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Insect-Microbial Interaction

Season-long microbial dynamics from the cuticle of rice weevil originating at food facilities after dispersal to novel food patches

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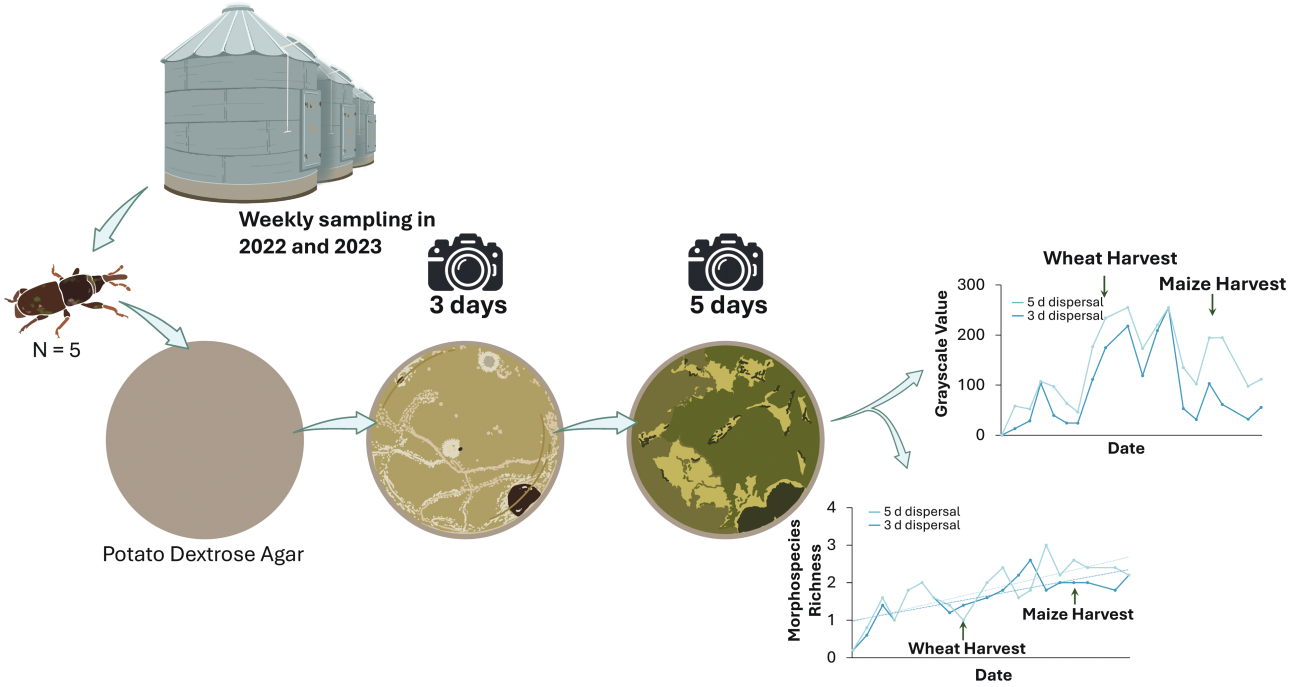
Stored-product insects may pose food safety concerns due to their capacity to vector harmful microbes. As climate change progresses, the propensity for vectoring may be affected by temperature. Vectoring capacity may also fluctuate over the season. Thus, we evaluated (i) how the area of microbial growth and morphospecies richness vary over the season from field-collected *Sitophilus oryzae* that were allowed to disperse onto a novel food patch habitat comprised of agar and (ii) whether temperature in the week preceding collection of *S. oryzae* affected growth and richness. On a weekly basis during 2022 and 2023, we introduced *S. oryzae* onto agar, and photographed patches at 3 and 5 d, characterizing growth with ImageJ and visually scoring richness. There was 1.4- to 1.6-fold more microbial growth in patches at 5 d compared to 3 d in both years. The greatest microbial growth consistently occurred from *S. oryzae* collected during the wheat and maize harvest in grain bins, while morphospecies richness increased progressively over time. We observed an 11-fold and 3-fold increase in the number of morphospecies at the end of the season compared to the beginning in 2022 and 2023. There was 2.1- to 316-fold more microbial growth during the wheat (Jun to Jul) and maize harvest (Sep to Oct) compared to early May. We found a positive exponential relationship between temperature in the field and microbial growth in both years. This study expands our understanding of insect–microbe interactions after harvest and highlights variable periods of risk by food facilities over the season.

Keywords: vectoring, plant pathogen, stored product, *Sitophilus oryzae*, behavioral ecology, dispersal

Graphical Abstract



Introduction

Inadequate management of grain after harvest leads to rapid deterioration of grain quality, decreasing weight of grain, as well as decreasing nutritional quality and germination with many of these changes catalyzed by insect feeding damage and damage due to microbial growth. The impact of insect feeding damage has been covered elsewhere (Magan et al. 2003, Magan and Aldred 2006). Microbial contamination in stored grain inflicts >10% losses (Käferstein 1990), as measured in developing countries. It is also associated with a variety of problems ranging from off-odor problems to food safety and security issues. For example, some species commonly found in stored grain, such as *Aspergillus* and *Penicillium*, produce carcinogenic mycotoxins that can have negative impacts on both human and animal health (Fleurat-Lessard 2017).

Even more important than the direct damage from microbes alone is the interaction between microbes and insects in the postharvest supply chain. There are health hazards associated with stored-product insect infestation as a result of the microbes that come with them (Hubert et al. 2018). Feeding activity of stored-product insects can exacerbate microbial growth and vice versa as both are known to increase temperature and humidity, creating more favorable environmental conditions (Dunkel 1988, Magan and Olsen 2004, Ponce et al. 2021). Volatiles from microbes can also attract stored-product insects. In a review of 43 articles, and 384 sets of tests involving 24 stored-product insects, Ponce et al. (2021) found that a total of 5 and 4 stored-product arthropods were significantly attracted or repelled by microbial cues, respectively, while 13 were unaffected or exhibited both attraction and repellency. Semiochemicals from fungal species, including *Aspergillus* and others, may elicit a behavioral response by multiple stored-product species (Ponce et al. 2022, Van Winkle et al. 2022). Moreover, stored-product insects can vector fungal and bacteria species. Prior work has found that *Lasioderma serricorne* (F.) (Coleoptera: Ptinidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) vectored 13 genera of fungi based on 59 sequences isolated from 2 species when introduced into novel food patches, with a total of 23% and 16% classified as *Aspergillus* and *Penicillium* spp., respectively (Ponce et al. 2024). In addition, stored-product insects can serve as physical vectors for bacteria that they encounter in the environment. For example, Sumino et al. (2010) identified bacteria from 21 different taxonomic families from food facilities in Japan, including several genera of microbes that could be mycotoxigenic depending on the strain, while Hubert et al. (2021) found environmental and feces-associated bacteria from 3 different species of stored-product mites. In addition, Hubert et al. (2018) found stored-product insects were important for public health and pathogen transmission. Live adult, larvae, and cast skins of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) yielded 4 *Aspergillus* species, including *A. flavus*, *A. niveus*, *A. terreus*, and *A. niger* (Bosly and Kawanna 2014). Moreover, the rapid growth of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) over 15 wk in bagged wheat resulted in increased temperature, CO_2 concentration, and bacterial growth compared to controls lacking the insect (Imura and Sinha 1984). Finally, Yezerski et al. (2005) found certain species of bacteria were introduced as the result of *Tribolium* infestation in flour. Therefore, and as Ponce et al. (2021, 2024) concluded, it is important to consider both stored-product insects and their associated fungi and bacteria, as they can act as mechanical vectors.

