

Biotype-Specific and Stable Induced Responses of Sorghum to Greenbug (*Schizaphis graminum*) Herbivory

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Research Article

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Abstract

Induced plant defenses influence herbivore performance and population dynamics, yet their temporal stability and biotype-specificity remain poorly understood in cereal–aphid systems. We evaluated local and systemic induced responses of sorghum (*Sorghum bicolor*) genotype ‘Tx2783’ to two *Schizaphis graminum* biotypes (E and K). Using clip-cage bioassays, demographic measurements, qualitative plant responses, and digital image analysis, we found that sorghum expresses stable resistance to biotype E and susceptibility to biotype K. Prior infestation did not alter subsequent aphid performance, and no evidence of induced obviolation or cross-biotype reversal was observed. These results clarify the role of induced defenses in sorghum and highlight the importance of biotype identity in shaping aphid population dynamics.

Introduction

Plants respond dynamically to herbivory through induced biochemical, physiological, and transcriptional changes that can alter herbivore performance and influence plant fitness. These induced defenses often vary over time, with resistance sometimes shifting toward susceptibility as tissues age or as defense metabolites degrade (Karban and Baldwin 1997; Underwood 2012). Such temporal variation has been documented in several systems, including soybean, where herbivory can trigger rapid induced resistance followed by delayed susceptibility.

The greenbug, *Schizaphis graminum* (Rondani), is a phloem-feeding aphid capable of causing severe economic losses in wheat, barley, oats, and sorghum. Greenbugs reproduce parthenogenetically, producing live nymphs that mature within a week under optimal temperatures (Burton and Brooks 1993). Their feeding induces chlorosis, necrosis, and red spotting due to salivary enzymes and toxins that disrupt chloroplast function, interfere with nutrient transport, and trigger oxidative stress (Nicholson et al. 2012; Smith et al. 2004). These physiological disruptions, combined with rapid reproductive capacity, make induced plant defenses a critical component of host resistance.

Greenbug populations are further complicated by the existence of multiple biotypes, historically defined by their ability to overcome specific resistance genes in small grains and sorghum. Since the 1960s, at least eight major biotypes (A–H) have been documented (Harvey and Hackerott 1969; Puterka et al. 1988). Biotype E became predominant across the Great Plains due to its broad host range and high intrinsic rate of increase (Porter et al. 1997). Biotype K, identified more recently, is notable for its virulence on certain sorghum genotypes (Armstrong et al. 2019). The emergence of new biotypes is driven by selection pressure from resistant cultivars, clonal reproduction enabling rapid fixation of virulence traits, gene flow among regional populations, and potentially endosymbiont-mediated gene transfer (Russell et al. 2013).

Sorghum (*Sorghum bicolor*) is a drought-tolerant C4 grass widely grown for grain, forage, and bioenergy. Its defense against aphids involves both constitutive mechanisms—such as epicuticular waxes, silica deposition, and secondary metabolites like dhurrin (Dreyer and Campbell 1987)—and induced

mechanisms involving salicylic acid (SA) and jasmonic acid (JA) signaling, flavonoid biosynthesis, reactive oxygen species (ROS), and callose deposition (Kiani and Szczepaniec 2018; Staley et al. 2011). Resistant sorghum genotypes rapidly upregulate defense genes within 24–48 hours of aphid feeding, reducing aphid reproduction and limiting population growth. Greenbug feeding can also induce cross-resistance to other aphids, including sugarcane aphid (*Melanaphis sacchari*) (Puri et al. 2023).

Despite extensive research on sorghum resistance genes, the temporal dynamics and biotype-specific nature of induced responses remain poorly understood. Sorghum genotype 'Tx2783' exhibits contrasting responses to biotypes E and K, providing an ideal system for examining whether prior infestation alters subsequent aphid performance, induces resistance or susceptibility, or triggers cross-biotype interactions. This study was conducted to characterize the timing, stability, and biotype-specificity of induced responses in sorghum. By integrating demographic measurements, qualitative plant responses, and digital damage assessments, we aimed to determine whether induced defenses contribute meaningfully to sorghum resistance and whether induction plays a role in shaping greenbug population dynamics across biotypes.

