Environmental Entomology, 49(1), 2020, 255–264
doi: 10.1093/ee/nvz151
Advance Access Publication Date: 18 December 2019
Research

Population Ecology

Temperature-Mediated Competition Between the Invasive Larger Grain Borer (Coleoptera: Bostrichidae) and the Cosmopolitan Maize Weevil (Coleoptera: Curculionidae)

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Subject Editor: Rodrigo Mercader

Received 22 August 2019; Editorial decision 29 November 2019

Abstract

Interspecific competition between agricultural pests may affect the species that can establish, and may also affect food production. *Prostephanus truncatus* (Horn), the larger grain borer, is endemic to Central America, but invaded Africa with disastrous consequences for maize production. Its main competitor is *Sitophilus zeamais* Motschulsky, the maize weevil, which is cosmopolitan. These insects co-occur in many regions of the world and both are threats to maize. However, the impact of competition between these two species is not well-understood, nor is its effect on grain quality or potential to limit *P. truncatus* invasion in new areas. The aims of our study were to evaluate the outcome of interspecific competition between *P. truncatus* and *S. zeamais* at four different temperatures on a fixed quantity of grain, and determine effects on progeny production, grain damage, and mold growth. We found that coexistence may be possible at a range of 25–30°C, but mixed colonies experienced a direct competitive cost compared to single-species colonies. *Prostephanus truncatus* performed better at warmer temperatures, while *S. zeamais* favored cooler temperatures. The majority of grain damage was the result of *P. truncatus* activity as opposed to *S. zeamais*. Finally, mold growth was greater where both species were present, and species of mold that produce aflatoxin were identified. Although there are an increasing number of areas where both of these species occur, our results suggest *P. truncatus* will be capable of destroying much more maize in a shorter period compared to *S. zeamais* at temperatures greater than 25°C.

Key words: interspecific competition, grain quality, development, *Sitophilus zeamais*, *Prostephanus truncatus*

In the developing world, one of the most important commodities grown for both human and animal consumption is maize (Badu-Apraku and Fakorede 2017). Together with wheat and rice, it provides 30% of the calories to more than 4.5 billion people in 94 developing countries (Shiferaw et al. 2011). However, in tropical regions of the world, especially in Africa, post-harvest losses of maize may exceed 40–70% of the entire crop produced by small stakeholders (Affognon et al. 2015), while in 2018 average total dry weight losses for much of Africa hovered at 17% (APHLIS 2019). These losses may be exacerbated by new threats posed by invasive stored product insects and climate change.

One such invasive species affecting maize production in Africa is the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). This species is endemic to Mexico and Central America, but was accidentally introduced into Tanzania in the late 1970s (Hodges et al. 1983) and subsequently quickly spread throughout Africa (Holst and Meikle 2003). As it spread, it adapted to feeding on cassava as an alternate host (Mushi 1983), which further facilitated its range expansion. Golob and Hodges (1982) recorded weight losses up to 30% for farm-stored maize, which was unprecedented compared to damage caused by other storage insects in such dry conditions. More generally, *P. truncatus* causes a mean estimated loss of between 7 and 41% of maize stored for 3 to 9 mo (Pantenius 1987, Keil 1988, Wright et al. 1993). At the same time, this species has been recorded as a wood borer in sub-Saharan forests, which seems to contribute further to its spread in the landscape (Borgemeister et al. 1998).

Before the arrival of *P. truncatus*, the major pest of maize in Africa was the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *Sitophilus zeamais* has been one of the

most economically damaging pests in African maize storage after harvest (Makundi et al. 2010). Both *P. truncatus* and *S. zeamais* are primary pests on maize, and develop internally (Hagstrum and Subramanyam 2006). *Sitophilus zeamais* co-occurs in many of the same places as *P. truncatus*, including throughout much of tropical Central and South America, as well as in Africa. However, it is generally more cosmopolitan, with a foothold in more temperate latitudes, because it can tolerate colder temperatures (Yakubu et al. 2011). It is likely that *S. zeamais* originated in Asia, but quickly adapted to maize once it gained access to maize from Mesoamerica (Corrêa et al. 2017). The crop losses attributable to *S. zeamais* have been estimated to reach 5% per year in Africa (Tyler and Boxall 1984).

In surveys on-farm, prior work has found that *P. truncatus* and *S. zeamais* will almost always occur in the same samples. For example, a case study performed in the Republic of Togo (W. Africa) found both species in surveys, but found that maize producers were most concerned about *P. truncatus* (Adda et al. 2002). Likewise, in a survey of maize stores in Benin, researchers found that most maize ears with *P. truncatus* also contained at least a few *S. zeamais* (Vowotor et al. 2005). Thus, it is likely that both species are competing for the same food sources in post-harvest environments.

Temperature has been previously shown to be an important abiotic factor affecting the outcome of competition among other stored product insects. For example, *Cryptolestes ferrungineus* (Stephens) (Coleoptera: Laemophloeidae) was found to be the superior competitor over *Cryptolestes pusillus* (Schönherr) (Coleoptera: Laemophloeidae) at 35 and 30°C but not at lower temperatures (White et al. 1995). Odeyemi (1997) found that the intensity of competition between *Dermestes maculatus* De Geer (Coleoptera: Dermestidae) and *Necrobia rufipes* (F.) (Coleoptera: Cleridae) was dependent on temperature, with exclusion of *N. rufipes* at lower temperatures and coexistence at higher ones. Prior work has determined that growth at 32°C and 80% RH is optimal for *P. truncatus* (Shires 1979), while 27°C and 40% RH are optimal for *Sitophilus* spp. (Longstaff 1981). This suggests that both *P. truncatus* and *S. zeamais* may co-exist under the same environmental conditions, but that one or the other may be favored at certain temperatures.

The effect of climate change on the post-harvest environment is often overlooked relative to its effect on pests in field and specialty crops (Moses et al. 2015). Direct effects of climate change may include changes in the growth and developmental cycles of pests, as well as their interactions with each other (Moses et al. 2015). Further, as the climate shifts, changes in temperature, humidity, rainfall, extreme weather, invasive species and other variables may require adaptation in the management of commodities in the post-harvest supply chain. However, some regions of the world may have limited ability to effectively combat shocks to existing management systems, meaning that adaptation strategies that work well in one area may fail or not be appropriate in another (Stathers et al. 2013). Management actions taken in a system may have unintended consequences such as favoring one post-harvest species over another, as *P. truncatus* and *S. zeamais* have been shown to respond dissimilarly to the same insecticide (e.g., Hodges 1986).

