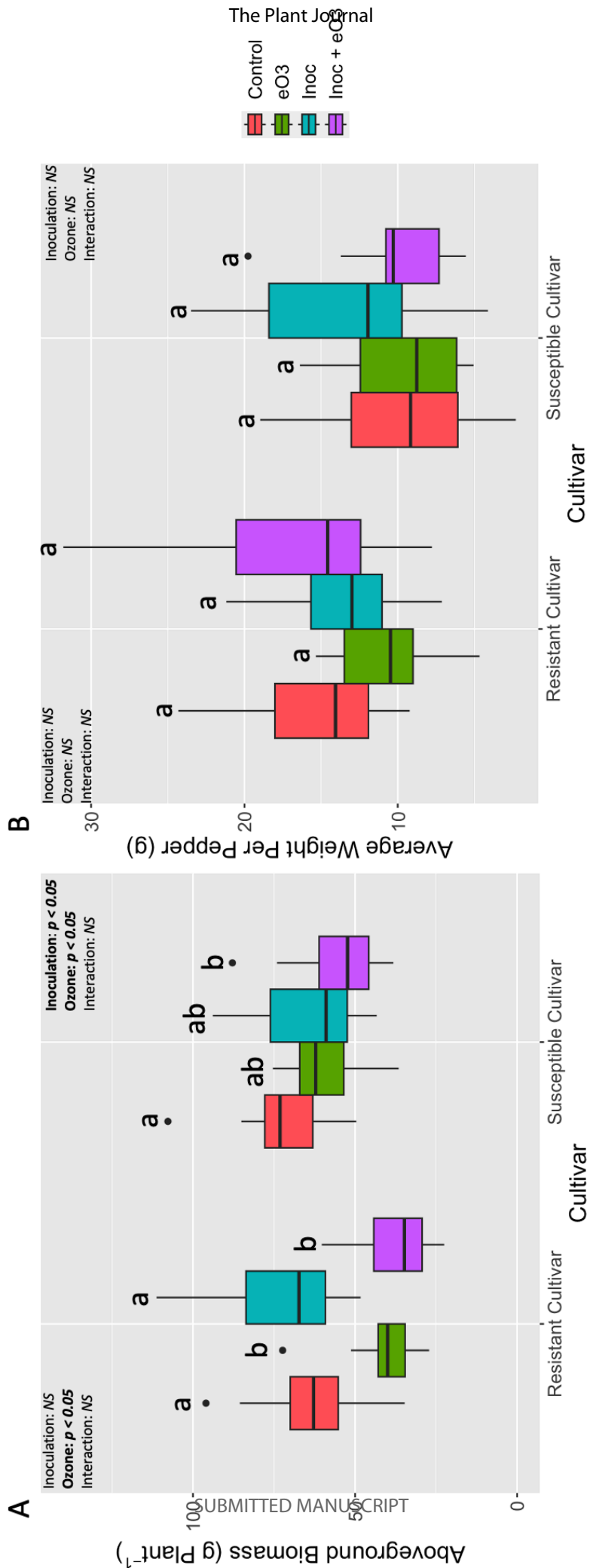
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