Materials and Methods

Plant Materials

Aphids were reared on two different plant species. Susceptible wheat ('Newton'), used to rear the primary greenbug colonies, was received from Sue Cambron, USDA-ARS, West Lafayette, IN, USA. A sorghum variety with a differential response to the two different greenbug colonies ('Tx2783') was received from USDA-GRIN (Accession No. PI 656001) (USDA Agricultural Research Service, 2022). All plants were planted in Jolly Gardener Proline 22G potting mix (Oldcastle Lawn & Garden Inc., Atlanta, GA). Twenty-five seeds were planted in 4-inch diameter round pots at 1-inch depth and treated with a Captan seed treatment to improve plant health and minimize damping-off symptoms. Individual plants were transplanted to either 3-inch square pots or 4-inch round pots before infestation. Uninfested sorghum plants were maintained in a growth chamber free of aphids at a constant 28°C with a 14hr:10hr (light: dark) photoperiod. Plants were watered and fertilized to minimize water and nutrient stresses.

Greenbug Populations & Maintenance

Two *S. graminum* populations, Biotype E and K, were received from Gary Puterka, USDA-ARS, Stillwater, OK, USA. Starting populations were originally reared on 'Newton' wheat kept in aphid-resistant cages and maintained in separate growth chambers set to 24°C:20°C with a 14hr:10hr (light:dark) photoperiod. Both populations were transferred to 'Tx2783' sorghum and maintained for at least two generations before experimental use. Colonies reared on sorghum were maintained in separate aphid-resistant cages in a growth chamber set at a constant 28°C with a 14hr:10hr (light:dark) photoperiod.

Local Induced Response Experiments

Experiment 1

To provide preliminary information on the effect of mixed infestations, four treatments were used to test for local induced responses. Two treatments were infested with inducer nymphs from each of biotypes E or K and two days later plants were infested with test nymphs of the same biotype (pure infestation). Two treatments were infested with inducer nymphs from either biotype E or K and two days later plants were infested with test nymphs of the other biotype (mixed infestation). Each treatment had three replicates each for a total of 12 experimental plants.

When the plants reached the two-leaf stage, 11 days after emergence (DAE), plants were fitted with a circular 2.5cm diameter clip cage near the midpoint of the 2nd leaf. Each cage featured a 0.5cm divider down the middle of the cage dividing the cage into two sections of adjacent 1cm long leaf lengths. The top round of the cage was fitted with rings of double-sided tape to adhere the top round of the cage to the upper portion of the leaf and the bottom round of the clip cage. A round of aphid-resistant mesh was secured to an upper portion of the top of the cage with a spot of hot glue, a round of double-sided tape, and three clips securing the bottom of the cage to the top of the cage. Treatments were groups in the same rows within a single flat after infestation.

The inducer infestation occurred at 11 DAE with 1-day old, age synchronized nymphs. Three nymphs were transferred with a fine brush into the section closest to the pseudostem for each treatment. On 13 DAE, 2 days after inducer infestation (DAII), nymphs in the inducer section were checked for survival and replaced with age synchronized cohorts if dead or missing. After inducer observations, three, one-day old age synchronized nymphs were transferred to the test section of each cage. Inducer and test aphid counts, and plant observations were taken at 2, 5, 7, and 9 DAII. Plant observations included a visual damage rating (0-100%) and qualitative response (resistant or susceptible). Susceptible plant responses were characterized by the presence of dark red spotting and flecking with chlorosis developing around the feeding sites, while a resistant response remained green and healthy. After the final observations at 9 DAII, the leaf was excised from the pseudo stem at the leaf sheath below the ligule, the cage sections borders were marked in black marker, the cages were removed from the leaf, remaining aphids were gently brushed off the leaf, and the leaf imaged for digital analysis.