To adequately address the risks posed by climate change, new knowledge about how temperature impacts post-harvest pests is required. The geographic distributions for a large variety of insects have been shifting polewards, and dispersal may be enhanced (Parmesan et al. 1999, Walther et al. 2002, Robinet and Roques 2010). For post-harvest systems, as the temperature increases, the geographic distributions of *P. truncatus* and *S. zeamais* may also change to include more temperate areas, as well as tropical regions of Asia, and additional areas of South America in the future (Arthur et al. 2019). As new *P. truncatus* populations become established, they are likely to encounter endemic *S. zeamais* in post-harvest food facilities. Indeed, prior work has found that although *Carpoglyphus lactis* (L.) (Acari: Astigmata) and *Sitophilus orzyae* (L.) (Coleoptera: Cucurlionidae) were historically pests of Southern Europe, they have moved northward to become problems for Middle Europe, which may possibly be linked to climate change (Stejskal et al. 2015).

Importantly, the presence of local competitors is a crucial parameter that heavily affects containment and establishment of invasive species. Under different temperature regimes, the outcome of competition between *P. truncatus* and *S. zeamais* may radically differ, with varying outcomes for grain quality and loss, and therefore stored product protection. In particular, the presence of both species may alter population dynamics for each and require different approaches for management. Given the lower optimum temperature for *S. zeamais* than *P. truncatus*, we expect *P. truncatus* to perform relatively better at higher temperatures. In addition, the presence of one or both species may alter the microbial community associated with grain by creating microhabitats conducive to the growth of different molds. As a consequence, the aims of our study were to evaluate the outcome of interspecific competition between *P. truncatus* and *S. zeamais* at different temperatures, and determine the repercussions for progeny production, grain damage, and presence of mold.

Materials and Methods

Insects

Adult *P. truncatus* and *S. zeamais* were obtained from insect colonies kept at the United States Department of Agriculture (USDA) Center for Grain and Animal Health Research facility in Manhattan, KS. Both insects were reared on whole kernels of maize and held in incubators under constant conditions at 27 ± 0.1°C (mean ± SE), 64 ± 1% RH, and 14:10 (L:D) h photoperiod for *S. zeamais* and at 27.2 ± 0.1°C, 37 ± 1% RH, and continuous darkness for *P. truncatus*.

Commodities

Untreated, clean, and uninfested yellow maize was used in the experiments. The moisture content of the grain, as determined by a moisture meter (GAC 2100, Dickey-John Corps, Auburn, IL), was approximately 16% for all treatments.

Competition Experiment

Plastic cylindrical vials (11 × 5 cm H:D) filled with 50 g of maize were used for the experiments. The treatments placed separately in each vial were: 1) 10 *P. truncatus* adults alone, 2) 10 *S. zeamais* adults alone, or 3) 10 adults of each species together. Although it is impossible to sex adults of these species without dissection, only healthy, actively moving adults were chosen for each replicate. A total of $n = 9$ replicate vials were created per treatment and temperature combination. The sets of vials containing all treatments were placed in environmental chambers at one of four temperatures, i.e., 20, 25, 30, and 35°C. In all temperatures, the RH level was 65%, and all chambers were programmed to continuous darkness to simulate a grain bin or the interior of a grain storage bag. This range of temperatures was chosen because the optimum temperature range for most stored product insects is 25–35°C (Fields 1992), while the lower developmental threshold for many stored product insects is around 18°C (Howe 1965). The vials were held together in plastic boxes with lids (38 × 26.5 × 14.5 cm, L:W:H) at the four different temperatures for a period of 65 d. At the end of the period, the vials were frozen at −4°C until they could be examined.

X-Ray Confirmation of Progeny

To assess the accuracy of progeny counts and account for the ability of these species to hide in grain, a total of 10 maize kernels were randomly subsampled for hidden *P. truncatus* and *S. zeamais* progeny (adults, pupae, larvae) by soft X-rays at 5× magnification (MX-20, Faxitron, Wheeling, IL). The images were digitally acquired using a computer and camera (BIOP_512X1024, Bioptics, Inc., Tucson, AZ) connected to the machine, and controlled with the software Vision (v. 2.2.5, Faxitron LLC, Wheeling, IL). The number of adults, pupae, and larvae in each kernel was recorded.

Insect Damage

After being held, the vials were opened and grain samples were evaluated for progeny production (e.g., number of adults), number of insect-damaged kernels (IDK; e.g., visually inspecting all grain in a vial), weight of IDK, number of undamaged kernels, weight of undamaged kernels, number of grains with more than 1 exit/entrance hole (e.g., also including grains with more than one area of chewing damage), and the weight of frass. To separate the progeny and frass from the maize, a 1.41 × 1.41 mm sieve placed on top of a 2 × 2 mm sieve was used in combination with agitation. Afterward, the frass, damaged, and undamaged kernels were weighed using a precision balance (LP 620P, Satorius AG, Göttingen, Germany). Progeny was defined as any adults present in the vials beyond the initial 10 insects at the conclusion of the experiment (e.g., original numbers of adults were subtracted from final progeny counts). In addition, though the maize used for this experiment was insect-free and kept in cold storage prior to experimentation, some kernels were chipped or broken in places, and thus the source grain was evaluated for the above grain quality measures to determine background rates of insect-mimicking damage.

Mold Damage

At the end of the competition experiment, after the grain had been placed into the freezer, but prior to sorting, we also evaluated mold growth by rating the grain in a vial on a scale of 1–4. The scale is defined as 1) no mold, 2) spotty, infrequent low levels of mold in sample, 3) limited areas of high mold infestation, 4) widespread and concentrated presence of mold throughout the sample. A level 4 infestation indicated that mold was visually spotted on nearly every kernel present in the vial and covered the majority of each kernel. We also further confirmed the mold rating as the grain was sorted, looking for visual mold growth on the kernels.