A variety of abiotic factors are known to affect the growth and persistence of microbes. For example, temperature may affect the growth of many microbes. Maximal growth of *Aspergillus flavus*, a key stored-product fungus, was found at 33 °C, whereas for other *Aspergillus* spp. the optimal temperature ranged between 30 and 37 °C (Holmquist et al. 1983, Bellí et al. 2004). Generally, increasing temperature (up to a point) results in faster microbial growth for many stored-product fungi. Relative humidity can also affect microbial growth. Germination time was the least for *Penicillium chrysogenum* at 60% RH (Lattab et al. 2012). Finally, grain moisture may also be a factor in microbial incidence and abundance, with higher values at grain with a moisture content of 19% compared to 15%. In addition, in a test of grain tempered to between 12% and 19% grain moisture in a stepwise fashion, Van

Winkle et al. (2022) found that there were significant changes in the volatiles emitted by grain, and that *Rhyzopertha dominica* (L.) (Coleoptera: Bostrichidae) was most responsive to grain incubated at moderate grain moisture of 15%. Therefore, abiotic factors, especially temperature, may be important to the growth of microbes and may be even more important with ongoing climate change and its effects on food facilities (Gerken and Morrison 2022).

We focused in this study on *S. oryzae* (Coleoptera: Curculionidae), which is a cosmopolitan and destructive internal-infesting pest of whole grain that has been previously shown to interact with vector microbes. Although the gut bacteria of *S. oryzae*, including *Bacillus* and *Pseudomonas* spp., were found capable of suppressing growth of *A. flavus* and of degrading aflatoxin B1 mycotoxin (Al-Saadi et al. 2024), other work has shown that *S. oryzae* can actually vector *Aspergillus* and *Penicillium* (Ponce et al. 2024). Thus, it seems that the gut microbes of *S. oryzae* have little effect on the external environment when not directly extracted, cultured, and applied to other fungi. In addition, in 50 stored wheat samples from India, *S. oryzae* was the dominant insect, and *A. flavus* was recorded in 87% of the insect-damaged wheat samples, but only in 25% of the insect-free controls (Sinha and Sinha 1990). Therefore, it appears that there is a close association between *S. oryzae* and the spread of fungi. However, up to this point, there has been no in-depth study on how the growth and species richness of microbes vectored by a stored-product insect may change over the course of the growing season. In addition, as some have pointed out, it is important not to just look at total microbial abundance, but microbial species richness as well (Fröhling et al. 2020). Consequently, the aims of our study were to evaluate (i) how the microbial growth and morphospecies richness vary over the course of a growing season from *S. oryzae* collected in the field and allowed to disperse onto a factitious novel food patch and (ii) whether temperature in the week preceding collection of *S. oryzae* affects microbial growth and species richness.

Materials and Methods

Trapping of Field-Collected *S. oryzae*

To capture field-collected *S. oryzae* to evaluate how microbial vectoring varies over the course of a season in 2022, 6 commercial pitfall traps (Storgard, Trécé Inc., Adair, Oklahoma) were baited with 5 g of wheat and the *S. oryzae* aggregation pheromone, (4*S*,5*R*)-5-hydroxy-4-methyl-3-heptanone (IL-703, Insects Limited, Westfield, Indiana), and deployed 10 m apart. In 2023, 14 pitfall traps (Storgard, Trécé Inc., Adair, Oklahoma) were deployed between grain storage bins, separated by 5 to 15 m and baited with the same stimuli plus approximately 100 g of cracked wheat. In addition, probe traps (WB Probe II, Storgard, Trécé Inc., Adair, Oklahoma) were taken in identical locations in adjacent grain bins, but >90% of individuals came from the pitfall traps. Trapping took place at the Kansas State University Agronomy Farm (39°12′23″N, 96°35′42″W), and occurred on a weekly basis from 11 May 2022 to 28 Sep 2022 and 17 May 2023 to 1 Nov 2023. Live *S. oryzae* from each trap were placed in a sterile Ziplock bag and brought back to USDA-ARS Center for Grain and Animal Health in a cooler. Live weevils were stored at 23 °C and 65% RH after brought back from the field. Within 24 h of being brought to the laboratory, *S. oryzae* were introduced to factitious, novel food patches as described below.

Linking With Weather Data

Weather data were obtained from the Kansas Mesonet system (https://mesonet.k-state.edu/) at a weather station located in the same location as the trapping on the Kansas State University Agronomy Farm (39°12′26″N, 96°35′42″W). Air temperature was measured at 1.98 m above ground level (HMP155 probe, Vaisala, Vantaa, Finland) inside of a non-aspirated radiation shield within an error range of ±0.1 °C. Data were acquired every hour with a microprocessor (CR3000 series, Campbell Scientific Inc., Logan, Utah), which accurately measures to the microvolt level and controls peripheral devices. Mean maximum and minimum temperature was compiled for each 7-day period preceding each date of collection for *S. oryzae* in 2022 and 2023, and it was linked to microbial growth measures detailed below from the same date.

Assessing Microbial Growth After Dispersal to Novel Food Patches

A total of $n = 5$ individual field-collected *S. oryzae* from each date were introduced individually onto Petri dishes (100 × 20 mm) composed of potato dextrose agar as a factitious, novel food patch for 3 and 5 d to mimic dispersal (following the methods of Ponce et al. 2024). This was done within the confines of a permitted BSL2 (Permit# IBC-1693) space using a biosafety cabinet (75 × 73 × 95 cm L:H:W, #302381101, Labconco, Kansas City, Missouri). After introduction of *S. oryzae*, the Petri dishes were placed in an environmental chamber (Percival Scientific, Perry, Iowa) set at constant conditions (30 °C, 65% RH, and 14:10 L:D cycle). Petri dishes were photographed at 3 and 5 d of *S. oryzae* foraging in the novel food patch with a 3D-imaging system (Cognisys Inc., Traverse City, Michigan) using an SLR camera (EOS 7D Mark II, Canon Inc., Tokyo, Japan) with a wide-angle lens (L series USM 17 to 40 mm, Canon Inc., Tokyo, Japan) and twin flash (MT-24X, Macro Twin Flash Lite, Canon Inc., Tokyo, Japan). Light was diffused using a partially cut frosted plastic jar (15.2 × 7.6 cm D:H). Images were processed using ImageJ v.1.53 (Wayne Rasband, National Institutes of Health) individually by first subtracting the background, then finding edges, and converting the image to binary (white/black). As needed, the erode and/or dilate function in ImageJ was sparingly used to make sure the binary image reflected microbial growth in the original image. A circle encompassing only the Petri dish was created and the mean grayscale, standard deviation of the grayscale value, and count of pixels were measured as a surrogate for microbial growth on the dishes. This allowed a quantitative measure of microbial growth by creating an average in each image. The mean grayscale value could range from 0 (full white), indicating no microbial growth, to 255 (full black), indicating full microbial growth on the entire dish. Increased microbial growth was defined as higher mean grayscale values. Finally, visually, microbial morphospecies (alpha) richness was assigned to each image by 2 observers given the number of unique morphospecies on the plate as a proxy for community complexity. Where these numbers varied by observer (which was rare), both observers discussed together and came to a consensus on the number of morphospecies present in the Petri dish. Microbial morphospecies have been used to assess diversity in other studies (Finlay et al. 2006, Telford et al. 2006).

Statistical Analysis

A general linear model was used to evaluate the microbial growth (eg grayscale value) and microbial morphospecies richness. Time in patch by weevils (3 or 5 d) and date of collection during the season were used as fixed, explanatory variables in the model. Residuals were inspected to ensure that assumptions of normality and homogeneity of variance were fulfilled, and where this deviated, a square root transformation was implemented for the continuous data,

which corrected any issues. Upon a significant result from the model, Tukey HSD was used for pairwise comparisons. For all data analysis, R Software was used (R Core Team 2024) with $\alpha = 0.05$. To generate the figures, *ggplot2* was used (Wickham 2016).

