

Improving management of the larger grain borer, *Prostephanus truncatus*, and the maize weevil,
Sitophilus zeamais

by

Hannah E. Quellhorst

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Abstract

The invasive larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) is a devastating stored product pest of maize and cassava, originating from Meso-America. Many outstanding areas of basic behavior and ecology remain to be assessed for *P. truncatus*, and its main cosmopolitan competitor, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae). This includes enhancing knowledge of *P. truncatus* and *S. zeamais* management through chemical control, improving understanding of interspecific competition in grain columns, and elucidating how these species potentially vector microbes. To enhance management through chemical control, I investigated the efficacy of a novel insecticide formulation (S-methoprene + deltamethrin + piperonyl butoxide; Gravista®) compared to an existing commercial standard formulation without synergist (Diacon IGR+®), and water-treated controls. We found significant multiple-fold mortality induction and movement reduction when applied as a grain protectant or surface treatment on concrete for 4–168 h compared to the control. In a more realistic test, we evaluated the recovery and horizontal dispersal capacity of *P. truncatus* and *S. zeamais* to food patches after 48 h in a dispersal apparatus with 15 g of maize. We found the percentage of *P. truncatus* and *S. zeamais* dispersing was significantly reduced by 90–97% or 5–25% after exposure to Gravista or Diacon IGR+ compared to the control. Further, we evaluated two other grain protectants (deltamethrin and pirimiphos-methyl) as surface treatments for maize to manage both species. Mortality of adults was nearly 100% for all treatments for both insecticides with *P. truncatus*, while progeny production was low. Mortality for *S. zeamais* remained low for deltamethrin but they were easily controlled by pirimiphos-methyl. These insects also co-occur in many regions of the world. Thus, I evaluated the outcome of interspecific competition between *P. truncatus* and *S. zeamais* between 25–35°C and found that coexistence may be

possible at a range of 25–30°C, but mixed colonies experienced a direct competitive cost.

Prostephanus truncatus than *S. zeamais* performed better at warmer temperatures. Even less information is available on the spatial dispersion of these species in grain columns, therefore we evaluated competition under three different densities (10–300 insects/kg) in monolayers of maize after 1 and 7 d in pure or mixed colonies. Both species generally aggregated together and were correlated to the same location as heterospecifics. When alone, *P. truncatus* created a clear path of destruction to the bottom of the monolayer, but when *S. zeamais* was present, damage was lessened and shifted upwards. Finally, the ability of both species to vector microbes when given the opportunity to forage on agar dishes (e.g., a novel food patch) after a 0, 24, or 72 h dispersal period was assessed. Both species readily vectored several plant pathogenic microbes, including 21 taxa from 11 genera, notably *Aspergillus*, *Penicillium*, and *Fusarium* spp. Increasing dispersal period resulted in a third less microbial growth by *S. zeamais* after 72 h. Colonization by *S. zeamais* resulted in 6.6-fold more microbial growth than *P. truncatus*. This highlights the importance of behavioral ecology in IPM and suggests new tactics for these economically important species.

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Approved by:

Co-Major Professor
Dr. Rob Morrison

Approved by:

Co-Major Professor
Dr. Kun Yan Zhu

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and found that coexistence may be possible at a range of 25–30°C, but mixed colonies experienced a direct competitive cost. *Prostephanus truncatus* than *S. zeamais* performed better at warmer temperatures. Even less information is available on the spatial dispersion of these species in grain columns, therefore we evaluated competition under three different densities (10–300 insects/kg) in monolayers of maize after 1 and 7 d in pure or mixed colonies. Both species generally aggregated together and were correlated to the same location as heterospecifics. When alone, *P. truncatus* created a clear path of destruction to the bottom of the monolayer, but when *S. zeamais* was present, damage was lessened and shifted upwards. Finally, the ability of both species to vector microbes when given the opportunity to forage on agar dishes (e.g., a novel food patch) after a 0, 24, or 72 h dispersal period was assessed. Both species readily vectored several plant pathogenic microbes, including 21 taxa from 11 genera, notably *Aspergillus*, *Penicillium*, and *Fusarium* spp. Increasing dispersal period resulted in a third less microbial growth by *S. zeamais* after 72 h. Colonization by *S. zeamais* resulted in 6.6-fold more microbial growth than *P. truncatus*. This highlights the importance of behavioral ecology in IPM and suggests new tactics for these economically important species.

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Dedication

First, I would like to dedicate this dissertation to Yahweh, for “by You, I have been upheld from birth; You are He who took me out of my mother’s womb. My praise shall be continually of you.” Secondly, I would like to dedicate this dissertation to anyone who has ever struggled with a chronic illness. Keep fighting.

**Chapter 1 - The biology, ecology, and management of the larger
grain borer, *Prostephanus truncatus* (Horn) (Coleoptera:
Bostrichidae)**

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Abstract

The invasive larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), originating from Meso-America, is a devastating stored product pest of maize (*Zea mays* L.) and cassava (*Manihot esculenta*). This species can destroy up to 40% of stored maize within four months. Despite four decades of research, *P. truncatus* has spread throughout Africa, while decimating maize and cassava production. Given the continuing problems with *P. truncatus*, the likelihood for its continued range expansion under climate change, and its status as a species of concern in many countries, the objective of this review was to provide an updated and comprehensive freely accessible record of the distribution and spread, biology, development, and ecology, host preferences, chemical ecology, detection, and monitoring, and management of *P. truncatus*. We conducted a search of the literature from 1911 to 2021 using Google Scholar and Web of Science to find all papers related to key search terms. We found that *P. truncatus* has been recorded in 36 countries across the globe, including 21 now in Africa. A recent predictive model found that the insect has been limited to tropical and subtropical regions but could likely spread to temperate regions as temperatures rise with climate change. Conspecifics respond to their two component, male-produced aggregation pheromone early after eclosion, but quickly switch to other cues as older adults. At close-range, *P. truncatus* may use food cues, but host volatiles are not involved in long-range host finding of commodities. Research on managing *P. truncatus* has mostly focused on chemical control to the detriment of other tactics, with the most promising tactic likely to be the different hermetic storage technologies. Many outstanding areas of basic behavior and ecology remain to be assessed for *P. truncatus*. We highlight specific areas that should be prioritized for further work in order to better manage and reduce the impact of this invasive insect pest.

Keywords: stored maize, stored products, behavior, biogeography, larger grain borer, integrated pest management

Introduction

Maize (*Zea mays* L.) is a cereal crop that feeds the world and is a major staple crop in Africa and Latin America. Together with rice and wheat, maize provides upwards of 60% of the daily caloric intake for people in many low and middle income countries (Awika, 2011). Africa consumes 30% of the world's maize, and 90% of the maize in Africa is consumed as food (Awika, 2011). In Latin America, Mexico consumes large quantities of maize, accounting for 45% of the daily caloric intake of residents (Awika, 2011). Even in more developed countries, maize and other cereals are important, comprising almost a third of the calories consumed (Awika, 2011). An equally important source of calories in Africa, Asia, and Latin America is cassava. In total, 13+ million ha of cassava (*Manihot esculenta*) are grown worldwide in subtropical and tropical regions, with more than 70% of that in Africa and Asia (El-Sharkawy 2003).

However, 10 to 30% of cereal crops, including maize and cassava, are lost to insects post-harvest, as these grains are harvested, stored, processed, and marketed to consumers (Pimentel, 1991). In Africa, where maize is of high importance for consumption, the major stored product insects include the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), and the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) (Tefera et al., 2011). Boxall (2002) estimated that weight loss in grain due to *P. truncatus* was between 34–40%, suggesting that this species

alone destroys and consumes considerable amounts of grain after only three months of storage on the farm. The mean weight loss in fermented and unfermented cassava root by *P. truncatus* was estimated to range between 52–74% (Hodges et al. 1985).

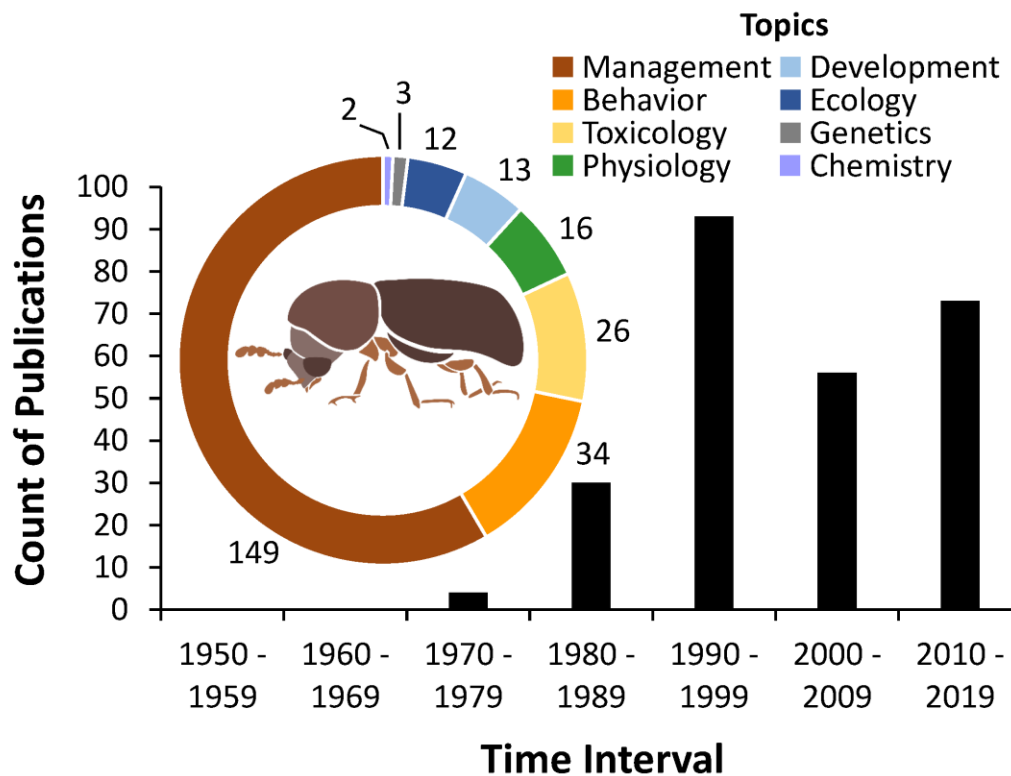
Prostephanus truncatus is a long-time pest of stored maize in Central America, and was first described in 1878 (Hodges, 1986). This insect was originally described as *Dinoderus truncatus* Horn and then revised to *Stephanopachys truncatus* (Horn) (Back and Cotton, 1938) before ending up at its current taxonomic location (Lesne, 1898). *Prostephanus truncatus* is native to Mexico and Central America, but was accidentally introduced into sub-Saharan Africa most probably through a refugee grain supply crisis in the 1970s in Tanzania although only confirmed in 1981 (Golob and Hodges, 1982; Hodges, 1986), it quickly spread throughout the equatorial region of Africa. The insect may infest the maize crop as it matures in the field right through to storage (Giles, 1975; Hodges et al., 1983a). After its introduction to Africa, it was found to readily and frequently attack dried cassava root and cassava chips, in addition to maize (Hodges et al., 1985).

Specific aspects of the biology and management of *P. truncatus* have been reviewed several times, initially by Hodges (1982), then by others in the 1980s and early 1990s (Wright and Spilman, 1983; Hodges, 1986; Markham et al., 1991). In the early 2000s, Hill et al. (2002) offered a limited review on *P. truncatus*, which focused solely on its ecology. Some of the reviews have been location specific. For example, Suma and Russo (2005) reviewed *P. truncatus* in Italy only, whereas Muatinte et al. (2014) reviewed *P. truncatus* only in its introduced range. Nansen et al (2002) reviewed the basic biology of *P. truncatus*, although that was about two decades ago, and needed updating. Finally, Muatinte et al. (2019) recently contributed an article on the control of *P. truncatus*. Importantly, while the article provided some concrete

recommendations for the management of *P. truncatus*, it is not as comprehensive as our contribution, which incorporates many aspects of basic biology and ecology of *P. truncatus*, including competition, movement, response to visual and olfactory stimuli, among other topics. Finally, that article appears hard to access and behind a pay wall, thus inaccessible to affected stakeholders unless they have access to the resources of a large library. Thus, now is an appropriate time to review the literature and provide an updated comprehensive resource on *P. truncatus* given 1) the increasing amount of literature amassed in recent years (Figure 1.1), 2) the perniciousness of the species, even after 40 years of research since its dispersal to Africa, 3) the likelihood for its continued range expansion under climate change, 4) its status as a species of concern in many countries, and 5) our desire to provide a freely accessible, comprehensive reference to stakeholders. Not surprisingly, before 1980s, the number of publications on *P. truncatus* is rather limited, and the increase after that period is attributed to its establishment and spread in Africa. The increase of the number of papers shown in Figure 1 is much higher during the 1980s and 1990s, and then this levels off, with most of the corresponding publications focused on control strategies. However, *P. truncatus* continues to spread and establish in new countries up to the present. In order to move the science forward, it is helpful to synthesize what is known about *P. truncatus* biology, spread, food sources, natural enemies, monitoring tools, and management strategies, as well as identify key gaps in the literature to better inform management and future research on *P. truncatus*.

Figure 1-1 Published studies on *Prostephanus truncatus* by decade from 1950 to 2019

This figure shows the published studies on *Prostephanus truncatus* by decade from 1950 to 2019 compiled via search using Web of Science and every species name synonym that *P. truncatus* has been assigned. The inset panel represents the overall focus of all 255 published articles.



Methods

A review of the literature was conducted using the databases Web of Science. Search terms included, but were not limited to, combinations of: “larger grain borer”, “*Prostephanus truncatus*”, “natural enemies”, “chemical ecology”, “volatiles”, “management”, “integrated pest management”, “distribution”, “occurrence”, “development”, “spread”, “food”, “insecticides”, “attraction”, among others. We found a total of 255 papers from 1950–2021 for the purposes of compiling Figure 1 from Web of Science, because this database cannot reach further back than 1950. Each article had to represent an original data manuscript on some aspect of biology,

ecology, taxonomy, or management of *P. truncatus* to be included. In cases where multiple categories may have been applicable, the category with the greater number of manuscript pages devoted to it in the contribution was judged to be the dominant and final assigned category. We assembled key tables from the information in these citations, including on documented food sources, behaviorally-active stimuli, trap designs, and establishment records for *P. truncatus* from pertinent literature. We categorized these papers into eight categories: management, behavior, toxicology, physiology, development, ecology, genetics, and chemistry. Taxonomy was also considered, but no articles dealt with taxonomy, so it was excluded from final figures. For the sake of being comprehensive, we additionally reviewed literature further back from 1911–2021 on *P. truncatus* with Google Scholar that may have been missed by the other database that also included review articles on *P. truncatus* and discussed these in the text of our contribution where relevant, but these were not included in Figure 1. To examine the spread of *P. truncatus* over time, the year of establishment based on locality descriptions in peer-reviewed contributions were mapped. In particular, occurrence records were color-coded by year, then plotted using R Software (R Core Team, 2019).

Distribution and Spread

Prostephanus truncatus did not gain attention as an economically significant pest of stored products such as maize until the early 1970s when it was discovered causing damage on maize and beans in Nicaragua (Giles and Leon, 1975; Giles, 1977). The historical range of *P. truncatus* is generally considered to be much of Mexico and Central America (Arthur et al., 2019). However, probably in the early 1970s, *P. truncatus* was accidentally introduced into Tanzania, and its presence there was confirmed in 1981. It began to rapidly expand its distribution throughout much of eastern and western Africa. This quickly sparked greater efforts

to understand this insect in the hopes of preventing it from devastating maize, the most important cereal crop in Africa.

Over the last 40 years, there have been various studies describing or modeling the distribution of *P. truncatus* (Golob and Hodges, 1982; Hodges et al., 1983a; Tigar et al., 1994a; Vowotor et al., 2005; Gueye et al., 2008; Nyagwaya et al., 2010; Arthur et al., 2019), but none really documents its spread over time. We have now summarized both the global distribution of *P. truncatus* and its spread over time (Figure 1.2; Table 1.1). Interestingly, *P. truncatus* failed to establish in Israel (Calderon and Donahaye, 1962) and Iraq (Al-Sousi et al., 1970). This makes sense because Arthur et al. (2019) found the primary climatic variables involved in determining the suitability of an area incorporated high temperature and high precipitation in various iterations, and both Israel and Iraq most obviously lack significant precipitation. In the first 10 years following the introduction of *P. truncatus* in Tanzania, the insect was quickly found in neighboring Kenya, Burundi, and Malawi. The insect was also introduced into West Africa, Togo, and spread out to neighboring countries like Ghana, Burkina Faso, and Nigeria (Table 1.1; Figure 1.2). By 1999, *P. truncatus* had spread down the coast from the East to South Africa. It was reportedly intercepted in Thailand (Sukprakarn, 1976), although Hodges (1986) underlined the need for confirmation of the finding since *P. truncatus* was previously confused with morphologically similar *Dinoderus* spp. endemic to the region. Unfortunately, to our knowledge, there was never any follow-up work to confirm the presence of *P. truncatus* in Thailand, so its status there remains unknown, though again models project that it is suitable for the species (Arthur et al., 2019). Since then, spread by *P. truncatus* has been dramatically slowed, with establishment in only three new African countries (Zimbabwe, Senegal, and Mozambique) in the last 20 years (Nyagwaya et al., 2010). The slowing spread in Africa may be the result of the

species running into the edges of its realized niche based on temperature and precipitation requirements, which appears to be recently validated (Arthur et al., 2019). While most records correspond to the continent of Africa, *P. truncatus* has been intercepted in a handful of other countries, including in Asia (e.g., Thailand) and Europe (e.g., Italy), but there has been no evidence of establishment in other areas. In most cases, these interceptions were likely due to the transport of wood materials.

Figure 1-2 Map showing spread and establishment of *P. truncatus*

This figure is a map showing the spread and establishment of *P. truncatus* from before 1980 until December 2020. Only the Americas and Africa are shown to better visualize countries where *P. truncatus* is currently located; it has not established on other continents.

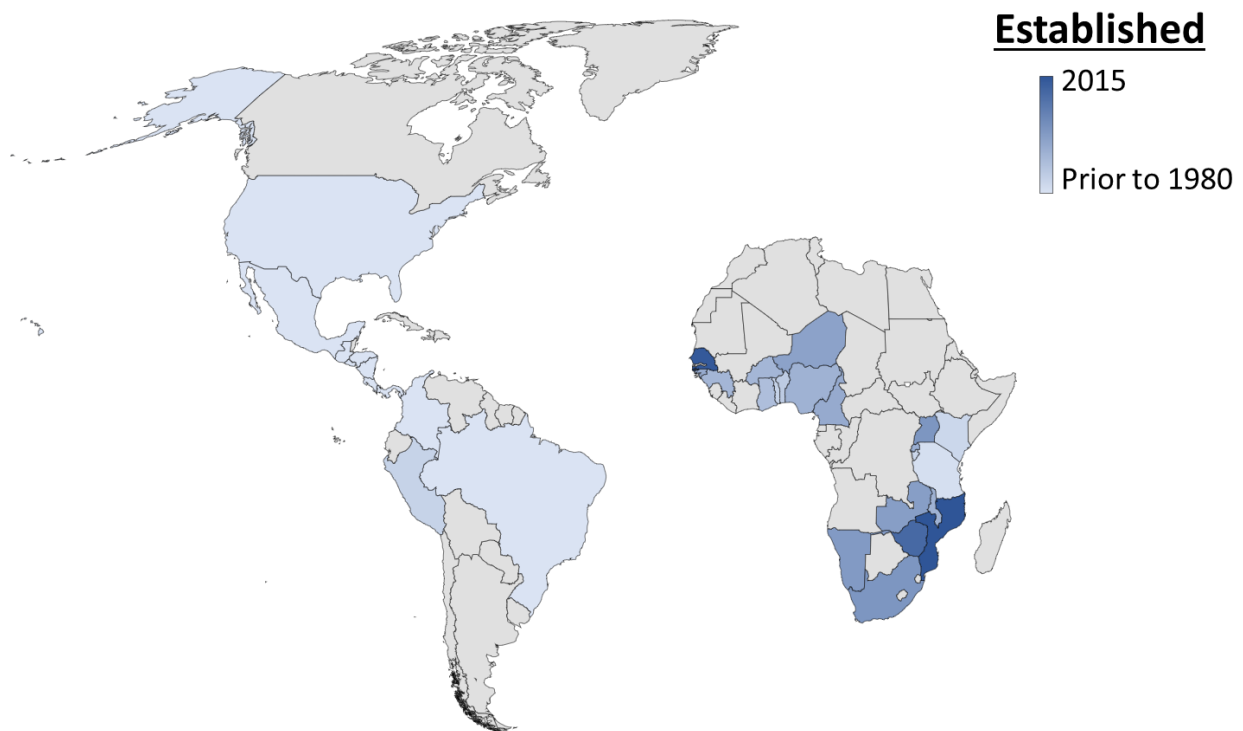


Table 1-1 Table of countries where *P. truncatus* has been intercepted and Established

Here we list the countries where *P. truncatus* has been intercepted and has established. Countries marked with an asterisk are those in which the species has been recorded, with no establishment.