Plant images were taken with a Nikon D610 DSLR camera with a Nikon 24-85mm f/3.5–4.5 G AF-S lens. The camera was set to manual mode with an F/25 aperture setting, a 4-second shutter speed, a focal length of 85mm, a custom white balance set with a white card, and an ISO of 100. The leaves were placed on a blue posterboard under ambient fluorescent lighting with two identical green to yellow color gradients and a label. The leaves were oriented with the upper surface visible and held flat on the posterboard with cage clips. The camera was set above the posterboard facing downwards and the shutter was released with a remote trigger. After the image was taken, the plant material was disposed of and autoclaved. The blue posterboard was periodically swept to remove plant and aphid debris.

Methods from previous canopy image analysis was adapted to estimate image damage for each leaf section (Richardson et al. 2001, Patton & Reicher 2007). Digital image analysis was performed in the software ImageJ version 1.53e (Schneider et al. 2012). Images were cropped to include only the leaf section in the marked area and blue background. Two area measurements were taken for each leaf section. Color thresholding was used to select the total leaf area and the green leaf area. To estimate the total leaf area, the color threshold parameters were set at a hue between 0 and 100, saturation between 0 and 255, and brightness between 1 and 255. To estimate the green leaf area, the hue was readjusted to between 45 and 100. The image damage (0-100%) was then estimated for each plant by taking the total leaf area subtracting the green leaf area, dividing by the total leaf area, and multiplying by 100.

Experiment 2

The second experiment replicated the general methods and the treatments from the first experiment but increased treatment replicates from 3 to 10 and implemented a randomized complete block design. Each block contained two replicates from each treatment and plants were placed in random positions in a single flat starting at 6 DAI. Flats were rotated daily to minimize any chamber effects.

The same two-section cages were placed in the center of the 3rd or 4th leaf and inducer infestation occurred at the three-leaf stage, 12 DAE, and the test infestation occurred at 2 DAI. For infestation, a single adult was placed in the given section on the day of infestation. The next day the adult and excess nymphs were removed to achieve the desired number of nymphs. Two nymphs were left in the inducer section and one nymph was left in the test section. If the adult did not lay nymphs, nymphs of the same age were transferred from a colony of age synchronized nymphs. Sections were checked daily until 6 DAI and dead or missing nymphs were replaced with new nymphs of the same age.

Aphid observations and counts were taken daily throughout the experiment to estimate days to reproduction, daily nymph production, day of adult death, and aphid escapes. Observed visual damage (0-100%) and qualitative response were taken at 6, 8, 10, and 13 DAI. At final observations on 13DAI, leaves were processed for imaging as previously described. Images were also analyzed as previously described, but the lower value for the green leaf area hue was set to 44.

Systemic Induced Response Experiment

The systemic susceptibility experiment used two, single-section cages placed on separate leaves. The experiment had 6 treatments. The treatment had three inducer options with no inducer, biotype E, or K, and two test options with either biotype E or K. The experiment utilized a complete randomized block design. Each treatment had 10 replicates placed in 5 blocks. Each block was placed in a single flat and contained two treatment replicates per flat after 6 DAI. Flats were rotated daily to minimize chamber effects. Cages were of the same design of the local induced response experiments, but the 0.5cm divider was removed to create a single section with a 2.5cm leaf section. Infestation methods and plant stage and age were the same as the local induced response experiment, with the cage design and

placement being the only difference. Inducer cages were placed near the base of the 3rd leaf and test cages were placed near the center of the 4th leaf. Aphid observations and counts were taken daily throughout the experiment to estimate days to reproduction, daily nymph production, intrinsic rate of increase, day of adult death, and aphid escapes. Observed visual damage (0-100%) and qualitative response were taken at 6, 8, 10, 13, and 15 DAIL. On 16 DAIL, test leaves were processed for imaging as previously described. Images were processed as previously described, but the lower value for the green leaf area hue was set to 43.

