

The stem galler *Eurosta solidaginis* induces both localized and systemic physiological changes in the leaves of *Solidago canadensis*

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Abstract

Eurosta solidaginis oviposits in the apical bud of *Solidago canadensis*, leading to the development of a stem gall. Whether the presence of a stem gall affects leaf physiology and whether such effects are beneficial or detrimental to *Solidago* are unknown. We examined the physiological effects of *Eurosta* infection on *Solidago* leaves at two locations on the stem: close to the gall and far from the gall (i.e., near the ground). Chlorophyll levels were not impacted by *Eurosta* infection, but stomatal density was higher in leaves from infected plants close to the gall, suggesting elevated CO₂ uptake capacity in infected plants. Starch concentration was lower in infected plants, but only in leaves far from the gall. Starch accumulates within the galls, which may act as such powerful carbon sinks that leaves near the ground cannot maintain their own starch reserves. Catalase activity was higher in infected plants but only close to the gall. Notwithstanding, cells within leaves close to the gall were not more resistant to membrane damage from exogenous H₂O₂. We propose that *Eurosta* induced higher catalase activity, but only within the chloroplasts, to protect itself from photosynthetic reactive oxygen species (ROS) from nearby leaves. Levels of salicylic acid (SA), which is involved in plant defense, were elevated in infected plants but only close to the gall, suggesting a localized defense response only. Overall, *Eurosta* maximizes its energy supply and minimizes its oxidative damage by modulating *Solidago* leaf physiology, thereby increasing its own fitness at the expense of its host.

Introduction

A considerable number of herbivorous insects (and mites) are known to induce the formation of galls—enlarged regions of plant tissue—in flowering plants (Desnitskiy et al. 2023), yet, according to Harris and Pitzchke (2020), “galls are an understudied phenomenon in plant developmental biology”. Several studies have been conducted to understand how insects induce gall formation. Broadly, galls are induced either by feeding or oviposition (Desnitskiy et al. 2023), with chemical inducers, such as auxins, cytokinins, and indole-3-acetic acid (IAA), being introduced to the plant via saliva or venom glands, respectively (Mapes and Davies 2001; Giron et al. 2016). Despite the morphological diversity of insect-induced galls, the adaptive significance of which remains unresolved (Stone and Schonrogge 2003), Takeda et al. (2019) showed that a common set of genes is upregulated in galls induced by a variety of insects, especially those genes regulating developmental processes. Several genes involved in gall formation are also involved in flower and fruit formation, suggesting that abhorrent induction of reproductive genes may also play some role in gall formation (Takeda et al. 2021). Gatjens-Boniche (2019) recently proposed that insects may additionally inject bacteria into their plant hosts, with these bacteria incorporating new genetic material into the plant genome, leading to altered development and gall formation.

Many researchers have examined how gall tissue compares to the surrounding non-gall tissue within infected plants. For example, Hartley (1998) compared gall and non-gall tissue in 11 different plant species and found lower nitrogen levels and higher phenolics levels in the former. Motta et al. (2005) showed that galls in *Tibouchina pulchra* had significantly higher contents of soluble phenols, tannins, lignin, fibers, soluble carbohydrates, and lipids, and significantly lower contents of flavonoids and organic nitrogen, compared to non-gall tissue. Additionally, Huang et al. (2014) showed that gall tissue from three unrelated plant species contained lower concentrations of both chlorophyll and carotenoids compared to ungalled tissue. More recently, Pandey and Kate (2024) showed that gall tissue from *Alstonia scholaris* contained high levels of aluminum, phosphorus, and carbon, as well as higher levels of plant secondary compounds, some of which have not been identified, compared to ungalled tissue. Therefore, it is abundantly clear that gall tissue has a different chemical composition from the surrounding ungalled tissue within the same plant, which likely contributes to the various proposed adaptive benefits of the galls in terms of insect survival (Price et al. 1987).

Galls might be expected to exert physiological changes in other regions of the plant as well. Indeed, a considerable amount of research suggests that galls act as a sink for many resources, thereby reducing resource availability in other plant tissues. For example, Larson and Whitman (1991) showed that galls acted as carbon sinks, competing against other carbon sinks, such as flowers. Galls act as such powerful resource sinks that even when the surrounding vasculature is disrupted, galls are still able to draw resources towards themselves (Giertych et al. 2023). This likely explains why seed output is often significantly reduced in galled plants (e.g., Sarratt et al. 2023). Little research has been conducted on the effect of galls on plant tissues beyond source-sink relationships. One study of the plant *Silphium integrifolium* revealed that its photosynthetic and water transport capacity were higher in galled plants, but only under drought conditions (Fay et al. 1993).

Given the paucity of research on the physiological effects of galls on other regions of the infected plant, in the present study, we examined chlorophyll content, starch content, stomatal density, catalase activity, ROS-induced cell membrane damage, and salicylic acid (SA) levels in naturally-growing populations of *Solidago canadensis* (hereafter *Solidago*) that displayed stem galls induced by *Eurosta solidaginis* (hereafter *Eurosta*). In particular, we addressed two important questions regarding the physiological impacts of stem galls on leaves: i) are any physiological changes observed in the leaves of galled plants restricted to those leaves adjacent to the gall location, or do they occur throughout the entire plant; and ii) do any physiological changes observed in the leaves of galled plants persist throughout the entire lifespan of the plant, or do they occur only in the period immediately following gall formation. We examined these characteristics in leaves from plants exhibiting galls and immediately adjacent plants without galls, thereby minimizing any effects of environmental variation on our findings. For each plant, we examined leaves at two positions along the stem—close to the gall and near the bottom of the plant, which is far from the gall

site—which allowed us to address our first research question. Moreover, we made measurements throughout the late summer and autumn, which allowed us to address our second research question. Understanding whether the physiological impacts of galls on other plant regions are widespread—both spatially and temporally—will help us assess the effectiveness of gall inducers as biological control agents (Harris and Shorthouse 2012).

Eurosta is a small, herbivorous insect that is widespread throughout much of North America and has been studied extensively for its freeze tolerance (Lee et al. 1995). Its entire life cycle occurs in association with *Solidago*, with specific host preferences having evolved in some regions (Abrahamson et al. 1989). After males and females mate, females deposit their eggs into the apical bud of goldenrod (Abrahamson and Weis 1997). The eggs develop into larvae that induce the formation of a gall in which they develop, feed, and overwinter, eventually undergoing pupation and emerging as adults in early spring.

Solidago is a perennial species that reproduces both sexually (via seeds) and clonally (via rhizomes and ramets; Huang et al. 2007). It is characterized by a large cluster of small, yellow flowers at the top of a stem that can be as high as 200cm. It is native to North America where it is considered a pest species in perennial gardens and crops (Werner et al. 1980), and it has become invasive in Europe and Asia (Tian et al. 2023). The key to the invasive nature of *Solidago* is its high reproductive allocation (Cheng et al. 2021) and the long-distance wind dispersal of its seeds (Zhang et al. 2022). Ren et al. (2022) have shown that climate warming is likely to accelerate the invasiveness of *Solidago*, which makes understanding its relationship with its galling insects even more important.