Country	Reference	Year
United States	Back and Cotton, 1922, Horn, 1887	1887, 1922
Guatemala	Zacker, 1926	1926
Brazil	Cotton and Good, 1937	1937
Costa Rica	Fisher, 1950	1950
Mexico	Quintana et al., 1960, Riley, 1984	1960, 1984
Israel*	Calderon and Donahaye, 1962	1962
Honduras	McGuire and Crandall, 1967	1967
Panama	McGuire and Crandall, 1967	1967
El Salvador	McGuire and Crandall, 1967	1967
Iraq*	Al Sousi et al., 1970	1970
Nicaragua	Giles and Leon, 1975	1974
Colombia	Posada et al., 1976	1976
Thailand*	Sukprakam, 1976	1976
Tanzania	Dunstan and Magazini, 1981	1981
Kenya	Kega and Warui, 1983	1983
Burundi	Kega and Warui, 1983, Gilman, 1984	1983, 1984
Peru	Wright, 1984	1984
Togo	Harnisch and Krall, 1984	1984
Benin	Anon, 1986	1986
Ghana	Dick et al., 1989	1987
Burkina Faso	Bosque-Perez et al., 1991	1991
Guinea	Kalivogui and Much, 1991	1988
Conakri		
Malawi	Munthali, 1992	1991
Nigeria	Pike et al., 1992	1992

Rwanda	Bonzi and Ntambabzi, 1993	1993
Cameroon‡	Hodges et al., 1994	1994
Guinea Bissau‡	Aman et al. 2007	1988
Democratic Republic of Congo (Zaire)‡	Hodges et al., 1994	1994
Niger	Adda et al., 1996	1994
Zambia	Milimo and Munene, 1993	1993
Namibia	Larsen, 1998	1998
Uganda	Opolot and Odong, 1999	1997
South Africa	Roux, 1999	1999
Italy*	Suma and Russo, 2005	2005
Zimbabwe	Nyagwaya et al., 2010	2005
Senegal	Gueye et al., 2008	2007
Mozambique	Muatinte and Cugala, 2015	2007

*Only interceptions of *P. truncatus*, not establishments

‡ Unconfirmed reports of establishment, but mentioned in prior literature.

In recent years, the focus has shifted to the creation of predictive models for understanding the distribution of *P. truncatus*. A model for native populations in Mexico (Tigar et al., 1994a) was created, which was preceded by a similar model that was created for introduced populations in East Africa (Tigar and Osborne, 1993). Much more recently, Arthur et al. (2019) modeled the potential range expansion of *P. truncatus* globally, and found it may be able to expand beyond the areas in which it is currently located.

Biology, Development, Ecology

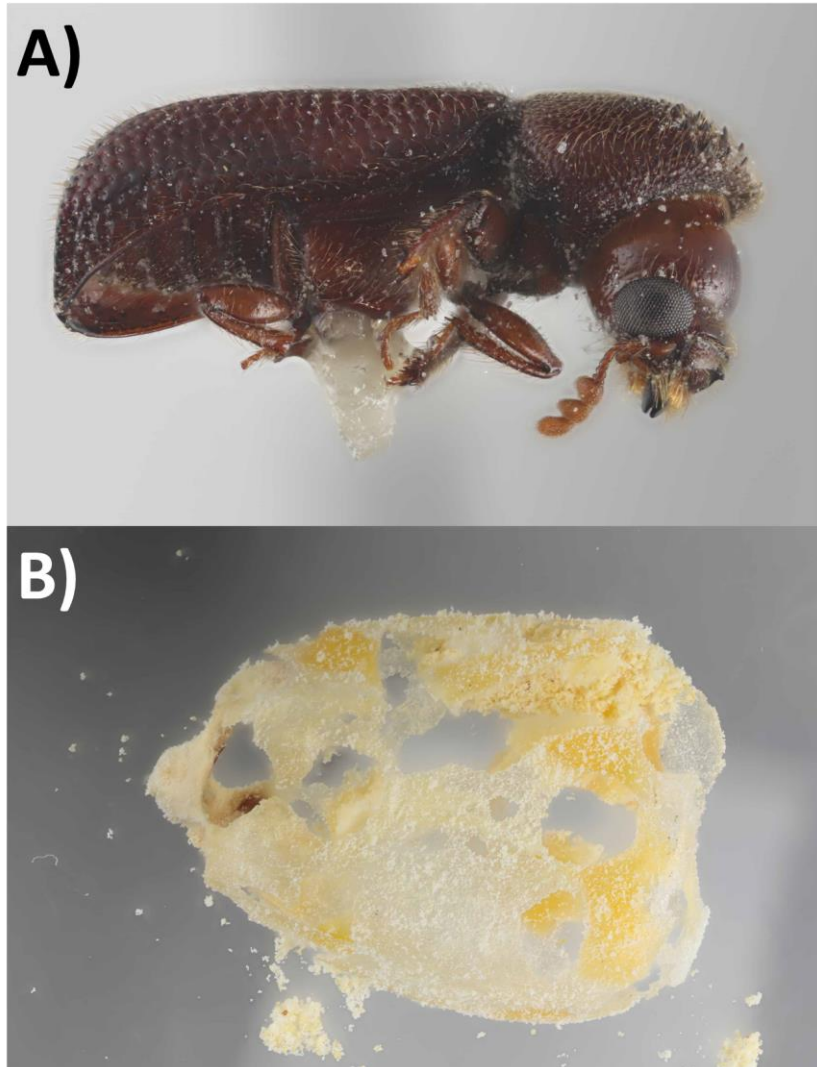
Life History

Survival and development of *P. truncatus* are possible under a range of temperatures and humidities. However, this species appears to thrive at temperatures that are higher than the optimum developmental temperatures for most stored product insects. For example, the optimal conditions for *P. truncatus* appear to be 32°C and 70-80% R.H. (Shires, 1979, 1980; Bell and Watters, 1982; Quellhorst et al., 2020). Nevertheless, population growth can occur at 25°C, even under the simultaneous presence of other species (Sakka and Athanassiou, 2018; Quellhorst et al., 2020). At 70% R.H., the lower developmental temperature for *P. truncatus* eggs, larvae, and pupae are estimated to be 11.4, 14.6, and 13.1°C, respectively (Subramanyam and Hagstrum, 1991). Averaging from eggs to adults, the lower developmental threshold for *P. truncatus* is estimated to be 15.05–15.29°C (Shires, 1979; Bell and Watters, 1982). *Prostephanus truncatus* requires 404–435 degree-days above those lower development thresholds to complete development from egg to adult (Subramanyam and Hagstrum, 1991). Shires (1979) reported that *P. truncatus* took approximately 37 d to develop at 27°C and 70% R.H. with females living approximately 61 d and males living approximately 45 d. At 32°C and 80% RH, the peak number of *P. truncatus* emerging was after a development period of 27 d (Shires, 1980). The lower lethal temperature threshold for adult *P. truncatus* was found to be -9°C for 4 h or -15°C for 30 min, while the upper lethal temperature thresholds were 45°C for 4 h and 49°C for 30 min (Machekano et al., 2020). The supercooling points for *P. truncatus* larvae and adults were -19.45°C and -16.65°C, respectively (Machekano et al., 2020).

Adult *P. truncatus* tend to produce large amounts of boring dust as they attack the maize grains. They also create large and noticeable holes, while often “skeletonizing” the grain (Figure

1.3). Adult females oviposit within the grain in “blind-ending” chambers, which have been chewed at a right angle from the main bored tunnel (Howard, 1983). Female beetles will lay approximately 20 eggs at a time and cover them with maize dust. There is a correlation between the number of eggs being laid and the maize dust produced (Howard, 1983). Therefore, it was postulated that the females might produce flour or frass for the benefit and development of the young larvae upon hatching, as has been documented for *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) (Potter, 1935; Howard 1983). However, this hypothesis has not been specifically or rigorously confirmed, and an alternative explanation may be that the food dust is useful for concealing the presence of larvae from predators in the postharvest environment. This should be evaluated in follow-up work, because it may reinforce existing messaging that sanitation is critical for appropriate integrated pest management (Morrison et al., 2019). Finally, the amount of food available may influence the number of eggs laid (Nyakunga, 1982).

Figure 1-3 Habitus 3D images of adult *P. truncatus* (A), and a damaged maize grain from *P. truncatus* feeding (B).



Movement

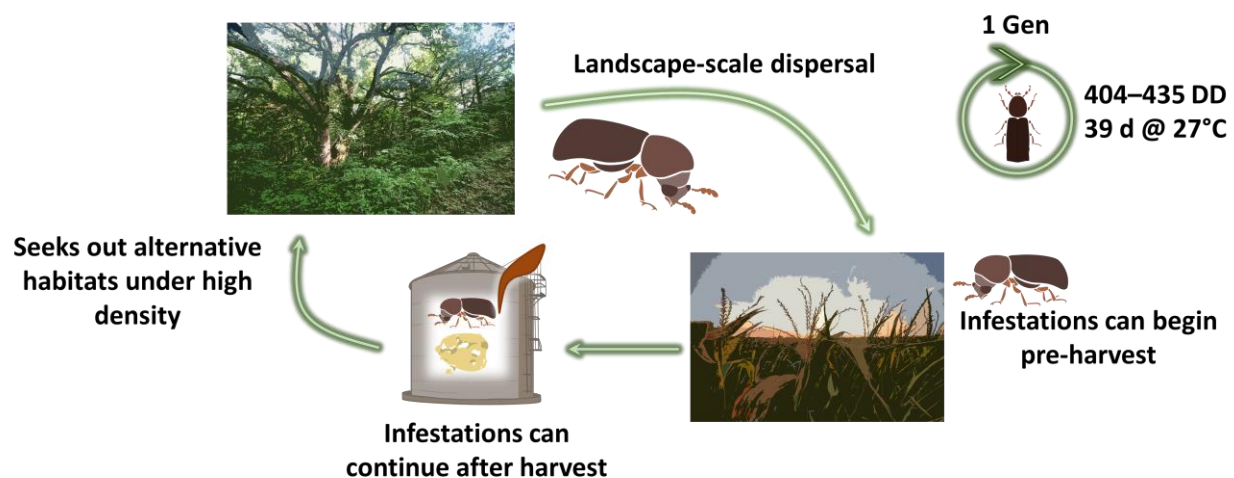
Overall, there has been a dearth of research on the fundamental movement ecology of *P. truncatus*. Nonetheless, it appears clear that *P. truncatus* is a landscape-level pest in the environments in which it occurs with frequent dispersal among forested habitats and agricultural landscapes (Figure 1.4). Prior work has suggested that conspecifics will disperse from forests associated with teak production to other forested areas and towards maize stores later in the season (Nansen et al., 2002b). Woody substrates, including 27 of 84 tested native and

agroforestry tree taxa, were found to be suitable as feeding and breeding hosts (Nang'ayo et al., 2002). That study showed that the breeding success of *P. truncatus* varied widely, showing no clear pattern by tree systematics or wood hardness; the greatest population increases were in the stem sapwood and occurred on wood samples collected and tested during the dry season.

Prostephanus truncatus captured in woody habitats had more lignin in their diets, whereas those in intensive agriculture settings had both starchy and lignin in their gut contents, suggesting dispersal and switching between hosts (Borgemeister et al., 1998b).

Figure 1-4 Behavioral dynamics of movement, infestation, and life cycle for *P. truncatus*

Behavioral dynamics of movement, infestation, and the life cycle for *Prostephanus truncatus* from published literature. Abbreviations = DD, degree-days; Gen, one full generation; density, population density.



Most of the research on movement of *P. truncatus* has been conducted on its flight behavior, but even in this case, the literature has been heavily biased towards simple pheromone-baited trapping studies in different habitats (discussed more in the *Traps* subsection below). Several abiotic factors have been investigated and found to be important in affecting the activity of *P. truncatus*. For instance, Nansen et al. (2001) found that daylength and minimum relative humidity (when below 75%) were positively correlated with flight activity, while minimum temperature was negatively associated with flight. Flight initiation by *P. truncatus* decreased

significantly with increasing wind speed, and a majority of beetles were found to fly at a low wind speed of 20 cm/s (Fadamiro, 1996). Follow-up work documented that *P. truncatus* generally flew upwind to the source of pheromone emissions, and also responded to the movement of visible patterns on the floor of a sustained flight tunnel, demonstrating directed taxis in response to external stimuli similar to lepidopterans (Fadamiro et al., 1998).

Relatively few biotic drivers of *P. truncatus* movement have been investigated. However, density was found to increase the rate at which conspecifics initiated flight, with high densities resulting in approximately a third of beetles flying (Scholz et al., 1997a). Conspecifics are clearly attracted to woody volatile host cues as well as cues from other wood-boring insects such as cerambycids (Borgemeister et al., 1998a). Few to no studies to our knowledge have investigated how physiology, starvation, host cues, predator cues, or other internal or external stimuli affect the movement ecology of *P. truncatus*. However, there is evidence suggesting that *P. truncatus* is not attracted to host volatiles from dried maize (Hodges et al., 1998; Fadamiro et al., 1998; Scholz et al., 1997a) and cassava (Scholz et al., 1997b). Finally, prior work has shown moderate heat (40-45°C) will drive *P. truncatus* out of grain (Hashem, 1990).

Intra- and Interspecific Competition

Grain stores are often a complex community of interacting insect pests. As a result, both competition among conspecifics as well as between *P. truncatus* and other species may be important in structuring interactions. For invasive insects like *P. truncatus*, which continues to colonize new regions, these interactions may be especially important as competition for resources may affect several other biological factors, such as population size, dispersal, developmental rate, and fitness. Once an invasive species is introduced into a new environment, its success will often depend on its ability to outcompete or coexist with the established species

in that environment (Sakka and Athanassiou, 2018). Most of the studies on this subject place two or more species together and assess grain damage and progeny production (Giga and Canhao, 1993; Sakka and Athanassiou, 2018; Quellhorst et al., 2020). However, there are few if any studies that view competition through an ecological lens, such as comparing population growth rates, R^* values from resource competition theory (Tilman, 1982), or other theoretically important values such as how carrying capacity for a species may be influenced by the presence of each player.

As *P. truncatus* spreads to new areas, the insect will inevitably come into contact with established species. Giga and Canhao (1993) were the first to highlight the importance of looking at the interactions between *P. truncatus* and other species. In particular, they studied both intraspecific and interspecific competition between *P. truncatus* and *S. zeamais* at 25 and 30 °C on maize, using a replacement series design (substitution). They found that both species were impacted negatively by competition. Effect size of competition increased with increasing population density and temperature. Further, they found that at 25 °C, *S. zeamais* was the superior competitor, while at 30 °C, *P. truncatus* had greater population growth compared to *S. zeamais*. Overall, the authors concluded that the outcome of the competition was inconclusive. Finally, that study documented that the presence of *S. zeamais* suppressed *P. truncatus* progeny production. This was later confirmed by Ngom et al. (2020), who found *S. zeamais* sought out grains infested by *P. truncatus*, while *P. truncatus* avoided previously infested grains by its competitor because *S. zeamais* larvae have the ability to consume competing larvae in a form of scramble competition. By contrast, under low population density, *P. truncatus* may just lay one egg per kernel and be at a competitive disadvantage and/or coexisting with other species (Guntrip et al., 1996), but under high density *P. truncatus* may oviposit multiple eggs per kernel

leading to “*P. truncatus* hotels” and the skeletonization of the kernel prior to a competitor reaching adulthood (Richards, 1947; Sharifi and Mills, 1971; Longstaff, 1981; Vowotor et al., 1998; Guedes et al., 2010).

In a follow-up study, Quellhorst et al. (2020) evaluated a greater range of temperatures and also looked at the interaction between *P. truncatus* and *S. zeamais*, including how their competition affected microbial growth in grain. The authors confirmed that the two insect species could coexist after two generations from 25–30°C. However, both *P. truncatus* and *S. zeamais* suffered competitive costs. The study also clarified the outcome of competition at higher temperatures (e.g., 35°C), finding that *P. truncatus* produced significantly more progeny and caused more damage compared to *S. zeamais*, while at the lower temperatures (e.g., 25–30°C) *S. zeamais* had nearly double the progeny production compared to *P. truncatus*. Despite this, *P. truncatus* was still responsible for most of the grain damage, even in this mixed colonies with far more *S. zeamais* progeny. Finally, they found that there was more mold growth in colonies with both species in an additive design compared to each species alone. There are relatively few other studies that looked at competition between these two species (Osipitan et al., 2012; Ngom et al., 2020), but they found similar results to the ones above. In considering the developmental time on competition dynamics, the shorter life cycle of *S. zeamais* compared to *P. truncatus* will mean higher local abundance of the former. The existence of higher populations of *S. zeamais* combined with the propensity of larvae to consume competing larvae in the same kernel may mean that *S. zeamais* will outcompete *P. truncatus* at temperatures from 25 to 30°C in the long-term, assuming food is limiting and there is no spatial niche partitioning. However, because several studies have documented that most damage results from *P. truncatus* when compared to other *Sitophilus* spp. (Quellhorst et al., 2020; Baliota et al., 2021), it is likely that sporadic

occurrence of *P. truncatus* in the field in response to aggregation pheromones early after emergence may seed infestations that disproportionately cause more damage. In the future, spatial niche differentiation should be specifically evaluated further as a mechanism of coexistence.

The interactions between *P. truncatus* and a handful of other stored product species have also been evaluated. In evaluating competition between *P. truncatus*, *R. dominica*, and *Dinoderus porcellus* Lesne (Coleoptera: Bostrichidae), *P. truncatus* was found to be the most abundant species on maize, but its presence benefited *D. porcellus* (Sakka and Athanassiou, 2018). Papanikolaou et al. (2018) suggest that interspecific competition may work to moderate the impact of *P. truncatus* on grain when niche partitioning is not possible. This highlights the importance of investigating the behavior of *P. truncatus* while in a grain mass.

The abiotic environment shapes development and competitive interactions in the postharvest environment. For example, Papanikolaou et al. (2018) found that as the RH increased, there were a higher percentage of live *P. truncatus*, while increasing temperatures and competition with *R. dominica* led to a decrease in the percentage of live insects. By contrast, Subramanyam and Hagstrum (1991) found that the development of *P. truncatus* eggs and pupae were similar regardless of whether RH was systematically varied between 40–90% RH in the laboratory. By contrast, they found temperature was far more important for development. However, in assessing the suitability of new areas for *P. truncatus*, annual precipitation was found to be important (Arthur et al., 2019), suggesting that in the field there may be an increased risk of water loss, especially considering the species' potential dispersal capacity and movement.

Intraspecific competition also shapes the grain environment for *P. truncatus*. In evaluating the effect of egg density in a single maize grain on the success of *P. truncatus* larvae,

prior work has found that a maximum of six conspecific adults can emerge, but this is also likely modulated by kernel size. The initial egg density on a grain did not significantly reduce the adult emergence weight, except in the case of the highest treatment, which consisted of grains artificially infested with eggs (e.g., 16 eggs/grain) (Vowoter et al., 1998). The authors found *P. truncatus* larvae reduced competition by dispersing to new grain under high density and that different *P. truncatus* instars fed on different types of grain tissues as they develop. Finally, Stathers et al. (2020) found that over a 4–6 month period of feeding by *P. truncatus*, conspecifics preferentially ate the germ and endosperm in grain, thereby reducing the fat, protein, iron, and zinc content relative to other nutrients. These mechanistic studies of behavior are an important component in filling research gaps with this species.

The majority of the work investigating competition between *P. truncatus* and other stored-products pests has been done in the laboratory. However, there are many conditions in the field, which are not adequately captured in the laboratory, and in the field, the occurrence of competition may also vary in time and space as commodities are stored. As a result, field and semi-field studies with *P. truncatus* and related species are imperative, but there have only been a handful of studies. For example, in the field, *P. truncatus* and *S. zeamais* were found to be sparsely aggregated in grain stores in Benin over two storage seasons (Vowotor et al., 2005). Those authors further found that ears with *S. zeamais* had no *P. truncatus*. There have also been a few other studies that monitored the population dynamics of *P. truncatus* in the field (Meikle et al., 1998a, 1998b). Meikle et al. (1998a) produced a simulation model, which could predict *P. truncatus* population growth during the season. However, while the model predicted most of the field data, it was found to overestimate population growth during the season and overall density late in the season. This study did not mention any interactions with *S. zeamais*. By contrast, *P.*

truncatus was found to be associated with the presence of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) in Zimbabwe (Nyabako et al., 2020). Thus, there is still more work to do in relating laboratory competition studies back to the field to develop a more comprehensive picture of competition under realistic circumstances.

However, there have been a large number of field storage monitoring studies that examine population sizes of varying numbers of species, different community compositions, different densities, and under different abiotic conditions. These often last for between 6-12 months (e.g., Baoua et al. 2015; Chigoverah and Mvumi 2016). One approach may be to compile this data under an ecological modelling framework to determine how competition may be structuring populations, and to identify other important factors modifying competitive parameters. Combining these different studies may also require a meta-analysis approach to be incorporated to adjust for different experimental designs. This may be a fruitful area of study going forward.