To identify the mold to genus, we used standard keys, and visual inspection of spores and hyphae under a high-powered dissecting microscope at 135 × magnification (SMZ18, Nikon Inc., Chiyoda, Japan). The fungus was tentatively identified using morphological characteristics (https://mycology.adelaide.edu.au/descriptions/hyphomycetes/aspergillus/). Samples appeared to be colonized by the same fungus in every instance, but to confirm, hyphae and spores were taken from two locations in three different vials. A piece of double-sided tape was applied to the mycelium to collect conidia and hyphae. The double-sided tape was then applied to a microscope slide. Conidial heads, size, shape, and roughness were all used to make a tentative identification. A more thorough identification of the same n = 6 samples was provided by sequencing. Fungi were removed from infested corn meal by scrapping visible mold infestations into microcentrifuge tubes and DNA was extracted using the Quick-DNA Fecal/Soil Microbe Miniprep Kit (D6010, Zymo Research, Irvine, CA). The ITS region of the fungal DNA was then amplified using polymerase chain reaction (PCR). Primers consisted of ITS4 5′-TCCTCCGCTTATTGATATGC-3′ and ITS5 5′-GGAAGTAAAAGTCGTAACAAGG-3′ (White et al. 1990). In each reaction, 1 µl of extracted DNA, 1 µl of each primer (10 µM), 9.5 µl of nuclease-free water, and 12.5 µl of master mix containing 50 units/ml of Taq DNA polymerase (Hot Start Taq 2X Master Mix, Promega, Madison, WI) were combined in a proprietary reaction buffer (pH 8.5). Briefly, the PCR program consisted of 2 min of initial denaturation at 95°C, followed by 30 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 1.5 min. Afterward, a final extension at 72°C for 5 min was performed, then PCR products were held chilled at 4°C. To clean up the samples, 5 µl of PCR products were mixed with 2 µl of ExoSAP-it (ThermoFisher Scientific, Waltham, MA), then placed in the thermal cycler at 37°C for 15 min, and ramped to 80°C for a further 15 min. Finally, the amplicons were sent for bidirectional sequencing on an ABI 3730XL instrument (Eurofins Scientific, Brussels, Belgium), and the resulting sequences were quality-filtered and aligned using Sequencher (v. 5.4.6, Gene Codes, Ann Arbor, MI). The consensus sequences were searched against NCBI's nucleotide database (nt) using the BLASTn algorithm (Altschul et al. 1997). In order to circumvent taxonomic misassignments, the consensus sequences were also checked against Michigan State's Ribosomal Database Project (RDP) that searches the UNITE Database (Wang et al. 2007). The consensus sequences were submitted to GenBank and under the accession numbers MN274948–MN274959.

Statistical Analysis

All data was log-transformed after inspecting preliminary residuals and performing Levene's test to fulfill the assumptions of normality and homogenous variances. A multivariate analysis of variance (MANOVA) was used to examine differences in progeny for both species (corrected by subtracting the number of adults initially used in experiment), using temperature, treatment, and their interaction as explanatory variables. Upon a significant result, sequential ANOVAs were performed for each response, following the same model form. Upon a significant result from the ANOVA, Tukey HSD was used for multiple comparisons. The same procedure was used for the progeny production determined by X-ray. The damage measures (insect-damaged kernels, weight of IDKs, number of kernels with more than 1 hole, weight of the frass) were analyzed jointly with MANOVA using the same model form as above to control the family-wise error rate. Upon a significant result from the overall model, sequential ANOVAs were performed for each response. If the ANOVA was significant, Tukey HSD was used for multiple comparisons. A Kruskal–Wallis test was used to analyze the main effects of competition treatment and temperature on mold damage ratings of grain. Pairwise comparisons employed χ^2 tests with Bonferroni correction. For the sake of visualization, the weight of the damaged kernels was represented as a percentage of the total grain in the vial (Harris and Lindblad 1978). However, for the analysis, the actual weight of the damaged kernels was used. In addition, percentage grain loss for each replicate vial was calculated by determining the weight per kernel for damaged grain, dividing it by the weight per kernel for undamaged grain, subtracting by one, and multiplying by 100. Grain loss was included as a response variable above and treated in the same manner. Unless otherwise noted, $\alpha = 0.05$, and R Statistical Software was used (R Core Team 2019).

Results

Progeny Production

The species present in the container had a significant effect on the overall number of progeny produced (MANOVA: Wilks Approx.

$F = 94.1$; df = 2, 100; $P < 0.0001$). For the combined species mixtures and *P. truncatus* alone, there were 30–31% more progeny produced, respectively, compared with *S. zeamais* alone. The temperature also had a significant influence on progeny production (Wilks Approx $F = 11.4$; df = 3, 100; $P < 0.0001$), with 4- to 5-fold more progeny produced as 25 and 30°C, respectively, compared with 20°C. Finally, there was a significant two-way interaction between treatment and temperature (Wilks Approx $F = 3.17$; df = 6, 100; $P < 0.001$).

Likewise, the number of *P. truncatus* progeny produced after 65 d was significantly affected by whether there was a competitor (ANOVA: $F = 205$; df = 2, 100; $P < 0.0001$; Fig. 1). In particular, 2.4-fold more *P. truncatus* progeny were produced when only conspecifics were present compared with combined species mixtures. The temperature significantly affected progeny production ($F = 17.8$; df = 3, 100; $P < 0.0001$), with 3.7- to 5.4-fold more progeny production at 25 and 30°C compared to 20°C, respectively. There was also a significant interaction between species mixture and temperature ($F = 2.54$; df = 6, 100; $P < 0.05$). This was likely a quantitative interaction, because *P. truncatus* always produced more progeny when alone compared with combined species mixtures, but the magnitude of the difference depended on temperature, with the greatest differences at 25 and 30°C (Fig. 1, Tukey HSD).