Several studies have investigated how *Eurosta* gall formation affects the morphology, ecology, and evolution of *Solidago*. Hartnett and Abrahamson (1979) showed that gall formation in *Solidago* increased stem growth, decreased rhizome production, and lowered seed output. Stinner and Abrahamson (1979) found that 7% of ramet energy production was utilized for the formation of the gall and the growth of *Eurosta*, which might account for decreased investment in reproductive output. McCrea and Abrahamson (1985) found that galled ramets become isolated from their clones in order to minimize the impact of resource drain induced by gall formation. More recently, Meyer et al. (2013) showed that gall presence did not impact pollinator visits. There have also been several studies regarding how *Eurosta* infection affects the physiology and biochemistry of *Solidago* plants. For example, Indigo et al. (2025) recently showed that natural dyes extracted from *Solidago* leaves and inflorescences had different colours in infected plants compared to uninfected controls. Additionally, Helms et al. (2013) showed that *Solidago* plants exposed to olfactory cues from *Eurosta* were less vulnerable to herbivory than unexposed controls, suggesting that *Solidago* must synthesize some kind of herbivore deterrent in the presence of *Eurosta*.

Our study has found that *Eurosta* gall formation, even though it occurs on the stem, has profound physiological effects on the leaves of *Solidago* plants, mostly those leaves in close proximity to the gall location. Broadly, our work provides a deeper understanding of how insects can alter the physiology of their hosts, even beyond the specific site of their interaction, a concept which remains sparsely understood. Specifically, our work provides new insight regarding how a plant species that is dominant in many North American habitats—sometimes to the detriment of other plant species (Eckberg et al. 2023)—is impacted by insect interactions, which could serve as the basis for the development of future control strategies.

Methods

Leaf sampling

The University of Toronto Scarborough is located in the easternmost part of Toronto, Ontario, Canada. *Solidago* is abundant in many campus and near-campus locations, including the Highland Creek Valley and Morningside Park, which are adjacent to the campus. For three consecutive years (2022, 2023, and 2024), from September to November, we harvested leaves from *Solidago* plants exhibiting galls and from uninfected plants in their immediate vicinity. Two leaves were harvested from each plant: one adjacent to the gall (“close” to the infection) and one at a location just above the ground (“far” from the infection). The leaves were placed in a plastic bag and transported to our laboratory for immediate analysis of several parameters. The leaf harvesting date was recorded in most instances in order to allow for analysis of seasonal changes in the parameters measured.

Chlorophyll content

Chlorophyll content of the leaves was assessed using the procedure described in Nisbett et al. (2024). Briefly, leaves were homogenized in 1.5mL of 100% acetone with a mortar and pestle at room temperature. The homogenate was pipetted into an Eppendorf tube and centrifuged at 10,000 RPM for 10 minutes at room temperature. The supernatant was removed and transferred to a new Eppendorf tube. To measure chlorophyll concentration, 200 μ L of supernatant was diluted into 3 mL of 100% acetone inside a glass cuvette. Samples were then placed inside of a spectrophotometer (Vintmax Visible Spectrophotometer 721) and their absorbance was measured at 662nm and 644nm, which allowed for determination of chlorophyll *a* and *b* concentrations using previously published equations (Holm, 1954). Absorbance values were

measured after zeroing the spectrophotometer using 100% acetone. Chlorophyll concentrations were standardized based on wet weight of the leaves.

Starch content

Starch content of *Solidago* leaves was assessed using a procedure modified from Berkholst and Gonzales (1989). A 2cm² section of leaf tissue was submerged in boiling water for 3 minutes within a test tube. After, the water was replaced with isopropyl alcohol. The test tubes were then placed within a pot kept inside an insulated cooler bag. 1.5L of boiling water was poured into the pot, which was then covered with aluminum foil, and the cooler bag was sealed. After 25 minutes, 1L of boiling water was added to the pot to ensure the temperature did not get too low. The temperature of the water within the pot ranged between 70–85°C. The tubes were removed from the pot after another 25 minutes. The isopropyl alcohol was poured out, and the leaf tissue were placed in a Petri dish. Two drops of Lugol's solution (J. Crow's) were applied to the leaf tissue. After 3 minutes, the Petri dishes were placed on a white background and photographed using an iPhone 11 camera from a distance of 25cm directly above. All samples were analyzed using the RGB analysis in ImageJ. The starch content in the leaves was quantified using a starch colorimetric index (SCI) formula used by Dinu et al. (2024), which compared the RGB values from the sample and the RGB values from a 100% potato starch solution to which Lugol's solution had been added. The SCI formula used was:

$$SCI = \frac{(R_{leaf} + G_{leaf} + B_{leaf})}{3} - \frac{(R_{starch} + G_{starch} + B_{starch})}{3}$$

Stomatal density

Images of leaf samples were captured at 400x magnification using an OMAX A35 camera mounted to an OMAX M83E compound microscope. The images were analyzed in ImageJ. The scale was set using a micrometer as reference (44.0454 pixels = 0.01mm). A 0.05mm² (1026.25 pixels x 1026.25 pixels) box was created, and any stomata that were fully or more than half in the box were counted. The box location was randomly generated by ImageJ to prevent selection bias. We then standardized the stomatal density per mm².

Catalase activity

Catalase activity of leaf tissue was determined using a procedure based on Iwase et al. (2013) and utilized previously in other plant species (e.g., flax; Santacroce et al. 2024). Leaf samples were weighed and homogenized in water at room temperature. The homogenate was centrifuged at 7000 rpm for 10 min at room temperature. Subsequently, 100 µL of supernatant was added to 100 µL of 1% (v/v) Triton X-100 (Biopharm) and 100 µL 3% (w/v) H₂O₂ within a 1.5 mL centrifuge tube. This sample tube was inverted gently to mix the reagents and allowed to sit at room temperature until apparent bubbling (indicative of catalase activity) had stopped. The height of the bubble layer formed was measured using digital calipers. The height of the bubble layer of a control tube processed in parallel was subtracted from the height of the bubble layer of the sample tube. The control tube contained 100 µL of 1% (v/v) Triton X-100, 100 µL of 3% (w/v) H₂O₂, and 100 µL of water (in place of supernatant). The height of the bubble layer was expressed on a weight-specific basis (i.e., mm/g fresh weight).