To understand whether there was a significant relationship between microbial growth (or richness) and time, dates were converted into Julian dates, and linearly regressed against microbial growth (grayscale value) and mean microbial morphospecies richness per date.

In addition, to understand whether maximum or minimum temperature in the week preceding collection significantly affected microbial growth in the laboratory, these variables were regressed using a nonlinear, exponential equation against the grayscale value and microbial morphospecies richness in 2022 and 2023. Separate models were created for each response variable and year. This involved the functions *nls* to calculate coefficients from the base R package and *ggplot2* to plot fitted lines. The formula used was $I = a \times e^{(b \times x)}$, where I is the predicted value of microbial growth, a and b are empirically derived coefficients from the data, and x represents maximum or minimum temperature. Tables for statistics were created using the *gt* (Iannone et al. 2024) and *gtExtras* package (Mock 2024).

Table 1. Statistical results of general linear model for microbial growth and species richness from field-collected *S. oryzae* dispersing into factitious, novel food patches

	Grayscale value			Morphospecies richness		
	df[a]	*F*	*P*	df[a]	*F*	*P*
2022						
timeinpatch	1	22.1	0.0001	1	2.21	0.2
date	18	11.1	0.0001	18	7.28	0.0001
timeinpatch:date	18	0.589	0.9	18	1.06	0.4
2023						
timeinpatch	1	16.3	0.0001	1	11.4	0.0001
date	24	5.94	0.0001	24	3.55	0.0001
timeinpatch:date	24	0.441	1	24	0.742	0.8

[a]Residual df for 2022 = 151, and for 2023 = 198.

Results

Microbial Growth Over Season From Field-Collected *S. oryzae*

Overall, there was a significant effect on microbial growth by the duration that *S. oryzae* foraged in a novel food patch (Table 1; Fig. 1). In particular, there was 1.4 to 1.6 more microbial growth in patches at 5 d compared to 3 d in 2023 and 2022, respectively (Fig. 1). In addition, date significantly affected the growth of microbes in novel food patches after the introduction of *S. oryzae* (Table 1). For example, in both 2022 and 2023, the greatest microbial growth occurred on *S. oryzae* collected during the time of wheat harvest and maize harvest from the field (Figs. 2–4). The lowest microbial growth was at the beginning of the season (Figs. 3 and 4; Supplementary Fig. 1). There was 2.1- to 316-fold more microbial growth during the wheat harvest, and 1.3- to 241-fold more microbial growth

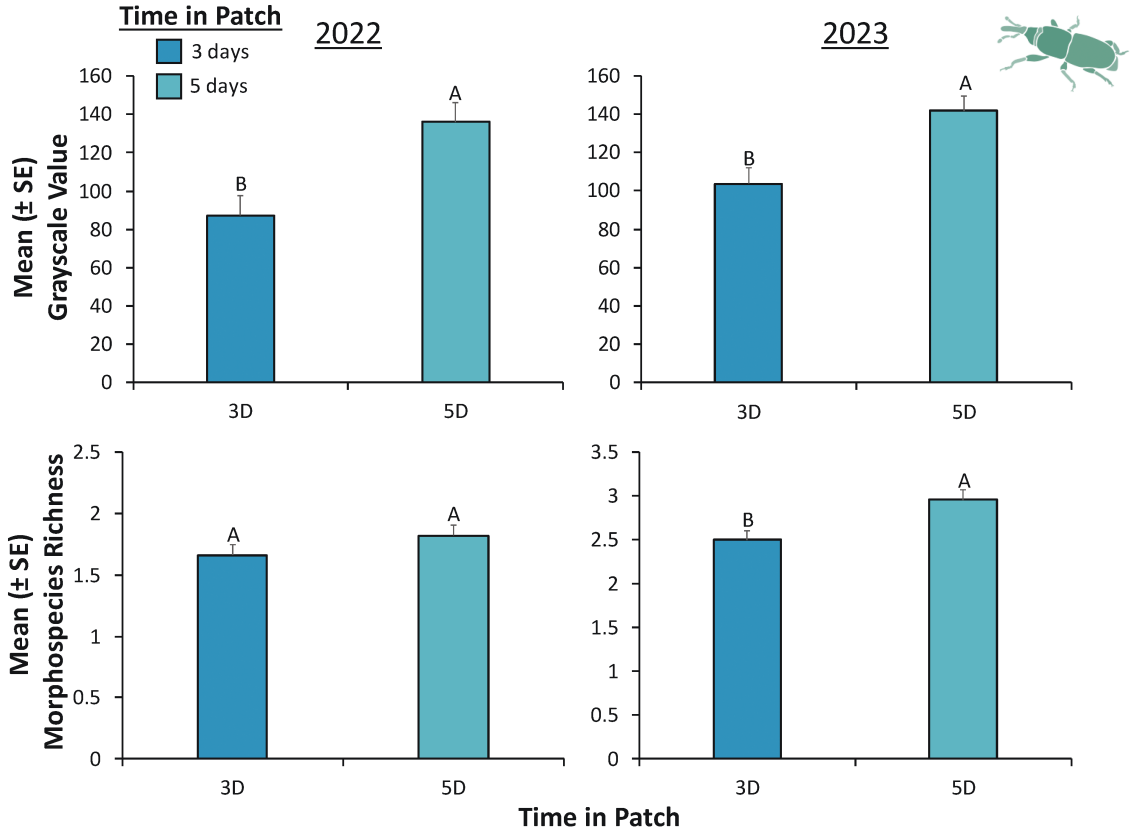


Fig. 1. Overall microbial growth (grayscale value ± SE, top panels) and microbial morphospecies richness (mean ± SE) in novel, factitious food patches after 3 and 5 d by field-collected *S. oryzae* from the Kansas State University Agronomy Farm in 2022 (right panels) and 2023 (left panels). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).

during the maize harvest compared to the beginning of the season in both years. Generally, there was not a significant linear relationship between microbial growth and Julian date over the course of the season (Supplementary Table 1; Supplementary Fig. 2). There was no significant interaction between date and foraging time in patch on microbial growth, as *S. oryzae* evaluated at 5 d had consistently greater microbial growth throughout the season in 2022 and 2023.

Microbial Species Richness Over Season From Field-Collected *S. oryzae*

The time spent foraging in a novel food patch by *S. oryzae* did not have a significant effect on microbial morphospecies richness in 2022, although it did affect morphospecies richness in 2023 (Table 1; Fig. 1). In 2023, there were 1.2-fold more morphospecies in novel foraging patches after the introduction of *S. oryzae* at 5 d compared

