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Abstract

Soybean gall midge, *Resseliella maxima* Gagné (Diptera: Cecidomyiidae), is a newly identified pest confirmed on soybean, *Glycine max* (L.) Merr. (Fabales: Fabaceae). To date, soybean gall midge has been found in Nebraska, Iowa, South Dakota, Minnesota, and Missouri, and has caused severe economic loss to commercial fields since 2018. Much is still unknown about this pest, so research efforts have been focused on biology and management. Larvae feed on the inside of the stem just above the soil line and are difficult to access and time-consuming to sample. In order to accelerate nondestructive sampling efforts, we developed an injury rating system to quantify the severity of plant injury from soybean gall midge larvae. Research plots from 2019 and 2020 in Iowa and Nebraska were evaluated for injury throughout the growing season and yield was measured. Our objective was to describe the

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relationship between injury severity and yield loss caused from soybean gall midge. A nonlinear regression model was developed to validate our injury rating system and to express the relationship between season long injury severity and yield loss. Results from our analysis indicate the injury rating system we developed correlates well with yield loss caused by larvae and may be an important tool for understanding the economic impact of this emergent pest of soybeans.

Keywords: crop protection, integrated pest management, field crop

Soybean, *Glycine max* (L.) Merr. (Fabales: Fabaceae), is the second most economically important crop in the United States, with over 33 million hectares planted in 2020 (USDA-NASS 2020). Observations of orange maggots in soybean stems were first reported in 2011 in Nebraska. In the following years, the frequency of observations of these larvae increased and sometimes resulted in plant death. In 2019, Gagné et al. (2019) determined the larvae present in soybean represented a new species, *Resseliella maxima* Gagné (Diptera: Cecidomyiidae), that has not been documented in other soybean production areas throughout the globe. Transect surveys have confirmed soybean gall midge in 140 counties throughout Iowa, Minnesota, Missouri, Nebraska, and South Dakota (McMechan and Hunt 2021, McMechan et al. 2021).

Soybean gall midge larvae feed on tissue inside the stem near the base of the plant (Gagné et al. 2019). Feeding injury disrupts nutrient and water movement (Dean et al. 2020) causing plants to wilt and potentially snap off at the feeding site. Larval infestations are typically heaviest at the field edge, with infestation levels dissipating towards the center of the field (McMechan et al. 2021). Soybean gall midge larval feeding may cause up to 100% yield loss on field edges (McMechan et al. 2021). Due to feeding location, larval feeding can go unnoticed until plants begin to experience stress. Sampling for larvae is destructive and time-consuming (i.e., splitting stalks with a knife or peeling back the outer layer), and not practical for agronomists and farmers.

Further adding to the complexity of this pest, soybean gall midge adults have nearly continuous emergence over 90 d, creating multiple, overlapping generations within a single growing season (McMechan et al. 2019, Hodgson and Helton 2021). Commercial fields and research plots are subject to season-long exposure to soybean gall midge colonization. Within fields, soybean gall midge infestations result in highly variable amounts of plant injury. Thus, the relationship between injury severity and overall yield loss from soybean gall midge infestations is not fully understood. The objective of this research was to create a model representing the relationship between the level of injury severity from soybean gall midge larval feeding and the yield loss it caused. To achieve this objective, experimental field plots were monitored throughout two growing seasons for injury caused by soybean gall midge infestations. Injury severity ratings and final yield measurements were used as inputs to express the relationship between injury severity and yield loss.

Materials and Methods

Data Collection and Plot Establishment

Data for this project were collected from small-plot research trials in 2019 and 2020. The trials were conducted on both university research farms and commercial farms located in Iowa (Cass and O'Brien Counties) and Nebraska (Cass, Lancaster, and Saunders Counties). To increase the likelihood of infestations, all trials were planted along field edges at locations where soybean gall midge injury had been observed in previous years. Preceding crops were soybean or maize, *Zea mays* L. (Poales: Poaceae), with adjacent fields planted to soybean. We collected data from 11 research trials, all of which were insecticide efficacy evaluations comparing seed-applied and foliar treatments. In Iowa, plots were four rows wide, 9.14 m long with 76.2 cm row spacing. Individual trials were conducted using a randomized complete block design. In Nebraska, plots were four rows wide and 9.14 m long with row spacing of 76.2 cm. Maturity groups ranged from 2.4 to 3.2 in Iowa and 2.7 to 3.2 in Nebraska. A summary of planting dates, varieties, seeding rates, and the numbers of treatments and replications used are summarized in **Table 1**.