H₂O₂-specific electrolyte leakage

When the cell membrane is damaged, the internal contents of the cell—including any electrolytes—are released into the extracellular environment. Quantification of the rate at which these electrolytes accumulate in the extracellular environment can be used to assess the rate at which a given treatment causes damage to the cell membrane (Hatsugai and Katagiri 2018) and has been used previously to assess leaf cell membrane susceptibility to exogenous H₂O₂ (Santacroce et al. 2024). Leaves were divided into two samples. One sample was submerged in a small jar containing 20 mL of distilled water (i.e., control medium), and the other sample was submerged in a small jar containing 20 mL of 30 mM H₂O₂ (i.e., experimental medium). The conductivity of the medium was immediately measured using a conductivity meter (Extech EC400). Subsequently, all jars were left at room temperature for 24 h, after which the conductivity of the medium was measured again. Then, all jars were submerged into a water bath continuously filled with boiling water for 60 min in order to completely damage all cell membranes within the leaf. After the jars had cooled down to room temperature, the conductivity of the medium was measured again. This conductivity represented the maximum electrolyte leakage. The percentage of electrolyte leakage that occurred during the 24-h exposure to exogenous H₂O₂, which was taken to represent to the susceptibility of cell membranes to H₂O₂-induced damage, was calculated as follows:

$$\% H_2O_2 - specific\ electrolyte\ leakage = \frac{(cond. H_2O_2@24h - cond. H_2O_2@0h)}{cond. H_2O_2@boiling} - \frac{(cond. water@24h - cond. water@0h)}{cond. water@boiling}$$

Salicylic Acid Concentration

Estimation of SA levels in leaf samples was based on a procedure modified from Warriar et al. (2013), which has been used previously to measure endogenous SA levels in plants (e.g., flax; Santacroce et al. 2024). Leaf samples were homogenized at room temperature in 1.5 mL of 100% acetone. The homogenate was pipetted into an Eppendorf tube and centrifuged at 7000 rpm for 10 min at room temperature. A 0.1% FeCl_3 solution was prepared by diluting 135 μL of 38% FeCl_3 stock solution (MG Chemicals) with 50 mL tap water. 300 μL of supernatant and 3 mL of the 0.1% FeCl_3 solution were mixed by inversion in a glass cuvette. Absorbance was measured at 540 nm using a 721-Vis Spectrophotometer. As a blank, 300 μL supernatant and 3 mL of tap water were combined and mixed in another glass cuvette. The absorbance value of this blank was subtracted from the absorbance value of the sample with FeCl_3 . Net absorbance values were converted to SA concentrations by constructing a standard curve using known SA concentrations ranging from 0 to 12 mM. SA concentrations were expressed on a fresh weight-specific basis (i.e., $\mu\text{mol/g}$ fresh weight).

Statistical Analysis

We used a linear model in R (version 4.3.0) to analyze the effect of infection status and leaf location, as well as their interaction, with sampling date as a covariate.

Results

Total leaf chlorophyll content did not differ between infected and uninfected plants, nor did it differ between leaves close to the infection site and leaves far from the infection site (Fig. 1a). Total chlorophyll content also did not change significantly over the course of the autumn season in any leaves (Fig. 1b).

Leaf starch content was evaluated by comparing the colour obtained when Lugol's solution was added to a decolorized leaf and the colour obtained when Lugol's solution was added to a pure starch solution. The resulting starch colour index (SCI) gives a value that correlates inversely with the leaf starch content. SCI was found to be significantly higher only for leaves from infected plants that were far from the gall, suggesting that starch levels are lowest in these leaves (Fig. 2a). As expected, SCI increased significantly over the course of the autumn (Fig. 2b), likely reflecting how the gradually decreasing daylength in autumn increasingly limited photosynthetic starch production. Notwithstanding the seasonal changes observed in leaf starch levels, they were consistently lower throughout the entire autumn in leaves far from the gall site in infected plants.

Stomata were examined on the abaxial side of all leaves only. Stomatal density was found to be significantly higher in leaves from infected plants but only at a location close to the infection site (Fig. 3a). In most plants, stomatal density decreased as the autumn season progressed, except for leaves near the base of the stem in uninfected plants, which showed a significant increase in stomatal density (Fig. 3b). Consequently, the higher stomatal density of leaves near the gall site of infected plants was only present until late October.

Catalase activity was significantly higher in the leaves from *Eurosta*-infected plants, but only for those leaves close to the infection site (Fig. 4a). Catalase activity in the leaves of all plants showed a significant decline over the duration of autumn, with the rate of decline being the same in all plants (Fig. 4b). Thus, the elevated catalase activity in leaves from infected plants close to the infection site was present at the start of autumn and persisted throughout the season.

Electrolyte leakage is a common metric for assessing the degree of membrane damage (and, by extension, cell death) caused by exposure to a particular condition. In the present study, *Solidago* leaves were exposed to either distilled water or H_2O_2 in order to determine to what degree *Solidago* leaves were susceptible to H_2O_2 damage specifically. H_2O_2 -specific electrolyte leakage was found to be the same for all leaves regardless of the infection status of their plant or their location on the stem (Fig. 5a). We did observe that H_2O_2 -specific electrolyte leakage increased over time throughout the autumn season, to the same degree in all leaf types (Fig. 5b).

SA concentration was significantly higher in the leaves from *Eurosta*-infected plants, but only for those leaves close to the infection site (Fig. 6a). SA concentration in the leaves of all plants showed a significant decline over the duration of autumn, with the rate of decline being the same in all leaf types (Fig. 6b). Thus, the elevated SA concentration in leaves from infected plants close to the infection site was present at the start of autumn and persisted throughout the season.

Discussion

Eurosta induces increased stomatal density in Solidago leaves in order to increase starch availability for itself at the expense of Solidago fitness

The overwintering survival of *Eurosta* larvae, as well as their reproductive output in the subsequent spring, depend on the size and rate of utilization of their pre-winter energy reserves (Irwin and Lee 2003). *Eurosta* are known to undergo diapause from early autumn until early spring,

during which they suppress their metabolic rate up to 80% (Irwin et al. 2001), thereby minimizing their rate of utilization of their energy reserves. Notwithstanding, it would still be beneficial for *Eurosta* to maximize the size of its energy reserves. The primary fuel reserve supporting metabolism during winter is glycogen (Storey and Storey 1986), with glycogen reserves accruing within *Eurosta* during late summer and early autumn, likely derived from the consumption of starch within the gall tissue. On this basis, it would be adaptive for *Eurosta* to induce physiological changes in *Solidago* leaves that maximize their photosynthesis rate, as this would permit *Eurosta* to accumulate a larger glycogen reserve. Two factors that affect the photosynthesis rate of leaves—and that could, at least theoretically, be modified by *Eurosta* larvae via chemical signaling—are chlorophyll concentration (Croft et al. 2017) and stomatal CO₂ uptake (Tanaka et al. 2013).

Given that *Eurosta*-induced galls on *Solidago* stems have a greenish appearance (JCLB, personal observations; Connor et al. 2012), they likely contain chlorophyll and should, therefore, be capable of photosynthesis; however, in other plant-insect systems (e.g., *Acacia longifolia* and *Trichilogaster acaciaelongifoliae*), it has been found that the survival of larvae within galls does not depend on photosynthesis by the gall tissue itself (Haiden et al. 2012), likely because gall tissue typically has lower chlorophyll levels than surrounding tissue (Huang et al. 2014; Huang et al. 2015). This finding suggests that leaf photosynthesis (and, thus, leaf chlorophyll content) is likely a key determinant of larval survival for gall-forming insects. On this basis, it might be expected that gall-forming insects would induce an increase in leaf chlorophyll concentrations in order to increase their survival likelihood. This would be possible for *Eurosta* given that females oviposit into the apical bud and, therefore, leaf development in the region adjacent to the gall occurs only after oviposition has taken place (Craig et al. 1993). Once eggs hatch, they develop into larvae and begin feeding on stem tissue, releasing phytohormones (esp. auxins, cytokinins, and indole-3-acetic acid [IAA]) in the process (Mapes and Davies 2001; Ponce 2021) that could alter adjacent leaf development. In the present study, however, we observed no difference in leaf chlorophyll concentration between infected and uninfected *Solidago* plants.