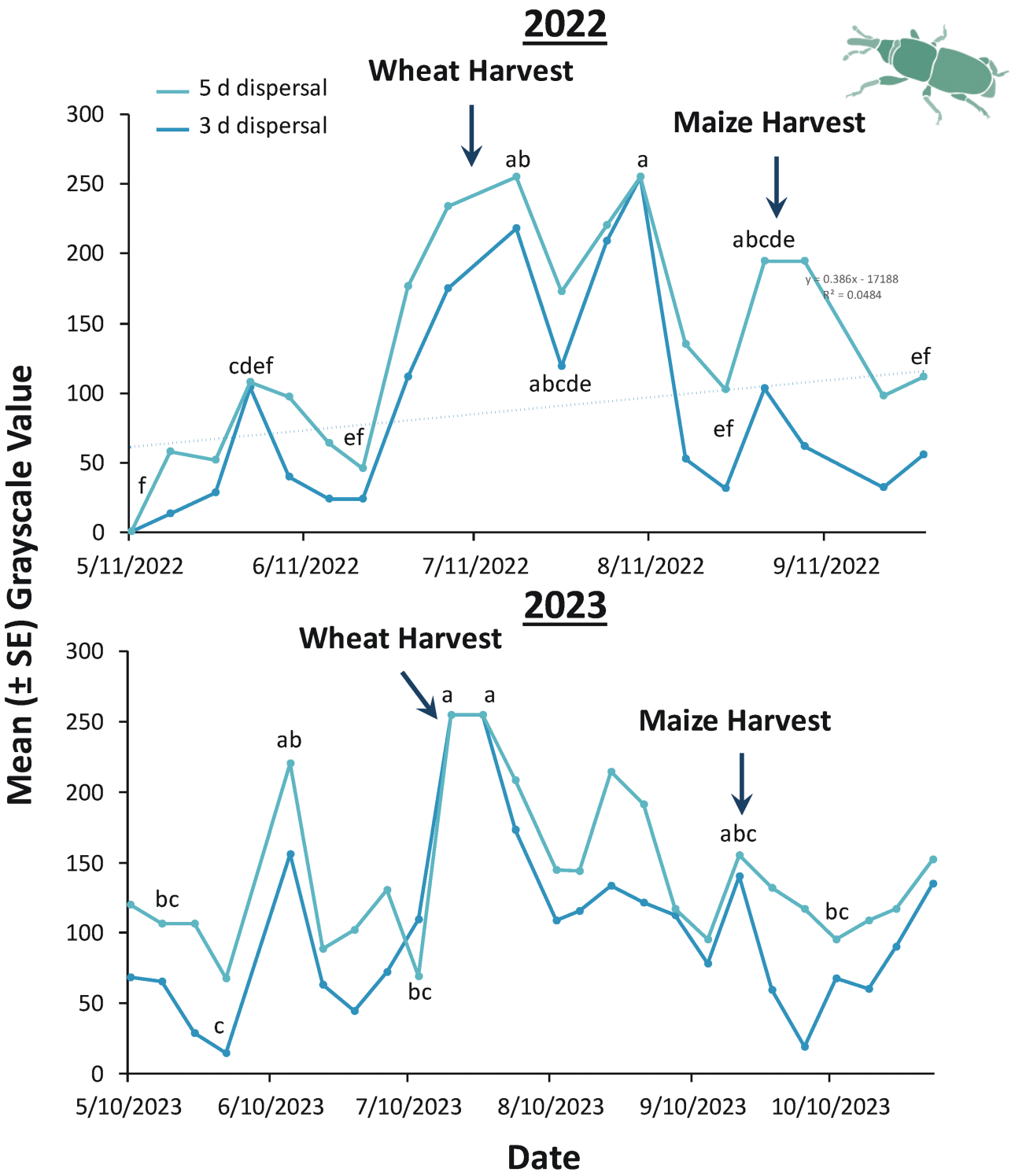


Fig. 2. Microbial growth (mean grayscale value ± SE) in novel, factitious food patches after 3 and 5 d by field-collected *S. oryzae* from the Kansas State University Agronomy Farm over the course of the growing season in 2022 (top panel) and 2023 (bottom panel). Dates with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).

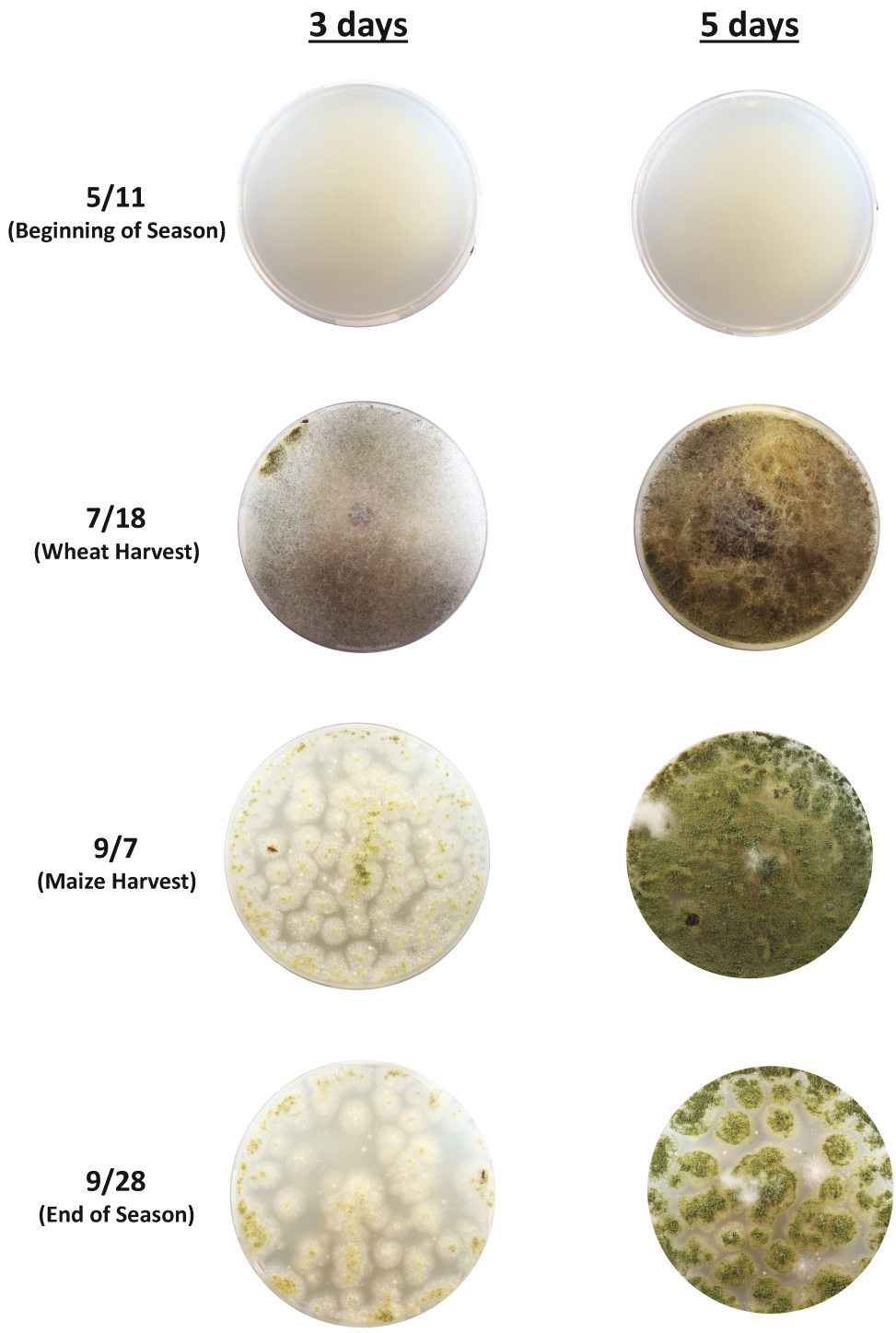


Fig. 3. Representative habitus images of microbial growth from *S. oryzae* introduced to factitious novel food patches composed of potato dextrose agar over the course of the season (5/11 to 9/28) in 2022 with snapshots at the beginning of the season, after wheat harvest, after maize harvest, and the end of the season.

to 3 d (Fig. 1). Date significantly affected microbial morphospecies richness in both 2022 and 2023. Especially in 2022, there was a consistent buildup of microbial morphospecies over the course of the season, with the lowest number of mean morphospecies on a date (0.2) at the beginning of the season and the highest mean number (2.2) at the end of the season, comprising an 11-fold increase (Fig. 5). To a lesser extent, the same pattern held in 2023 though with more variability. The lowest mean morphospecies richness (1.2) was at the beginning of the season in 2023, while the highest mean morphospecies richness was at the end of the season (4), comprising a 3.3-fold increase. Indeed, there was generally a positive linear relationship between microbial morphospecies richness and Julian date in both 2022 and 2023 (Supplementary Table 1; Supplementary Fig. 2). Some dates may have had similar morphospecies richness, but there was similar (or dissimilar) species composition, depending on time in the season (Fig. 6).