Statistical Analysis

Observations for days to nymph production, daily nymph production for the first three full reproductive days, final aphid population, and image damage were analyzed for the second local induced response experiment and systemically induced response experiment. In addition, the intrinsic rate of increase was estimated for the systemically induced response experiment using the methods described by Wyatt & White (1977):

$$r_m = 0.738(\log_e M_d)/d.$$

For both experiments, observations for days to nymph production, daily nymph production, and final aphid population were excluded from analysis if the original test nymph died or cage escapes were suspected before observations. If the original test nymph died before reproduction, the image damage was excluded from the analysis. Data were analyzed with a two-way analysis of variance (ANOVA) using the PROC GLM procedure from SAS software (SAS 9.04, SAS institute, Cary, NC, 2020). Type III model statistics were reported for all ANOVA results. The treatment and block served as independent variables with the interaction term also included. Using the LSMEANS statement, the least significant differences ($\alpha = 0.05$) were used to separate response means. Response means and standard error by treatment were calculated using PROC MEANS procedure.

Discussion

This study provides new insight into the dynamics of induced resistance in sorghum–greenbug interactions and clarifies how biotype-specific responses shape aphid performance. Although induced plant defenses have been documented across numerous systems, their timing, stability, and specificity remain incompletely understood, particularly in cereal crops. Our findings demonstrate that sorghum genotype ‘Tx2783’ expresses consistent resistance to greenbug biotype E and susceptibility to biotype K, and that these responses remain stable regardless of prior infestation. This stability contrasts with systems such as soybean, where induced resistance can decay into susceptibility over time (Karban and Baldwin 1997; Underwood 2012), and suggests that sorghum’s induced defenses may be more durable or less prone to temporal shifts.

The absence of induced obviation or cross-biotype reversal in sorghum is notable. In some plant–herbivore systems, prior herbivory can either enhance or suppress subsequent resistance, depending on

the herbivore species, feeding guild, and timing of attack (Stout et al. 2006; Pieterse and Dicke 2007). In contrast, sorghum's response to greenbug appears genetically fixed and biotype-specific, with no evidence that early infestation alters later aphid performance. This aligns with transcriptomic studies showing rapid and robust activation of defense pathways in resistant sorghum genotypes following aphid feeding (Kiani and Szczepaniec 2018).

The demographic differences observed between biotypes E and K further reinforce the biological significance of biotype-specific resistance. Biotype E exhibited lower reproduction, slower development, and reduced intrinsic rate of increase compared to biotype K, consistent with earlier work showing that biotype E performs poorly on resistant cultivars (Harvey and Hackerott 1969; Porter et al. 1997). Biotype K's superior performance on 'Tx2783' suggests that it possesses virulence traits enabling it to overcome the plant's resistance mechanisms. The lack of a measurable fitness cost associated with virulence (Puterka et al. 1988) may facilitate the persistence and spread of virulent biotypes such as K in sorghum-growing regions.

Although greenbug feeding has been shown to induce cross-resistance to other aphids (Puri et al. 2023), our results indicate that such induction does not extend across greenbug biotypes. This suggests that sorghum's induced defenses may be highly specific to particular herbivore species or biotypes, reflecting fine-scale evolutionary interactions between sorghum and its aphid pests. The specificity observed here underscores the importance of understanding biotype diversity when developing durable resistance strategies.

Concluding Synthesis

Taken together, these results demonstrate that sorghum's responses to greenbug herbivory are shaped by stable, biotype-specific interactions rather than by transient induction effects or cross-biotype signaling. By showing that prior infestation does not alter subsequent aphid performance, this work clarifies a previously unresolved aspect of sorghum–aphid ecology and provides evidence that induced defenses in sorghum may operate differently from those documented in other systems where resistance decays or susceptibility emerges over time. These insights will be valuable for future efforts to develop sorghum cultivars with broad and durable resistance to aphid pests.