Given that chlorophyll concentration was preserved in infected *Solidago* leaves, if *Eurosta* could induce an increase in stomatal density, it might increase starch production by infected *Solidago* plants through increasing leaf CO₂ uptake and, thus, photosynthesis rate. Indeed, in the present study, we found that stomatal density was significantly higher in leaves from infected *Solidago* plants, but only for leaves close to the gall. This would suggest that whatever hormones and/or signaling molecules are synthesized and released by *Eurosta* only have a localized impact on leaf development. This likely reflects that the stomatal density of already mature leaves is fixed and only newly developing leaves can respond to insect cues (Shimada et al. 2011). While direct measurements of leaf photosynthesis rate in infected and uninfected *Solidago* leaves will be needed to confirm that increased stomatal density leads to higher photosynthetic starch production, we believe that it is a reasonable proposition given our findings. The higher stomatal density in *Eurosta*-infected plants might also increase their water transpiration rate, though this was not measured in the present study. Williams et al. (2004) showed that, concomitant with their increasing cold tolerance during autumn, *Eurosta* also greatly reduce their water loss rate; therefore, they would not likely be negatively impacted by any increase in transpiration rate caused by their induction of increased stomatal density.

While the starch content of leaves near the gall in infected plants was similar to that of leaves at the same position in uninfected plants, the starch content of leaves near the base of infected plants was lower than that of leaves near the base of uninfected plants. Given that photosynthetic starch production is likely elevated in infected plants, as rationalized above, we propose two possible explanations for this observation, both of which are based on the notion that the inner region of the gall contains an abundance of starch (Murakami et al. 2021). First, sucrose produced within the leaves above gall is being taken up into the gall tissue in order to support the energetic needs of the developing insect larva, leaving less sucrose to be transported to the lower leaves, where it is converted into starch. Consistent with this idea, McCrea et al. (1985) showed that radiolabelled carbon (¹⁴C) added to *S. altissima* plants as CO₂ through leaves above the gall location was less likely to be transported to the roots compared to ¹⁴C added to plants through leaves below the gall location, with the degree of ¹⁴C transport reduction being correlated with gall size. The main drawback of this explanation is that most studies suggest that mature leaves, even older ones, are a source—rather than a sink—for photosynthetically-fixed carbon (Lemoine et al. 2013). If this notion is true for *Solidago* leaves, then our second explanation may be more reasonable. Galls have been described as “phloem parasites” (Larson and Whitham 1997) because they act as such strong sinks for photosynthetically-fixed carbon. It is possible that the lower leaves of *Solidago* are exporting more sucrose than they can produce due to shading from upper leaves, thereby leading to net starch depletion over time. It should be noted the reduced starch levels in the lower leaves of infected *Solidago* plants could be observed as early as late summer, and in the absence of any reduction in chlorophyll concentration, which seems to preclude the notion that infected *Solidago* plants commence the senescence of their lower leaves—and remobilization of their resources—prematurely.

Regardless of the underlying reason why the lower leaves of infected *Solidago* plants have lower starch levels, this reduction in starch content might have significant ecological implications. As leaves undergo senescence prior to winter, their starch is remobilized to the roots (Wojciechowska et al. 2020), serving as an energy source to power overwintering metabolism in the roots but also as a source of energy for shoot growth in the spring. Wyka (1999) showed that, in *Oxytropis sericea*, plants that were intentionally shaded to minimize their carbohydrate content showed less leaf growth in the following spring and were less likely to flower. This suggests that *Eurosta* infection may reduce the *Solidago* fitness by siphoning starch away from its leaves, leaving less starch available to be transported into the roots for overwintering storage. This might explain why *Eurosta* does not typically infect all *Solidago* plants within a given population, with infection rates generally not

exceeding 50% (Cronin and Abrahamson 2011): higher infection rates may lead to shrinking plant (host) populations, which would limit host availability for *Eurosta* and eventually compromise its own fitness.

Eurosta induces increased catalase activity in Solidago leaves, which does not protect Solidago leaves from oxidative damage but may protect Eurosta from photosynthetic ROS production

Catalase activity was significantly higher in leaves from infected *Solidago* plants, but only near the site of infection, suggesting a localized rather than systemic response. Despite elevated catalase activity in leaves near the infection site, these leaves were not more resistant to the damaging effects of exogenous H_2O_2 , at least as assessed via the electrolyte leakage assay. One possible interpretation of these findings is that *Eurosta*, upon establishing its infection, releases signaling molecules that cause upregulation of catalase activity for its own protection against oxidative stress. The photosynthetic electron transport system produces a large amount of ROS during light exposure (Ivanov and Khorobrykh 2003), which could migrate towards the gall (via “ROS waves; Zandalinas et al. 2020) and subject the developing larva to elevated oxidative stress. By inducing increased catalase expression in *Solidago* leaves, *Eurosta* would minimize its degree of oxidative damage by stimulating greater ROS degradation within the leaves. Given the likely energetic costs associated with the maintenance of catalase (and other antioxidant) activity (Pamplona and Costantini 2011), inducing *Solidago* to synthesize catalase rather than synthesizing catalase itself allows *Eurosta* to increase survivability without compromising energetic investment in its growth. The notion that *Eurosta* induces its host plant to synthesize compounds for its own protection—and not the protection of the plant—is not unprecedented. Indeed, a previous study in *Solidago altissima* found that phenolic compounds were elevated in gall tissue compared to adjacent stem tissue, likely having been induced by *Eurosta* in order to minimize its chances of pathogen infection (Abrahamson et al. 1991).

The catalase activity of *Solidago* leaves declined over the course of the autumn season. As autumn progresses, the leaves become less valuable to the plant as light intensity and daylength decrease; therefore, it seems justifiable that the plant would invest less energy in protecting its leaves from oxidative stress. Notwithstanding, not all studies of seasonal variation in catalase activity in plants has observed this gradual decline. For example, Badiani et al. (1996) showed that catalase activity gradually increased in wheat leaves over time since their emergence except in the last 10–20 days prior to senescence. Consistent with the observed decrease in catalase activity over the autumn, we also observed that H_2O_2 -specific electrolyte leakage increased over the same period. This inverse relationship between catalase activity and H_2O_2 -specific electrolyte leakage suggests that, as leaf catalase activity declines over the season, the leaves become more susceptible to oxidative damage.