Host Preferences

Host and Habitat Preferences: Natural Hosts

Prostephanus truncatus belongs to a family of wood-boring insects, and was initially described as a wood borer in the early 20th Century (Chittenden, 1911). The species is regularly captured in traps placed in forests and wooded savannahs in both its native and introduced range (Tigar et al. 1994b; Nansen et al. 2002b; Nansen et al. 2004). In particular, a high abundance of *P. truncatus* has been found in teak tree (*Tectona grandis* L.f.) forests, where the insects then dispersed to other areas of the forest and into agricultural areas (Nansen et al., 2002b). This finding suggests that movement is from forests to agricultural areas including stores of grain, rather than from these stores and into forests (Nansen et al., 2002b). Further, *P. truncatus* is

frequently present in branches that have been girdled by Cerambycidae, suggesting that natural populations of *P. truncatus* may target already damaged or stressed woody hosts as part of its realized niche to reproduce and feed (Nang'ayo, 1993; Ramirez-Martinez et al. 1994; Borgemeister et al., 1998a; Table 1.2). To date, cerambycid-girdled branches are the only niche in the natural environment outside of the stored products environment where *P. truncatus* has been found reproducing. In Mexico, *P. truncatus* was found with its natural enemy *Teretrius* (*Teretriosoma*) *nigrescens* (Lewis) (Coleoptera: Histeridae) in branches of *Spondias purpurea* L. (Anacardiaceae) and *Bursera fagaroides* (Kunth) Engl. (Burseraceae) girdled by the cerambycid *Oncideres albomarginata* Thomson (Ramirez-Martinez et al., 1994). In Kenya, *P. truncatus* was found associated with branches of *Commiphora riparia* Engl. (*C. mildbraedii* Engl.) (Burseraceae) and *C. africana* Engl. girdled by *Paranaleptis reticulata* (Thomson) (Coleoptera: Cerambycidae) and a second unidentified cerambycid (Nang'ayo 1996). In Benin, a small number of *P. truncatus* were found in branches of *Lannea nigritana* (Scott Elliot) Keay (Anacardiaceae) girdled by *Analeptes trifasciata* F. (Coleoptera: Cerambycidae). Hodges (1986) and Hill et al. (2002) mentioned that *P. truncatus* has also been found boring into the wood of dwellings and grain storage structures, but no data or citations were provided to support this.

Table 1-2 List of wild and stored product hosts of *P. truncatus*

List of assessed wild and stored product hosts for survival, reproduction, and presence of *P. truncatus* in the laboratory and field.

Host Plant Type	Scale	Host Plant Name	Survival	Progeny Production	Girdled ?	Girdling Species	Country	Citation
Woody	Field	<i>Spondias purpurea</i>	- ^a	-	Yes	<i>Oncideres albomarginata</i>	Mexico	Ramirez-martinez et al., 1994
Woody	Field	<i>Bursera fagaroides</i>	-	-	Yes	<i>Oncideres albomarginata</i>	Mexico	Ramirez-martinez et al., 1994
Woody	Field	<i>Commiphora riparia</i>	-	-	Yes	<i>Paranaleptis reticulata</i>	Kenya	Nang'ayo et al., 1996
Woody	Field	<i>Commiphora africana</i>	-	-	Yes	<i>Paranaleptis reticulata</i>	Kenya	Nang'ayo et al., 1996
Woody	Field	<i>Lannea nigritana</i>	-	-	Yes	<i>Analeptes trifasciata</i>	Benin	Borgemeister et al., 1998a
Woody	Field	<i>Tectona grandis</i>	-	-	Yes	Unknown	Benin	Nansen et al., 2004
Woody	Lab	<i>Acacia mellifera</i>	-	Yes	-	-	Kenya	Nang'ayo et al., 1993

Woody	Lab	<i>Commiphora campestris</i>	-	Yes	-	-	Kenya	Nang'ayo et al., 1993
Woody	Lab	<i>Cassia abbreviata</i>	-	Yes	-	-	Kenya	Nang'ayo et al., 1993
Woody	Lab	<i>Cassia siamea</i>	-	Yes	-	-	Kenya	Nang'ayo et al., 1993
Woody	Lab	<i>Acacia polyacantha</i>	-	Yes	-	-	Kenya	Nang'ayo et al., 1993
Woody	Lab	<i>Calliandra calothyrsus</i>	-	Yes	-	-	Kenya	Nang'ayo et al., 1993
Woody	Lab	<i>Leucaena diversifolia</i>	-	Yes	-	-	Kenya	Nang'ayo et al., 1993
Woody	Lab	<i>Leucaena leucocephata</i>	-	Yes	-	-	Kenya	Nang'ayo et al., 1993
Woody	Lab	<i>Leucaena shamonii</i>	-	Yes	-	-	Kenya	Nang'ayo et al., 1993
Woody	Lab	<i>Prosopis chilensis</i>	-	Yes	-	-	Kenya	Nang'ayo et al., 1993
Woody	Lab	<i>Commiphora baluensis</i>	-	Yes	-	-	Kenya	Nang'ayo et al., 1993
Woody	Lab	<i>Commiphora rostrata</i>	-	Yes	-	-	Kenya	Nang'ayo et al., 1993

Woody	Lab	<i>Delonix lata</i>	-	Yes	-	-	Kenya	Nang'ayo et al., 1993
Woody	Lab	<i>Lamnea sp.</i>	-	Yes	-	-	Kenya	Nang'ayo et al., 1993
Woody	Lab	<i>Pachira quinate</i> (predrilled)	Yes	No	-	-	Germany	Detmers 1990
Woody	Lab	<i>Prunus spinosa</i>	Yes	No	-	-	Germany	Detmers 1990
Woody	Lab	<i>Pinus sp.</i>	Yes	No	-	-	Germany	Detmers 1990
Woody	Lab	<i>Agave sisalana</i>	Yes	No	-	-	Germany	Detmers, 1990
Woody	Field	<i>Brachystegia spiciformis</i>	-	-	-	-	Mozambique	Muatinte and Van den Berg 2019
Woody	Field	<i>Colophospermum mopane</i>	-	-	-	-	Mozambique	Muatinte and Van den Berg 2019
Woody	Field	<i>Strychnos spinosa</i>	-	-	-	-	Mozambique	Muatinte and Van den Berg 2019
Woody	Lab	<i>Terminalia sericea</i>	-	No	-	-	Mozambique	Muatinte and Van den Berg 2019
Woody	Lab	<i>Androstachys johnsonii</i>	-	Yes	-	-	Mozambique	Muatinte and Van den Berg 2019

Woody	Lab	<i>Julbernardia globiflora</i>	-	Yes	-	-	Mozambique	Muatinte and Van den Berg 2019
Woody	Lab	<i>Brachystegia boehmii</i>	-	Yes	-	-	Mozambique	Muatinte and Van den Berg 2019
Woody	Lab	<i>Brachystegia utilis</i>	-	Yes	-	-	Mozambique	Muatinte and Van den Berg 2019
Woody	Lab	<i>Cassia abbreviata</i>	-	Yes	-	-	Mozambique	Muatinte and Van den Berg 2019
Woody	Lab	<i>Piliostigma thommingii</i>	-	No	-	-	Mozambique	Muatinte and Van den Berg 2019
Woody	Lab	<i>Philenoptera violacea</i>	-	Yes	-	-	Mozambique	Muatinte and Van den Berg 2019
Woody	Lab	<i>Sacacia xanthophloea</i>	-	Yes	-	-	Mozambique	Muatinte and Van den Berg 2019
Woody	Lab	<i>Ziziphus mauritiana</i>	-	Yes	-	-	Mozambique	Muatinte and Van den Berg 2019
Grass Stalks	Lab	<i>Acroceras macrum</i>	-	Yes	-	-	Mozambique	Muatinte and Van den Berg 2019
Grass Stalks	Lab	<i>Aristida congesta</i>	-	No	-	-	Mozambique	Muatinte and Van den Berg 2019
Grass Stalks	Lab	<i>Digitaria eriantha</i>	-	No	-	-	Mozambique	Muatinte and Van den Berg 2019

Grass Stalks	Lab	<i>Eulalia villosa</i>	-	Yes	-	-	Mozam- bique	Muatinte and Van den Berg 2019
Grass Stalks	Lab	<i>Hyparrhenia hirta</i>	-	Yes	-	-	Mozam- bique	Muatinte and Van den Berg 2019
Grass Stalks	Lab	<i>Pennisetum glaucum</i>	-	Yes	-	-	Mozam- bique	Muatinte and Van den Berg 2019
Grass Stalks	Lab	<i>Zea mays</i>	-	Yes	-	-	Mozam- bique	Muatinte and Van den Berg 2019
Durable Commodity	Lab	Barley (peeled)	No	No	-	-	Greece	Athanassiou et al. 2017a
Durable Commodity	Lab	Barley (whole flour)	Yes	Yes	-	-	Greece	Athanassiou et al. 2017a
Durable Commodity	Lab	Barley (whole)	No	No	-	-	Greece	Athanassiou et al. 2017a
Durable Commodity	Lab	Cassava chips (dried)	Yes	Yes	-	-	Togo	Helbig and Schulz 1996

Durable Commodity	Field	Cassava roots (fermented dried)	Yes	Yes	-	-	Tanzania	Hodges et al. 1985
Durable Commodity	Field	Cassava roots (unfermented dried)	Yes	Yes	-	-	Tanzania	Hodges et al. 1985
Durable Commodity	Lab	Cassava wood	Yes	Yes	-	-	Togo	Helbig and Schulz 1996
Durable Commodity	Lab	Maize (5-100% cracked)	Yes	Yes ^b	-	-	Greece	Athanassiou et al. 2017b
Durable Commodity	Lab	Maize (flour)	Yes	Yes	-	-	Greece	Athanassiou et al. 2017b
Durable Commodity	Lab	Maize (intact)	Yes	Yes	-	-	Greece	Athanassiou et al. 2017b
Durable Commodity	Semi- field	Maize (on cob)	Yes	Yes	-	-	Tanzania	Golob and Hanks 1990

Durable Commodity	Lab	Oat flakes (whole)	No	No	-	-	Greece	Athanassiou et al. 2017a
Durable Commodity	Lab	Oats (peeled)	No	No	-	-	Greece	Athanassiou et al. 2017a
Durable Commodity	Lab	Oats (whole)	No	No	-	-	Greece	Athanassiou et al. 2017a
Durable Commodity	Lab	Pasta	No	No	-	-	Greece	Athanassiou et al. 2017a
Durable Commodity	Lab	Rice (peeled)	Yes	No	-	-	Greece	Athanassiou et al. 2017a
Durable Commodity	Lab	Rice (rough)	No	No	-	-	Greece	Athanassiou et al. 2017a
Durable Commodity	Lab	Rye (whole)	No	No	-	-	Greece	Athanassiou et al. 2017a

Durable Commodity	Lab	Semolina	No	No	-	-	Greece	Athanassiou et al. 2017a
Durable Commodity	Lab	Triticale (whole)	Yes	Yes ^c	-	-	Greece	Athanassiou et al. 2017a
Durable Commodity	Lab	Wheat (whole)	No	No	-	-	Greece	Athanassiou et al. 2017b
Durable Commodity	Lab	Wheat flour (white hard)	No	No	-	-	Greece	Athanassiou et al. 2017b
Durable Commodity	Lab	Wheat flour (white soft)	No	No	-	-	Greece	Athanassiou et al. 2017b
Durable Commodity	Lab	Wheat flour (whole hard)	No	No	-	-	Greece	Athanassiou et al. 2017b
Durable Commodity	Lab	Wheat flour (whole soft)	No	No	-	-	Greece	Athanassiou et al. 2017b

^a Hyphen indicates not applicable or not assessed; ^bReproduction only
found in the 100% cracked maize treatment; ^cOnly marginal reproduction

There have been several efforts made to identify woody hosts of *P. truncatus* (Detmers, 1988; Laborius, 1990; Helbig et al., 1992; Nang'ayo, 1993, 1996; Espinal et al., 1996; Nang'ayo et al., 2002; Nansen et al., 2004). For example, the earliest study tested 15 tropical and 10 temperate tree species in the laboratory and found no reproduction by *P. truncatus* (Detmers, 1988). However, *P. truncatus* was then found to reproduce in the stems of cassava and the flamboyant tree, *Delonix regia* (Detmers et al., 1990). In that work, *P. truncatus* performed much better on cassava stems than on the flamboyant tree stems, suggesting that the softer woods with higher starch content were responsible for this outcome. Further work in Kenya found that of the 81 local tree species tested, 23 were found favorable to *P. truncatus* for reproduction (Nang'ayo et al. 1993). Later, Nang'ayo (1996) investigated the idea that starchy soft woody substrates were preferred to other substrates, but found no relationship between the host and the concentration of starch in the wood. In a particularly illuminating study on the diet composition of *P. truncatus*, Borgemeister et al. (1998b) trapped *P. truncatus* in Benin over a 12-month period in a primary forest, a peri-urban area, and a region with intensive agriculture, analyzing the gut contents of beetles. They found *P. truncatus* with both lignin and starch, which were indicators of feeding on woody hosts and starchy hosts (e.g., maize and dried cassava), respectively. Forested locations resulted in capture of beetles with mostly lignin, the peri-urban areas had beetles with a high proportion of starch, and the agricultural site consisted of beetles with equal proportions of lignin and starch in their midguts. This suggests that at agricultural sites, there may be frequent dispersal of *P. truncatus* between wooded landscapes and postharvest environments, and thus frequent immigration of new individuals. These are the kinds of studies that are needed to determine the host and habitat preferences of *P. truncatus*, but which have largely been lacking up to this point.

As some have noted, *P. truncatus* readily attacks and causes damage to stored timber and timber products (Wright, 1984). It is very common for homes and storage structures in Sub-Saharan Africa to incorporate wood, and it remains an open question whether this wood provides an important refuge from which *P. truncatus* can locally invade grain stores. In a survey of almost 300 smallholders in Zimbabwe, *P. truncatus* was reported attacking wooden items, crib poles, plastic, leather, and textiles in nontrivial amounts (Nyagwaya et al., 2010). If infestations of wood are noticed in or near a grain storage site, it may be worth taking preventative management steps. Despite the many attempts over the years to discover the hosts of *P. truncatus*, there are still many unanswered questions about natural host refugia in the landscape, including: 1) does *P. truncatus* have preferred woody hosts in its natural host range, and do these vary from its introduced host range, 2) in which microhabitats does *P. truncatus* spend its time when away from grain stores, 3) once in or near a food store, does *P. truncatus* remain in nearby wood structures after grain has been moved out, or is there two-way movement from the grain store to wild refugia, 4) what is the role of resident populations and year-round sanitation at grain stores (e.g., Morrison et al., 2019), and 5) what is the role of meta-population ecology in the population dynamics of *P. truncatus*? The answers to these questions may result in improved recommendations regarding habitat management to deal with current populations or target biosurveillance to vulnerable locations in the future.

Host Preferences: Stored Products

Despite the presence of natural refugia, *P. truncatus* remains a significant threat to food security by attacking important stored products. Specifically, *P. truncatus* is a pest of maize, in stores, on the cob in the field, and found in/on cribs and platforms during drying, causing serious pre-storage infestations (Delgado and Hernandez Luna, 1951; Ramirez Genel, 1960b; Quintana

et al., 1960; Giles and Leon, 1975; Giles 1975; Hodges et al., 1983a). The species has been known to infest grains in the field with high moisture content (e.g., 40–50%) (Giles 1975). While larvae target different seed tissues, adults feed indiscriminately on every part of the grain (Ramirez and Silver, 1983; Howard, 1983). Very few *P. truncatus* raised exclusively on germ flour ended up surviving (Howard, 1983). However, maize flour was found to be a superior food source for development compared to intact maize kernels, because it resulted in lower mortality of larvae (Athanassiou et al., 2017).

Varietal differences affect the susceptibility of maize to *P. truncatus*. Harder grains suffer less damage compared to softer grains (Bell and Watters, 1982; Ramirez and Silver, 1983; Howard, 1983, 1984). Maize stored on the cob often suffers more damage than shelled grain (Chittenden, 1911; Golob et al., 1985), with up to three times the population growth rate compared to loose grains (Cowley et al., 1980; Howard, 1983). A possible mechanism is that *P. truncatus* performs better when it has some leverage to bore into its host (Cowley et al., 1980), and tightly packed grains on a cob allow *P. truncatus* to make contiguous tunnels (Bell and Watters 1982).

As cassava is increasingly adopted as a climate-smart crop in sub-Saharan Africa (Pushpalatha and Gangadharan, 2020), the spread of *P. truncatus* imperils its profitable production during the postharvest storage period of the value chain. Both fermented and unfermented dried cassava roots experience significant weight loss during storage as a result of *P. truncatus* feeding (Hodges et al., 1985), while losses in bagged cassava chips was around 8% (Hell et al., 2014). As a result, along with maize, cassava represents a vulnerable commodity susceptible to *P. truncatus* attack at a time when climate change is increasing reliance on its use.

A variety of other commodities are considered inferior food sources for *P. truncatus*. Cowpea, haricot beans, cocoa, coffee beans, hard winter wheat, and rough rice all failed to serve as reproductive hosts for *P. truncatus* (Hodges, 1986; Athanassiou et al., 2017). By contrast, Shires (1977) found that *P. truncatus* could develop on a soft variety of wheat, but reproductive output was diminished. Grain size may also significantly affect susceptibility to *P. truncatus* (Hodges, 1986). For example, it was found that Tanzanian varieties of sorghum with small grain size escaped infestation by *P. truncatus* (Hodges et al., 1983a). Hodges et al. (1983a) postulated that smaller sorghum grains were able to escape attack by *P. truncatus* because of the body size of the species, but this still needs to be confirmed. In fact, although adults of this species are able to make holes on small grains, offspring emergence is not possible (Athanassiou et al., 2017; Sakka and Athanassiou; 2018). However, *P. truncatus*, has been found to survive on dried chips of plantain and cassava (Isah et al., 2009, 2012). Other work has found *P. truncatus* infested and damaged dry cassava chips, wheat, rice, chickpea, sweet potatoes, sorghum, and several other legumes (Roux, 1999). More recently, among whole barley, peeled barley, maize, whole oats, peeled oats, peeled rice, rough rice, rye, triticale, wheat, and among a large variety of flours (Table 1.2), only whole maize or maize flour were suitable for the development of *P. truncatus* (Athanassiou et al. 2017). That study also found that *P. truncatus* performed better on cracked maize grains compared to intact grains. Thus, the inability of *P. truncatus* to produce offspring in non-maize grains, and also on non-maize flour, suggests that grain size may not be the only reason for the suitability of maize over small grains, and allelopathic interactions with non-maize commodities may also contribute (Athanassiou et al., 2017). Hagstrum and Subramanyam (2009) list nine different hosts/commodities on which this species has been reported, but this list refers to records, rather than establishment and progeny production capacity. Considering the

devastating effects of this species to stored maize, additional experimental work is needed to demonstrate the likelihood of host switching of this species to small grains, such as wheat, sorghum, and rice, which will further increase its importance for global food security.

Chemical Ecology, Detection, and Monitoring

Pheromones

Prostephanus truncatus may aggregate on a food source in response to the major male-produced aggregation pheromone component known as Trunc-call 1 (T1), which consists of (2*E*)-2-methyl-2-pentenoate (Hodges et al., 1984). Both males and females respond to this compound in the laboratory using electroantennographic and behavioral bioassays (Cork et al., 1991). Under identical conditions in the laboratory, *P. truncatus* produces 0.001% the amount of pheromone produced by *R. dominica*. Later work found that Trunc-call 2 (T2), 1-methylethyl (*E*)-2,4-dimethyl-2,4-heptadienoate, is a minor component of the aggregation pheromone and synergizes response to T1 (Dendy et al. 1989; Hodges et al. 1998). Initially, fundamental work looking at the T1 and T2 components found that the specific ratio of each did not matter as much as whether both were present (Dendy et al. 1989). Nonetheless, later work actually found polythene capsules with a 2:1 ratio of T1:T2 captured almost a third more beetles in the field compared to a 1:1 ratio (Hodges et al., 2004). Small populations, even as low as a single male, may be sufficient to trigger high infestations later in the storage season (Scholz et al., 1997a). In fact, a single male inside a storage site may start emitting aggregation pheromones, which brings in conspecifics if they are flying by shortly after eclosion (Figure 4).

There are a variety of factors that may affect aggregation pheromone production by male *P. truncatus*. For example, the presence of females or maize that had been infested with females resulted in significant inhibition of pheromone production (Smith et al., 1996), suggesting a role

for the aggregation pheromone in attracting mates. Thus, there appears to be an as-yet-to-be-identified residue produced by females on grain that suppresses future production of pheromone by males. On average, males released 1.9 and 0.5 $\mu\text{g}\cdot\text{d}^{-1}$ of the T1 and T2 aggregation pheromone components, respectively, though there is significant variation among males in how much pheromone and in what ratio it is produced, with possibly two different release strategies (Hodges et al., 2002). These differences among males are consistent and may be sexually selected by females (Birkinshaw and Smith, 2000).

In terms of behavior, *P. truncatus* are attracted towards their aggregation pheromone 2 d after eclosion, then attraction gradually and significantly decreases up to 10 d later, with younger beetles responding quicker and arrested for longer in the vicinity of the aggregation pheromone than older beetles (Boughton and Fadamiro, 1996). These authors predicted that young beetles may initially use the pheromone to locate food, but may then stop responding to the pheromone as they age and as immediate nutritional needs are met, with reproduction becoming correspondingly more important. Indeed, other work showed that once *P. truncatus* has located a food source, it is not lured away to pheromone-baited traps, even at higher densities, suggesting that there is some brief window during which *P. truncatus* may primarily respond to its pheromone, with short-range food cues becoming more important as beetles age (Pike, 1993). This is a common pattern observed in stored product species that are long-lived at the adult stage (Trematerra, 1997; Maille et al., 2021). Intermediate doses of pheromone (e.g., 0.2 mg) were found to increase the speed that *P. truncatus* initiated flight, and minimized their phototaxis (Fadamiro 1996). By contrast, the suitability of the resource (e.g., diet), sex, mating status, prior exposure to pheromone, and nutritional status did not affect the response of *P. truncatus* to its aggregation pheromone (Fadamiro and Wyatt, 1996). Using sticky cards baited with both

aggregation pheromone components, Tigar et al. (1993) found that the two main peaks of flight activity were around dawn and dusk, with the latter comprising the larger peak. *Prostephanus truncatus* was found to orient to its pheromone similarly to predictions based on studies on moths (Fadamiro et al., 1998). It is interesting to note that the aggregation pheromones of *R. dominica* and *P. truncatus* are cross-attractive to both species (Hodges et al., 1983b); *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) was also found to be significantly attracted to a commercial lure containing *P. truncatus* pheromone (Losey, 2015). Finally, Edde and Phillips (2006) found that deployment of the aggregation pheromone for *P. truncatus* captured a large number of *Prostephanus punctatus* (Say) (Coleoptera: Bostrichidae), a wood-boring congener of *P. truncatus* in Oklahoma, USA.