Declarations

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Author Contribution

C.W. collected data and prepared figures and tables. B.S. conceived project, edited figures and tables, and wrote manuscript. All author reviewed the manuscript.

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Tables

Table 1

Mean ($\pm$ SE) days to nymph production, daily nymph production, final aphid population, and image damage from the second local induced response experiment test aphid section

Inducer biotype	Test biotype	Days to nymph production (d)	Daily nymph production†	Final aphid population	Image damage (0-100%)
E	E 1	6.6 $\pm$ 0.2 a	1.6 $\pm$ 0.1 b (26)	8.3 $\pm$ 0.7 b (8)	0.1 $\pm$ 0.03 b
K	E 4	6.7 $\pm$ 0.3 a	1.8 $\pm$ 0.2 b (28)	7.9 $\pm$ 0.5 b (9)	1.0 $\pm$ 0.78 b
K	K 2	5.4 $\pm$ 0.2 b (9) ‡	3.1 $\pm$ 0.2 a (27)	16.6 $\pm$ 1.0 a (8)	57.8 $\pm$ 5.4 a (9)
E	K 3	5.8 $\pm$ 0.3 ab	3.0 $\pm$ 0.2 a	17.3 $\pm$ 1.8 a (9)	46.2 $\pm$ 5.5 a
Column means with the same lowercase letters are not significantly different; $P > 0.05$, LSD (Tukey or Tukey-Kramer Adjustment) † Daily nymph production from the first three full days of nymph production; $n = 30$ ‡ Parenthesis indicate means with excluded data with values equal to number of replicates (n)					

Table 2

Mean ($\pm$ SE) days to nymph production, daily nymph production, intrinsic rate of increase, final aphid population, and image damage from the systemic induced response experiment test aphid cage

Inducer biotype	Test biotype	Days to nymph production (d)	Daily nymph production†	Intrinsic rate of increase	Final aphid population	Image damage (0-100%)
None	E	5.8 $\pm$ 0.2 a	2.8 $\pm$ 0.2 b (29) ‡	0.36 $\pm$ 0.02 b (7)	27.1 $\pm$ 4.5 b (7)	0.1 $\pm$ 0.04 b
E	E	5.9 $\pm$ 0.1 a	2.9 $\pm$ 0.2 b (29)	0.36 $\pm$ 0.01 b (9)	26.1 $\pm$ 3.7 b (9)	0.5 $\pm$ 0.41 b
K	E	5.8 $\pm$ 0.1 a	2.8 $\pm$ 0.2 b (29)	0.36 $\pm$ 0.02 b (9)	25.9 $\pm$ 4.4 b (9)	0.6 $\pm$ 0.19 b
None	K	5.1 $\pm$ 0.1 b	4.1 $\pm$ 0.2 a	0.48 $\pm$ 0.02 a	98.9 $\pm$ 9.7 a	35.4 $\pm$ 4.96 a
K	K	5.0 $\pm$ 0.1 b	4.4 $\pm$ 0.2 a	0.49 $\pm$ 0.01 a	109.0 $\pm$ 8.9 a	37.9 $\pm$ 5.16 a
E	K	5.1 $\pm$ 0.1 b	4.8 $\pm$ 0.2 a	0.51 $\pm$ 0.01 a	115.5 $\pm$ 7.4 a	38.8 $\pm$ 4.67 a
Column means with the same lowercase letters are not significantly different; $P > 0.05$, LSD (Tukey or Tukey-Kramer Adjustment) † Daily nymph production from the first three full days of nymph production; $n = 30$ ‡ Parenthesis indicate means with excluded data with values equal to number of replicates (n)						