In the same vein, the number of *S. zeamais* progeny produced also varied, depending on whether it was alone or combined with *P. truncatus* ($F = 249$; df = 2, 100; $P < 0.0001$; Fig. 1). For instance, when *S. zeamais* was alone, 31% more progeny were produced at the end of 65 d than when it was in a mixed-species colony. Similarly, temperature also significantly affected progeny production ($F = 6.52$; df = 3, 100; $P < 0.001$), with 4- to 5-fold more progeny produced at 25 and 30°C, respectively, compared with 20 and 35°C. There was also a significant two-way interaction between species mixture and temperature on progeny production ($F = 3.91$; df = 6, 100; $P < 0.01$). Again, this was a quantitative interaction, with the greatest difference in *S. zeamais* progeny production between single and mixed colonies at 25°C (Fig. 1, Tukey HSD).

To confirm the number of progeny produced, X-rays of the grain were taken. Both the species present (MANOVA: Wilks $F = 3.85$; df = 2, 23; $P < 0.01$; Fig. 2) and the temperature ($F = 51.2$; df = 3, 23; $P < 0.0001$), but not their interaction ($F = 1.76$; df = 6, 23; $P = 0.06$), affected the progeny found through X-rays. Overall, average of 0.13 adults were found per replicate and kernel, with neither the species present ($F = 3.16$; df = 2, 23; $P = 0.06$) nor the interaction with temperature ($F = 0.68$; df = 6, 23; $P = 0.67$) affecting the number found. However, the temperature did affect the number of adults found ($F = 8.01$; df = 3, 23; $P < 0.001$; Fig. 2A), with 12-fold and fivefold more adults found at 25°C compared to the lowest and highest temperature, respectively. For every treatment except one, the percent of adults missed by the progeny counts was less than 10% (Fig. 2A).

The number of pupae found through X-rays was significantly affected by species present ($F = 4.62$; df = 2, 23; $P < 0.05$), with 2.8-times more pupae found in *S. zeamais* colonies than in *P. truncatus* colonies. Temperature also significantly affected the number of pupae present ($F = 3.79$; df = 3, 23; $P < 0.05$), with four times and two times more pupae present at 30°C than 20 or 35°C. There was no significant interaction between species present and temperature on pupae ($F = 2.22$; df = 6, 23; $P = 0.07$).

The number of larvae found through X-rays was not affected by species present ($F = 0.25$; df = 2, 23; $P = 0.78$), temperature ($F = 0.85$; df = 3, 23; $P = 0.48$), or their interaction ($F = 1.81$; df = 6, 23; $P = 0.14$).

Grain Quality

Prior to the beginning of the trials, there was an average of 23 ± 6 kernels with defects (insect or otherwise) amounting to an average weight of 5.6 ± 1 g, with zero kernels containing more than one

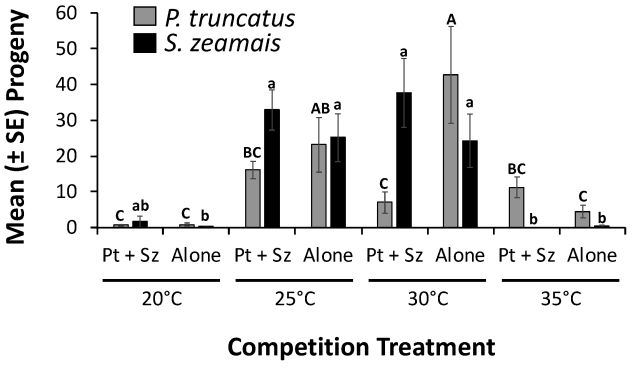

Fig. 1. The mean (SE) progeny produced after 65 d by combined species mixtures ('Pt + Sz') of *S. zeamais* (black bars) and *P. truncatus* (gray bars) or each species in isolation ('Alone') under different temperatures. Lower case letters represent pairwise comparisons within results for *S. zeamais,* while upper case letters represent pairwise comparisons among treatments for *P. truncatus*. Bars with shared letters of the same type are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).

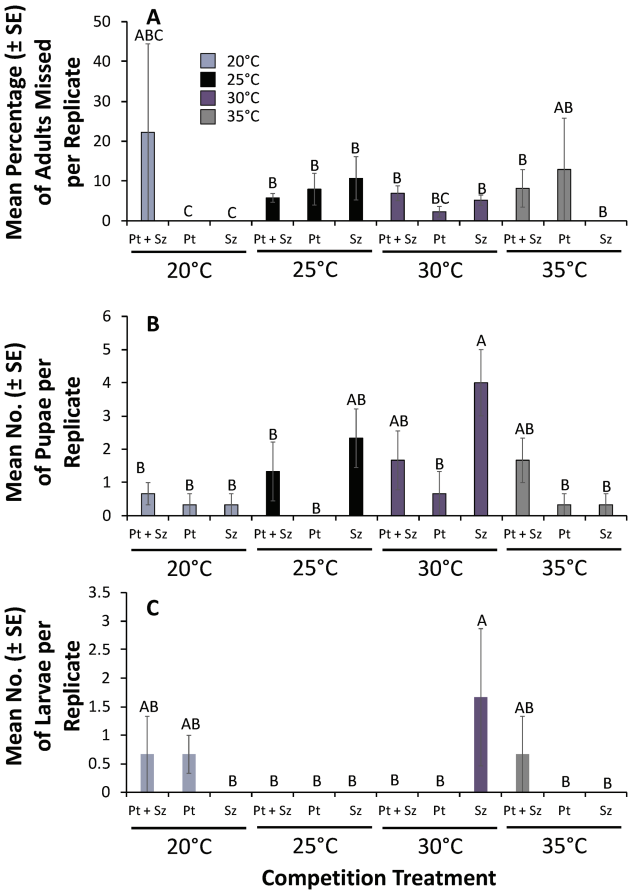

Fig. 2. Confirmation of life stage abundance through the use of soft X-rays in the laboratory after 65 d for (A) mean percentage of adults that were missed per replicate, (B) mean pupae per replicate, and (C) mean larvae per replicate. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).

defect, and no frass present. Importantly, grain damage measures were significantly affected by the interspecific competition treatment (MANOVA: Wilks Approx. $F = 19.7$; df = 2, 100; $P < 0.0001$) and temperature (Wilks Approx. $F = 2.89$; df = 3, 100; $P < 0.001$). There was also an interaction between the variables (Wilks Approx. $F = 3.27$; df = 6, 100; $P < 0.0001$).