Host Stimuli

There is conflicting evidence about the utility of host volatiles on taxis and attraction of *P. truncatus*. At close range, host volatiles appear to have a larger effect on *P. truncatus* than when tested at long range. For example, Fadamiro et al. (1998) found that upwind flight of conspecifics was only mediated by the aggregation pheromone of *P. truncatus*, but not various blends of maize volatiles. Likewise, Hodges et al. (1998) found that a combination of maize volatiles (e.g., hexanoic acid, nonanoic acid, nonanal, decanal, 2-phenylethanol, and vanillin) with T1 and T2 did not improve or synergize trap captures of *P. truncatus*. Wind tunnel experiments with *P. truncatus* demonstrated that conspecifics were not attracted to woody plant volatiles or maize grains (Fadamiro, 1995). In the introduced and invaded range, it was found that there was no long-range attraction to shelled maize, cobs, or dried cassava in the field (Wright, 1993; Tigar et al., 1994b). Arnold et al. (2015) found that crushed white maize was attractive in both odor and color for *S. zeamais*, but not for *R. dominica* and *P. truncatus*. Thus,

studies generally do not support the role of host stimuli in long-range attraction or orientation of adult *P. truncatus*.

There appears to be ample evidence that *P. truncatus* uses host stimuli at close-range, but these cues act mostly in arrestment of individuals. This has primarily been demonstrated through choice and olfactometer assays. For example, *P. truncatus* was found to be most attracted to the maize variety Synth-C, and least attracted by a suite of other varieties, including Across-Pool, Obatampa, SWAN, Tzee-White, and Tzee-Yellow, over short distances (Ngom and Sembène, 2020). Detmers (1990) found that over short distances, *P. truncatus* is attracted to maize grains, whereas Wright et al. (1993) found that dried cassava was arresting at short distances. Pike (1993) also found that the extracted volatiles of both maize grains and cassava were attractive in a laboratory bioassay. In a study by Scholz et al. (1997b), the odors of maize, cassava chips, wheat, cowpea, and woody plant species were tested in 4-way olfactometer experiments. They found that *P. truncatus* adults from maize stores or reared on maize in the laboratory were attracted to odors from starchy commodities (cassava and maize) and to some woody plant species, but *P. truncatus* captured from savannah or forested ecosystems were not attracted to the same odors. Follow-up work using an olfactometer demonstrated that males placed on *Commiphora riparia* Engl. and *Commiphora africana* (A. Rich.) (Burseraceae) were just as arresting as males placed on maize (Scholz et al., 1998b). Two more studies by Scholz et al., (1997b, 1998b) showed that *P. truncatus* was attracted to both damaged and intact wood from branches of *Sarracenia purpurea* L. (Sarraceniaceae). Likewise, in a study by Scholz et al. (1998b), *P. truncatus* captured from maize-growing areas were slightly more attracted to maize volatiles in olfactometer experiments compared to insects captured in forested habitats. This finding suggests that prior experience or pre-conditioning of the insects on one host could

influence their attraction to new host materials as they disperse. *Prostephanus truncatus* significantly preferred volatiles from cerambycid frass, as well as girdled and mechanically damaged *Lannea nigritana* (Sc. Elliot) Keay (Anacardiaceae) twigs in a 4-way olfactometer compared to controls (Borgemeister et al., 1998a). Overall, host odors may be important at short range to *P. truncatus*, but not at long range, and may only have arresting properties as opposed to effects on orientation or flight. Studies on host volatiles and their impact on the behavioral response of *P. truncatus* strongly suggests that host volatiles do not play a primary role in dispersal and taxis to commodities by conspecifics.

Visual Stimuli

Very few studies have investigated the visual preferences of *P. truncatus*. De Groote et al. (2017) evaluated grain size and color preference of *P. truncatus* and *S. zeamais*, but their experimental design only included a no-choice assay, making conclusions about preference on either topic difficult. Other work has found that yellow is attractive to *S. zeamais*, but neither that color, nor green, blue, and cream affected arrestment time of *P. truncatus* or *R. dominica* (Arnold et al., 2015). Fadamiro (1995) found that *P. truncatus* is positively phototactic. However, the only reference to the evaluation of light traps for *P. truncatus* is to an unpublished study with no data provided in Hodges (2002), which mentioned that five attempts showed no captures by *P. truncatus* to light-based traps. Thus, little to no work has investigated whether there is a preference for certain shapes or light stimuli, which may be important for a species that appears to regularly use trees as a host. Other arboreal insects have been found to have a preference for dark silhouettes that mimic tree shapes and ultraviolet light (Leskey et al., 2012; Leskey et al., 2015).

Traps

Trapping and monitoring for *P. truncatus* adults may be particularly difficult because they are diffusely distributed among forested (e.g., wild) habitats and maize stores. The most common trap designs for *P. truncatus* have been delta sticky cards and funnel traps (Table 1.3). In a study evaluating three designs, two flight traps captured significantly more *P. truncatus* than a pitfall trap for walking beetles, and authors concluded that conspecifics fly directly to a pheromone source, thus it is more economical to monitor adults using flight traps (Dendy et al., 1989). Another study found that delta traps baited with 1 mg of T1 + T2 captured many-fold more *P. truncatus* than traps baited with one maize cob and one male *P. truncatus* (Scholz et al., 1997a). In a survey of five Mexican regions using crevice traps, *P. truncatus* was more abundant in drier regions, including Guanajuato, La Laguna, and Jalisco. Out of five alternate trap designs, an 8-funnel Lindgren trap with both aggregation pheromone components was found to be the most effective trap, capturing 6-, 4-, 2-, and 2-fold more *P. truncatus* than a sticky open box, wing trap, short strips over a funnel, and long strips over a funnel, respectively (Leos-Martinez et al., 1995)

Table 1-3 Summary of major trap types, lures formulations, and parameters for monitoring *P. truncatus* globally.

Trap Type	Supplier	Trap Dimensions	Trap Height (m)	Trap Spacing (m)	Time of Trapping	Killing Mechanism	Lure Stimuli	Stimuli Ratio	Lure Dispensers	Trapping Interval	Habitat	Location	Citation
Double-sided Sticky Cards	Phercon II, Trece, Inc.	539 cm ²	1.08	80	-	Sticky glue	2 mg of T1 & T2	1:01	Cylindrical polythene vials	14 d	-	Mexico	Tigar et al. 1993
Delta sticky trap	Phercon II, Trece, Inc.	539 cm ²	-	100	30 March - 8 Jun 1995	Sticky glue	2 mg of T1 & T2	1:01	Cylindrical polythene vials	7 d	IITA Station	Benin	Scholz et al. 1997
Flight funnel trap	Agrisense, Ltd., UK	9 cm at top, 4 cm at bottom	1.5-2	500	1 Oct 1995 - 30 April 1996	Sticky glue	2 mg of T1 & T2 +/- 40 mg maize volatiles	1:1, 1:0, or 0:1	Poly-ethylene vials or rubber septa	7 d	Along road	Kenya	Hodges et al. 1998
Crevice trap	DIY	9 cm cardboard square with 1.5 cm well	Ground level	2.7	1 Sep 1995 - 30 April 1996	1 g of permethrin and 2.5 g pimplipos methyl a.i./m2	2 mg of T1 & T2 +/- 40 mg maize volatiles	1:1, 1:0, or 0:1	Poly-ethylene vials or rubber septa	7 d	Maize cribs	Kenya	Hodges et al. 1998
Wing trap	Similar to Pherocon 1C, Trece, Inc.	-	0.6	40-60	22 April 1991- 31 Dec 1991	Sticky glue	25 uL of T1:T2, or each individually	1:01	Poly-ethylene microcentrifuge tubes with snap caps	7 d	Ware-house	Mexico	Leos-Martinez et al. 1995
Box Trap	Similar to Pherocon II, Trece, Inc.	-	0.6	40-60	22 April 1991- 31 Dec 1991	Sticky glue	25 uL of T1:T2, or each individually	1:01	Poly-ethylene microcentrifuge tubes with snap caps	7 d	Ware-house	Mexico	Leos-Martinez et al. 1995
Delta sticky trap	Agrisense BCS, Ltd.	-	0.6	40-60	22 April 1991- 31 Dec 1991	Sticky glue	25 uL of T1:T2, or each individually	1:01	Poly-ethylene microcentrifuge tubes with snap caps	7 d	Ware-house	Mexico	Leos-Martinez et al. 1995
Lindgren 8-Funnel Trap	Lindgren 1983	90 cm length	0.6	40-60	22 April 1991- 31 Dec 1991	Drowning	25 uL of T1:T2, or each individually	1:01	Poly-ethylene micro-centrifuge tubes with snap caps	7 d	Ware-house	Mexico	Leos-Martinez et al. 1995

Lindgren 4-Funnel Trap	Lindgren 1983	-	0.6	40-60	22 April 1991-31 Dec 1991	Drowning	25 uL of T1:T2, or each individually	1:01	Poly-ethylene micro-centrifuge tubes with snap caps	7 d	Ware-house	Mexico	Leos-Martinez et al. 1995
Funnel Trap	Japanese Beetle Trap	15 × 11 cm funnel with 4, 10 cm vertical crossvanes	2	>135	20 Jan - 15 April 1994	Filter paper w/5ml of 10% soln of 25 g/L deltamethrin	2 mg of T1:T2	1:01	Poly-ethylene capsules	1-2 wk lure changes/ 1 d trap changes	Food Facility	Ghana	Compton et al. 1997
Box Trap	Pherocon II, Trece, Inc.	170 cm ²	2	>135	20 Jan - 15 April 1994	Sticky glue	2 mg of T1:T2	1:01	Poly-ethylene capsules	1-2 wk lure changes/ 1 d trap changes	Food Facility	Ghana	Compton et al. 1997
Delta sticky trap	Pink Bollworm Trap	540 cm ²	2	>135	20 Jan - 15 April 1994	Sticky glue	2 mg of T1:T2	1:01	Poly-ethylene capsules	1-2 wk lure changes/ 1 d trap changes	Food Facility	Ghana	Compton et al. 1997
Pitfall/Pot Trap	DIY	57 × 76 mm (D at base:top)	Height of maize ears	6-Mar	25 May 1988 - 11 June 1988	Sticky glue	2 mg of T1:T2	1:04	Poly-ethylene vial	8 d lure / 5, 8, 13, 16 d trap changes	Maize fields	Tan-zania	Dendy et al. 1989
Delta sticky trap	Commercial	295 × 106 mm	Height of Maize Ears	6-Mar	25 May 1988 - 11 June 1988	Sticky glue	2 mg of T1:T2	1:04	Poly-ethylene vial	8 d lure / 5, 8, 13, 16 d trap changes	Maize fields	Tan-zania	Dendy et al. 1989
Flight funnel trap	Graham 1970	296 × 210 mm	Height of Maize Ears	6-Mar	25 May 1988 - 11 June 1988	Drowning	2 mg of T1:T2	1:04	Poly-ethylene vial	8 d lure / 5, 8, 13, 16 d trap changes	Maize fields	Tan-zania	Dendy et al. 1989
Funnel Trap	Japanese Beetle Trap, Trece, Inc.	15 × 11 cm funnel with 4 10 cm vertical crossvanes	1.5-2 m	150	-	Drowning	2, 3, 4, or 6 mg of T1:T2	1:1, or 2:1	polythene vial	5 d	Teak Wood-land	Ghana	Hodges et al. 2004
Funnel Trap	Japanese Beetle Trap, Trece, Inc.	15 × 11 cm funnel with 4 10 cm vertical crossvanes	-	-	1 Nov 1999 - 30 Oct 2000	0.1 cm ³ w/25% Actellic Super	1 mg of T1:T2	1:01	polythene vial	7 d lure / 16-26 d trap changes	Porch of house	Ghana	Birkinshaw et al. 2004
Lindgren 4-Funnel Trap	Phero Tech, Delta	20 cm D × 57.15 cm height	1.7 m	20	Variable (Summer)	Dichlorvos	10 mg of T1:T2	1:01	rubber septum	7 d/7 d	Second-ary growth forest and food facilities	OK, US	Edde and Phillips 2006

There has also been some work on the optimal timing of trap and lure replacement for *P. truncatus*. Using vials of synthetic aggregation pheromone and sticky cards, a two-week trapping period was found to have captured the majority of *P. truncatus* compared to shorter or longer intervals (Tigar et al., 1993). Further work has confirmed that the standard 2 mg polythene vials with a 1:1 ratio of T1:T2 typically lasted about two weeks (Hodges et al., 2004).

In characterizing optimal trapping periods diurnally and seasonally for *P. truncatus*, a study with Japanese beetle-style traps found that most conspecifics were captured from 5:30–8:30 (minor peak) and 17:30–20:30 (major peak) in Mexico, Kenya, and Ghana (Tigar et al., 1994a; Giles et al., 1995; Birkinshaw et al., 2004), and the major seasonal peaks of *P. truncatus* were May–June and November–December in Ghana (Birkinshaw et al., 2004). Manu et al. (2018) investigated the population dynamics of *P. truncatus* in and around warehouses in Ghana. They found that *P. truncatus* was not prevalent in the warehouse but was more commonly encountered outside the warehouses, on farm, and in pre-storage granaries. The authors of this study were using pheromone and/or kairomone-baited traps (e.g., Storgard II™ and Dome™ configurations, Trécé Inc.) for monitoring, so it is possible that this study further confirms the findings of Pike (1993), regarding the involvement of food sources in the attraction and colonization of *P. truncatus* at close range.

Other sampling methods

Direct sampling of commodities for presence of *P. truncatus* is generally inadvisable. Direct sampling may be performed through visual inspection of maize cobs or by probe sampling shelled maize or dried cassava (Walker and Farrell, 2003). However, if inspecting unhusked maize cobs, each cob should be dehusked, then rapidly tapped against a white tray to count the number of *P. truncatus* dislodged. The number of insects dislodged should be multiplied by 2 (if

all are alive, weighted for ongoing infestation) or 1.5 (if all are dead, discounted for historical infestation) and added to the number of *T. nigrescens* after multiplying predator numbers by 1.3 (whose presence may be an indirect indicator of ongoing issues) to get the total number of *P. truncatus* (Compton and Sherington, 1999). All multiplication factors were empirically derived. Sampling effort and number of samples depends on the commodity, level of infestation, and the certainty one would like in knowing an infestation exists, assuming one in fact does. For example, in order to detect a 0.1% infestation of *P. truncatus* in cobs with 86% probability, one would need to sample 2000 cobs, while to detect a 5% infestation with 99% certainty, one would need to inspect just 100 cobs (Hodges, 2002). Generally, it is best to take the largest sample that circumstances will allow in as many locations in the grain store as possible, even at depth, since *P. truncatus* can often be found at the bottom of grain masses (Wright, 1993; Wekesa, 1994; Hodges et al., 1999). Conspecifics will most easily be detectable several months after harvest (Hodges, 2002), but it would be most useful to catch an infestation earlier shortly after harvest. Inspecting grain as it is loaded into bags may aid this effort. Because *P. truncatus* bores into grain, hints of infestation may include traces of dust at entrances to burrows or on the outside surface of bags; under especially high infestation, a distinct rasping sound can be heard by the multitude of *P. truncatus* feeding in a grain store.

However, there are significant disadvantages to direct sampling or the use of probe traps. One of these is the fact that *P. truncatus* generally occurs at the bottom of bagged commodities, and the design of most probe traps prevents sampling the bottom 5 cm of commodities, thereby preventing the development of an accurate estimate of infestation (Nyabako et al., 2020). In many cases, *P. truncatus* are highly aggregated and sporadic (Vowotor et al., 2005), which also

complicates direct sampling. As a result, the use of direct sampling and probe traps may present difficulties in providing a quantitative measure of infestation.

Management

Given the threat that the larger grain borer poses to the world's maize supply, it is integral that we fully use as many tools in the integrated pest management (IPM) toolkit as possible. Traditionally, *P. truncatus* management has focused on the use of insecticides to reduce pest infestations to below economic threshold levels. However, the sometimes harmful side effects of insecticides on human health and the residues left on commodities are of increasing concern to consumers (Agboola, 1992; Batte et al., 2007). This issue coupled with the development of insecticide resistance has made the search for alternative tools an important focus in the 1980s and continuing through the present.

Host Plant & Varietal Resistance

One important aspect of IPM is host plant resistance. This tactic may reduce or completely eliminate the need for insecticides, allow for maintaining the effectiveness of our current chemical tools, while protecting our environment, human health, and minimizing non-target effects (Smith and Clement, 2012). Host plant resistance takes advantage of plant secondary metabolites or enhanced physical characteristics of the grain kernel to obviate attack by *P. truncatus* and other stored product pests. Thus, this tactic is attractive in a variety of contexts and countries. For example, the seed of an insect-resistant variety of cereal can be purchased with little need for other inputs such as insecticides (Smith and Clement, 2012). Host plant resistance has been explored extensively for field crops but has been far less common in the post-harvest sector of the value chain for important cereal crops. Indeed, it is often a challenge to

ensure breeders consider both preharvest and postharvest qualities when developing new resistant varieties.

Increased host commodity resistance will allow grains to be stored for long periods of storage with little or no damage. There are several key characteristics of grains that may provide resistance to stored products pests (Ahmed and Yusuf, 2007). One of these are physical barriers that hinders the insects from accessing the grain, such as the husk protecting maize on the cob (Giles and Ashman, 1971; Kossou et al., 1993). The husk on the cob forces *P. truncatus* to move between the cob and husk in order to gain access to the grain (Mahafuzi, 2018). The longer the leaves completely cover the maize ear, the longer it is protected from insect and microbial attack. Thus, breeding for high-yielding maize varieties may have increased susceptibility to *P. truncatus*. These high-yielding hybrid maize varieties may have shorter husk coverage of the cob, which makes the grain less protected more vulnerable to attack. Another characteristic is the hardness of the grain and pericarp thickness and/or toughness, with harder grains less susceptible to insect chewing or boring (García-Lara et al., 2004; García-Lara and Bergvinson, 2014; Jansen, 1977; Kossou et al., 1993; Mwololo et al., 2013). Other potential traits to be exploited include the size of the grains to control development, the nutritional profile of the grain, and the presence of secondary metabolic compounds that may inhibit oviposition and/or larval development (Ahmed and Yusuf, 2007). Overall, there may be three main bases for resistance against stored product insects, including anatomical traits (e.g., kernel hardness, pericarp thickness, endosperm qualities), biochemical traits (upregulated acids, peroxidases, etc.), and genetic traits (Lopez-Castillo et al., 2018). Importantly, it appears that especially anatomical and genetic traits (which may partially encode biochemical traits) may be especially durable over typical storage periods,

including in countries such as Malawi, where work on breeding for resistance to larger grain borer and maize weevil in maize has met with some success (Matewele, 2014).

There have been relatively few studies on using host plant resistance as a tool to combat *P. truncatus* specifically. Resistance is often assessed using the Dobie Index of Susceptibility (Dobie, 1974). Caribbean land races of maize were investigated for resistance to *P. truncatus* and 19 were identified with resistance due to grain dust produced after 140 days of insect infestation (Kumar, 2002). Lower reproductive capacity was documented on the resistant varieties, thereby demonstrating antibiosis. Others have found that the phenolic acid in the maize was strongly correlated with the hardness of the grain, and may ultimately have a toxic effect on *S. zeamais* and *P. truncatus* larvae attempting to develop in the grain (Arnason et al., 1992; Classen et al., 1990; García-Lara et al., 2004; Oloyede-Kamiyo and Adetumbi, 2017; Santiago et al., 2013). Further, other authors evaluated 100 different maize genotypes, and found significant differentiation among the biophysical traits of the maize genotypes, which resulted in reliable differences in the amount of dust, damaged grains, and progeny produced by *P. truncatus* (Mwololo et al., 2010). The authors in that study concluded that their data offered the opportunity for the development of resistant maize varieties to feeding by *P. truncatus* during storage, and recommended that molecular techniques be used in the future to study the resistant maize varieties. A separate study tested 54 maize hybrids for resistance to *S. zeamais* and *P. truncatus* (Tefera et al., 2011). They found eight hybrids with resistant properties, which showed a large reduction in the grain damage caused and progeny production by both species. Finally, García-Lara and Bergvinson (2013) investigated landraces of maize for resistance to both *S. zeamais* and *P. truncatus*. The authors identified several novel landraces with a high level of resistance to *S. zeamais* and *P. truncatus* from 24 different Latin American countries. They

found the best source for *S. zeamais* resistance was found in the Antilles and the best *P. truncatus* resistance was found in Southern Mexico (García-Lara and Bergvinson, 2013). Collectively, these studies suggest that host plant resistance appears to be a viable option for IPM of both *S. zeamais* and *P. truncatus*. However, it appears relatively more work has been done to develop resistant varieties of maize to control *S. zeamais* compared to *P. truncatus* (e.g., Derera et al., 2001; Abebe et al., 2009). This may be due to the more recent pest status of *P. truncatus* in much of Africa and the fact that the insect is only USDA's Animal and Plant Health Inspection Service (APHIS)-designated pest of concern in the USA, with very few colonies approved and available for study. Considering the extremely large variety of hybrids and genetic lines of maize, future work should address maize hybrid groups that are most commonly used in areas where *P. truncatus* is not currently present in order to forestall the further spread of this species, and draw the inferences necessary for a genetics-oriented control strategy.