Figures

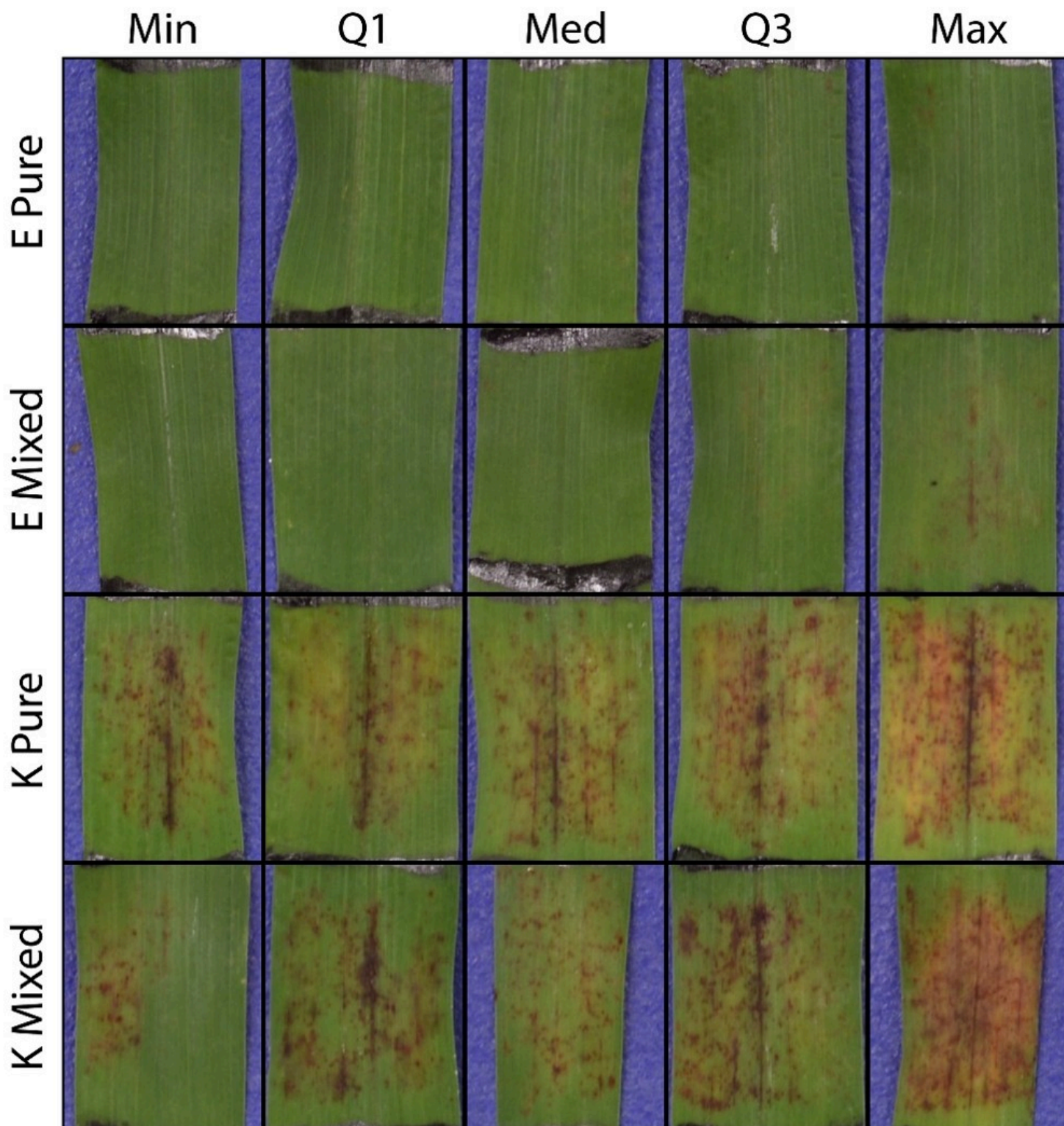


Figure 1

Cropped images of test sections from the first local induced response experiment. Images are arranged by image damage for each treatment.