To measure plots for injury severity from soybean gall midge larval feeding, we developed a visual rating system. Soybean gall midge larvae are small in size, 0.5 to 3.0 mm long, and also feed within the plant stem (Gagné et al. 2019). Therefore, assessing plant injury based on larval abundance would require destructive sampling and would

Table 1. Summary of trials included in the generation of the area under the severity progress curve (AUSPC)

Year	*State*	*County*	*Variety*	*Planting date*	*Planting Population (seeds ha^{-1})*	*Harvest date*	*Treatments*	*Replications*	*Observations*	*Max AUSPC value*
2019	Iowa	Cass	S24-K2	15 May	345,947	14 Oct	22	4	88	6,325.0
			AG30X9	15 May	345,947	14 Oct	6	4	24	5,575.0
2020	Iowa	Cass	AG32X8	23 Apr	345,947	6 Oct	24	4	96	1,537.5
		O'Brien	AG32X8	24 Apr	345,947	30 Sep	24	4	96	606.3
2020	Nebraska	Cass	P28A42X	3 May	365,674	28 Sep	8	4	32	1,331.3
		Lancaster	P28A42X	2 May	365,674	23 Sep	4	4	16	4,028.1
			AG32X8	7 May	373,129	23 Sep	7	4	28	3,584.4
			S27-M8X-GH2788X	7 May	373,129	23 Sep	11	4	44	3,112.5
			P28A42X	12 May	365,674	23 Sep	4	4	16	4,412.5
		Saunders	P28A42X	3 May	365,674	22 Sep	8	4	32	3,300.0
			P28A42X	3 May	396,141	22 Sep	10	4	40	3,925.0

be difficult to quantify accurately, especially in the field. For these reasons, visually rating plots was determined to be the most efficient method for evaluating injury levels. All plots were evaluated for injury throughout the growing season and were rated on a 5-point scale, which ranges from 0 to 4 depending on the percentage of plants showing visual injury symptoms (wilting, lodged, or dead) caused by soybean gall midge infestations. Individual injury rating values with the related approximate percentage of total injured plants (±12.5%) is described as: 0 = 0%, 1 = 25%, 2 = 50%, 3 = 75%, and 4 = 100%. Ultimately, we wanted this injury rating to be used by practitioners and researchers, and therefore the transformation to increments seemed more intuitive and potentially more efficient than reporting injury in percentages. The linearity of the injury rating system allows users to further break down the ratings for greater precision. In some instances, we incorporated ratings in increments of 0.25 to document relatively small differences between plots. Various injury rating levels were used to evaluate soybean gall midge feeding injury (**Fig. 1**). All plots were given a weekly injury rating until plant senescence.

The center two rows of each plot were harvested with an ALMACO (Nevada, IA) Specialized Plot Combine (model SPC40). Yields were determined by weighing grain with a hopper that rested on a digital scale sensor custom-designed for each combine. Yields were corrected to 13% moisture and reported in kilograms per hectare.

Data Transformation

A method for assessing crop exposure to a persistent biotic stressor was suggested by Van der Plank (1963) in the form of an area under the disease progress curve. This method has been adopted widely by plant pathologists to express disease intensity over time (Jeger and Viljanen-Rollinson 2001). The concept has been used by entomologists as well, most notably to describe season-long crop exposure to aphids (e.g., Hanafi et al. 1989, Ragsdale et al. 2007). We adapted this method as an area under the severity progress curve to quantify season-long exposure to soybean gall midge feeding injury by transforming our discrete injury ratings into a cumulative injury value. First, injury ratings were expressed on a 0 to 100 scale, in which the new value equals the approximate percentage of total plot injury represented