The total number of damaged kernels were significantly affected by the species present (ANOVA: $F = 4.77$; df = 2, 100; $P < 0.01$). There were 1.77- to 2-fold more damaged grain kernels in containers with *P. truncatus* alone and both species, respectively, compared with *S. zeamais* alone (Fig. 3A; Supp Fig. 1A [online only]). However, colonies that contained *S. zeamais* alone had two times more insect-damaged kernels than controls without any insects (data not shown). Importantly, the number of damaged kernels in the combined species treatment was most similar to the damage observed in colonies that contained *P. truncatus* alone. Likewise, the temperature significantly affected the number of damaged kernels ($F = 5.34$; df = 3, 100; $P < 0.01$), with the number of damaged kernels peaking at 25 and 30°C. At those two temperatures, there were 1.5-times more damaged kernels than at 20°C, which had the fewest damaged kernels. There was no interaction between species and temperature ($F = 0.88$; df = 6, 100; $P = 0.53$), suggesting that main effects did not vary at levels of the opposite variable.

There were similar results for the weight of damaged kernels (Fig. 3B; Supp Fig. 1B [online only]). The beetle species present significantly affected the weight of damaged kernels ($F = 9.38$; df = 2, 100; $P < 0.0001$), with 74–84% greater damage observed in colonies that contained *P. truncatus* alone and the combined colonies containing both species, respectively, compared with *S. zeamais* alone. Again, weight of damaged kernels in combined colonies of species was most similar to *P. truncatus* alone (Fig. 3B; Supp Fig. 1B [online only]). The temperature also significantly affected the weight of damaged grain produced ($F = 7.31$; df = 3, 100; $P < 0.001$). In particular, there was a 56–60% greater weight of damaged grain at 25–30°C, respectively, compared with 20°C. However, the interaction was not significant between species and temperature ($F = 0.49$; df = 6, 100; $P = 0.82$).

Likewise, the species present had a significant effect on the number of kernels with more than one hole in them ($F = 69.7$; df = 2, 100; $P < 0.0001$), with a 11- to 13-fold higher number of more severely damaged kernels in treatments containing *P. truncatus* alone or in mixed-species colonies, respectively, compared with *S. zeamais* alone (Fig. 3C; Supp Fig. 1C [online only]). Importantly, the number of kernels with more than one hole was most similar between the combined species treatment and *P. truncatus* alone. While temperature did not significantly affect the number of kernels with more than hole caused by insects ($F = 0.54$; df = 3, 100; $P = 0.66$), there was a significant species × temperature interaction ($F = 4.66$; df = 6, 100; $P < 0.001$). For example, at higher temperatures (30–35°C) there were more kernels with greater than one hole in the combined species treatment compared to *P. truncatus* colonies alone (Fig. 3C, Tukey HSD; Supp. Fig. 1C [online only]), while at lower temperatures (20–25°C) there were fewer or the same number of kernels.