Residual Insecticides

Most of the studies available for the control of *P. truncatus* are focused on the efficacy of different contact insecticides, as these can be easily adopted and used at the farm and smallholder scale. However, before the introduction of this species in Africa, there were only limited data regarding the application of these compounds, with most studies evaluating organochlorine compounds, which are no longer in use, while there were also some indications that organophosphate compounds (OPs) could control *P. truncatus* at elevated concentrations (Delgado and Hernández Luna, 1951; Giles, 1984; Ramirez Genel, 1960). The initial response to the rapid appearance and spread of *P. truncatus* in Africa was to adopt insecticides that were most effective for the established major stored product insect of maize, *S. zeamais*, including mostly traditional OPs and pyrethroids at the time (Golob, 2002). In the years that followed the

initial spread of *P. truncatus* into Africa, there were numerous published studies on the efficacy of contact insecticides for active ingredients that were in prior use. Initial laboratory, semi-field and field tests from that period revealed the tolerance of this species to specific active ingredients that easily controlled other stored product insects such as *S. zeamais*. For example, Golob et al. (1983) found that pyrethroids were superior to OPs for the control of *P. truncatus*, and suggested that the former should be used in their place. Considering that *S. zeamais* could not be controlled easily with pyrethroids in Africa (Golob, 2002), researchers investigated the use of various “cocktails” of OPs and pyrethroids (Golob et al., 1985; Dales and Golob, 1997), and ended up recommending their use to farmers in order to achieve control of both species with a single application of the mixture. These cocktails became the basis for registered commercially available formulations, that provide good efficacy against a wide range of stored product insect pests, without changing the cost of the application much (Golob, 2002; Stathers et al., 2002a,b, 2008; Machingura, 2019; Mlambo et al., 2018).

The reduced effect of OPs on *P. truncatus* compared to *S. zeamais* likely relates to the natural susceptibility by each species, as well as the decreased efficacy of OPs in warm, tropical climates, and not due to development of resistance. For example, Rumbos et al. (2013) found that the OP pirimiphos-methyl was less effective against *P. truncatus* and *R. dominica* when applied as a grain protectant relative to other stored product beetle species in the laboratory. However, when pirimiphos-methyl was applied on surfaces, its efficacy against *R. dominica* was high in the laboratory (Rumbos et al., 2014). For direct application on grains, low efficacy levels have been also reported for the OP chlorpyrifos-methyl, against both *P. truncatus* and *R. dominica* laboratory and field (Arthur, 1992; Guedes et al., 1996; Golob, 2002; Pozidi-Metaxa and Athanassiou, 2013). Nevertheless, Biliwa and Richter (1989) showed that pirimiphos-methyl

and deltamethrin provided better results than the combinations of other active ingredients at concentrations that were comparable with the already labeled rates. The withdrawal of chlorpyrifos and chlorpyrifos-methyl, for use either alone or in combination with other active ingredients, and a possible withdrawal of pirimiphos-methyl in the future, is expected to leave a gap in grain protection, given the wide range of target species of these insecticides at the post-harvest stages of durable agricultural commodities. Still, this gap might not be critical in the case of *P. truncatus*, as this species can be better controlled with a wide variety of pyrethroids, often combined with the synergist piperonyl butoxide (PBO) (Golob et al., 1985; Golob, 2002; Pozidi-Metaxa and Athanassiou, 2013; Quellhorst et al., 2021).

Interestingly, the occurrence of resistance by *P. truncatus* to different synthetic insecticides that are currently in use has not been investigated extensively, despite the large number of studies that have examined the insecticidal efficacy of various active ingredients. Early evidence suggested that the species can develop resistance to OPs and pyrethroids, but the resistance mechanisms, and overall frequency of resistance are poorly understood (Golob and Hanks, 1990; Haubruge, 1991; Golob, 2002). Hence, the evaluation of resistance of *P. truncatus* is an important gap that needs to be closed, in order to design an appropriate IPM approach that uses available active ingredients on a rotating basis. One reason for this gap could be the phytosanitary restrictions on rearing this species, as it is only available in a handful of laboratories throughout the globe. Since resistance studies are generally more demanding than insecticide evaluations, it is likely this work cannot be performed by the vast majority of research groups that work with stored maize.

Moreover, newer compounds have reached the registration and market stage for grain protection or surface treatments, that are less toxic to mammals, and have at least the same

efficacy level with the traditional insecticides. One of the most recently introduced compounds in the market for grain protection is the bacterial insecticide spinosad, which exploits metabolites of the actinomycete *Sacharopolyspora spinosa* Mertz and Yao (Actinonycetales: Pseudonocardiaaceae) (Hertlein et al., 2011). Despite the fact that spinosad is only moderately effective for some key stored product insect species, it has shown remarkable efficacy for the control of both *P. truncatus* and *R. dominica*, and hence, spinosad can be used effectively for these species as a replacement of OPs and pyrethroids (Hertlein et al., 2011; Pozidi-Metaxa and Athanassiou, 2013). An early study by Mutambuki et al. (2012) with spinosad dust, applied at 0.7 and 1.4 ppm performed much better against *P. truncatus*, in comparison with pirimiphos-methyl + permethrin. Follow-up studies with liquid spinosad demonstrated its efficacy for the control of *P. truncatus* on maize, at concentrations between 0.1 and 1 ppm (Hertlein et al., 2011; Athanassiou and Kavallieratos, 2014; Pozidi-Metaxa and Athanassiou, 2013). Therefore, the current registration of spinosad as a grain protectant is expected to provide solutions to the implications of controlling *P. truncatus* on maize, especially in populations that cannot be controlled easily with traditional contact insecticides. Efficacy of spinosad against *P. truncatus* and other species is much less dependent on temperature and relative humidity, in contrast with other types of insecticides, particularly diatomaceous earths or OPs, where abiotic conditions may seriously impair their efficacy (Athanassiou et al., 2008; Hertlein et al., 2011). There is also a newer compound among this group of bacterial insecticides, spinetoram, which has also been found to be effective against *P. truncatus* (Vassilakos et al., 2012; Athanassiou and Kavallieratos, 2014) .

Other novel compounds have been tested with success, including ones with dissimilar modes of action. For example, the insect growth regulator (IGR) S-methoprene was found to be

effective as a grain protectant for many stored product insect species, including *R. dominica*, but there are no data available for *P. truncatus* regarding the application of this insecticide alone (Wijayarathne et al., 2018). However, other IGRs such as the juvenile hormone analogues, fenoxycarb and pyriproxyfen, and the chitin synthesis inhibitors, diflubenzuron, flufenoxuron, lufenuron, and triflumuron, could notably suppress progeny production of *P. truncatus* when applied on maize (Kavallieratos et al., 2012). Given that S-methoprene has been registered in many countries as a grain protectant, and has an extremely low mammalian toxicity, additional work is needed to evaluate this growth regulator for *P. truncatus* as well.

Most studies investigating the application of newer insecticides against *P. truncatus* are focused on their application on maize, and far fewer evaluate residual control on surfaces. Although the focus on commodity treatments is considered as a priority, residual surface treatment protocols should also be developed, considering the phytosanitary importance of the species. Tsaganou et al. (2014) found that the neonicotinoid thiamethoxam was effective for *P. truncatus*, and induced delayed mortality on *P. truncatus* adults when removed from the treated substrate, even after short exposures. Nevertheless, in that work the authors indicated that thiamethoxam was less effective than the pyrethroid alpha-cypermethrin. Quellhorst et al. (2021) also found significant mortality as well as sublethal reduction in movement for *P. truncatus* exposed to concrete surfaces treated with the novel formulation Gravita® that contains deltamethrin + methoprene + PBO. Doganay et al. (2018) found that the application of thiamethoxam and alpha-cypermethrin was effective against *P. truncatus* adults, with efficacy improved on plastic and concrete surfaces compared to linen. This is an important consideration, as plastic woven propylene is currently in use for bagged stored maize in many parts of the world.

The efficacy of insecticides for control of *P. truncatus* has also been evaluated on other kinds of surfaces. For example, Kavallieratos et al. (2017b) found that the pyrrole chlorfenapyr and pirimiphos-methyl were more effective than alpha-cypermethrin for the control of adults of this species, when applied on polypropylene bags, but overall all three insecticides provided satisfactory results in reducing the number of holes in the bags. Treated bags, either coated or impregnated, can provide a viable storage solution in the case of farm-stored maize, and probably for other durable commodities that are infested by *P. truncatus* (Kavallieratos et al., 2017a; Otidotun et al., 2017). Kavallieratos et al. (2017a) evaluated bags that were incorporated with deltamethrin and found that these bags could cause high (e.g. >94%) mortality of *P. truncatus* adults after a short exposure interval (< 5 d) when exposed on the outer surface of the bag, but much less when exposed on the inner surface; nonetheless, *P. truncatus* adults still chewed through the bags (Kavallieratos et al., 2017a; Otidotun et al., 2017).

Gaseous Insecticides

Paradoxically, there are disproportionately few data for the use of fumigation against this species, compared with other major insect species of stored products. Golob (2002) lists a number of trials that supported the efficacy of methyl bromide against *P. truncatus*, but this insecticide is no longer in use, with the exception of specific quarantine and pre-shipment treatments (Bell, 2000; Cox, 2017). Otidotun et al. (2017) found that phosphine successfully managed infestation of *P. truncatus* and *S. zeamais* in stored bags of maize but subsequent re-infestation could not be completely avoided after extended periods. The application of phosphine for 72 h at concentrations that exceeded 400 ppm in transport containers caused <90% mortality in *P. truncatus* adults, while for other stored product species, all individuals were found dead (Athanassiou et al., 2016). Early works suggest that phosphine was used as an emergency

remedy when this species was first introduced in Africa, but the storage structures in the fumigated areas were often not suitable for such applications (Krall, 1984; Taylor and Harris, 1989; Brice and Golob, 1999; Golob, 2002). Taylor and Harris (1989) suggested that this species can be controlled with phosphine concentrations and exposure intervals that are comparable to those that are used for other major stored product insects. In facilities with proper sealing in the sub-Saharan Africa, complete control of this species can be achieved with the application of 2 g/MT of maize for 5 d (Taylor and Harris, 1994). The relative evaluation of different *P. truncatus* strains from native and introduced parts of its range should be a priority to assess potential resistance development from the continuous use of phosphine (Nayak et al., 2020). The way that maize is stored in Africa by farmers, e.g., in bags or in small structures, is an additional consideration in the use and limitation of phosphine, for both efficacy and safety.

The data for the efficacy of other gases against *P. truncatus*, such as carbon dioxide and nitrogen, are extremely few. Most data are about parameters that are associated with hermetic storage, so refer to the effect of low oxygen levels, and indirectly, elevated carbon dioxide. Quezada et al. (2006) evaluated the efficacy of hermetic storage for the control of *P. truncatus*, which resulted in a rapid decline of oxygen level to <1% in 3 d, and reported 100% mortality in 6–9 d, depending on the grain moisture content. Diara and Amoah (2019) tested factors that affect the control of *P. truncatus* on maize stored in SuperGrainPro® hermetic bags and reported that during storage, there was an increase in temperature, a decrease in relative humidity and moisture content of the grain, along with a drop in the oxygen level. The decrease in RH was a presumed result of temperature changes. In addition, the authors found that hermetic bags provided long-term protection against *P. truncatus* infestations, and also prevented fungal growth (Diara and Amoah, 2019). In shelled maize stored in hermetic bags, Njoroge et al. (2014)

found that during a six month storage period, population growth of *P. truncatus* was notably delayed, while seed germination remained high. Extensive studies in different parts of Africa have confirmed the efficacy of hermetic bags for the control of *P. truncatus* under a wide range of conditions and scenarios (Baoua et al., 2014; Hell et al., 2014; Njoroge et al., 2014; Nwaubani et al., 2020). Nwaubani et al. (2020) found that hermetic bags were superior to other control methods on maize, not only for *P. truncatus*, but also for other major insect species, such as *S. zeamais* and psocids. Nevertheless, using hermetic bags for protection of cassava chips during an eight-month period, Hell et al. (2014) found that these bags were unable to control natural or augmented infestations of *P. truncatus* or *Dinoderus* spp., which are also considered emerging bostrychid pests of durable stored products, including maize (Sakka and Athanassiou, 2018). In that work, Hell et al. (2014) attributed this failure in insect control to the fact that during their trials, oxygen level remained at high levels which allowed insect survival. The ability of *P. truncatus* to easily penetrate different materials, including plastics, may greatly contribute to failures in hermetic storage in bags, especially in already infested products. In contrast to maize where only small interstitial spaces remain among grains, large cassava pieces allowed large gaps in the commodity, which Hell et al. (2014) concluded had prevented the rapid formation of anoxia. Hence, the success of hermetic bags seems to be commodity mediated, and should be taken into account in on- and off-farm storage. Considering the limitations in using contact insecticides on cassava, as compared with raw grains, the protection of this commodity from *P. truncatus* infestations should be based on the use of alternative techniques such as controlled or modified atmospheres.

Biological control

Immediately after the introduction of *P. truncatus* in Africa, a suite of studies focused on the use of biocontrol agents to manage the species under field conditions (Meikle et al., 2002) (Table 4). Nevertheless, in contrast with most other stored product insect species, *P. truncatus* is also a wood borer (Borgemeister et al., 1998b), which complicates its biological control, as natural enemies are not equally effective in all environments, especially those that are radically different, such as stored-product and forested ecosystems.

Initial surveys in the native range of *P. truncatus* (e.g., Meso-America) indicated that there was a dearth of natural enemies associated with *P. truncatus*, and those that were found consisted mostly of species with a cosmopolitan distribution (Table 1.4). Thus, the introduction of these natural enemies was not considered necessary in Africa, since most of these species were already established (Helbig, 1998; Meikle et al., 2002). Nonetheless, during the initial spread of *P. truncatus* in Africa, there was a concerted effort to enhance distribution and predation by *T. nigrescens*, which was associated with *P. truncatus* in Mexico and elsewhere in its native range (Haines, 1981; Rees, 1991, 1987). This predator is considered endemic and very common in Meso-America, and was found to be effective against *P. truncatus* in that range (Boeye, 1990; Markham et al., 1994a, 1994b). The adults and larvae of this predator feed on the eggs and larvae of *P. truncatus*, but its diet also includes other stored product species, such as *S. zeamais* (Holst and Meikle, 2003). *Teretrius nigrescens* readily hunts and subdues *P. truncatus*, and this ability was expected to be an asset in improving biocontrol to reduce pest populations outside storage facilities in Africa (Rees, 1985, 1987). The species were released in different ecological zones of sub-Saharan Africa in the 1990s, while numerous laboratory studies focused on maximizing biological parameters to improve its efficacy (Rees, 1991; Markham et al.,

1994a, 1994b; Hodges and Dobson, 1998; Scholz et al., 1998; Meikle et al., 2002; Holst and Meikle, 2003).

Table 1-4 List of natural enemies of *P. truncatus*

Order	Family	Species	Type	Stage		Importance	Origin	Citation
				Attacked				
Coleoptera	Histeridae	<i>Teretrius [=Teretriosoma] nigrescens</i> Lewis	Predator	adults		High	Mexico	Haines 1991
Hemiptera	Anthoridae	<i>Xylocoris flavipes</i> (Reuter)	Predator	larvae		Low	Cosmopolitan	Helbig 1999
Hemiptera	Reduviidae	<i>Alleocranum biannulipes</i> Montrouzier and Signoret	Predator	larvae & pupae		Low	Cosmopolitan	Loko et al. 2020
Hymenoptera	Bethylidae	<i>Cephalonomia tarsalis</i> (Ashmead)	Parasitoid	larvae		Low	Cosmopolitan	Helbig 1998
Hymenoptera	Bethylidae	<i>Cephalonomia waterstoni</i> Gahan	Parasitoid	larvae		Low	Cosmopolitan	Helbig 1998
Hymenoptera	Bethylidae	<i>Holepyris sylvanidis</i> Brèthes	Parasitoid	larvae		Low	Cosmopolitan	Helbig 1998
Hymenoptera	Bethylidae	<i>Plastonoxus westwoodi</i> (Kieffer)	Parasitoid	larvae		Low	Cosmopolitan	Helbig 1998
Hymenoptera	Bethylidae	<i>Plastonoxus</i> sp.	Parasitoid	larvae		Low	Togo	Helbig 1998
Hymenoptera	Encyrtidae	<i>Cerchysiella</i> sp.	Parasitoid	larvae		Low	Togo	Helbig 1998
Hymenoptera	Eulophidae	<i>Pediobius furvus</i> (Gahan)	Parasitoid	pupae		Low	Cosmopolitan	Helbig 1998
Hymenoptera	Pteromalidae	<i>Lariophagus distinguendus</i> Förster	Parasitoid	larvae		Low	Cosmopolitan	Brower 1990
Hymenoptera	Pteromalidae	<i>Pteromalus cerealellae</i> (Ashmead)	Parasitoid	larvae		Low	Cosmopolitan	Brower 1990
Hymenoptera	Pteromalidae	<i>Theocolax elegans</i> (Westwood)	Parasitoid	larvae		Low	Cosmopolitan	Helbig 1998
Hymenoptera	Pteromalidae	<i>Anisopteromalus calandrae</i> (Howard)	Parasitoid	larvae		Moderate	Cosmopolitan	Helbig 1998

Prior work found that *T. nigrescens* responded positively to the *P. truncatus* aggregation pheromone, and supported its active hunting strategy (Hodges and Dobson, 1998; Holst and Meikle, 2003). The predator *T. nigrescens* was found to be broadly attracted to the frass of *P. truncatus*, which ended up stimulating greater oviposition, and this attraction did not degrade during long-term storage, nor could it be destroyed by baking grain to 150°C (Stewart-Jones et al., 2004). Moreover, larval *T. nigrescens* spent more time, moved a greater distance, and showed a higher angular velocity in zones where the dust and frass from *P. truncatus* had been added after tracking the movement for 45 min with Ethovision (Stewart-Jones et al., 2006). In addition, adult *T. nigrescens* was found to show higher arrestment when exposed to the dust and frass of *P. truncatus* than those from *R. dominica*, *D. minutus*, *S. zeamais*, *S. oryzae*, *T. castaneum*, or *T. confusum* in two different laboratory assays (Stewart-Jones et al., 2007).

Nevertheless, despite high initial expectations, it soon became clear that this predator was only moderately effective, which sparked a long-lasting debate in the scientific community as to the root causes (Holst and Meikle, 2003). For instance, *T. nigrescens* also attacks other wood-, shoot- and root-borers, which may serve as alternate food sources for the species, reducing its efficacy against *P. truncatus* (Rees, 1990; Meikle et al., 2002). The concurrent presence and high abundance of *S. zeamais* at many food facilities may comprise an additional limitation in its efficacy against *P. truncatus* populations (Meikle et al., 2002). These disadvantages may outweigh the possible benefits that the presence of alternative prey species may have in maintaining predator populations of *T. nigrescens* in periods where *P. truncatus* populations are low (Holst and Meikle, 2003). It has also been postulated that this predator may have limitations in adapting to novel release environments that differ substantially from its Neotropical origin (Rees, 1990; Holst and Meikle, 2003). In many of the cases examined, *T. nigrescens* was not

able to control *P. truncatus* populations, and after multiple release attempts and field studies in Africa that lasted for more than ten years, this classical biological control effort was considered unsuccessful (Meikle et al., 2002; Holst and Meikle, 2003; but, see the following for a different perspective: Bell et al., 1999; Mutlu, 1998). Regardless of the shortcomings, the use of *T. nigrescens* is an important advancement for smallholder farmers, as its establishment will reduce the time and money that are needed to utilize other control methods. The predator may also serve as an especially important supplemental source of control in natural forested habitats of *P. truncatus* to reduce field populations as opposed to their use around grain storage facilities. An open question includes the actual magnitude of reduction that *T. nigrescens* contributes in natural habitats, which may be amenable to answering through population genetics approaches and/or population modeling. Other predators that attack *P. truncatus* have also been found, which include *Calliodis* spp. (Hemiptera: Lyctocoridae) and *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae) (Meikle et al., 2002), but these species were not considered sufficiently effective biocontrol agents for *P. truncatus* (Boeye, 1990; Helbig, 1999; Meikle et al., 2002).