Of course, given this seasonal relationship between catalase and H_2O_2 -specific electrolyte leakage, we were surprised that the increase in catalase activity observed in *Solidago* leaves close to the site of infection did not also make these leaves less susceptible to exogenous oxidative stress. Our proposed explanation for this lack of correlation is that the increased catalase expression induced by *Eurosta* infection may only occur within the chloroplasts. Such a targeted increase in catalase expression would neutralize ROS within their major site of production, thereby preventing them from diffusing to the larvae within the gall but would also preserve the structural integrity of the photosynthetic apparatus, thereby ensuring that the leaves can produce enough carbohydrate to meet the energetic demands of the developing *Eurosta* larva. When we exposed the leaves to exogenous H_2O_2 , it reached all regions of the plant cells—not only the chloroplasts—allowing it to cause damage to the cell membrane, which is not better protected even in leaves close to the infection site. Indeed, our finding is reminiscent of animal studies showing that maximum lifespan can be increased by targeted catalase expression within the mitochondria (Schriner et al. 2005) but not by the broad distribution of increased antioxidant activity (Page et al. 2010).

Elevated levels of SA in leaves close to the infection site suggest that Solidago plants activate a localized defense response only or that Eurosta induces localized SA production to protect itself from pathogens

SA is a primarily a pathogen defense hormone in plants (Ding and Ding 2020), though other roles have been demonstrated (Rivas-San Vicente and Plasencia 2011). In other plant species that are infected by gall-forming insects, SA levels have been shown to correlate with resistance to gall induction. For example, among four species of pine (*Pinus spp.*), those that were resistant to gall formation contained a significant amount of SA, whereas those that were susceptible to gall formation did not (Son et al. 1999). Similarly, willow (*Salix viminalis*) plants that were resistant to gall induction by the gall midge (*Dasineura marginemtorquens*) had significantly higher SA levels than conspecifics that were susceptible to gall induction (Ollerstam and Larson 2003). In the present study, we did not measure SA concentrations until after infections had taken place, so we cannot assess whether interindividual variation in SA concentration may determine which plants within a population are likely to become infected by *Eurosta*. However, our data suggest that *Eurosta* infection likely triggers a localized defense response in *Solidago*, as SA levels were elevated in the leaves of infected plants that were close to the site of infection.

We were surprised that SA levels were not also found to be elevated in the distal leaves of infected plants. The vast majority of studies on plant pathogen infection indicate that SA plays a role in systemic acquired resistance (SAR). For example, Gaffney et al. (1993) showed that tobacco plants infected with tobacco mosaic virus (TMV) showed elevated SA levels in uninfected leaves that protected them against subsequent TMV infection. Of course, *Eurosta* is a unique invader because, following its spring emergence, adult females only live for 5 days (Uhler 1951);

therefore, there is only a short period during each season in which *Solidago* plants can become infected with *Eurosta*. This likely explains why, although the occurrence of multiple galls on a single *Solidago* stem has been reported (Cane and Kurczewski 1976), it is, at least in our experience, very rare. On this basis, the development of SAR may be futile in *Solidago* in response to *Eurosta* infection. Moreover, Wise and Abrahamson (2017) showed that, under certain environmental conditions, the cost of resistance to *Eurosta* infection exceeds its benefit, in terms of reproductive output, so, once infected, mounting a systemic defense response against further infection may actually cause increased harm to the plant. Alternatively, the localized increase in SA in the leaves near the gall location may have been induced by *Eurosta* rather than initiated by *Solidago*. Wilson (1995) showed that wasp-induced galls in oak leaves exhibited significant mortality from fungal pathogens, likely because these pathogens depleted the nutrients within the gall tissue, thereby depriving the wasp of energy. Though, to our knowledge, no studies have demonstrated fungal infection of *Eurosta* galls in *Solidago*, a localized SA induction by *Eurosta* could minimize the chances of fungal (or other pathogens) invasion of its gall.

In leaves from all *Solidago* plants, we observed a gradual decline in SA levels from early September to late November. This finding makes sense in light of the role of SA in pathogen defense, as plants should invest less energy in defending leaves from pathogens as their leaves get closer to senescence. Indeed, this idea is consistent with Griebel and Zeier (2008) who showed that increases in SA levels in response to a pathogen attack was more pronounced during periods of high light, yet we examined SA levels during autumn, when solar intensity and day length are decreasing. Our findings contrast with Zhang et al. (2024), who found that SA levels were significantly higher in leaves during later growing stages compared to earlier growing stages, although, in their study, measurements were made from May to September. Linked to its role in pathogen defence (and abiotic stress resistance, more broadly), SA is also known to promote flowering in many species, perhaps to transition stressed plants to reproductive growth before stress depletes their energy reserves (Martinez et al. 2004). Thus, it is possible that the declining SA levels observed in *Solidago* leaves in the present study reflects that our sampling occurred after flowering had already taken place.

Conclusions

As Chen et al. (2020) noted, “it has been a long-standing question as to whether the interaction between gall-forming insects and their host plants is merely parasitic or whether it may also benefit the host.” Our work suggests that *Eurosta* are parasitic on *Solidago*, inducing physiological changes—predominantly in the leaves adjacent to the gall—that benefit the insect at the expense of the plant. By understanding the physiological effects that *Eurosta* infection imposes upon *Solidago* leaves, we can better explain the ecological effects that *Eurosta* infection imposes upon *Solidago*. Future research should seek to elucidate how the occurrence and magnitude of these physiological changes may be influenced by environmental parameters. Indeed, it is well known that *Eurosta* infection of *Solidago* is patchy, with some populations experiencing high infection rates and other populations experiencing no infections. Most of the current hypotheses to explain this distribution pattern are ecology-based. For example, Confer and Orloff (1990) showed that *Eurosta* galls are less frequently observed near forest edges, which they proposed was due to increased bird predation risk in these regions. However, photosynthesis rates are typically lower in plants at the forest edge (Svriz et al. 2014), so *Eurosta* may avoid ovipositing in forest edge plants as such plants may not be able to supply its larvae with sufficient starch to survive the winter. Thus, we encourage more research on the physiological nature of the relationship between gall insects and their plant hosts.

Declarations

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Author Contributions

JCLB conceived the study and wrote the manuscript. MJC, MA, and SHK collected leaf samples and conducted the assays.