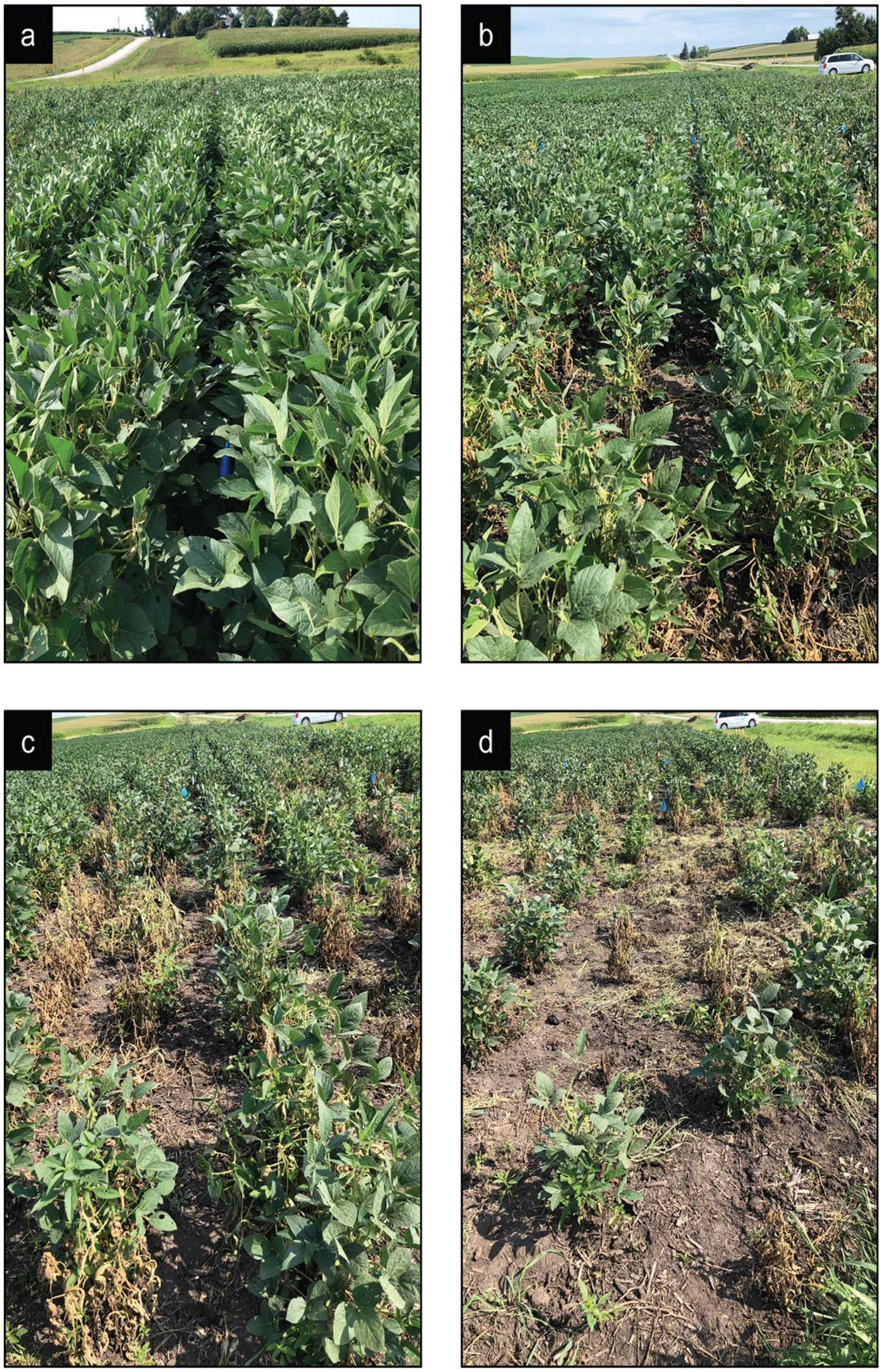

Fig. 1. Representative photographs depicting the various rating levels used to assess soybean gall midge feeding severity: (a) 0 or 1, (b) 2, (c) 3, and (d) 4. Each photograph was taken from the center front of a four-row plot 9.14 m in length. Distinct photographs for ratings of 0 and 1 were not provided because these plots often appear similar from this vantage point and require closer examination of the plants within the plot to assign a value.