There are several parasitoid species that have been found to parasitize *P. truncatus*. In Meso-America, Boeye (1988, 1990) found a number of the parasitoids *Anisopteromalus calandrae* (Ashmead) and *Theocolax* (= *Choetospila*) *elegans* (Westwood) (Hymenoptera: Pteromalidae), which were able to suppress *P. truncatus* populations. These species, especially *A. calandrae*, have been evaluated extensively for the control of many internal feeders, such as *Sitophilus* spp. and *R. dominica* (Schöller and Flinn, 2000; Schöller et al., 2018). Additional studies showed that *P. truncatus* can be parasitized by *Lariophagus distinguendus* (Förster) and *Pteromalus cerealellae* (Walker) (Hymenoptera: Pteromalidae). Nevertheless, some species, although able to parasitize, have a reduced impact on *P. truncatus* populations, as it has been

noted in the case of *P. cerealellae* or *T. elegans* (IITA, 1997; Helbig, 1998). On the other hand, *A. calandrae* provided high parasitism rates on *P. truncatus* larvae at elevated temperatures, and has been proposed as a target for further evaluation as a biocontrol agent when *T. nigrescens* is not effective (Bonu-Ire et al., 2015). In a survey in Togo, Helbig (1998) found nine species of hymenopterous parasitoids that were associated with maize in facilities where *P. truncatus* was present, with *A. calandrae* and *T. elegans* being the most abundant. In laboratory trials, it was found that *A. calandrae* significantly suppressed *P. truncatus* numbers, but *T. elegans* had no effect (Helbig, 1998). Brower (1990) tested four parasitoids, *A. calandrae*, *L. distinguendus*, *P. cerealellae* and *T. elegans*, and found that the efficacy of each parasitoid against *P. truncatus* was strongly influenced by the host size, suggesting that different parasitoids exhibited different preferences in host ages/sizes, while the author proposed that the simultaneous use of more than one parasitoid species might be most effective in improving biological control for *P. truncatus*.

Entomopathogens are considered promising biocontrol agents in stored product protection, and some of them can usually be applied in the same way that conventional insecticides are (Rumbos and Athanassiou, 2017a, 2017b). They can also be used to stop the spread of pest outbreaks (Meikle et al., 2001, 2002). The use of entomopathogenic fungi for the control of *P. truncatus* has been extensively tested at the laboratory scale, and to a lesser extent, in field tests. For example, Smith et al. (1999) formulated conidia of *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) with pheromone, and reported a satisfactory level of efficacy against *P. truncatus* adults, despite slightly reduced conidial viability from the pheromone, which is the only behaviorally-based strategy that has been evaluated for *P. truncatus* beyond monitoring (Morrison et al., 2021). Moreover, a combination of wood ash with conidia of *B. bassiana* provided high mortality levels of *P. truncatus*, and

while there was no synergism in mortality by combining ash and the conidia on the parental adults, the application of both did significantly suppress progeny production at low rates of conidial application, possibly by decreasing conidia adhesion to the grain and increasing egg mortality (Smith et al., 2006). A lot of data have demonstrated the beneficial effect of combining inert materials with entomopathogenic fungi for several stored product insect species (e.g., Lord et al., 2007; Rumbos and Athanassiou, 2017a). In field experiments in Benin, Meikle et al. (2001) noted that the application of *B. bassiana* was able to reduce the infestation caused by *P. truncatus*, but insect densities remained high in artificially infested maize, suggesting that the fungus could not completely protect the commodity from already established populations. In screening isolates of entomopathogenic fungi from Ethiopia, Kassa et al. (2002) found 13 isolates that belonged to *B. bassiana*, *Beauveria brongniantii* (Saccardo) Petch, *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) and *Paecilomyces* spp. (Eurotiales: Trichocomaceae). *Prostephanus truncatus* was highly susceptible to all isolates, which provided 98–100 % mortality after 2.9 and 4.1 days (Kassa et al., 2002). Bourassa et al. (2001) tested *M. anisopliae* and *B. bassiana* isolates that originated from sub-Saharan Africa, and recorded >90 % of *P. truncatus* after 14 d of exposure. Interestingly, in that study, the authors noted that *T. nigrescens* was far less susceptible than *P. truncatus* to the same isolates, which highlights the need to investigate the potential of the simultaneous use of both micro- and macro-biocontrol agents. More recent research in Ghana demonstrated the efficacy of an isolate of *B. bassiana* originating from the UK (IMI 389521) for the control of immature *P. truncatus* on maize (Acheampong et al., 2016). In that study, the fungus was formulated with the electrostatically charged dust Entostat® and Kaolinite®, which improved conidial attachment and penetration of the insect's cuticle. This technique, i.e., the combination of conidia with

Entostat, has proved effective in the case of other stored product insect species in both laboratory and semi-field tests (Athanassiou et al., 2017; Rumbos and Athanassiou, 2017a). Similarly, Nboyine et al. (2015) found that the application of low doses of IMI 389521 was lethal to *P. truncatus*, but not to *T. nigrescens*, and suggested that the combined use of the two biocontrol agents merits additional investigation. Overall, it seems that the application of entomopathogenic fungi, especially *B. bassiana* might be viable for large scale applications during maize storage; however, there are no relevant data available for other commodities that are infested by *P. truncatus*.

In contrast with entomopathogenic fungi, the data on the efficacy for other entomopathogens, i.e., nematodes, bacteria, viruses and protozoa, on *P. truncatus* are particularly limited. In a review on the potential use of entomopathogenic nematodes in stored product protection, Rumbos and Athanassiou (2017a) documented the absence of published data on this topic. Henning-Helbig (1994) referred to the occurrence of a parasitic alveolate *Mattesia* sp. (Neogregarinoida: Lipotrophidae) that increased mortality of *P. truncatus* under laboratory conditions, but the overall impact of this pathogen to population growth of *P. truncatus* was considered insignificant when compared to entomopathogenic fungi (Meikle et al., 2002). Schulz and Laborius (1986) noted that bacterial contribution in suppressing *P. truncatus* populations is limited, and again underscored the promise of entomopathogenic fungi. However, the focus on the efficacy of entomopathogenic fungi may inadvertently lead researchers to overlook other pathogen options, such as viruses or bacteria that merit further experimentation. Furthermore, the degree that these pathogens would be applicable in large scale applications in developing countries has not been examined with the required detail.

Inert materials

Two of the most commonly used inert materials in stored product protection are diatomaceous earths (DEs) and ash (Athanassiou and Arthur, 2018). DEs are particularly important in certain areas of sub-Saharan Africa where farmers often have no access to conventional insecticides, or lack the necessary application equipment and training to effectively use conventional insecticides. Studies on farmers' acceptance of DEs in Tanzania and Zimbabwe demonstrated that smallholders appreciated the efficacy of DEs, as well as their ease-of-use (Stathers et al., 2002a,b; Stathers et al., 2008). Meanwhile, DEs have far lower mammalian toxicity than most of the conventional insecticides currently used for managing stored product pests in Africa (Stathers et al., 2008; Athanassiou and Arthur, 2018). Stathers et al. (2002a,b) noted that commercially available DEs could successfully suppress *P. truncatus* population growth, at concentrations that ranged between 0.1 to 1 % (w/w) of the total grain mass. In a series of semi-field studies in Ghana and Kenya, different DEs were found to be effective, especially during the dry season, or in the absence of humid conditions (Giles et al., 1995; Golob, 2002). In general, it is well established that increased levels of moisture/humidity are likely to decrease the efficacy of DEs, and thus, DE applications may not be effective in humid areas in Africa (Korunic, 1998; Subramanyam and Roesli, 2000; Stathers et al. 2004; Vayias and Athanassiou, 2004). Athanassiou et al. (2007) tested, under laboratory conditions, the insecticidal effect of three DE formulations, at concentrations that ranged between 500 and 1500 ppm (mg/kg of grain) on *P. truncatus* adults; these formulations included Protect-It (a DE-based formulation), PyriSec (a combination of DE with natural pyrethrum and PBO), and DEA-P (a mixture of DE with abamectin). The authors found that DEA-P was much more effective than the other two formulations against *P. truncatus*. By contrast, Stathers et al. (2002a) found there

was no additional effect of adding a DE to small amounts of the pyrethroid deltamethrin on mortality of *P. truncatus* or production of F₁ progeny. These contrasting results suggests further work on elucidating the combined effect of DE with a conventional insecticide is required.

DEs have varying efficacy when applied on different commodities (Subramanyam and Roesli, 2000; Athanassiou and Kavallieratos, 2005; Kavallieratos et al., 2005, 2018). Kavallieratos et al. (2018) found that some DEs performed better on certain maize hybrids as compared with others, in terms of both mortality and progeny production capacity of *P. truncatus*. In general, adherence of DEs on the insect cuticle is generally lower in maize than in small grains, which is expected to reduce efficacy (Athanassiou and Kavallieratos, 2005; Kavallieratos et al., 2018). Overall, the efficacy of DEs against this species increased with increasing temperature, but decreased with increasing relative humidity, and there is variable efficacy among DEs (Stathers et al., 2004; Athanassiou et al., 2007; Kavallieratos et al., 2017a). Stathers et al. (2004) found that the susceptibility of *P. truncatus* to DE-treated maize was inversely related with storage time, indicating that this species is less susceptible to DEs than other species that infest stored maize. In laboratory experiments, Machekano et al. (2017) found that a combination of DE with spinosad or deltamethrin was very effective for the control of *P. truncatus* on maize, at reduced application rates, as compared with the application of these insecticides alone. Zhanda et al. (2020) reported similar findings for different enhanced DEs, containing sesame oil, spinosad or pyrethrins, while Kavallieratos et al. (2018) found the same for DEA-P. Finally, the adherence ratio of DEs to maize was found to vary among different types of products and maize hybrids (Kavallieratos et al., 2018).

One other promising inert material group for stored product protection is zeolites (Eroglu et al., 2017). To our knowledge, there are no data available for the use of zeolites for the control

of *P. truncatus*, despite the fact that numerous zeolites have been shown to be effective for the control of other stored product insects (Rumbos et al., 2016; Yao, 2016). As siliceous materials, zeolites are considered to have a similar mode of action to DEs (Eroglu et al., 2017). For a silica-based formulation, Mutambuki (2013) tested different doses of silica dust (0.25-50 mg/50 g of maize) and found that the efficacy of this formulation against *P. truncatus* and *S. zeamais* was moderated by the higher moisture content of the grains. Additional data showed that different synthetic silica aerogels were effective at relatively low concentrations (Barbosa et al., 1994; Golob, 2002; Muatinte et al., 2014).

Older dust formulations that have been traditionally evaluated in stored product protection are amorphous materials such as different ash types, clay, lime, and salts (Golob, 1997). For volcanic ash, Ramírez Genel (1962) reported high efficacy levels against *P. truncatus* when ash was admixed with maize at 1%. Similarly, other ash types, such as wood ash and paddy husk ash, were effective on *P. truncatus* but at extremely high concentrations (e.g., when admixed at 30% rate by weight of grain being protected) (Golob and Hanks, 1990; Golob, 2002). In addition, when using ash, worker and consumer safety must be considered as it is a caustic material; common worker safety precautions include eye protection, gloves, long sleeves, and masks. Ash and other inert materials, including DEs, can be used successfully in combination with fungi against *P. truncatus* (Smith et al., 2006; Rumbos and Athanassiou, 2017a). Nevertheless, access to inert dusts by farmers has not been quantified in detail.

Botanicals and plant extracts

Plant derivatives and botanicals have been traditionally used by farmers, perhaps for centuries (Bouda et al., 2001); they have also been extensively used against *P. truncatus*. One benefit is that some of these agents are more accessible by farmers and are easier to apply than

conventional insecticides. Botanicals and plant extracts are usually applied at elevated concentrations, and their efficacy may generally be lower in comparison with conventional control methods, but their use is a promising alternative at the local farm-scale level, as they are simply admixed with the commodity during storage. Nwaubani et al. (2020) used *Piper guineense* Schumacher (Piperales: Piperaceae), known also as Benin pepper, and found that, although this application was not as effective as the use of hermetic bags, insecticide-impregnated bags, DE or permethrin, *P. guineense* suppressed insects on maize compared with the untreated controls. A screening of plant extracts showed the insecticidal potential of many against *P. truncatus*, including *Ricinus communis* L. (Malpighiales: Euphorbiaceae), *Gliricidia sepium* (Jacquin) Steudel (Fabales: Fabaceae), *Lavandula angustifolia* Miller (Lamiales: Lamiaceae) and many others (Schulz and Laborius, 1986; Rodríguez Lagunes, 1987; Markham et al., 1991; Golob, 2002). There is even more data on the use of botanicals in Africa. Wormseed, *Dysphania* (= *Chenopodium*) *ambrosioides* (L.) Mosyakin & Clemants (Caryophyllales: Amaranthaceae) was found effective against *P. truncatus* infestations, when applied on maize at 0.8 % by weight (Tapondjou et al., 2002). Muatinte et al. (2019) found nine plant species with insecticidal properties against *P. truncatus* in Cameroon, Kenya and Ghana. Earlier, Osipitan et al. (2010) examined seven plant species for their insecticidal value against *P. truncatus*, and found that the extracts of neem, *Azadirachta indica* A. Jussieu (Sapindales: Meliaceae) and tobacco, *Nicotiana tabacum* L. (Solanales: Solanaceae) were superior than the extracts of the other five plant species in causing mortality of *P. truncatus* individuals. However, when *A. indica* and *N. tabacum* were compared with pirimiphos-methyl, the latter proved much more effective in controlling *P. truncatus* (Osipitan et al., 2010). Mukanga et al. (2010) tested five plant species for the same purpose and also showed that neem was effective for the control

of *P. truncatus* larvae. For different extracts, a search in Scopus (August 2020) indicated more than 30 papers published so far with “*Prostephanus truncatus*/larger grain borer” and “botanicals/plant extracts/plant species” in their title, with their number increasing in the recent years. A recent study by Kavallieratos et al. (2020) examined furanosesquiterpene isofuranodiene, which is a very common terpenoid in several plant species, and confirmed its effectiveness for the control of *P. truncatus*, and another invasive species of stored products, the khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae). This study showed that specific compounds of plant species could be further exploited or even capitalized towards the development of commercial formulations. However, the synthesis and characterization of these compounds may lay beyond the “real world” needs of small holder farmers in Africa, who are likely to have access to certain plants, but not to specialized formulations that are based on specific compounds.

While botanicals are a promising alternative for control of *P. truncatus* over the use of conventional insecticides, there are certain shortcomings in their use and evaluation that should be taken into account. These include the lack of standardized synthesis of botanicals, the high concentrations that these plant parts must be applied in order to have high efficacy, the risk of plant overharvesting when botanicals are used to treat large areas or with frequent application, and above all, the absence of detailed studies on their safety profile, including effects on human health and interactions with the environment (Belmain and Stevenson, 2001; Athanassiou et al., 2015). Another important limitation in many of these studies is that the sensory properties of end products are often not assessed, which can be an issue when mixing commodities with aromatic and fragrant plant secondary compounds; this is a topic that has been noted elsewhere in the literature (Morrison et al., 2021). Finally, the cost to benefit ratio in the use of botanicals is rarely

considered, but may not be trivial for smallholders who do not have much liquidity in income. Because of their accessibility, farmers in Africa can easily use plant extracts; this makes the need for additional research on the use of botanicals essential, especially at the local farm-scale level.

Extreme Temperatures, Aeration, and Grain Chilling

There is not much data available on the use of extreme temperatures for the control of *P. truncatus*, probably due to the fact that the application of either cold or heat is energetically costly, and typically makes more sense later in the supply chain to protect higher value commodities like processed products rather than raw grains. Golob and Hodges (1982) considered *P. truncatus* particularly tolerant to elevated temperatures, but McFarlane (1988) demonstrated that exposures to 50°C for 1 hour provided high mortality, which was not far from the thermal disinfestation procedures followed for other stored product insect species, generally hovering between 45–60°C (Fields, 1992). As traditionally smoked storage facilities in Africa do not reach these levels, solar power has been proposed as a means of heat treatment (Murdock and Shade, 1991; Golob, 2002). Sun drying of the commodity has also some effect, but does not provide complete control (Boxall and Compton, 1996). Prior work has shown that grain heated to 45°C will drive *P. truncatus* out of a grain mass (Hashem, 1990). In a recent study, Machekano et al. (2020) showed that *P. truncatus* adults had a higher thermal plasticity than larvae for both heat and cold. The authors suggested that exposure to temperatures between 45.5 and 47°C for 4 and 2 h, respectively, is effective for killing *P. truncatus*. Moreover, exposure to -9 and -15°C, for 4 and 0.5 h, respectively, also provided high mortality. In contrast to heat, third instar larvae were found to be more tolerant than adults to cold, with the supercooling point of former found to be -19.5°C, while the latter's supercooling point was -16.7°C (Machekano et al., 2020). Thus,

P. truncatus may also be controlled using low temperatures at levels that are effective for other stored product insect species (Fields, 1992).

Related to work with extreme temperatures, there has been disproportionately few data generated on using aeration or chilling for the control of *P. truncatus*. In a paper correlating the global distribution of this species with temperature increase, Arthur et al. (2019) noted that aeration may not be effective in controlling *P. truncatus* in Africa, due to the rapid population growth of this species, which probably equally applies for *S. zeamais* as well. Nonetheless, aeration may still slow down the development of *P. truncatus* in facilities with appropriate equipment for its application (Admire et al., 2014).

While *P. truncatus* can complete its life cycle under a wide range of conditions (Watters, 1984), storage under low temperatures and at low moisture content or relative humidity are expected to have the lowest infestations. However, this species can easily infest and colonize maize that is relatively dry (e.g., 11.2% grain moisture) (Golob and Hodges, 1982; Athanassiou et al., 2007). Although the rapid population growth of *P. truncatus* and the humid conditions in certain areas of Africa may restrict the feasibility of aeration or chilling, the application of these techniques may seriously delay population growth and the subsequent infestations. McFarlane (1988) reported that in regions where natural aeration can be combined with natural cooling, e.g., lower overnight temperatures, *P. truncatus* activity is likely to be reduced. The development and reproduction of *P. truncatus* below 20°C or 40% RH is reduced (Shires, 1980, 1979; Watters, 1984; McFarlane, 1989, 1988). In most of its natural range, these conditions may not be feasible for maize storage. However, as modeling the efficacy of aeration for other species and regions has shown, there may be added benefits to aeration in areas away from the coast or in upland or

mountainous regions where nighttime temperatures may be cooler (Arthur et al., 2020; Morrison et al., 2020).

Packaging

Packaging and physical barriers of different varieties appear highly susceptible to boring by *P. truncatus*. This insect appears to make exploratory holes into different substrates as it forages. *Prostephanus truncatus* has been observed boring into wooden structures, beans, groundnuts, and even plastics (Chittenden, 1911; Giles, 1975; Hodges et al., 1983a; Howard, 1983; Ramirez and Silver, 1983). The individuals have been documented abandoning unsuitable substrates after boring in 1-2 cm, with no signs of reproduction (Hodges, 1994). Li (1988) found that adult *P. truncatus* could penetrate plastic with 35 mm thickness. Fleurat-Lessard and Serrano (1990) found that *P. truncatus* could penetrate 30–300 µm polyethylene films.

Hermetic bags have been evaluated for the control of *P. truncatus* in laboratory, semi-field and field tests (Njoroge et al., 2014; Garcia-Lara et al., 2020; Nwaubani et al., 2020). Another study looked at the ability of Purdue Improved Crop Storage (PICS) bags to control *P. truncatus* on cassava and found that *P. truncatus* already present inside a bag could break out (Hell et al., 2014). Mutambuki et al. (2014) used PICS bags containing maize infested with *P. truncatus* and *S. zeamais*, and found that after 12 weeks of storage, carbon dioxide content exceeded 10%, while oxygen levels dropped below 10%; after 16 weeks there was an increase of oxygen, which was attributed to the penetration of the bags by *P. truncatus* adults. However, in a comparative study with other methods in Africa, Nwaubani et al. (2020) demonstrated that the PICS triple layer hermetic storage bags were more effective at controlling *P. truncatus* than the other control methods, including the application of contact insecticides. Likewise, Njoroge et al. (2014) found that triple bag hermetic storage was successful at preventing damage by and also

induced mortality of *P. truncatus* over a 6-month period of storage in Kenya. Similarly, other hermetic bags, such as the Super Grain Pro bags, were found to be effective against *P. truncatus* and suitable for long-term storage (Nhamucho et al., 2017; Singano et al., 2020). In one study that evaluated hermetic storage bags against three stored product insects, the authors found that *P. truncatus* and *R. dominica* could puncture Super Grain Pro bags after about three to six weeks (García-Lara et al., 2013). The ability of this species to easily penetrate plastics should be seriously considered when designing IPM programs, since it can negatively affect hermetic storage based on plastic bags. Other studies confirm the importance of this consideration, showing hermetic bags may allow *P. truncatus* to continue causing grain damage in bagged-stored maize, and they may falsely provide the impression that hermetic storage is effective against the species when they are not (Baoua et al., 2014; Chigoverah and Mvumi, 2018; Ognakossan et al., 2013). Further, Chigoverah and Mvumi (2018) compared four different hermetic plastic bag types and reported that all four were susceptible to penetration by *P. truncatus*, confirming its ability to penetrate plastics, regardless of their specific type. As hermetic technology continues to innovate, there is the possibility for improvement. Recently, Garcia-Lara et al. (2020) assessed two improved hermetic technologies (e.g., sBag, an improved bag, and Bioxilo™, a hermetic plastic silo). The authors found both were highly successful at preventing damage over 12 months of storage in areas of Mexico with high populations of *P. truncatus* compared to the much higher damage found in traditional storage during the same period. There is a continued need for field validation of these improved hermetic and packaging technologies as well as more studies on this insect's ability to bore through various materials in no choice tests and in choice tests. Finally, it is unknown how the foraging response of *P. truncatus* is modulated by volatiles emitted from packaging.