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Figures

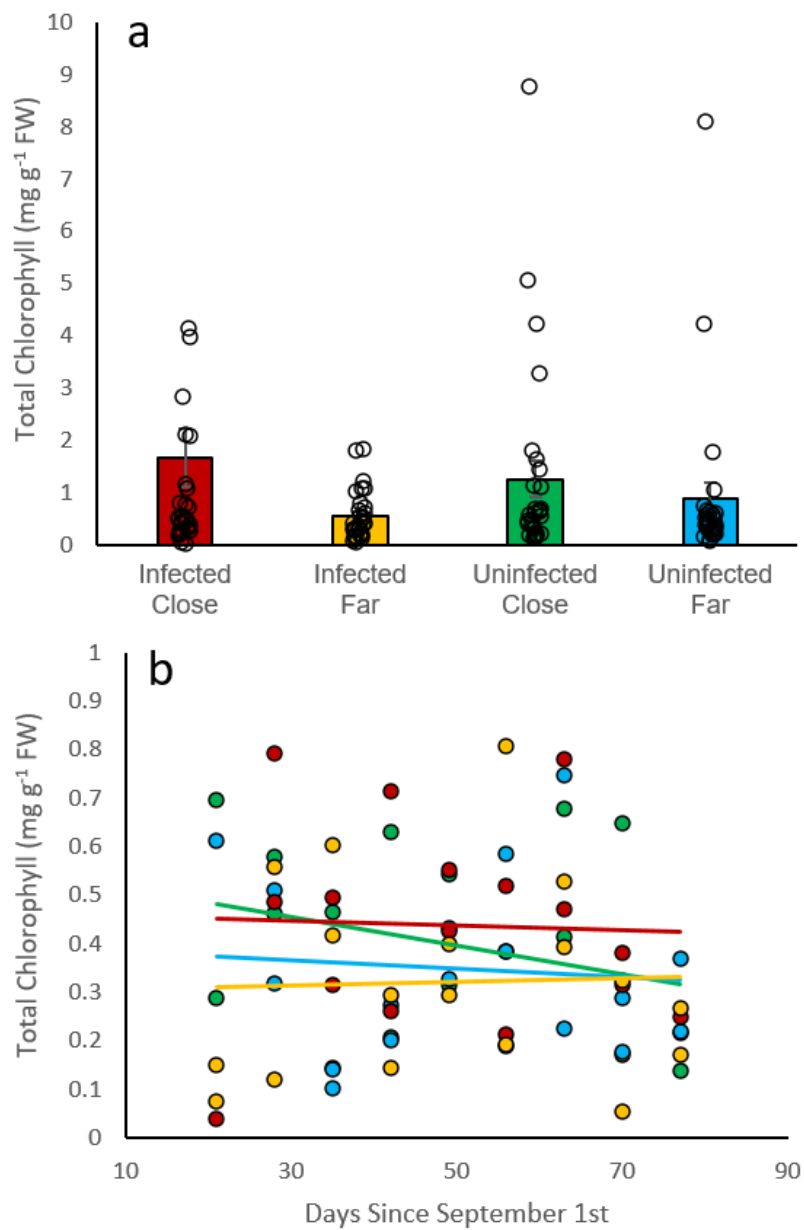


Figure 1

Total chlorophyll content in *Solidago* leaves. (a) Leaves were collected from *Eurosta*-infected plants and uninfected controls in the same location. From each plant, leaves were collected from a location close to the infection site and at a location near the bottom of the stem (i.e., far from the infection site). Bars represent mean total chlorophyll content $\pm$ SEM. Dots represent individual data points. (b) In 2024 only, leaves were collected on various days throughout the autumn season in order to assess whether any differences among leaves (due to infection status and/or stem location) were transient or persistent. Dot and line colours correspond to those in panel (a). There was no significant difference in chlorophyll content among leaf types, and there was no significant change in chlorophyll content over the course of the autumn.

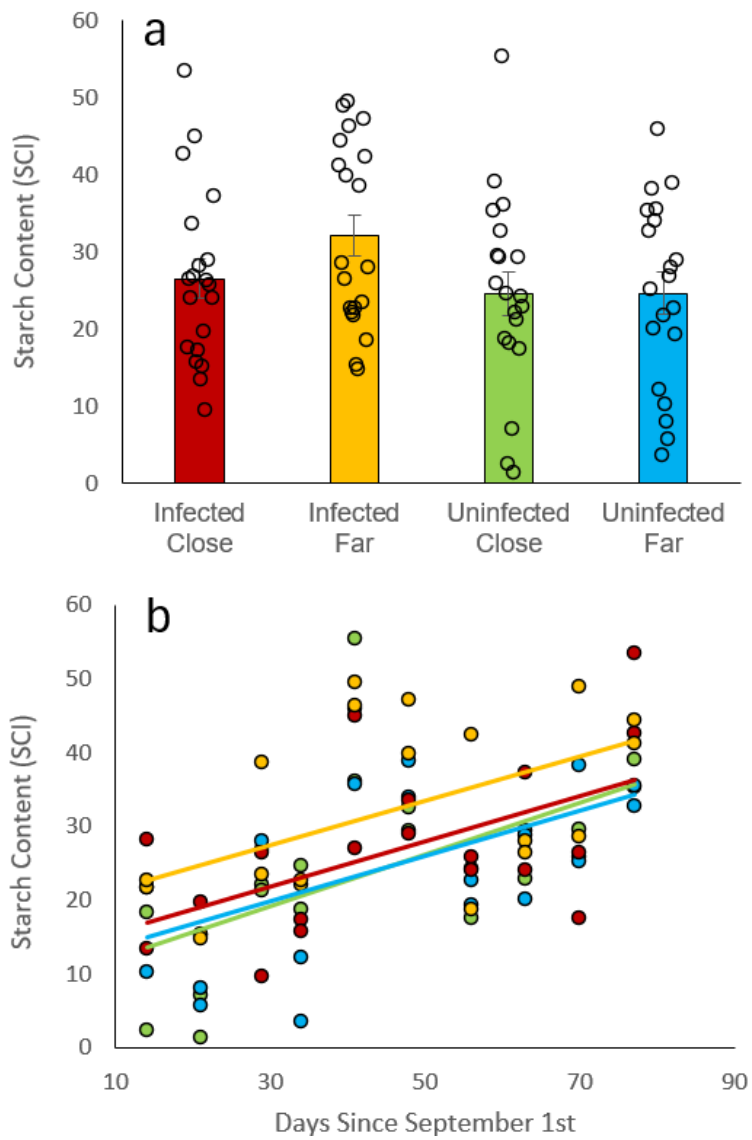


Figure 2

Starch content in *Solidago* leaves. See Figure 1 for explanation of data formatting. Starch colour index (SCI) is inversely related to the starch concentration within the leaves. SCI was significantly higher in "Infected Far" leaves compared to all other leaf types. In addition, SCI increased significantly over the autumn season in all leaf types.