Relationship Between Temperature and Microbial Growth or Richness Over Season From Field-Collected *S. oryzae*

There was a positive exponential relationship between the maximum and minimum temperature preceding collection of *S. oryzae* in the field and microbial growth in both 2022 and 2023 (Table 2; Figs. 7 and 8). This was true for a weekly maximum temperature range of 20 to 35 °C and a minimum temperature range of 10 to 22.5 °C (Fig. 7). Likewise, there was a positive exponential relationship between the weekly maximum temperature preceding collection of *S. oryzae* in 2022, but not the weekly minimum temperature (Table 2; Fig. 7). However, because the relationship between weekly minimum temperature and microbial richness appeared to be linear (Fig. 7), we re-analyzed with a linear regression and found a significant effect of minimum temperature on microbial richness in 2022 by *S. oryzae* in novel food patches ($F_{1,36} = 7.54$; $R^2 = 0.36$; $P < 0.0001$;

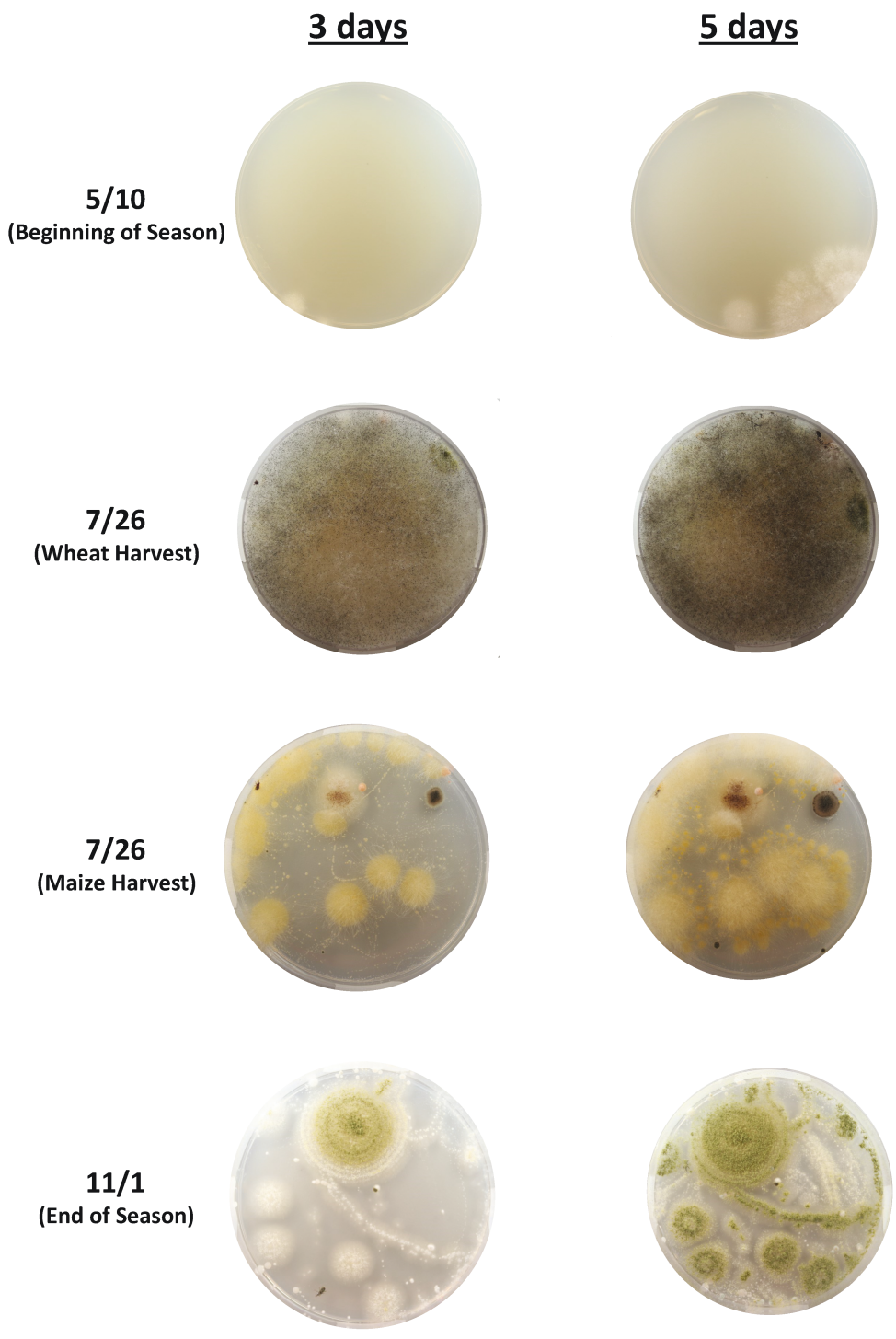


Fig. 4. Representative habitus images of microbial growth from *S. oryzae* introduced to factitious novel food patches composed of potato dextrose agar over the course of the season (5/10 to 11/1) in 2023 with snapshots at the beginning of the season, after wheat harvest, after maize harvest, and the end of the season.