by our injury rating system. For example, a 2 on our injury rating scale would be transformed to 50. Next, the average of two sequential transformed values were multiplied by the number of days between the two dates the injury ratings were collected. This number was added to the previous cumulative injury value. This process was repeated for each individual injury rating through the last rating to obtain a final severity progression curve value. This technique can be expressed using the formula

$$\sum_{i=1}^{n} (x_{i,i-1} \times d_i)$$

where n is the number of sampling dates, $x_{i,i-1}$ is the average transformed injury rating between sequential sampling dates i and $i-1$, and d_i is the number of days since the previous sampling date.

Plots were established in multiple locations and years. As a result, yield potentials due to different environments had to be normalized across locations and years. To account for variation in yield potential, plot yields were expressed as proportions relative to the maximum possible yield at the location plots were established (Catangui et al. 2009). Maximum yield for a given trial was estimated by examining historical yields for the field in which a trial was conducted. Historical yields were provided by the grower, and the highest yield from the three years preceding the trial year was selected.

Regression Analysis

To express the relationship between injury severity and yield loss, a nonlinear logistic decay regression model in the form of

$$y_i = \frac{1}{1 + e^{(bx_i - a)} + \varepsilon_i}$$

where y_i is the proportion of maximum yield associated with observation i, x_i is the severity progression curve value associated with observation i, b and a are regression parameters associated with the function's rate of decay and midpoint, e is Euler's number (2.71828), and ε_i is the random error term associated with observation i. The error term is assumed to have an independent normal distribution with a mean of 0 and constant variance. The nls function of the R package stats (version 3.6.2) in RStudio (version 1.2.5033) (RStudio, Inc., Boston, MA) was used to determine least-squares parameter estimates for *b* and *a*. Based on visual inspection of the data, initial starting values for estimating parameters were 0.001 (*b*) and 2.5 (*a*).

Outliers in the data were identified if the standardized residual for a given observation resulting from an initial model fit was greater than or equal to three. Seven outliers were identified and removed from our analysis due to the tendency of outliers to influence a fitted function disproportionately when using the least-squares method (Kutner et al. 2005). Residuals (e_i) were assessed graphically to determine whether assumptions related to the error term were met. A histogram (**Fig. 2**) and quantile plot (**Fig. 3**) of standardized residuals confirmed a generally normal distribution with a mean of 0, and a plot of sequential residuals (e_{i+1} vs. e_i) indicating the error term was uncorrelated (**Fig. 4**). The predict NLS function of the R package propagate (version 1.0-6)