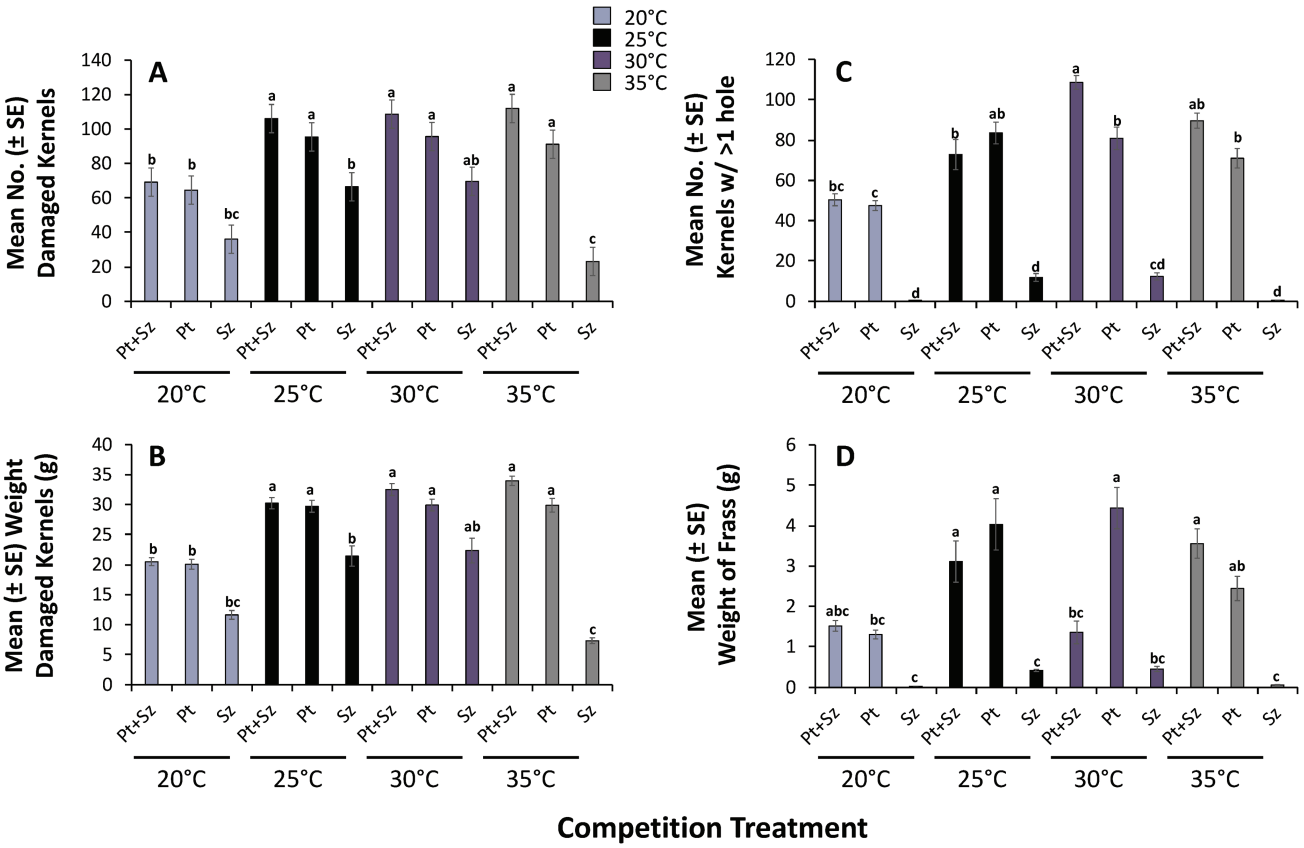

Fig. 3. The effect of combined species mixtures (Pt + Sz), *S. zeamais* alone (Sz), and *P. truncatus* alone (Pt) on maize grain quality at 20°C (lavender bars), 25°C (black bars), 30°C (dark purple bars), and 35°C (gray bars) after 65 d, with particular reference to (A) the mean number of kernels with any signs of insect damage, (B) the mean corresponding weight of the damaged grain, (C) the mean number of kernels with more than one exit or entry hole, and (D) the weight of the insect frass produced. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). See online for colour version of this figure.

The weight of frass was also significantly affected by the species present ($F = 23.1$; df = 2, 100; $P < 0.0001$; Fig. 3D; Supp Fig. 1D [online only]). For instance, there was 9- to 13-fold more frass in the colonies containing *P. truncatus* alone and combined species mixtures, respectively, compared to when *S. zeamais* was alone. As with the other damage measures, the amount of frass produced was most similar between the *P. truncatus* and combined species mixtures. Similar to the severity of kernel damage, temperature did not affect the weight of frass present ($F = 2.20$; df = 3, 100; $P = 0.09$). However, there was a significant two-way interaction between the variables ($F = 8.71$; df = 6, 100; $P < 0.0001$). In particular, the amount of frass was significantly lower or the same in the combined species mixture compared with *P. truncatus* alone at lower and moderate temperatures (20–30°C), whereas it was higher in the combined species mixture at the warmest of the temperature range (35°C; Fig. 3D, Tukey HSD; Supp Fig. 1D [online only]).

The percentage of grain attacked was affected by the insect species present ($F = 138$; df = 2, 100; $P < 0.0001$; Fig. 4), with *S. zeamais* attacking only half as much of the grain as *P. truncatus* or mixed colonies. In addition, temperature significantly affected the amount of grain attacked ($F = 63.0$; df = 3, 100; $P < 0.0001$), with 1.7- to 1.8-fold more grain attacked at 25 and 30°C compared to 20°C. Finally, there was a significant interaction between these two variables ($F = 9.03$; df = 6, 100; $P < 0.0001$; Tukey HSD, Fig. 4), with *P. truncatus* attacking much more of the grain, especially at temperatures above 20°C.

Finally, the percentage grain loss did not differ by species present in colonies ($F = 2.70$; df = 2, 100; $P = 0.07$; Fig. 4) nor by temperature ($F = 1.13$; df = 3, 100; $P = 0.34$). However, there was a significant species by temperature interaction ($F = 2.79$; df = 6, 100; $P < 0.05$), though grain loss was mostly similar between species at different temperatures.

Mold Identity and Damage

The ITS sequences from every sample were identical and they all had highest scoring BLASTn matches to *Aspergillus flavus* with 100% similarity (Eurotiales: Trichocomaceae). A total of 83% of the sequences were most similar in the RDP database to *Aspergillus flavus* or its corresponding reproductive stage (anamorph) *Petromyces*.

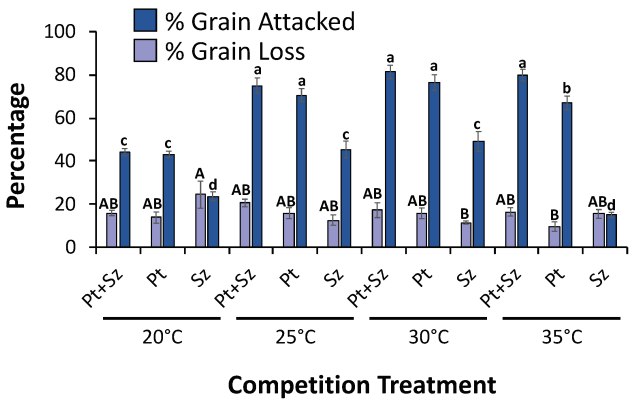

Fig. 4. The effect of combined species mixtures (Pt + Sz), *S. zeamais* alone (Sz), and *P. truncatus* alone (Pt) at 20°C (lavender bars), 25°C (black bars), 30°C (dark purple bars), and 35°C (gray bars) on percentage of grain attacked in each competition treatment (blue bars), and percentage grain loss in each treatment after 65 d (purple bars). Case of letters denotes comparisons within a response variable. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). See online for colour version of this figure.

One of the sequences was classified as *Talaromyces islandicus* (Trichomaceae). Morphological observations (mycelium color and conidial characteristics) of the mold provided further indication that the fungal populations in the vials were dominated by the presence of *Aspergillus* spp.

The insect species present significantly affected mold ratings (Kruskal–Wallis: $H = 12.1$; df = 2; $P < 0.001$; Fig. 5A). The *P. truncatus* alone was most similar to the control, with very low levels of mold. By contrast, the combined species mixture had the highest mold ratings followed by colonies that contained *S. zeamais* colonies alone. The combined species treatments had a much greater total number of insects and therefore greater amounts of frass production, which may have contributed to the growth of the mold. The temperature also significantly affected the level of mold ($H = 37.5$; df = 3; $P < 0.0001$), with the higher mold ratings observed at the two temperatures with the greatest insect activity (e.g., 25 and 30°C; Fig. 5B, Tukey HSD).

Discussion

In this study, we systematically evaluated the competition between the cosmopolitan *S. zeamais* and invasive *P. truncatus* over a wide but biologically realistic temperature range. Importantly, only *P. truncatus* experienced a cost to competition with *S. zeamais* at 30°C, but not the other temperatures. At other temperatures, costs of competition to both species appeared to be negligible. In contrast, a prior study considered how density affected competition of *S. zeamais* and *P. truncatus* at those two temperatures, and found that the former was the superior competitor at the lower temperature, while coexistence was likely at the higher temperature (Giga and Canhao 1993).