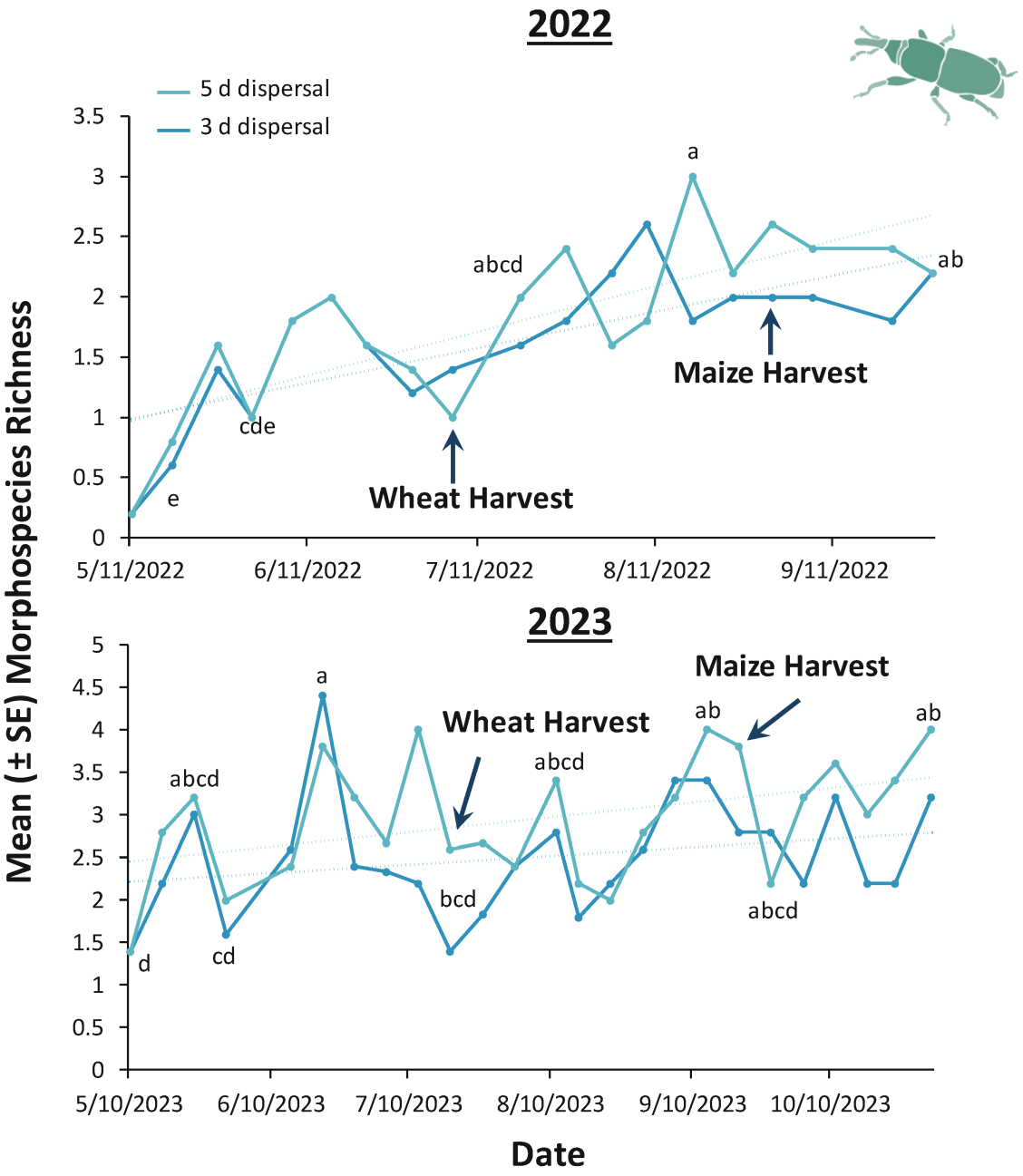


Fig. 5. Microbial morphospecies richness (±SE) in novel, factitious food patches after 3 and 5 d by field-collected *S. oryzae* from the Kansas State University Agronomy Farm over the course of the growing season in 2022 (top panel) and 2023 (bottom panel). Dates with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).

Supplementary Fig. 3). By contrast, in 2023, there was not a significant effect of maximum temperature on microbial morphospecies richness, but minimum temperature significantly affected richness (Fig. 8).

Discussion

We evaluated the seasonal dynamics (eg microbial growth and morphospecies richness) of field-collected microbes after the dispersal of *S. oryzae* to novel food patches. We found that microbial growth spiked during the harvest periods for wheat and maize in storage. This may suggest that crops colonized by various fungi in the field have a significant effect on what grows and is spread by insects in grain storage environments. It also delineates high-risk periods for microbial growth in bulk storage, which coincides with the harvest of major agronomic crops in the area. Prior work has described how the harvest of crops can result in profound changes in the microbiota of these commodities in storage, but *Aspergillus* and *Penicillium* may persist in storage conditions, especially when the crop is dry (Lacey 1989). We have taken this a step further, and shown that the harvest of crops may be linked to the microbial community that may be harbored on and spread by the insect community.

We found *S. oryzae* consistently vector fungi throughout the season (there was not a single date or plate without fungi after introduction of individuals). Prior work has speculated that *Sitophilus* spp. promotes the growth of storage fungi in whole grain via a combination of inoculation during oviposition and by larval metabolic and feeding activities (Dunkel 1988). However, on these

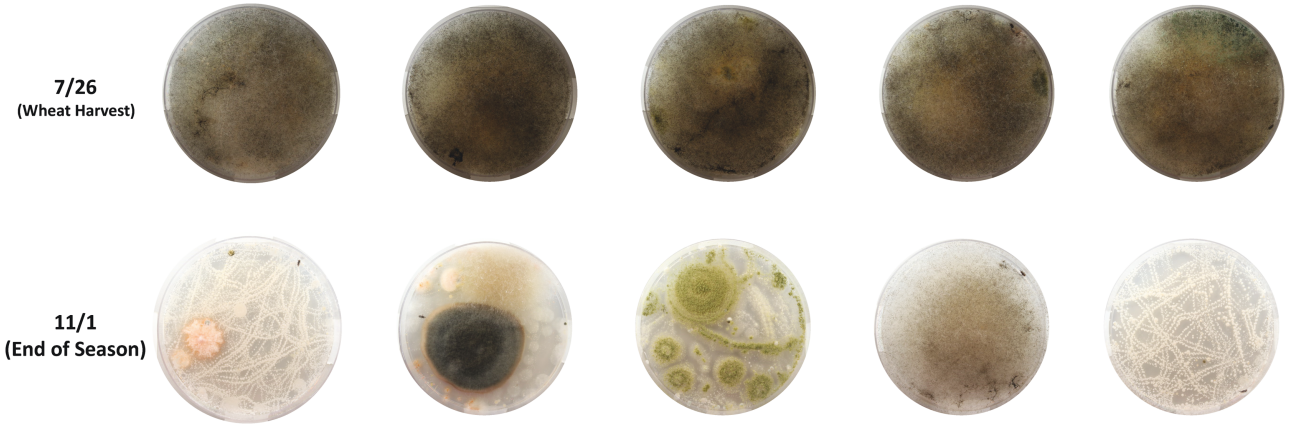


Fig. 6. Representative habitus images of morphospecies richness from *S. oryzae* introduced to factitious novel food patches composed of potato dextrose agar from 7/26 and 11/1 in 2023 exhibiting the same community composition or diverse community composition among individuals.

Table 2. Nonlinear regression analysis for microbial growth and species richness from field-collected *S. oryzae* dispersing into factitious, novel food patches against the mean maximum or minimum temperature in the week preceding collection

	Grayscale value						Morphospecies richness					
Model	*a*	*b*	df	*F*	*P*	R^2	*a*	*b*	df	*F*	*P*	R^2
2022												
Max	12.4	0.0722	36	4.13	0.05	0.103	0.225	0.0665	36	18.7	0.00012	0.342
Min	16	0.108	34	6.95	0.013	0.17	1.08	0.0275	34	2.21	0.15	0.061
2023												
Max	42.6	0.0346	48	4.13	0.048	0.0793	3.72	−0.0108	48	2.67	0.11	0.0528
Min	51.8	0.0516	48	7.64	0.0081	0.137	3.48	−0.0162	48	5.07	0.029	0.0955

The exponential function was used, $I = a * e^{b * \max (\text{or } \min)}$, where I is microbial growth or species richness, a and b are fitted empirically from data, and max/min is the temperature in the week preceding collection.

agar plates (factitious food patches) there were neither oviposition sites, nor larvae or feeding sites. Thus, we may conclude that the spread of fungi in this study was the result of either adult excreting processes or by contact of tarsi or other parts of the body with the substrate. Future work should link these behavioral processes with the resulting fungal community.

Further, we found that there was greater microbial growth after 5 d compared with 3 d. This is likely from microbes having more time to grow at suitable temperatures in the chamber. Ponce et al. (2024) and Quellhorst et al. (unpublished data) found similar patterns in microbial growth from 3 to 5 d on agar plates after the introduction of *S. oryzae*, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), and *S. zeamais* with the greatest growth present at 5 d. Microbial growth was found to be fit well with a polynomial equation using growth curve data, provided the lag phase was excluded (Ram et al. 2019). We also found slightly greater morphospecies richness at 5 d compared to 3 d. It is possible some microbes could be slow-growing species; thus, they would not have been apparent until more time had passed. In any case, taking images at multiple time points was useful in determining microbial growth as well as morphospecies richness.