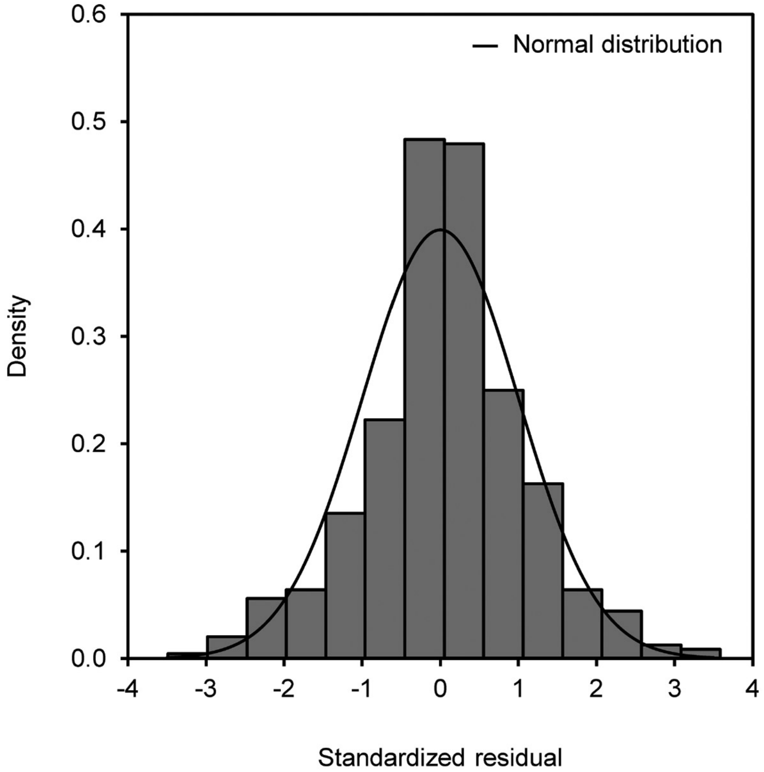

Fig. 2. Histogram of standardized residuals resulting from the nonlinear least squares regression model. An overlaying normal distribution is provided for comparison.

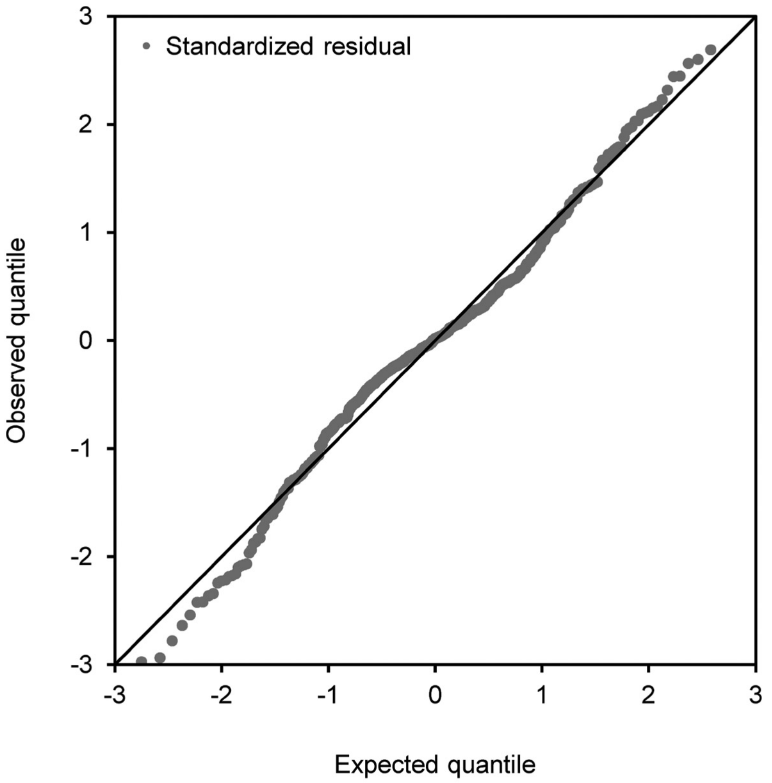

Fig. 3. Quantile plot depicting the observed quantiles of standardized residuals resulting from the nonlinear least squares regression model and their expected quantiles under an assumption of normality.

was used to estimate 95% confidence and prediction intervals for the model (**Fig. 5**). Examination of the distribution of observations about the fitted model in this figure did not reveal a substantial departure from the assumption of constant variance.

The experiments included in this analysis were sampled for injury caused by soybean gall midge on a weekly basis (when averaged across all trials, the mean interval between sampling dates was 7.2 d). To understand how sampling frequency may have affected the calculation of injury severity (i.e., severity progression curve) values, a posthoc comparison was performed where the original severity progression curve values derived from the full set of sampling dates was compared with severity progression curve values calculated from two sets of sampling dates where individual dates were omitted systematically to produce longer sampling intervals. The first of these was