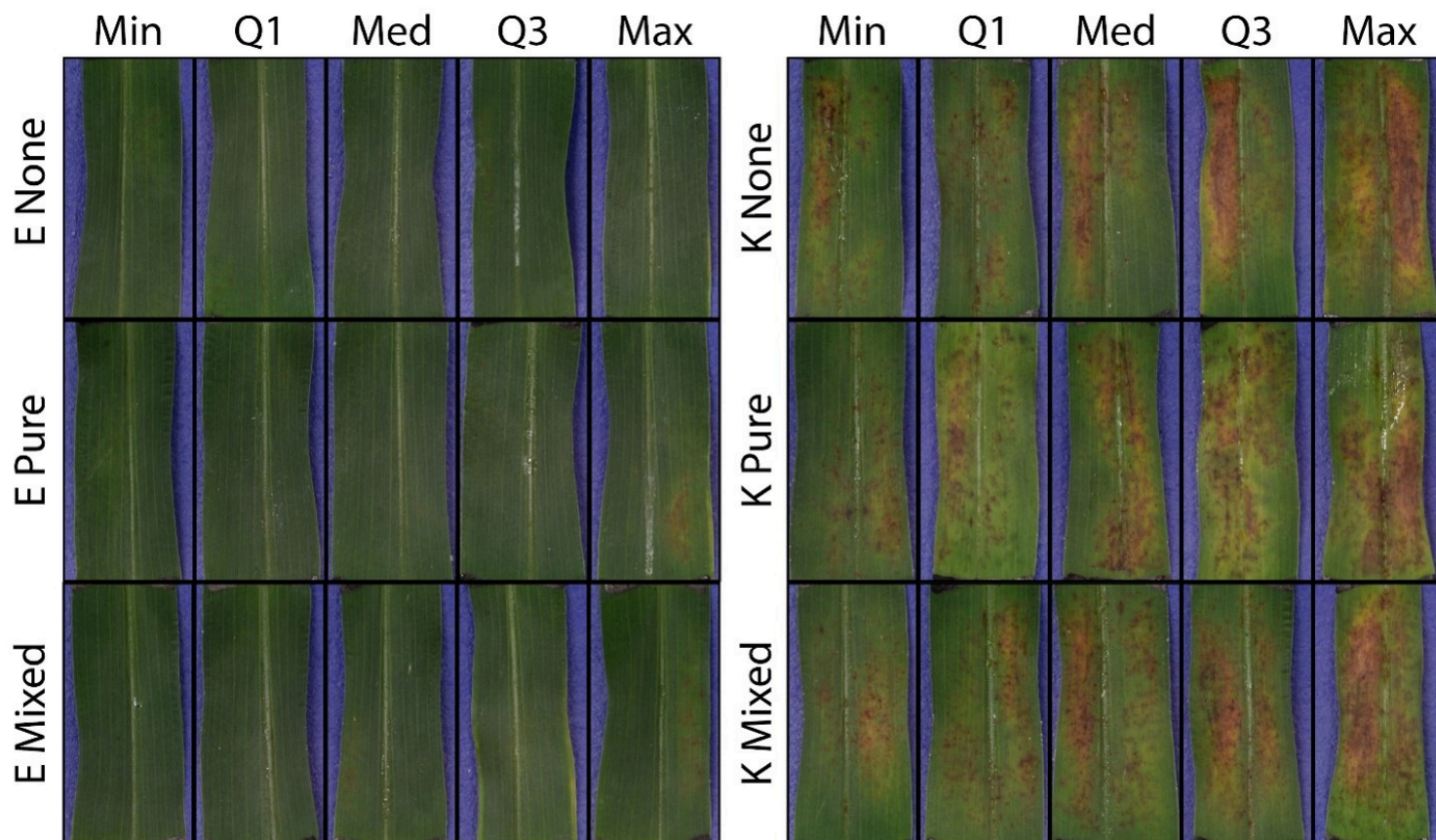


Figure 2

Cropped images of test sections from the second local induced response experiment. Images are selected and arranged by the min, Q1, median, Q3, and max image damage for each treatment.