In addition, we have found that the microbial morphospecies richness introduced by *S. oryzae* into novel food patches increases over the course of the season. The average lifespan for male and female *S. oryzae* is 60 to 77 d on rice (Kaundal et al. 2023). Thus, because this is a long-lived species as adults, it is logical that fungi would build up on the cuticle of *S. oryzae* as they navigate their external environment and the internal environment in bulk storage, especially with periodic harvests of major crops. This may be why we found that the highest number of morphospecies from the dispersal of *S. oryzae* to a novel food patch occurred at the end of the season. Prior work allowing *S. oryzae* to disperse to novel food patches suggests that most of these fungi belong to *Aspergillus*, *Penicillium*, and *Fusarium* spp. (Ponce et al. 2024). In addition, other work has found *T. castaneum* harbor the pathogenic bacteria, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli*, *Enterobacter* spp., and pathogenic fungi, *A. flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium* spp., *Fusarium* spp., and *Rhizopus oryzae* (Kumari et al. 2011). Moreover, stored-product insects were found to also be associated with antibiotic-resistant and potentially virulent enterococci (Lakshmikantha et al. 2006). However, future work should use high-throughput sequencing to identify which fungi were present over the course of the season.

While it is not surprising that increasing temperatures resulted in increased microbial growth (Magan and Lacey 1988), it is surprising that higher temperatures in the field in the week preceding collection of insects could be linked to higher microbial growth in the laboratory. This was true both for the weekly maximum and minimum temperature. Generally, when crops are harvested and placed into storage, they are very warm (Arthur et al. 2020), which can result in the proliferation of storage microbes. While grain aeration may be employed to cool the grain mass even in warmer climates (Morrison et al. 2022), this was not in use by the food facility in the current study. Hot spots of microflora and insects may form after harvest, resulting in elevating the temperature of the grain to between 14 to 34 °C, but in extreme cases, the temperature

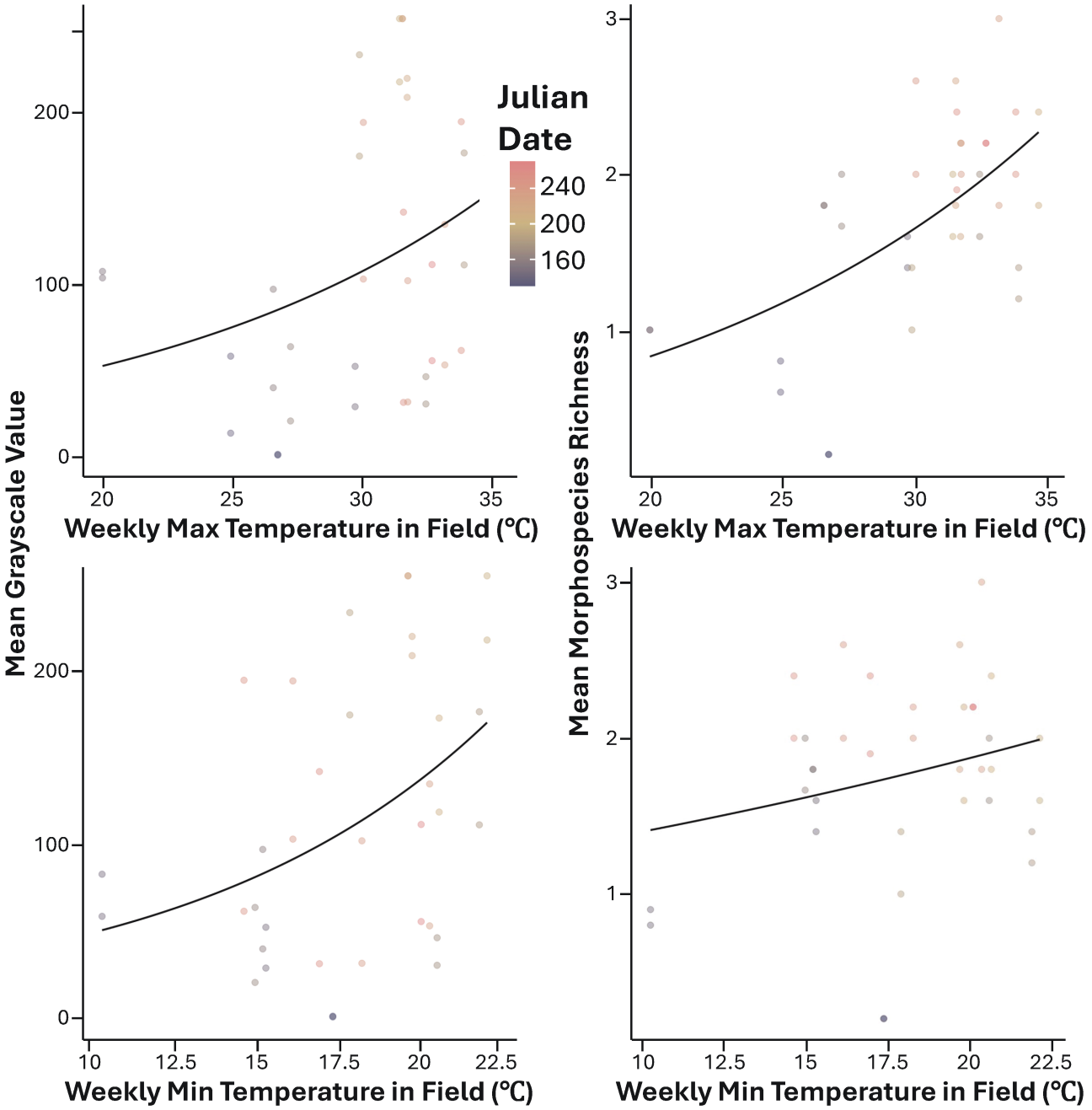


Fig. 7. Microbial growth (grayscale value ± SE, left panels) and microbial morphospecies richness (±SE, right panels) from 2022 regressed against the mean maximum temperature (top panels) or mean minimum temperature (bottom panels) in the week preceding collection of *S. oryzae* in the field at the Kansas State University Agronomy Farm. Samples are colored according to Julian date, with cool colors representing collections earlier in the year, and warmer colors representing dates later in the year. The formula used was $I = a \times e^{(b \times x)}$, where I is the predicted value of microbial growth or morphospecies richness, a and b are empirically derived coefficients from the data, and x represents maximum or minimum temperature. Coefficients can be found in Table 2.

in the grain can reach up to 400 °C (Jian and Jayas 2012a), especially in soybeans and faba beans (Muir 2000). The proliferation of microbes in grain during these key points in the season may result in fungi already germinating and/or present in higher abundances, which translated to noticeable bumps in microbial growth after the introduction of *S. oryzae* into novel food patches during these key points in the season. Importantly, over a 24 h to 72 h period of increasing dispersal opportunity, there was no appreciable decrease in the microbial growth in novel food patches after the introduction of *S. oryzae* (Ponce et al. 2024). This suggests that higher temperatures carry an increased food safety risk from the subsequent activity of insects.

Temperature effects are even more important when factoring climate change into the equation. Even in buffered environments, climate change is expected to result in more insect generations per year and elevated temperatures for longer periods of the year (Gerken and Morrison 2022). Practically, this may mean that there are higher maximum and minimum temperatures for a greater period of time in food facilities, extending the risk window for immigrating stored-product insects that may bring microflora along with them. This may