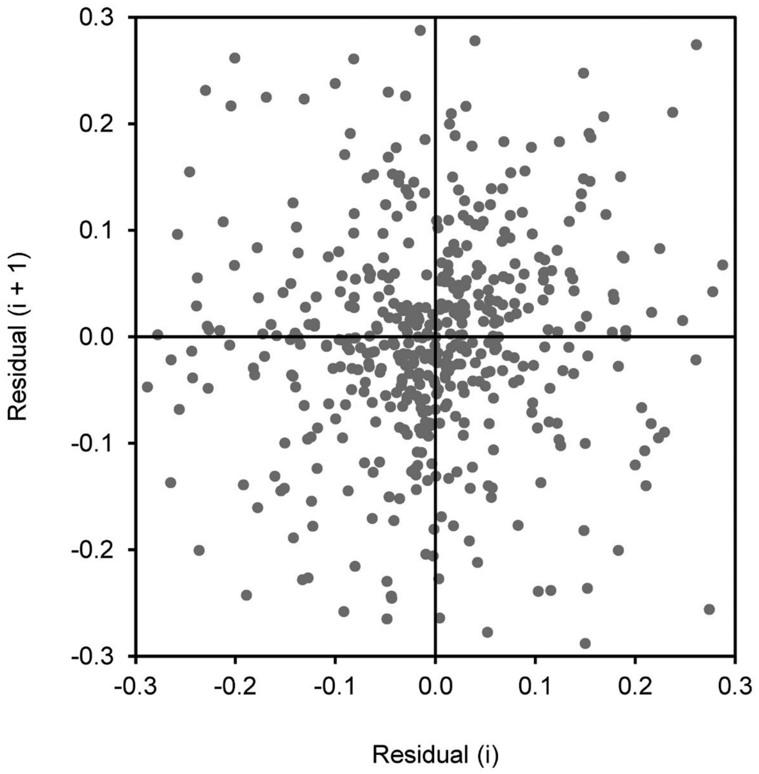

Fig. 4. Sequential plot of residuals resulting from the nonlinear least squares regression model used to confirm the assumption of an uncorrelated error term.

termed the 'reduced' set of sampling dates and included only ratings performed on alternating dates (mean sampling interval = 14.4 d). The second of these was termed the "minimal" set of sampling dates and included only ratings performed on every third date (mean sampling interval = 21.6 d). For any given plot, the final sampling date represents the maximum level of injury caused by soybean gall midge; therefore, to produce the reduced and minimal sets of sampling dates, the final date was always included, and sampling dates were omitted in reverse chronological order.

Results

For this model, a total of 505 observations (excluding outliers) were used to estimate injury severity and associated yield loss. Initial injury

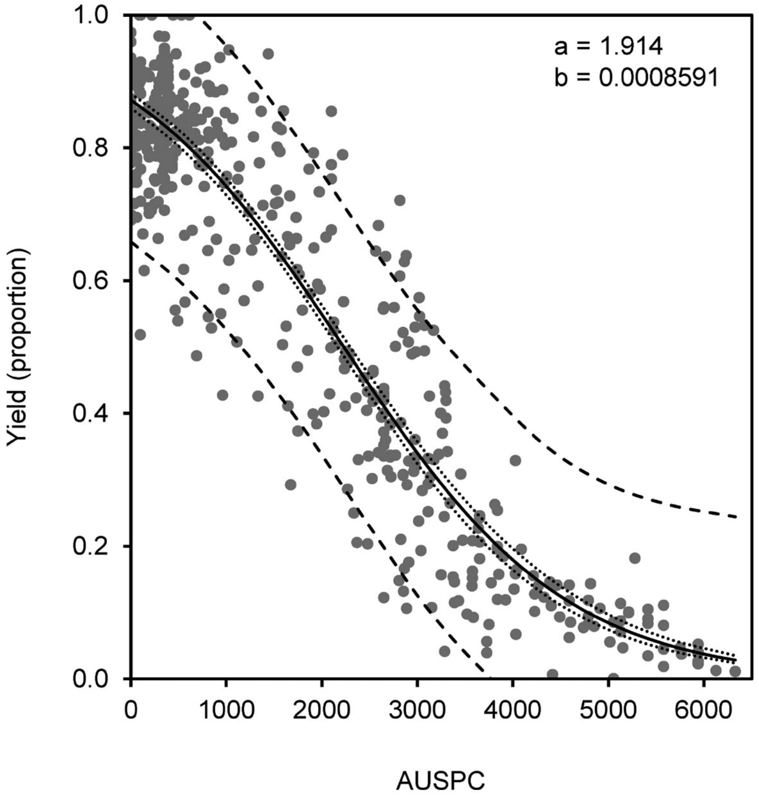

Fig. 5. Area under the severity progress curve (AUSPC) model with 95% confidence and prediction intervals.