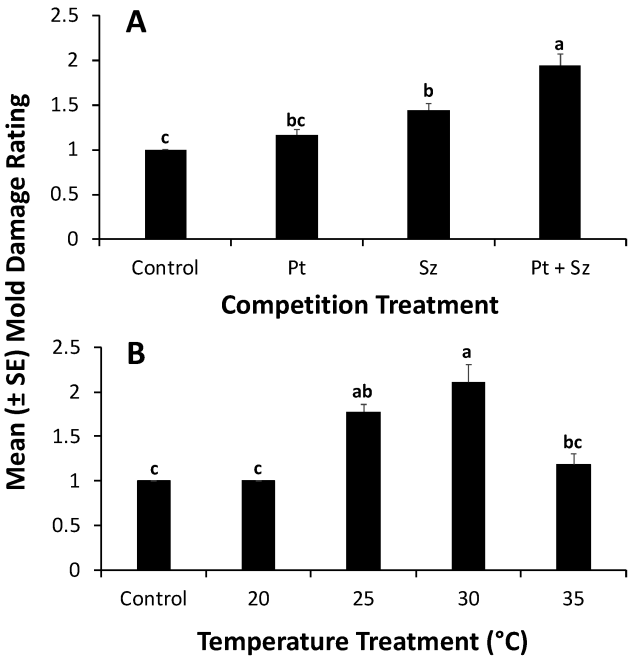

Fig. 5. The average effect of (A) combined species mixtures (Pt + Sz), *S. zeamais* alone (Sz), and *P. truncatus* alone (Pt), and (B) varying temperature on mold growth in maize over 65 d per vial. Grain was classified according to the following scale: 1—no presence of mold, 2—minor and spatially limited mold infestation, 3—moderate mold infestation, and 4—widespread and severe mold contamination throughout grain. Bars with shared letters are not significantly different from each other (Chi-squared tests, Bonferroni correction).

Importantly, that study only qualitatively evaluated the outcome of competition using a substitutive approach. In our study, we quantitatively found that *P. truncatus* produced twice as many progeny at 35°C compared with *S. zeamais*, suggesting that at extremely warm temperatures, *P. truncatus* has a clear advantage, even if it does not produce as many progeny in absolute terms as at lower temperatures. This is also in alignment with prior work, which has shown that the temperature optimum is 32°C for *P. truncatus* (Shires 1980, Bell and Watters 1982), and 30°C for *S. zeamais* (Throne 1994). Indeed, at 30°C *S. zeamais* had double the number of progeny compared to *P. truncatus* when in mixed colony treatments.

Despite *S. zeamais* producing more progeny at intermediate temperatures (e.g., 25 and 30°C) in mixed colonies, we found that most of the damage in the combined treatment is likely the result of *P. truncatus* activity. This finding is clear considering that the levels of damage in mixed colonies did not differ significantly from colonies where *P. truncatus* was reared alone while the levels of damage in colonies where *S. zeamais* was reared alone were relatively small. This finding aligns well with several prior studies. For example, after a 90-d period on 200 g of maize, *S. zeamais* caused 6.9% weight loss to the commodity, while feeding by *P. truncatus* had resulted in a weight loss of 67.1% (Tefera et al. 2011). Furthermore, prior research has shown that over a 37 d period, *S. zeamais* feeding results in a mean 18.3% weight loss per kernel (Adams 1976), while weight loss for batches of stored maize cobs ranged between 0 and 56% for *P. truncatus* (Compton and Sherington 1999). The presence of frass seem to be highly beneficial in the case of *P. truncatus* development (Hodges 1986, Athanassiou et al. 2017a), in contrast with the common belief that primary grain colonizers are not positively affected by the presence of grain dust or dockage. In a recent population growth study, Athanassiou et al. (2017b) demonstrated that the number of *P. truncatus* adults was higher in vials that contained 100% cracked maize kernels, as compared to 100% intact kernels. The higher damage created by *P. truncatus* may be partly attributable to its biology, which involves much more extensive feeding by adults than for *S. zeamais*. By contrast, adult feeding by *S. zeamais* is minimal, and most of the damage is the result of individual exit holes on kernels after pupation and emergence as adults.

One of our damage measures, e.g., the number of kernels with more than one area of damage, appeared very different depending on species. For example, damage for *S. zeamais* primarily consisted of small circular, discrete exit holes, while damage for *P. truncatus* generally consisted of irregular chewing damage. However, regardless of species-specific appearance, the goal of this measure was to evaluate the relative severity of damage on grain kernels, which was adequately captured by considering multiple areas of injury on a grain kernel. Our study demonstrated that treatments in which *P. truncatus* were present exhibited the greatest intensity of damage to the grain.

There are two possible limitations to this study. One of these limitations is that our study species were originally reared under different conditions (14:10 (L:D) h for *S. zeamais* compared with complete darkness for *P. truncatus*) as mandated by the USDA's APHIS-permitted use for retaining the species. However, our experiment is similar to a 'common garden' experiment, whereby populations under two different growing regimes are placed under similar conditions and their performance is compared (e.g., Messina et al. 1996). While systematic studies on the effect of photoperiod on the biology of stored product insects are relatively rare, prior work with *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), the lesser grain borer, has demonstrated that photoperiod does not affect egg hatch, larval-pupal development, or oviposition by young females (Aslam et al. 1994). While the effects of changed photoperiod on the outcome of competition cannot be ruled out, we expect that it has not had a larger impact compared to temperature, which is the more relevant variable for stored product insect development.

Another possible limitation of the study is that it was impossible to sex adults for *P. truncatus* and *S. zeamais*, making the number of females and males in each treatment somewhat variable. However, if this had a strong effect relative to our treatments, we would expect random oscillations in the outcome of treatments regardless of our other factors (e.g., temperature and species combination), which we did not observe. The variability in number of each sex was likely mitigated by the high number of replicates per treatment combination as well as the fact that populations were given sufficient time to complete two generations. At 30°C, it takes *P. truncatus* 32 d to undergo a generation (Guntrip et al. 1996), while it takes *S. zeamais* an average of 33 d at the same temperature (Throne 1994). Throne (1994) found that even though only five S. *zeamais* females were placed in a jar with maize, the resulting progeny from the F_1 generation was not significantly different from a 1:1 sex ratio. Undergoing a second-generation would have likely corrected a previous imbalance in sex ratio, allowing for reasonable interpretation of the outcome of competition. Finally, our study design is in alignment with prior competition experiments in stored products, which also used unsexed adults for *P. truncatus*, *S. zeamais*, and related species (e.g., Sakka and Athanassiou 2018; Athanassiou et al. 2017c).