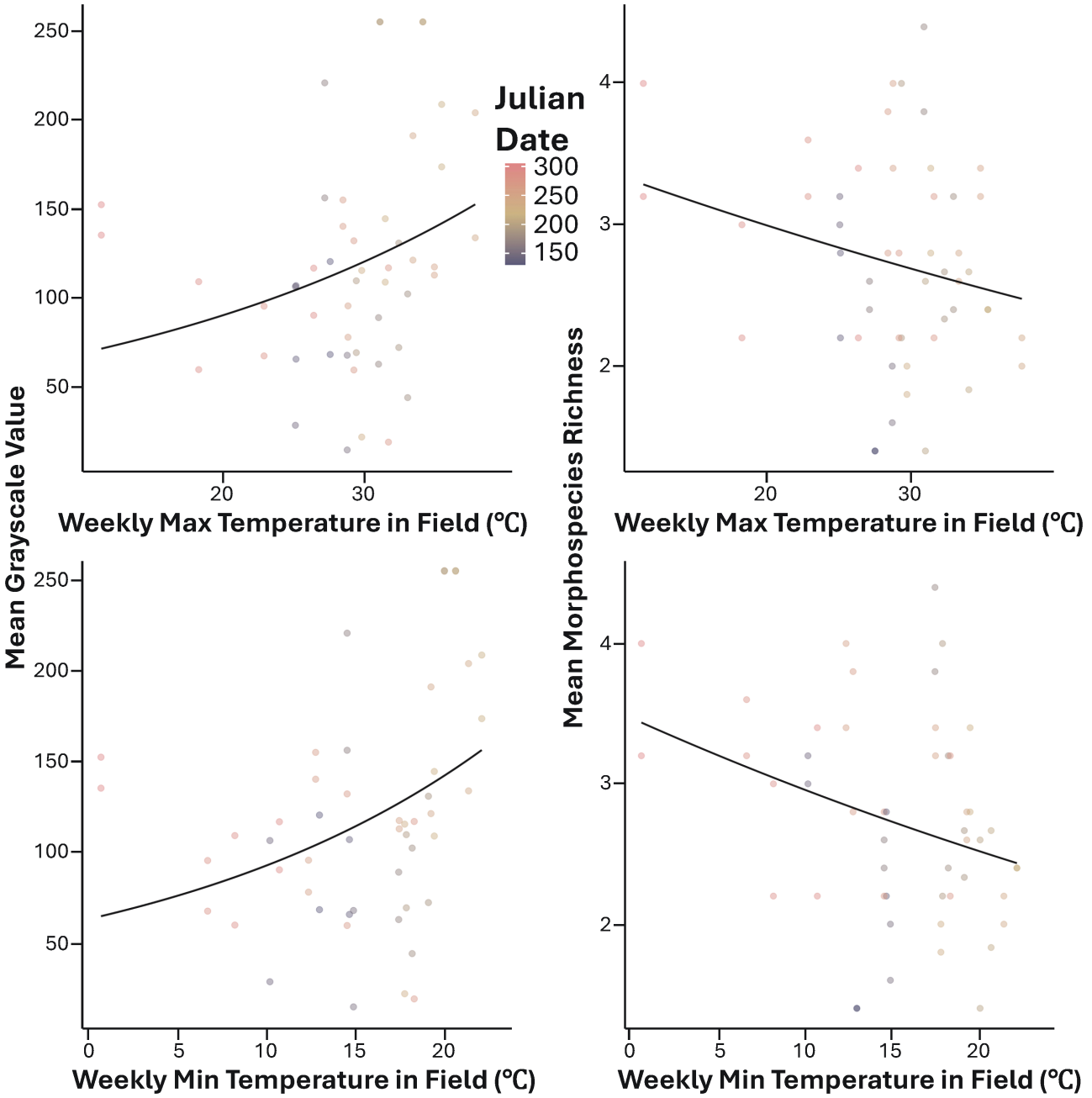


Fig. 8. Microbial growth (grayscale value ± SE, left panels) and microbial morphospecies richness (±SE, right panels) from 2023 regressed against the mean maximum temperature (top panels) or mean minimum temperature (bottom panels) in the week preceding collection of *S. oryzae* in the field at the Kansas State University Agronomy Farm. Samples are colored according to Julian date, with cool colors representing collections earlier in the year, and warmer colors representing dates later in the year. The formula used was $I = a \times e^{(b \times x)}$, where I is the predicted value of microbial growth or morphospecies richness, a and b are empirically derived coefficients from the data, and x represents maximum or minimum temperature. Coefficients can be found in Table 2.

be compounded by higher grain temperatures, which may serve as perfect incubators for microbial germination. Generally, when grain is harvested, it is warm in the summer, so warm grain is transferred to a silo (Jian and Jayas 2012b), but then there is a gradual decrease in grain bulk temperature, which is commonly known as hysteresis (eg Yao et al. 2020). During a season, this likely means that microbial growth may peak shortly after harvest, as we witnessed in this study even for the insects from the grain. As the climate warms, this initial warmth may be even greater, thus exacerbating microbial growth. Thus, the interaction between insects like *S. oryzae*, and the microflora in the environment becomes even more important, and will continue to increase going forward.

A limitation of this study is the fact that we did not sequence and/or identify the specific fungi vectored by *S. oryzae*. However, this was beyond the scope of the current study given how many samples it would have produced. Nonetheless, we know from prior work using this same potato dextrose method at these 2 time points that *Sitophilus* spp. likely vectors *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor* sp., and *Rhizopus* (Ponce et al. 2022, 2024, Quellhorst 2023, Quellhorst et al. unpublished data). Other studies have also described *Sitophilus* spp. as vectoring *A. flavus* (Barry et al. 1985), *Penicillium* spp. (Dix and All 1987), but also the human pathogens *Escherichia*, *Serratia*, *Streptococcus*, *Bacillus*, *Klebsiella*, and *Salmonella* (Husted et al. 1969, Crumrine et al. 1971). In the future, we would like to

look at the community composition of microbes on the cuticle of *S. oryzae* through time.

Several other studies have clearly demonstrated that fungi and bacteria of concern are associated with *T. castaneum*, *Tyrophagus putrescentiae*, *L. serricorne*, and various other stored-product insects (Lakshmikantha et al. 2006, Sumino et al. 2010, Kumari et al. 2011, Erban et al. 2016, Ponce et al. 2021, 2022). Future work should be invested toward examining (i) whether and how the microbial community from other stored-product insects fluctuates in time, (ii) evaluate the vectoring capacity of other stored-product insects for which there is little information, and (iii) perform manipulative experiments examining a range of temperatures on microbial vectoring capacity by stored-product insects. Overall, our study has demonstrated the importance of the temporal component to the vectoring capacity of *S. oryzae*, which is worth considering for other species.

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Author contributions

William Morrison (Conceptualization [lead], Data curation [equal], Formal analysis [lead], Funding acquisition [lead], Project administration [lead], Resources [lead], Supervision [lead], Visualization [lead], Writing—original draft [lead], Writing—review & editing [equal]), Marco Ponce (Conceptualization [lead], Data curation [equal], Investigation [lead], Methodology [lead], Writing—review & editing [equal]), Joseph Castaldi (Data curation [equal], Investigation [equal], Methodology [equal], Writing—review & editing [equal]), Avery James (Data curation [equal], Investigation [equal], Methodology [equal], Writing—review & editing [equal]), Jenna Moreland (Data curation [supporting], Investigation [supporting], Writing—review & editing [supporting]), Ian Stoll (Data curation [equal], Investigation [equal], Methodology [equal], Writing—review & editing [supporting]), Jennifer Abshire (Data curation [equal], Investigation [equal], Methodology [equal], Project administration [supporting], Writing—original draft [supporting], Writing—review & editing [equal]), Tania Kim (Funding acquisition [supporting], Methodology [supporting], Project administration [equal], Resources [supporting], Supervision [equal], Writing—review & editing [equal]), and Alison Gerken (Conceptualization [supporting], Investigation [supporting], Methodology [supporting], Writing—review & editing [equal])

Supplementary material

Supplementary material is available at *Environmental Entomology* online.

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Conflicts of interest. None declared.

Data availability

Data has been deposited per USDA regulations as follows: Morrison, III William; Ponce, Marco A.; Castaldi, Joseph; James, Avery; Stoll, Ian; Moreland, Jenna; Abshire, Jennifer; Kim, Tania N.; Gerken, Alison R. (2024). Data from: Season-long microbial dynamics from the cuticle of rice weevil originating at food facilities after dispersal to novel food patches. Ag Data Commons. Dataset. https://doi.org/10.15482/USDA.ADC/27276897.v1

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