severity ratings were collected weekly between 2 July (Nebraska 2020) and 11 July (Iowa 2019), and ended between 28 August (Nebraska 2020) and 10 September (Iowa 2019). The number of weekly ratings collected varied across locations, ranging from 8 to 10 collection dates per trial. Severity progression curve values ranged from 0 (Iowa 2020) to 6,325 (Iowa 2019) with a mean of 1,731, and yield proportions ranged from 0.00 (Iowa 2019) to 1.00 (Iowa 2019, 2020; and Nebraska 2020) with a mean of 0.59. Least-squares parameter estimates resulted in $b = 0.0008591$ (standard error = 0.0002293, t = 37.46, and $P < 0.0001$) and $a = 1.914$ (standard error = 0.05114, t = 37.44, and $P < 0.0001$). The nonlinear regression model is expressed in Fig. 5.

Severity progression curve values calculated from the reduced and minimal sets of sampling dates were highly correlated with those derived from the full set of sampling dates (**Fig. 6**a). However, severity progression curve values from the minimal set of sampling dates

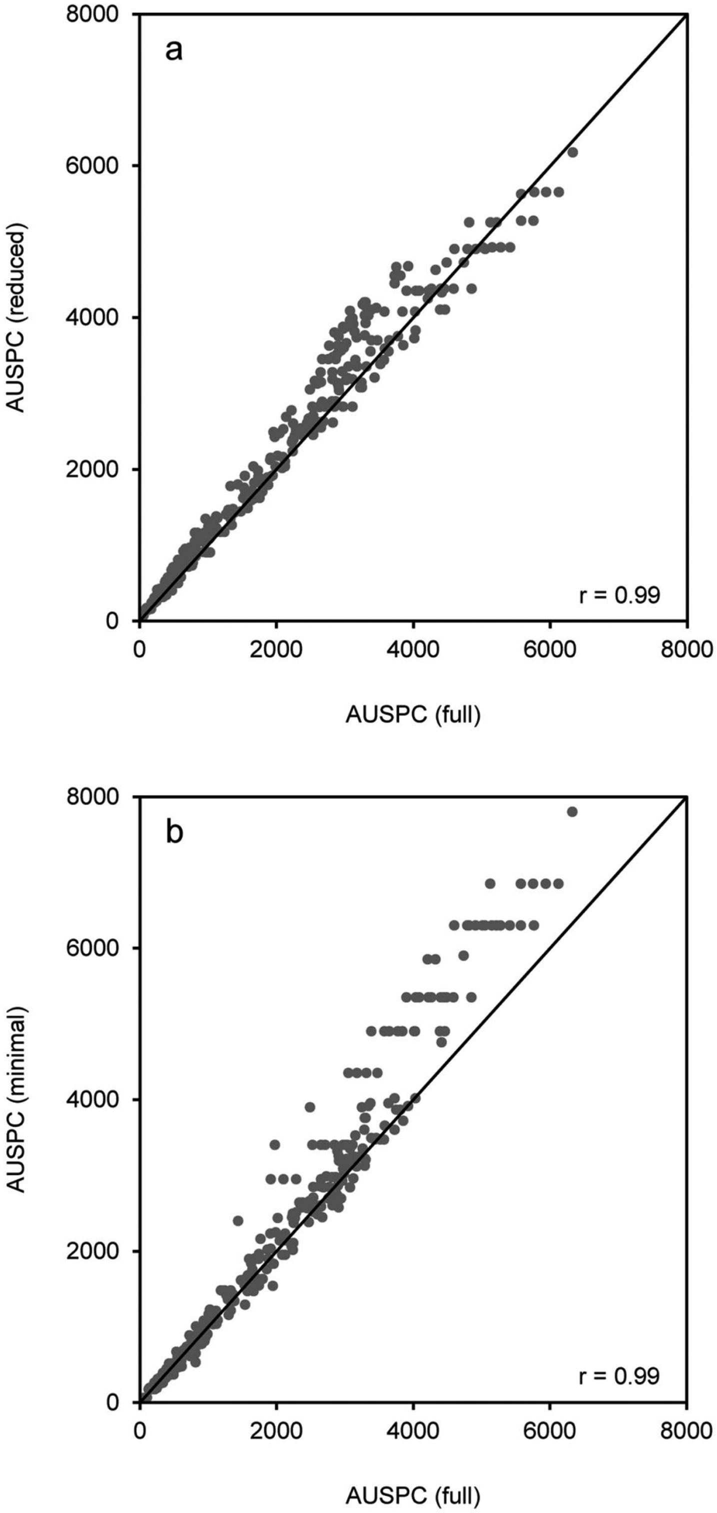

Fig. 6. Correlation between area under the severity progress curve (AUSPC) values derived from the full set of sampling dates and those calculated from the (a) reduced and (b) minimal sets of sampling dates.

displayed a tendency to overestimate injury severity, especially for values above approximately 3,000 (Fig. 6b). While a longer sampling interval may be desirable to increase efficiency, this finding suggest that intervals longer than approximately 2 weeks may be less accurate when injury severity is moderate to high. Future work could be conducted to optimize sampling efficiency while accounting for factors like time spent rating and precision of severity progression curve values calculated under varying sampling schedules.

Discussion

This study is the first to describe the relationship between plant injury severity and yield loss from soybean gall midge. The primary goal was to improve our understanding of the potential injury and yield loss that can occur from season long exposure to soybean gall midge larval infestations. Due to this pest being a new species, understanding the injury potential of soybean gall midge will aid in the development of pest management strategies. This model can be used to predict the potential loss of yield at a given level of injury severity during the season. Predicting the yield loss associated with larval feeding allows for a more complete understanding of the economic impact of this pest and can serve as the foundation for determining whether potential control measures will be economically justifiable.