The growth of mold was significantly elevated for single *S. zeamais* colonies, and highest in combined mixtures of species. This supports the hypothesis that insects may change the microclimate in grain to benefit microbial growth. In particular, the increased activity of insect individuals may have positively affected moisture content due to respiration. In the combined species treatment, there were twice as many individuals as in the single-species colonies, suggesting that at least in part, mold growth may be affected by the number of insects infesting a commodity in addition to the species identity. It has been firmly established that many insects use microbially-produced volatiles for a range of functions, including habitat location, mate-finding, and foraging (Davis et al. 2013), showing a range from attraction to repulsion even for stored product insects (Pierce et al. 1991, Herrera et al. 2015). The only fungi identified in our samples were in the family Trichomaceae, the majority of which were *Aspergillus flavus*. Many strains of this species can produce aflatoxins, and can adversely affect the quality of grain during storage (Cotty et al. 1994). Furthermore, *Aspergillus flavus* has been found more commonly in insect-infested wheat samples, than uninfested samples (Sinha and Sinha 1990). Our results highlight the importance of insect–microbe interactions and suggest that further studies should elucidate the role of these two groups in affecting grain quality as well as their reciprocal relationships.

The growing threat of climate change is beginning to imperil agricultural productivity. As the climate changes, so too do challenges for the post-harvest supply chain. The different temperatures under which competition occurred in our study mimicked increasing temperatures, including the 1.5°C increase by 2050 predicted by the Intergovernmental Panel on Climate Change (Allen et al. 2018). Under the warmest conditions, *P. truncatus* produced twofold more progeny than *S. zeamais*. Neither species performed well under the lowest temperature; although both species produced progeny at intermediate temperatures, *S. zeamais* seemed to have the advantage at these temperatures. Because *P. truncatus* has primarily been a tropical species, it may be possible that its optimal range may shift polewards under various climate-warming scenarios. With changing abiotic conditions, and subsequent

altered biotic interactions, there may be a confluence of factors that adversely affect grain quality in temperate regions more than has historically been the case. This will require agriculture to adapt by effectively adopting alternative mitigation strategies in order to maintain productivity (Howden et al. 2007).

While we have directly assessed competitive ability in this study, there may be biological sieves that result in the realized niche of both species being somewhat different in the post-harvest environment. For example, some research has suggested that the species may partition the bulk storage environment, with *P. truncatus* seeking out grain closer to the bottom of a mass to leverage the added compaction in chewing through grain (Vowotor et al. 2005). Spatial and temporal niche differentiation have been documented as important processes in other systems (Albrecht and Gotelli 2001). Variation in temperature, humidity, and pressure gradients in a grain bin or bag may result in spatial segregation of these two species. At the field-scale, postharvest facilities usually comprise multiple buildings spanning across large areas and may have different commodities present in each part or may have different sections with commodities in different phases of processing. Preferences by each species may result in habitat partitioning along these or other niche axes. This may enable coexistence under certain abiotic conditions in grain stores where it would not otherwise be possible for *S. zeamais* and *P. truncatus*. There may also be intraspecific semiochemicals that affect distribution of one or both species. For example, it is known that the presence of female *P. truncatus* results in the suppression of the male-produced aggregation pheromone emissions (Smith et al. 1996).

There has been an abundance of prior research in evaluating the competition between other stored product insect species, probably due to the fact that these experiments can be carried out with ease in laboratory conditions. These studies suggest that the outcome of competition is often temperature and species-dependent, and that the superior competitor may not always be the most damaging species. For example, Athanassiou et al. (2014) found that there was no difference in the number of *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae) when reared at 25°C by itself or with one of two other psocids. However, when *L. bostrychophila* was reared at 30°C, it was the dominant species. Irshad and Talpur (1993) found that both *R. dominica* and *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), the Angoumois grain moth, suffered a cost when reared with other species compared to when reared alone, while *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle, actually benefited from competition in mixed colonies on whole grain because of feeding by primary pests. Similar to the results in this study, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), the khapra beetle, was found to have contributed more to total frass production and grain loss than *R. dominica*, even though *R. dominica* was the superior competitor (Kavallieratos et al. 2017). In a competition study among three species of the genus *Sitophilus*, including *S. zeamais*, *S. oryzae* was found to relatively produce both the greatest number of progeny, and cause the most damage (Athanassiou et al. 2017c). Thus, the context, species identity, and temperature may be important factors in shaping competitive interactions in the postharvest environment.

Overall, our study suggests a range of temperatures with coexistence between *S. zeamais* and *P. truncatus*. However, *P. truncatus* is expected to perform better under higher temperatures, while *S. zeamais* is expected to perform better under lower temperatures. In a warming climate, *P. truncatus* may be favored, and its increased capacity for causing damage should be of concern to managers of food facilities in the post-harvest supply chain. The threat of future post-harvest losses from *P. truncatus* may mean that new adaptation strategies will be required, including evaluating and optimizing new chemical, cultural, and biological control tactics. Performing this work prior to the invasion of new areas will be of benefit to stakeholders in the food industry. Finally, future work must more comprehensively evaluate insect-microbe interactions and their effect on grain quality, as well as potential niche partitioning between these two species to gain a better grasp on their realized niches in grain stores.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

Acknowledgments

We would like to thank the excellent technical assistance of Kathy Leonard (USDA-ARS). This work was funded, in part, by a National Institute of Food and Agriculture's Crop Protection and Pest Management Program Grant #2017-70006-27262. The use of trade names is for the purposes of providing scientific information only, and does not constitute endorsement by the Kansas State University, the University of Thessaly or the United States Department of Agriculture. The Kansas State University, University of Thessaly, and USDA are equal opportunity employers.

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