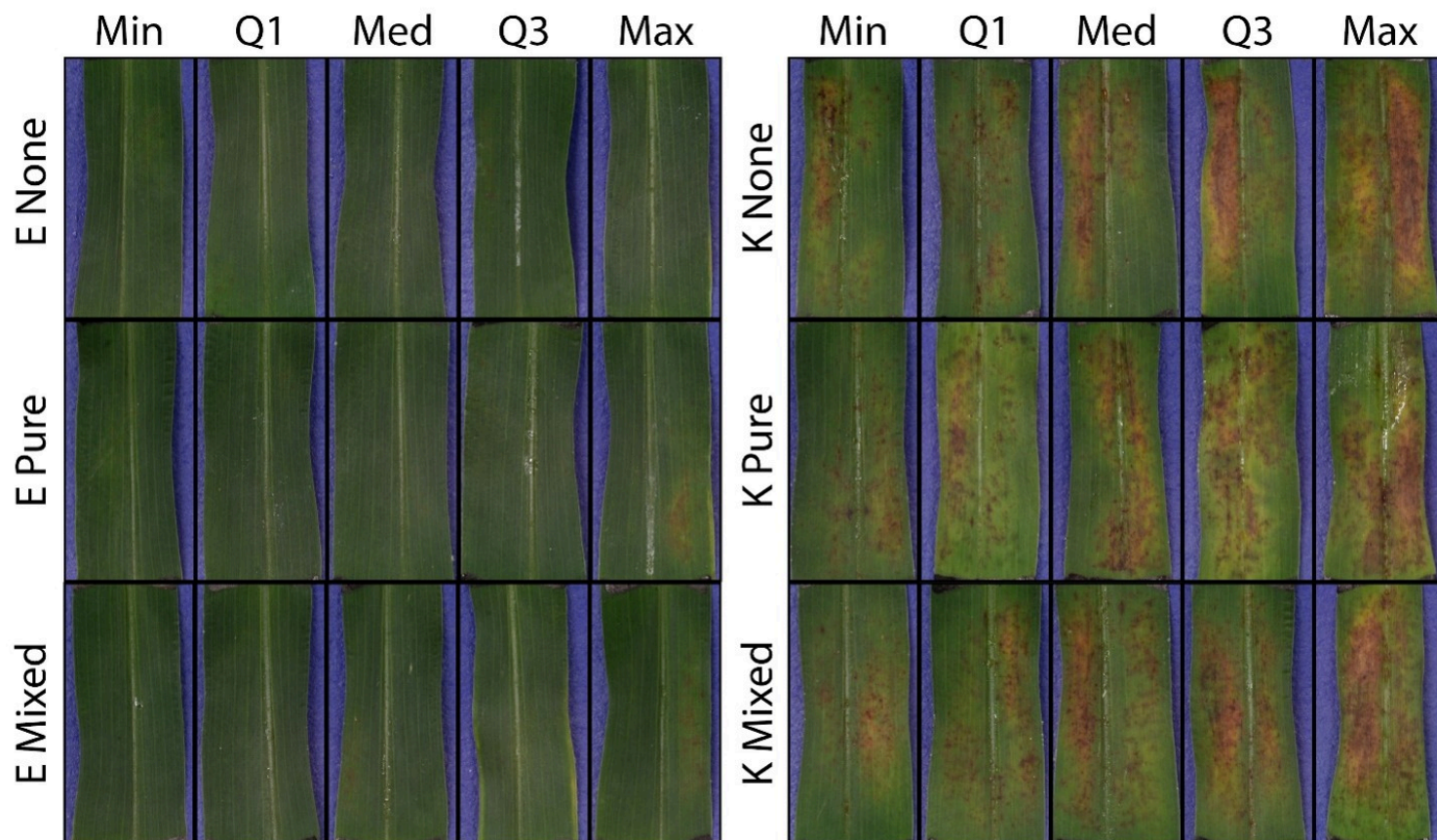


Figure 3

Cropped images of test sections from the systemic induced response experiment. Images are selected and arranged by the min, Q1, median, Q3, and max image damage for each treatment.