Another goal of this research was to develop an injury rating system to quantify injury caused by soybean gall midge. The results from our model indicate that our injury rating system is closely associated with observed yield loss and soybean gall midge injury levels. We believe that our injury rating system is a useful tool in assessing and recording plant injury as a result from soybean gall midge larval feeding. Key advantages of the rating system we designed are that the ratings are based on relatively conspicuous signs of feeding injury (e.g., wilted, lodged, or dead plants) and that ratings are nondestructive in nature, meaning that repeated, season-long observations can be performed on the same plants used to estimate yield at the end of the growing season.

We hypothesize that the injury severity approach outlined in this project may also be used to describe the relationships of yield loss and insect injury in other pest systems that also produce a drawn out, season-long effect on the crop. The inputs for this project simply rely on injury ratings recorded throughout the duration of injury presence and final yield. With these data collection methods and analysis, the associated yield loss with an extended feeding period in other insects, with overlapping, multigenerational life cycles, can be better understood.

Potential factors that were not explored in this study may have an impact on the model. While environmental variability in soil characteristics, local weather conditions, and soybean variety were accounted for indirectly by expressing yield loss for each plot as a proportion of an estimated maximum for the trial site, it may be possible that these factors play an important role in the likelihood and/or severity of soybean gall midge injury. Further experiments should be conducted to determine which factors may affect the severity of yield loss associated with injury caused by this pest. Geographically, our analysis includes only five counties in two states, while soybean gall midge infestations have been confirmed in 114 counties in five states (McMechan et al. 2021). Efforts should be made in the future to include data inputs from a wider geographic region to validate our model. Yet another factor to consider is that all trials included in this project were planted along field edges, where other biotic and abiotic stressors are known to decrease yields (Sara et al. 2013, Nguyen and Nansen 2018, Carlesso et al. 2019). Additional research is needed to better understand the dynamics between field edge stressors, soybean gall midge injury, and yield loss to help identify effective strategies for this pest's control.

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