One way to ameliorate the problem of *P. truncatus* boring through packaging is to use bags that contain insecticides (Kavallieratos et al., 2017a; Doganay et al., 2018). Some of these insecticides have an immediate effect after contact, and they immobilize insects quickly, reducing the damage to the bag itself. These bags are expected to kill the adults that are in contact with the plastic surface, reducing the holes in the bag that will lead to increasing oxygen levels. The combination of both techniques, i.e., hermetic and insecticide-impregnated bags, is a recent development that has been evaluated with very promising results (Baributsa and Ignacio, 2020). Other controlled release materials, such as long-lasting insecticide-incorporated netting, have shown great promise for a variety of major stored product species, and should be expanded to include testing against *P. truncatus* (Morrison et al., 2018; Scheff et al., 2020; Wilkins et al., 2020a; Wilkins et al., 2020b).

Integration of Pest Management Tactics

It is clear in many cases *P. truncatus* co-occurs with other species, and management programs need to be able to target multiple species simultaneously. In most case, these tactics can be viewed as layering on multiple hurdles to pest infestation. Some of the hurdles will be more or less expensive, and some will require more or less knowledge to apply successfully. Among these, on the low cost, low knowledge input would likely be the use of contact insecticides and inert materials, as these techniques can be easily adopted by the farmers, without the need of specialized equipment. Ultimately, stakeholders must be able to respond flexibly to the local situation, and conditions will undoubtedly vary greatly from, for example, a large postharvest facility in Mexico to smallholders in Sub-Saharan Africa. The adoption of most techniques by farmers requires intensive training, which will result in visible results in the reduction of the post-harvest losses (Stathers et al., 2002a, 2008; Nyabako et al., 2020).

Future Needs

Nearly four decades have passed since the first major efforts to study *P. truncatus*. Since then, this destructive and invasive pest has spread across continents, traveling from its native range in central America and invading a vast region in Africa. *Prostephanus truncatus* has also been intercepted in many other areas, though the pest was not able to establish. Arthur et al. (2019) makes clear that even more areas may be suitable for *P. truncatus* colonization, such as the southern USA, Southeast Asia, and more parts of South America.

The origins of *P. truncatus* pest were initially uncertain, and yet it became very clear that this insect did not share the same long-standing preference for stored commodities to the same degree as with many other stored product insect pests. *Prostephanus truncatus* originally evolved as a wood borer, and as such there was a great need for research on the host preferences and its host-finding behavior. It is clear *P. truncatus* is a facultative wood borer that will primarily attack maize and cassava with little interest in small grains.

It is possible that many researchers in stored product entomology considered the studies on key biology characteristics of *P. truncatus* definitive and completed, and the subsequent focus after the 1980s on chemical control of *P. truncatus* supports this view. However, there has been a renewed push towards ecologically-informed management and behaviorally-based tactics [e.g., Morrison et al., (2021)] to manage stored product pests. There are still many outstanding basic ecological questions to be asked about *P. truncatus*, which may signal a renaissance on the research of this species. Some of these research gaps are as follows. Modeling and documenting the effects of climate change on shifts in the distribution of *P. truncatus* will become increasingly important, as they may result in a radically shifted range and increased grain damage. There have been very few recent attempts to determine the spread and distribution of

this insect. While we report the known occurrences of the insect here to date, there was no new trapping data available to update its distribution. Further, biosurveillance on the periphery of *P. truncatus*' current range will be increasingly important to determine whether its range may be shifting and to protect the biosecurity of the postharvest supply chain in new areas of colonization. An improved understanding of the population dynamics of *P. truncatus* is required, including host-switching behavior. There is also an important need to assess new wild and woody host plant species that may play a role in the survival and distribution of *P. truncatus* outside grain stores. In addition, there is a need to empirically follow-up and quantify interactions between *P. truncatus* and its main predator in its introduced range in forested landscapes. This information will result in habitat management recommendations to reduce presence of *P. truncatus* in and around grain stores and adjacent landscapes. Further, a mechanistic understanding of coexistence and exclusion on fine spatial scales with other stored product insect competitors will be useful for assessing how communities may change with the range expansion of *P. truncatus*. It will additionally be useful to enhance understanding of dispersal and immigration in the landscape and at food facilities, including elucidating flight capacity, and dispersal behavior after application of management tactics. Future studies should evaluate how physiology, starvation, host cues, predator cues, or other internal or external stimuli affect the movement ecology of *P. truncatus* in the laboratory, semi-field, and field. Finally, key cultural control and habitat management methods deserve increased consideration, including aeration, grain chilling, heat, controlled release materials, and packaging. A focus on the links between field and food stores (including release-recapture studies) to increase knowledge of the population ecology and behavior of *P. truncatus* will be paramount for moving forward.

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**Chapter 2 - Exposure to a novel insecticide formulation on maize
and concrete reduces movement by the stored product pests,
Prostephanus truncatus Horn and *Sitophilus zeamais* Motschulsky**

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Abstract

Two major stored products pests of maize are the *Prostephanus truncatus* Horn (larger grain borer) and *Sitophilus zeamais* Motschulsky (maize weevil). Under climate change, *P. truncatus* may be expected to shift its distribution northward farther into the United States (US). Thus, there is a critical need to develop diversified chemical control tools in the post-harvest supply chain for these two species. In this study, we investigated the efficacy of a novel insecticide formulation, containing the insect growth regulator, S-methoprene, combined with the pyrethroid, deltamethrin and the synergist piperonyl butoxide (Central Life Science, Schaumburg, IL, US), compared to the existing commercial standard formulation without synergist, and controls at inducing direct mortality and sublethal changes in movement on treated grains as a grain protectant, and on concrete as a surface treatment. Mortality of adults was assessed visually, while movement was tracked with a network camera coupled with Ethovision software that automatically recorded velocity and distance moved by both species, after continuous exposure on treated material for 4–168 h. The novel formulation significantly induced mortality while reducing distance and velocity moved by multiple-fold compared to controls for exposed adults even after relatively brief exposure periods. In fact, the novel formulation was just as effective as the older formulation, but used only a fraction of the active ingredients, thus it may be more cost-effective. Overall, the novel insecticide formulation is a promising tool for controlling *S. zeamais* and *P. truncatus* in bulk storage and around other food facilities.

Keywords: Deltamethrin, Methoprene, Larger Grain Borer, Maize Weevil, Integrated Pest Management

Introduction

Cereal grains comprise 30–60% of the daily caloric intake for humans around the globe. The top three most important cereal crops are maize, rice and wheat (Awika 2011). Maize is a major staple crop and preferred in southern and eastern Africa, Central America, and Mexico (Ranum et al. 2014; Awika 2011). It was reported that persons in Mexico consume 267 g of maize per day (Awika 2011).

However, 10–30% of these important cereal crops, such as maize, are lost to insect pests post-harvest as these grains are harvested, stored, processed, and marketed to consumers (Pimentel et al. 1991). The major stored product pests of maize are the larger grain borer, *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae) and the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *Prostephanus truncatus* is a long-time pest of stored maize in Mexico, the US, and parts of Central and South America (reviewed in Quellhorst et al. 2021), and was first described in 1878 (Hodges 1986). However, the insect has also become an invasive species in Africa from 1981 onwards (Golob and Hodges 1982), where it is estimated by Boxall (2002) to cause 34–40% in losses after only 3 months of storage.

The Southern US is at the very edge of the current range of *P. truncatus*, but the species represents an emerging biosecurity threat for maize production in the country (Arthur et al. 2019). In fact, Arthur et al. (2019) documented *P. truncatus* in three Mexican states directly adjacent to the US, including Nuevo Leon, Sonora, and Tamaulipas, with the closest site located less than 200 km (or a 3 h drive) from Arizona. While *P. truncatus* has been documented in maize facilities in southern states like Texas (e.g., records found in the research collection at Texas A&M; pers. comm. D. Ludwick), as well as California and Arizona (Cotton and Good 1937; Dormond 1998), it has not yet been detected in the breadbasket of the US, such as Kansas

and adjacent areas (Quellhorst et al. 2021). The distributions of agricultural insects are predicted to shift under climate change (Macfadyen et al. 2018), and this is often in a polewards direction. As a result, under a changing climate, *P. truncatus* may be expected to become increasingly problematic at higher latitudes in the US. As it does so, it will encounter endemic *S. zeamais* with increasing frequency, and both may contribute to maize damage (Quellhorst et al. 2020).

Given the threat posed by *P. truncatus* and *S. zeamais* to the biosecurity of the US and thus the world's maize supply, it is critical that we expand the integrated pest management chemical toolbox to combat them. Traditionally, stored product insect control has focused on the use of fumigants to reduce consumer complaints, such as phosphine. However, the worker safety in applying fumigants is a concern, and consumers are increasingly demanding products with fewer insecticide inputs (Batte et al. 2007). This issue coupled with a stark rise in the global resistance to phosphine (Nayak et al. 2020) has made the search for additional alternative chemical tools an important focus.

Methoprene was first registered for use after harvest in the US under the name Diacon® in the 1980s (Arthur 2004). The first commercial formulation of deltamethrin plus methoprene used in the US was Diacon IGR+ ® (Central Life Sciences, Schaumburg, IL, US). Diacon IGR+ contains 0.05 kg of active ingredient [AI]/L of deltamethrin and 0.12 kg [AI]/L of the insect growth regulator (IGR) methoprene. It can be applied at two commercially labeled rates, 0.5 ppm deltamethrin + 1.25 ppm methoprene, or 1.0 ppm deltamethrin + 2.5 ppm methoprene. For species such as *P. truncatus* and *Rhyzopertha dominica* Fabricius (Coleoptera, Bostrichidae), methoprene alone is enough to control these insect species because the eggs are laid outside the grain kernel by mated females (Athanassiou et al. 2010a,b). Thus, there is a brief time where the larva is exposed to the insecticide. However, for species like *S. zeamais*, the addition of

deltamethrin is needed to control adults because this species lays eggs inside the grain kernels, thereby protecting them from methoprene exposures (Athanassiou et al. 2010a,b).

Gravista® (Central Life Sciences, Schaumburg, IL, US) is a new formulation of the Diacon IGR+ that consists of an IGR (2.85% methoprene, 0.027 kg [AI]/L), combined with the pyrethroid, 1.2% deltamethrin (0.012 kg [AI]/L) and a synergist, 33.3% piperonyl butoxide (0.32 kg [AI]/L). The main difference between the two insecticides (IGR + and Gravista) is the addition of a synergist. Synergists such as piperonyl butoxide (a cytochrome P450 detoxification enzyme inhibitor) allow for less active ingredient (deltamethrin and methoprene) to be incorporated in the product, as in this case, and may also help to control resistant insect populations.

While mortality is a common measurement for assessing the efficacy of an insecticide, it provides an incomplete picture of the full efficacy of a compound. In addition, Guedes et al. (2011) noted the importance of assessing the sublethal effects of exposure to insecticides in order to develop a comprehensive assessment of exposure to insecticides. This can be done using a variety of methodology, but one standard methodology is to use video-tracking coupled with Ethovision® software (Noldus et al. 2002; Noldus et al. 2001; Morrison et al. 2018). Prior work has shown deltamethrin, when applied as an emulsifiable concentrate, can significantly decrease the walking ability of *S. zeamais* (Vélez et al. 2019), while no work to our knowledge has assessed sublethal effects of deltamethrin on *P. truncatus*. Further, little work has specifically been done with Diacon IGR+ or Gravista. Understanding the effects of sublethal toxicity on the behavior of insects may help to provide a better understanding of the efficacy of the insecticide in question and its ability to prevent movement and further damage after contact. Thus, our aims were to (i) characterize sublethal changes in movement over time after exposure to an existing

formulation (IGR+®) and newer formulation (Gravista®) as a grain protectant on maize and residual spray on concrete surfaces, and (ii) quantify direct mortality from these compounds.

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 $27 \pm 0.1^\circ\text{C}$ (mean $\pm$ SE), $64 \pm 1\%$ RH, and 14:10 (L:D) h photoperiod for *S. zeamais* and at $27.2 \pm 0.1^\circ\text{C}$, $37 \pm 1\%$ RH, and continuous darkness for *P. truncatus* as mandated by the applicable USDA-APHIS PPQ-approved permit (P526-191021-020).

Insecticide Formulations and Spray Rates on Concrete

There were two insecticides in this study: an existing formulation (tradename: Diacon IGR+ ®; Central Life Sciences, Schaumburg, IL, USA), and a new formulation with synergist and less concentrated active ingredients (tradename: Gravista®). Diacon IGR+ contains 11.4% methoprene and 4.75% deltamethrin, labeled for application at 0.12 kg AI/L and 0.05 kg AI/L, respectively. Label directions for application as a residual surface treatment give a range of 28.5 mL AI/L–171 mL AI/L H₂O to cover 94 m² for both compounds. To obtain the maximum possible effect, we employed the maximum labeled rate in this study (Table 2.1), namely 24 mg AI/m² for deltamethrin and 57 mg AI/m² for methoprene. This corresponded to 0.3 ml of the formulation in 25 ml H₂O, sprayed at the rate of 0.3 ml per 50.3 cm² arena, using separate artist's air brush (Badger 100 series, Badger Corporation, Franklin Park, IL, US) for each treatment.

Each replicate was evenly applied to the concrete dish. The new Gravista formulation has one labeled rate, namely 684 ml formulation/L H₂O to cover 92.9 m². Scaling down, we mixed 0.5 ml of the new formulation in 10 ml H₂O. This was sprayed at the same rate as the other compound. Distilled water was used for the control arenas at 0.3 mL per arena. The arenas were allowed to dry overnight (~8 h) prior to use in experiments.

Table 2-1 Summary of label, mixing, and application rates for insecticides used in residual assay

Assay	Max Label Rate (ppm)				Application Rate (per kg or per m²)		Mixing Rate (mL of product)		Amount (mL) applied to 5 kg maize or 93 m² surface	
									Diacon	Gravista
	Diacon IGR +		Gravista ^a		Diacon IGR+ ^b	Gravista ^c	Diacon IGR+	Gravista ^a		
	Deltamethri n	Methopren e	Deltamethri n	Methopren e						
Maize	1	2.5	0.5	1.15	0.02 ^d	0.05 ^d	0.68 ^d	0.6 ^d	3.7	3.7
Concrete	1	2.5	0.5	1.15	0.48 ^e	1.94 ^e	0.3 ^e	0.5 ^e	45	180

^aAlso contains 13.35 ppm of piperonyl butoxide synergist

^bMixed in 25 mL H₂O

^cMixed in 10 mL H₂O

^dAssumes 532 mL (Diacon IGR+) or 1062 mL (Gravista) of product is mixed in 5 gal (=18 920 mL) of water to cover 1000 bushels of corn (=25 454 kg).

^eAssumes 45 mL (Diacon IGR +) or 180 mL (Gravista) of product is mixed in 1 gal (3784 mL) of water to cover 1000 ft² (93 m²)

Direct Mortality on Concrete

Concrete arenas were prepared for testing by first mixing a slurry of water and Rockite cement in a large pitcher (Rockite, Cleveland, OH, US; as published prior in Arthur 2008). Briefly, water was added until a thick paste consistency was achieved. The mixture then sat for 1 min before it was stirred vigorously, making sure any remaining clumps of dry Rockite were hydrated. Once the mixture was a loose, batter-like consistency, 8.5 ml of the slurry was poured into plastic 100 × 20 mm Petri dishes (area of the bottom: 62 cm²; hereafter, arenas). The slurry was poured to approximately 1.27 cm thick. The cement dishes were then left at room temperature to cure for 24 h. The three treatments were sprayed onto the concrete arenas included: control (distilled water), an existing insecticide (Diacon IGR+: 11.40% S-methoprene + 4.75% deltamethrin), and a novel formulation (Gravista: 2.85% S-methoprene + 1.20% deltamethrin + Synergist, 33.30% piperonyl butoxide). There were 6 exposure times: 4, 8, 24, 48, 72, 168 h. A total of 270 individuals per species (*P. truncatus* and *S. zeamais*) were collected, placed in cohorts of 15 individuals per concrete-filled Petri dish. There were n = 3 replicate arenas per treatment combination.

After exposure, individual *P. truncatus* and *S. zeamais* were removed and placed into clean Petri dish arenas and evaluated for condition. Using a stereomicroscope (SMZ-18, Nikon Inc., Tokyo, Japan) under 60× magnification, *P. truncatus* and *S. zeamais* were classified as alive (moving normally, is able to right itself when flipped over, no twitching), affected (moving sluggishly or erratically, unable to right itself, twitching of antennae or legs may be present), or dead (completely immobile even after prodding) according to prior published definitions (Morrison et al. 2018).

Spray Rates on Treated Maize

Untreated, clean, and uninfested yellow maize was used in the experiments. Grain was sourced from a local farmer in northeast Kansas, US. The highest labeled rate for Diacon IGR+ on maize is 1 ppm deltamethrin and 2.5 ppm of methoprene, which is what we used in this study by adding 0.70 ml of the insecticide to 25 ml of H₂O. After mixing, 0.5 mL was applied to 700 g lots of maize, and this was done three separate times, one for each replicate. For Gravista, the labeled rate on maize is 0.5 ppm deltamethrin and 1.15 ppm of methoprene, which is mixed by adding 0.56 mL of insecticide to 10 mL of H₂O. After mixing, 0.5 mL was applied to 700 g lots of maize, using separate artist's air brush (Badger 100 series, Badger Corporation, Franklin Park, IL, US; designed to spray a line 3.2–50.8 mm wide with materials of high viscosity) at a pressure of 0.70 kg/cm² for each treatment, and this was repeated three separate times, one for each replicate.

Insect Mortality on Treated Maize

To assess direct mortality on treated maize for application in bulk storage, we followed the same protocol as above for the concrete arenas with a few modifications. Specifically, the experiment was conducted with three bioassay replicates ($n = 3$); each with 18 combinations of three treatments (IGR+, Gravista, and H₂O Control) and six exposure times (4, 8, 24, 48, 72, 168 h) of each treatment. For each bioassay replicate, 700 g of maize were treated with each insecticide or H₂O (e.g., control) as described above. Each of 18 vials (120-mL volume) was filled with 100 g of treated maize and 30 adult insects of either species, and the insects were maintained at $27 \pm 0.02^{\circ}\text{C}$. Only 15 of the 30 insects in each vial were randomly selected and evaluated for direct toxicity at each of six exposure times. Extra insects were provided in these experiments to account for a subset of individuals that may bore completely into the kernel (e.g.,

especially *P. truncatus*), which would prevent them from being classified and counted. To recover the insects, the grain was sieved (No. 10, 2.0 x 2.0 mm mesh, W.S Tyler, Mentor, OH, US), and shaken to maximize the number of insects falling into the collection pan.

Insect Mobility on Concrete Arenas and in Treated Maize

To assess insecticide effect on insect mobility in both of the bioassays above, a random subset individuals classified as either alive or affected were tracked with a network camera (Basler AG, Ahrensburg, Germany) coupled with Ethovision XT (v. 14.0, Noldus Software, Leesburg, VA, US). Individuals of each species were placed individually into arenas consisting of 90 mm Petri dishes lined with 85 mm Whatman #1 filter (GE Healthcare, Chicago, IL, USA). The camera was placed 80 cm above the arenas, and trials lasted 30 min. At the end of the trial, the total distance moved (cm) and the average instantaneous velocity (i.e., speed with a direction, cm/s) was recorded. To account for cursor bounce, an input filter was applied that discarded the accumulated distance if it was greater than 5 cm per s. Each trial was manually checked for irregularities, and any with suspicious accumulations of distance were re-run. There were a total of $n = 15$ replicate individuals per treatment combination, totaling to 234 h of video for 468 individuals per species and assay.

Statistical Analysis

A generalized linear model (GLM) based on a quasibinomial to account for overdispersion in the dataset (Aho, 2017) was used to assess differences in percentage of mortality, with a separate model used for each species and assay. Fixed, explanatory variables included insecticide (Diacon IGR+, Gravista, and control), exposure time (0, 4, 8, 24, 48, 72, 168 h), and their interaction. Likelihood ratio tests were conducted to calculate the χ^2 -value and determine significance of each variable in a model with (alternative hypothesis) or without the

variable (null hypothesis) in question. Upon a significant result from the GLM, Tukey HSD was used for multiple comparisons and implemented with the `glht` function from the `multcomp` package (Hothorn 2008). Unless otherwise specified, $\alpha = 0.05$, and R Statistical Software was used (R Core Team 2019).

To analyze the data from the insecticide effect on insect mobility, a special case of a GLM was used based on a Gaussian distribution (e.g. general linear model) with distance moved and velocity as the response variables to account for the fact that each response was continuous, unbounded, and normally distributed (Bolker et al. 2009). A single model was created for each assay and species. The model used insecticide, exposure time, and their interaction as fixed, explanatory variables. Upon a significant result from the general linear model, Tukey HSD was used for multiple comparisons.

Results

Mortality on Concrete

On concrete, the insecticide had a significant overall effect on mortality (percent of individuals affected and dead) compared to the control for both *S. zeamais* and *P. truncatus* (Table 2.2; Figure 2.1). However, mortality was not significantly different for the two insecticide formulations, IGR+ and Gravista. The exposure time also significantly affected the condition of adults of both *S. zeamais* and *P. truncatus*, with more adults dead at 72 and 168 h than at other periods (Figure 2.2). *Prostephanus truncatus* was slightly more susceptible to both insecticide formulations with no “alive” individuals (all affected or dead) compared to some *S. zeamais* individuals remaining alive. Mortality reached 33–37% for insecticide-exposed *S. zeamais*, while it was 40–47% for insecticide-exposed *P. truncatus* by 168 h. Nonetheless, the majority of individuals were simply still affected. There was no significant interaction between exposure

time and insecticide among the health conditions and species for the concrete assay, except for the percent of affected adult *S. zeamais*, where there were initially more alive adults after treatment with Gravista (Figure 2.2).