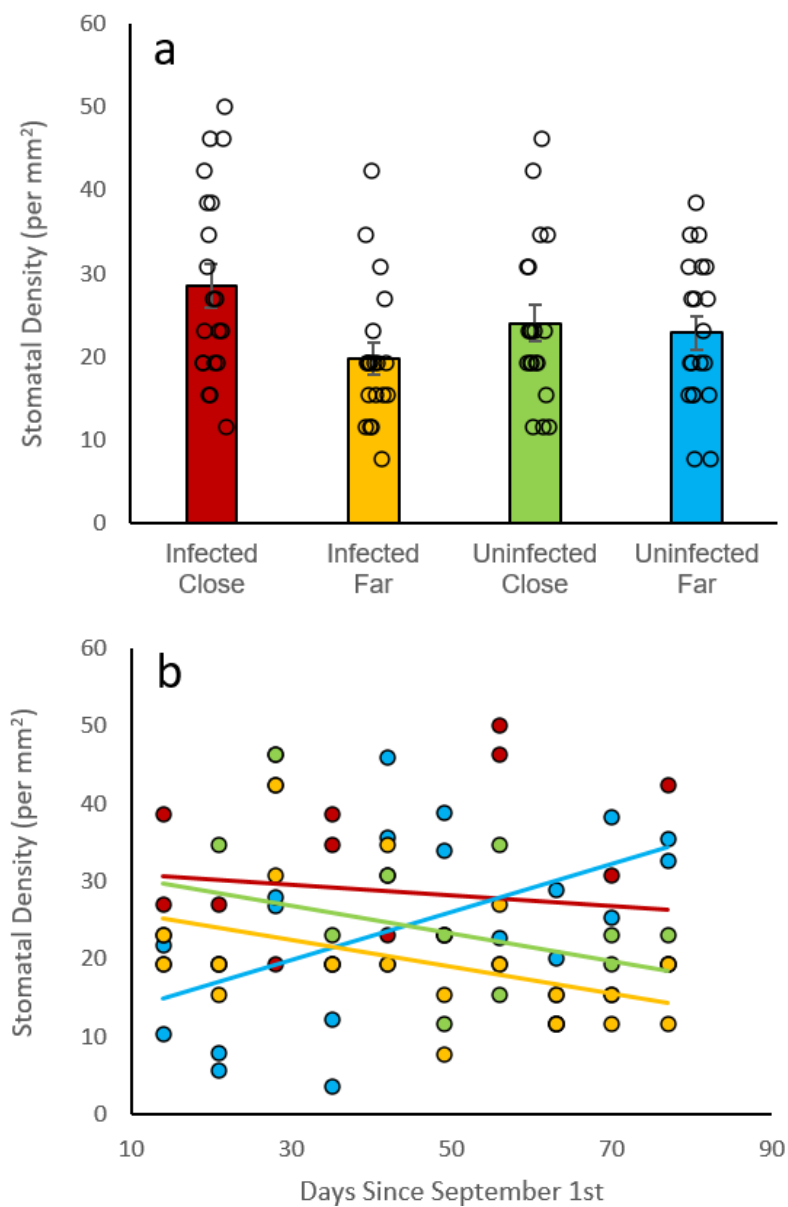


Figure 3

Stomatal density in *Solidago* leaves. See Figure 1 for explanation of data formatting. Stomatal density was measured on the abaxial surface. Stomatal density was significantly higher in "Infected Close" leaves compared to all other leaf types. In addition, stomatal density decreased significantly in all leaf types over the autumn season except for the "Uninfected Far" plants, where it increased significantly.

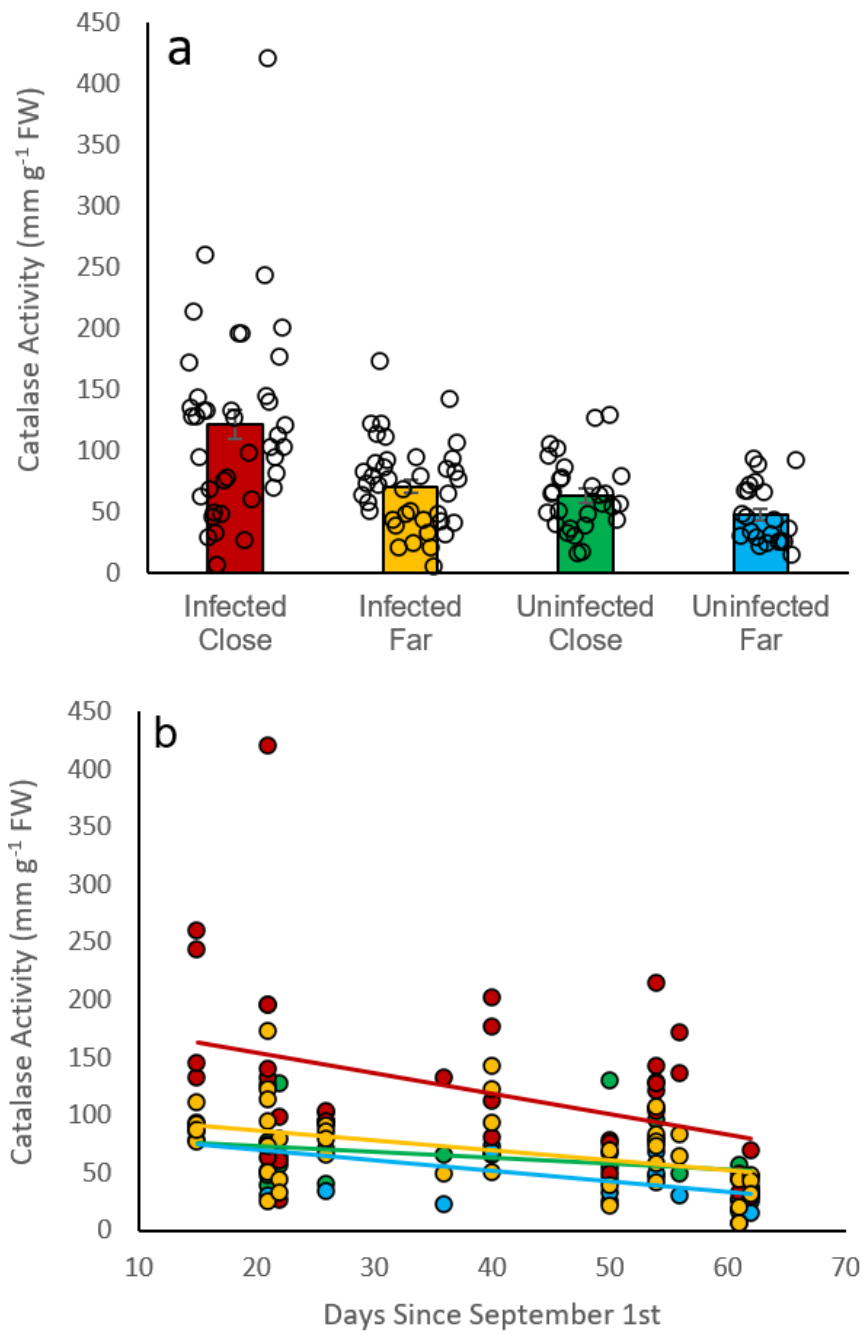


Figure 4

Catalase activity in *Solidago* leaves. See Figure 1 for explanation of data formatting. Catalase activity was significantly higher in “Infected Close” leaves compared to all other leaf types. In addition, for all leaf types, catalase activity declined significantly over the course of the autumn.

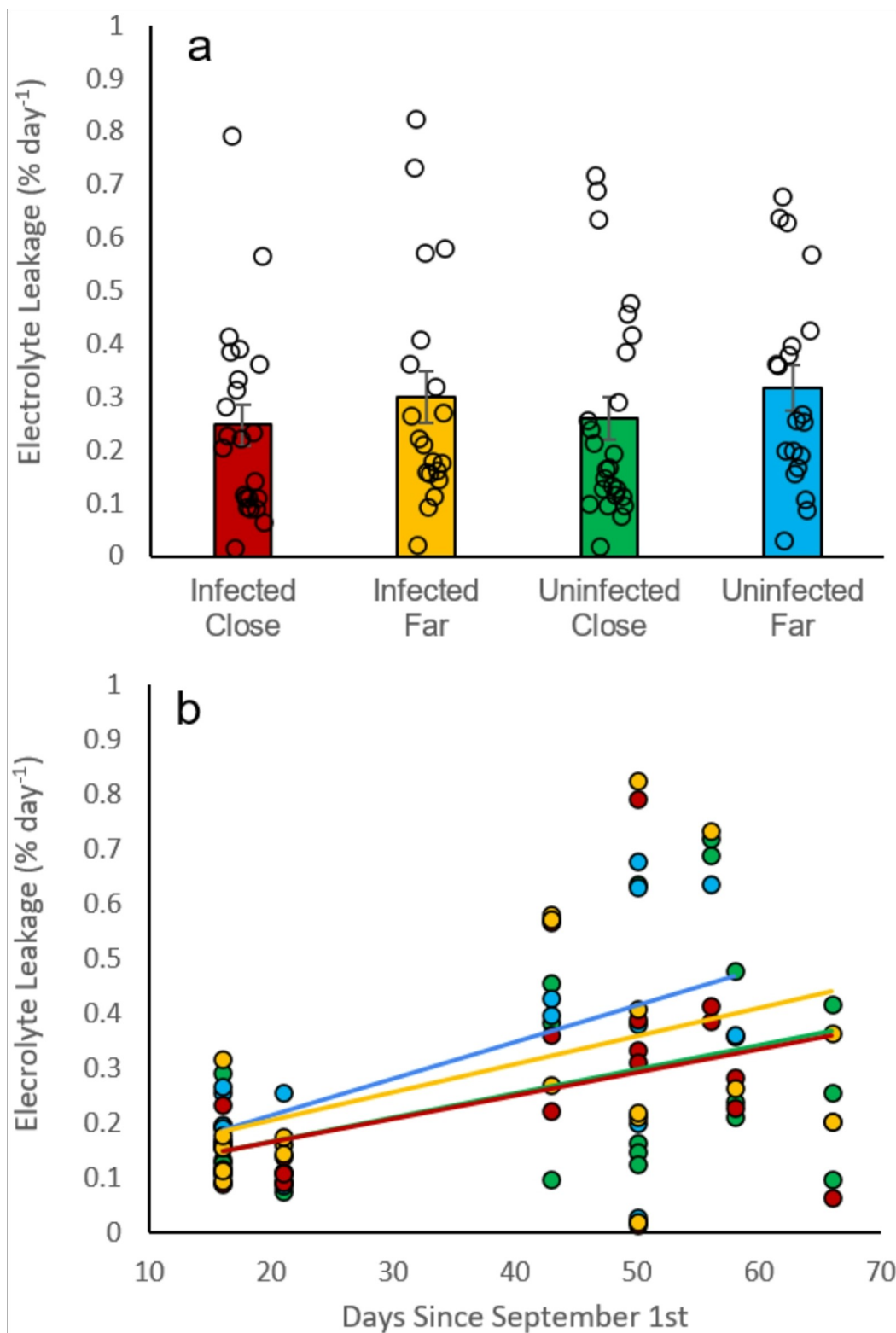


Figure 5

H₂O₂-specific electrolyte leakage in *Solidago* leaves. See Figure 1 for explanation of data formatting. Electrolyte leakage represents the percentage of the total leaf electrolytes that leaked out of leaves during 24 hours of exposure to H₂O₂ (compared to 24 hours of exposure to water). Electrolyte leakage was similar among all leaf types, but it increased significantly over the autumn season in all leaf types.

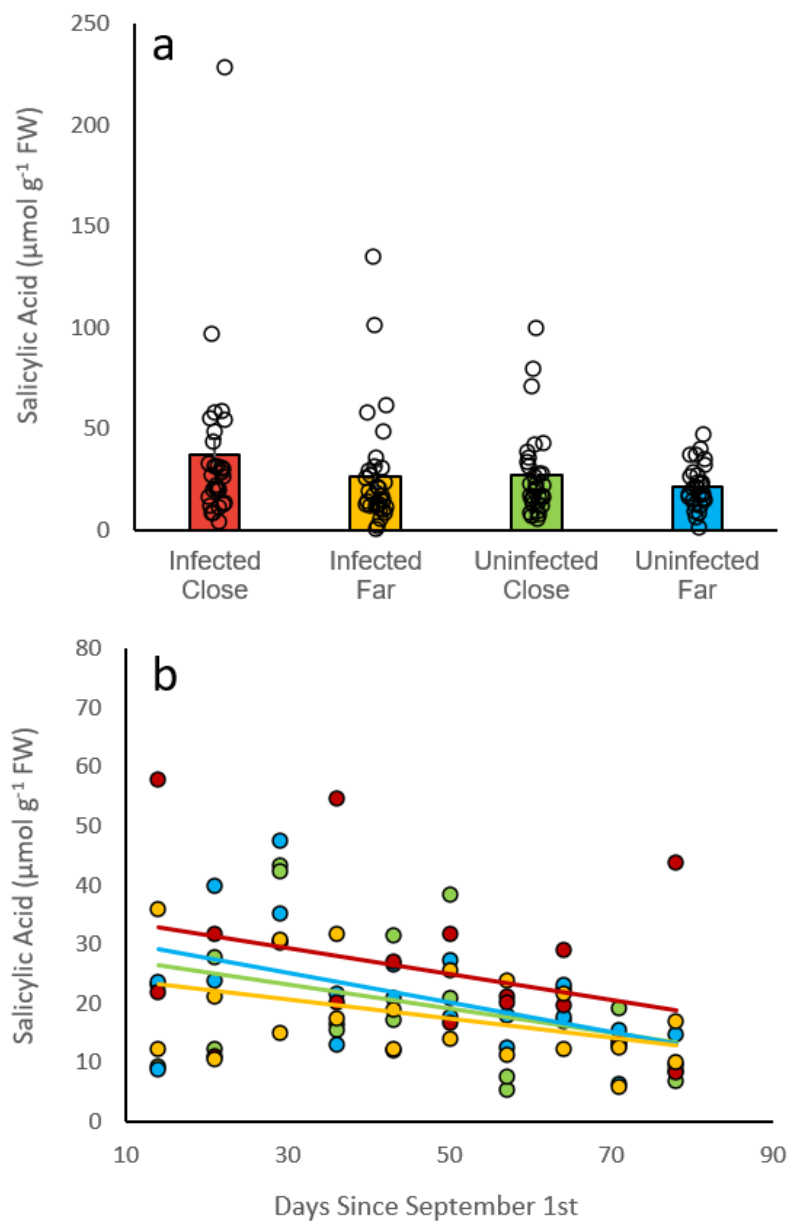


Figure 6

Salicylic acid (SA) concentration in *Solidago* leaves. See Figure 1 for explanation of data formatting. SA levels were significantly higher in “Infected Close” leaves compared to all other leaf types. In addition, SA levels declined over the course of the autumn season (data for 2023 only) in all leaf types.