Table 2-2 Summary of statistical GLM model results for mortality of *S. zeamais* and *P. truncatus* in the concrete assay and maize assay.

Factor	Concrete Assay			Maize Assay		
	df	χ^2	P	df	χ^2	P
<u><i>S. zeamais</i></u>						
<i>Alive</i>						
Insecticide	2	582	0.0001	2	2421	0.0001
Exposure	5	6.95	0.22	5	1.99	0.85
Interaction	10	85.9	0.0001	10	61.1	0.0001
<i>Affected</i>						
Insecticide	2	2509	0.0001	2	509	0.0001
Exposure	5	58.6	0.0001	5	25.7	0.001
Interaction	10	19.9	0.05	10	1.01	0.99
<i>Dead</i>						
Insecticide	2	20.3	0.0001	2	19.2	0.0001
Exposure	5	84.0	0.0001	5	104	0.0001
Interaction	10	11.8	0.30	10	4.22	0.94
<u><i>P. truncatus</i></u>						
Insecticide	2	837709	0.0001	2	113944	0.0001
Exposure	5	15.0	0.05	5	15.0	0.05
Interaction	10	0.00	1.00	10	0.00	1.00
<i>Affected</i>						
Insecticide	2	2573	0.0001	2	529	0.0001
Exposure	5	103	0.0001	5	42.7	0.0001
Interaction	10	2.35	0.99	10	2.75	0.99
<i>Dead</i>						

Insecticide	2	30.9	0.0001	2	30.5	0.0001
Exposure	5	60.6	0.0001	5	87	0.0001
Interaction	10	5.11	0.88	10	4.79	0.90

Figure 2-1 Overall percent mortality induced by Gravista and Diacon IGR+ on concrete

Overall percent mortality induced by an existing (methoprene + deltamethrin, IGR+) and novel (methoprene + deltamethrin + synergist, Gravista) commercial formulation of insecticide when applied against *S. zeamais* (top panel) and *P. truncatus* (bottom panel) on concrete arenas after exposure for 4–168 h, resulting in alive (light purple), affected (dark purple), or dead (black) individuals.

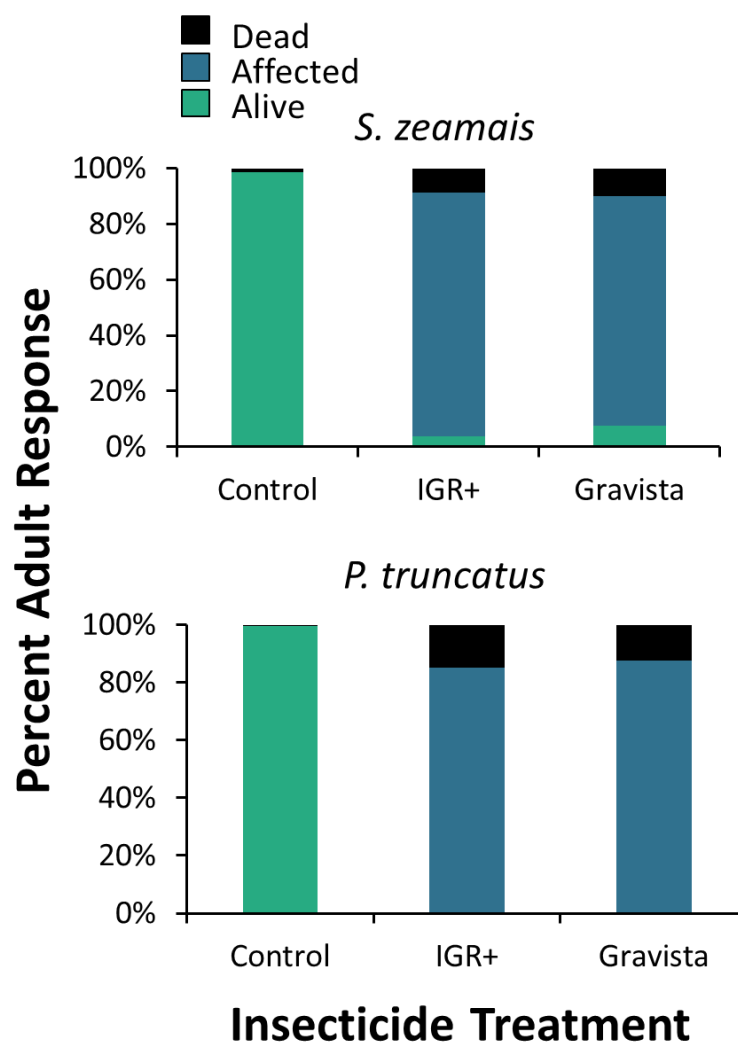
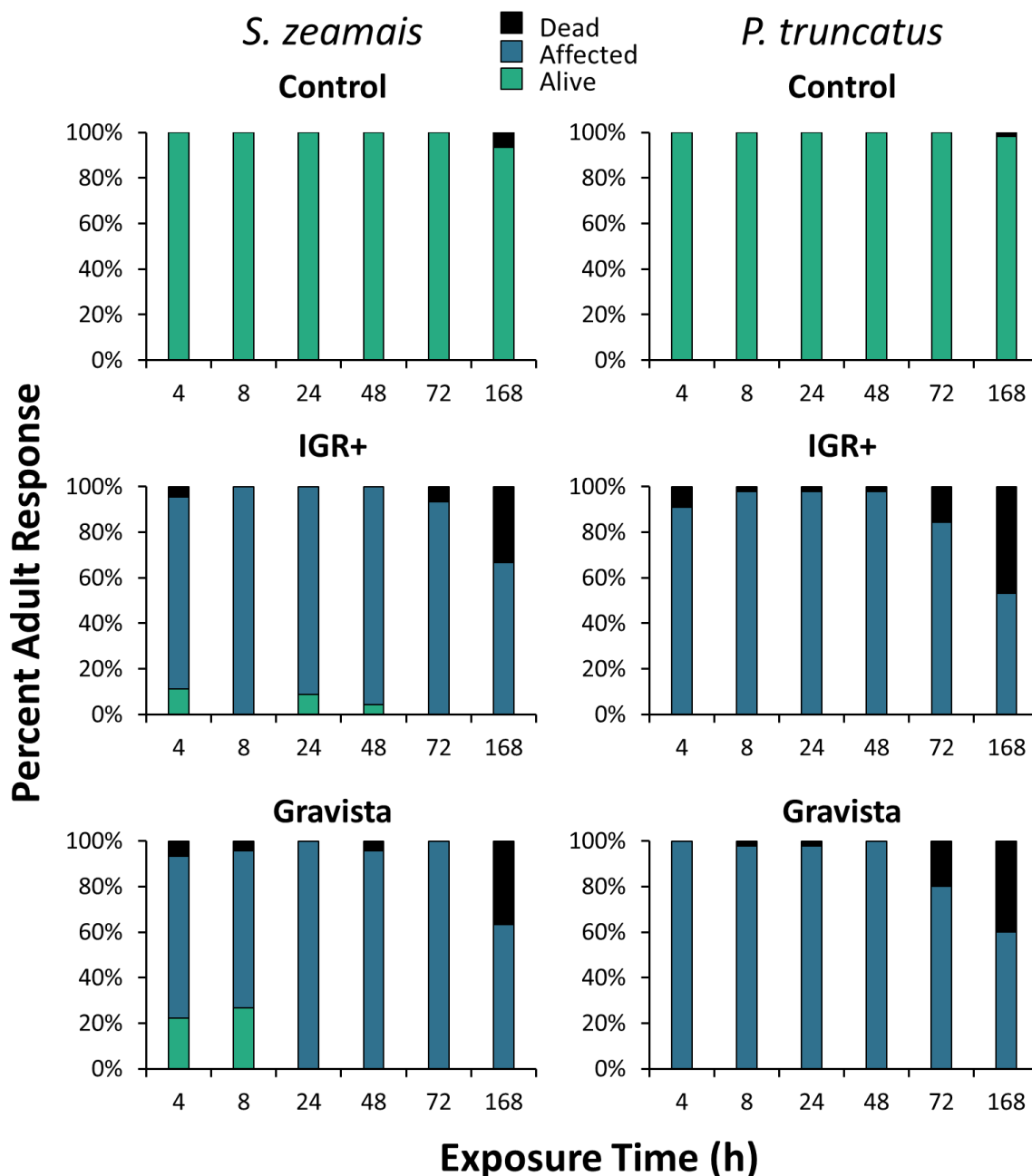


Figure 2-2 Percent mortality induced by Gravista and Diacon IGR+ on concrete by exposure time

Percent mortality induced by an existing (methoprene + deltamethrin, IGR+) and novel (methoprene + deltamethrin + synergist, Gravista) commercial formulation of insecticide when applied against *S. zeamais* (left column) and *P. truncatus* (right column) on concrete arenas broken down by exposure time, resulting in alive (light purple), affected (dark purple), or dead (black) individuals.



Mobility Effects on Concrete

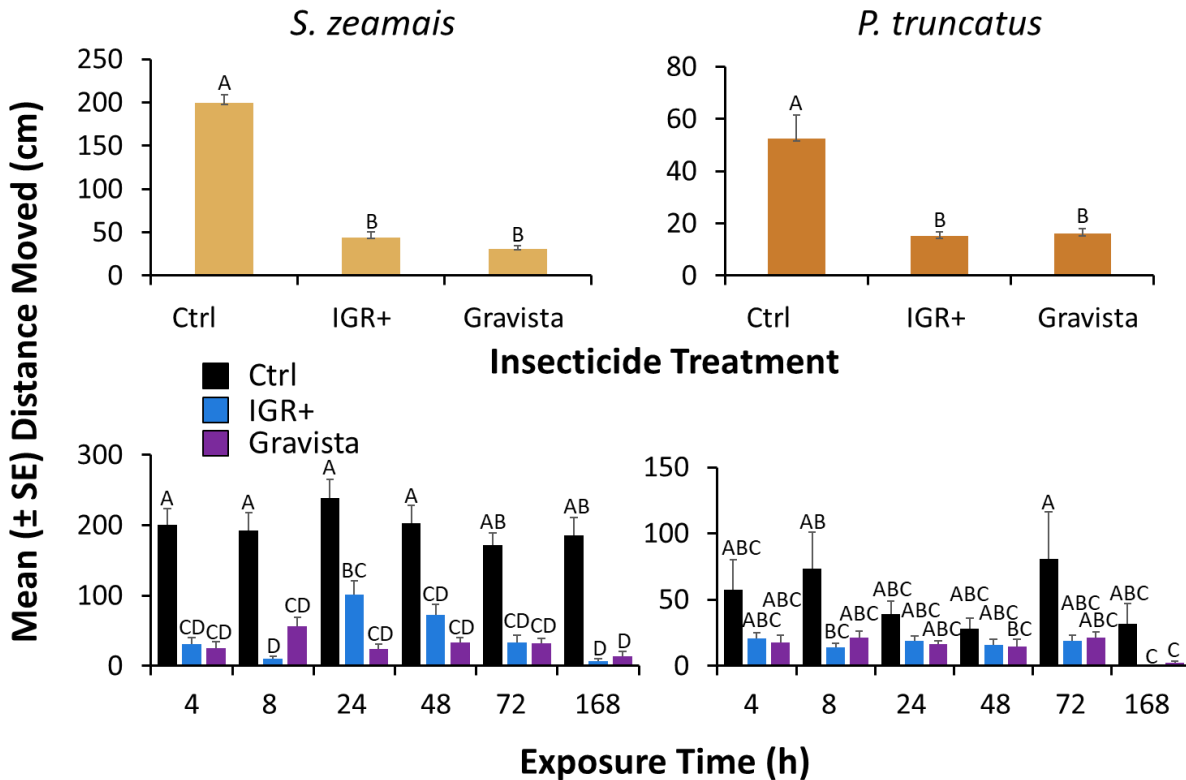
On concrete, the distance moved by *S. zeamais* was significantly affected by exposure to both insecticide formulations (IGR+ and Gravista; Table 2.3). IGR+-exposed *S. zeamais* showed a nearly 5-fold decrease in movement compared to the control, while Gravista-exposed *S. zeamais* exhibited a nearly 7-fold decrease (Figure 2.3). Exposure time significantly affected the distance moved by *S. zeamais*, with adults moving 1.8-fold less 168 h after exposure compared to 24 h after exposure. The interaction between insecticide and exposure time was not significant for *S. zeamais*.

Table 2-3 Summary of statistical model results for GLM of distance moved by *S. zeamais* and *P. truncatus* in the after exposure in the concrete assay and maize assay.

Factor	Concrete Assay			Maize Assay		
	df	F	P	df	F	P
<i>S. zeamais</i>						
Insecticide	2	23.5	0.0001	2	103	0.0001
Exposure	5	3.71	0.01	5	14.8	0.0001
Interaction	10	1.47	0.15	10	10.3	0.0001
<i>P. truncatus</i>						
Insecticide	2	50.2	0.0001	2	219	0.0001
Exposure	5	13.8	0.0001	5	11.9	0.0001
Interaction	10	3.36	0.0001	10	2.32	0.01

Figure 2-3 Distance moved ($\pm$ SE) by *S. zeamais* and *P. truncatus* after exposure to Gravista and Diacon IGR+ on concrete

Distance moved ($\pm$ SE) by *S. zeamais* (left column) and *P. truncatus* (right column) after application of an existing (methoprene + deltamethrin, IGR+) and novel (methoprene + deltamethrin + synergist, Gravista) commercial formulation of insecticide to concrete compared to a control overall (top panels) or broken down by exposure time (bottom panels). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).



Moreover, on concrete, the distance moved by *P. truncatus* was also significantly affected by exposure to both insecticide formulations (Table 2.3). IGR+-exposed *P. truncatus* saw a nearly 4-fold decrease in distance moved compared to the control. Gravista-exposed *P. truncatus* saw a 3-fold decrease in movement compared to the control (Figure 2.3). *Sitophilus zeamais* moved an average of 200 cm compared to 53 cm by *P. truncatus*. Thus, a greater decrease in movement can be seen for both insecticide formulations for *S. zeamais*. Exposure time significantly affected the distance moved by *P. truncatus*, with adults moving 3.6-fold farther 72 h after exposure than 168 h after exposure. The interaction between insecticide and exposure time was significant (Table 2.3), with less movement in control individuals at 24 and 48 h compared to 4, 8, and 72 h (Figure 2.3).

On concrete, the velocity by *S. zeamais* was significantly affected by exposure to both insecticide formulations (IGR+ and Gravista) compared to controls (Table 2.4). IGR+-exposed *S.*

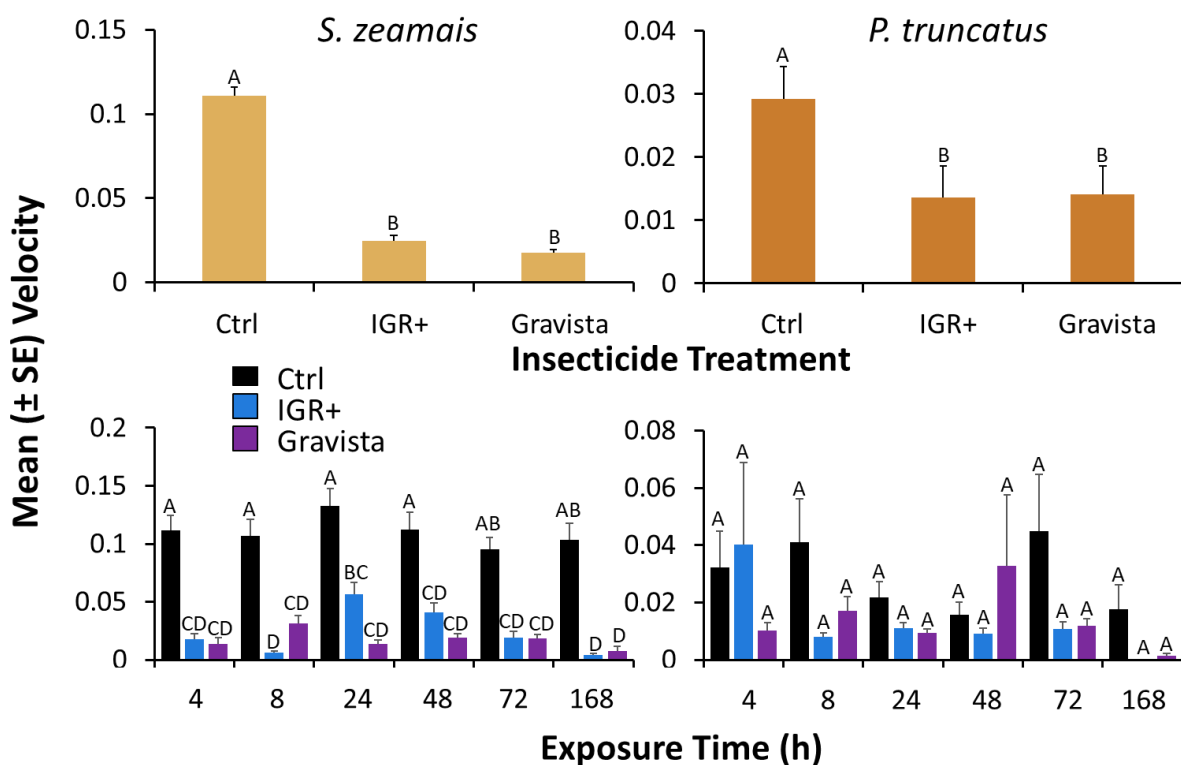
zeamais exhibited a nearly 5-fold decrease in velocity compared to the control, while Gravista-exposed *S. zeamais* showed a nearly 7-fold decrease (Figure 2.4). Furthermore, exposure time significantly altered the velocity by *S. zeamais*, with adults moving 1.8-fold slower 168 h after exposure compared to 72 h afterwards. The interaction between insecticide and exposure time had a significant effect on the velocity (Table 2.4), with *S. zeamais* moving 1.4–14-fold faster 24 h after exposure to IGR+ compared to 4 and 168 h (Figure 2.4).

Table 2-4 Summary of statistical model results for GLM of velocity by *S. zeamais* and *P. truncatus* in the after exposure in the concrete assay and maize assay.

Factor	Concrete Assay			Maize Assay		
	df	F	P	df	F	P
<i>S. zeamais</i>						
Insecticide	2	21.8	0.0001	2	78.8	0.0001
Exposure	5	4.91	0.001	5	29.1	0.01
Interaction	10	3.75	0.001	10	6.07	0.15
<i>P. truncatus</i>						
Insecticide	2	2.97	0.05	2	2.90	0.05
Exposure	5	0.811	0.54	5	0.07	0.99
Interaction	10	2.08	0.05	10	2.42	0.01

Figure 2-4 Velocity ($\pm$ SE) by *S. zeamais* and *P. truncatus* after exposure to Gravista and Diacon IGR+ on concrete

Velocity ($\pm$ SE) by *S. zeamais* (left column) and *P. truncatus* (right column) after application of an existing (methoprene + deltamethrin, IGR+) and novel (methoprene + deltamethrin + synergist, Gravista) commercial formulation of insecticide to concrete compared to a control overall (top panels) or broken down by exposure time (bottom panels). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).



Similarly, the velocity by *P. truncatus* was significantly affected by exposure to IGR+ and Gravista compared to a control (Table 2.4). IGR+- and Gravista-exposed *P. truncatus* showed a 2-fold decrease in velocity compared to the control. Again, it should be noted that, *S. zeamais* is a much more mobile species compared to *P. truncatus*, with control *S. zeamais* velocity on average 0.11 cm/s compared to 0.03 cm/s by *P. truncatus*. Thus, the greater decrease in velocity can be seen for both insecticide formulations for *S. zeamais*. By contrast, exposure time did not significantly affect the distance moved by *P. truncatus*. There was no significant interaction between the insecticide and exposure time on velocity.

Mortality on Maize

The overall mortality on maize was significantly different after exposure to both IGR+ and Gravista compared to the control for both *S. zeamais* and *P. truncatus* (Table 2.2; Figure 2.5). However, mortality (percent dead and percent affected) was not significantly different for

the two insecticide formulations, IGR+ and Gravista. Exposure time significantly affected mortality by both species also (Figure 2.6), but there was no significant interaction between insecticide and exposure time on the percent of affected and percent of dead adults for either species (Table 2.2). As exposure time increased, so too did the number of dead adults, which reached 47–67% and 42–53% for insecticide-exposed *S. zeamais* and *P. truncatus* by 168 h, respectively. *Prostephanus truncatus* was slightly more susceptible to both insecticide formulations with no “alive” individuals (all affected or dead) compared to the *S. zeamais*. *Sitophilus zeamais* was more susceptible on maize compared to exposure on concrete with less than 1% left “alive” compared to nearly 8% “alive” on concrete.

Figure 2-5 Overall percent mortality after exposure to Gravista and Diacon IGR+ on maize

Overall percent mortality induced by an existing (methoprene + deltamethrin, IGR+) and novel (methoprene + deltamethrin + synergist, Gravista) commercial formulation of insecticide when applied against *S. zeamais* (top panel) and *P. truncatus* (bottom panel) as a grain protectant on maize after exposure for 4–168 h, resulting in alive (light purple), affected (dark purple), or dead (black) individuals.

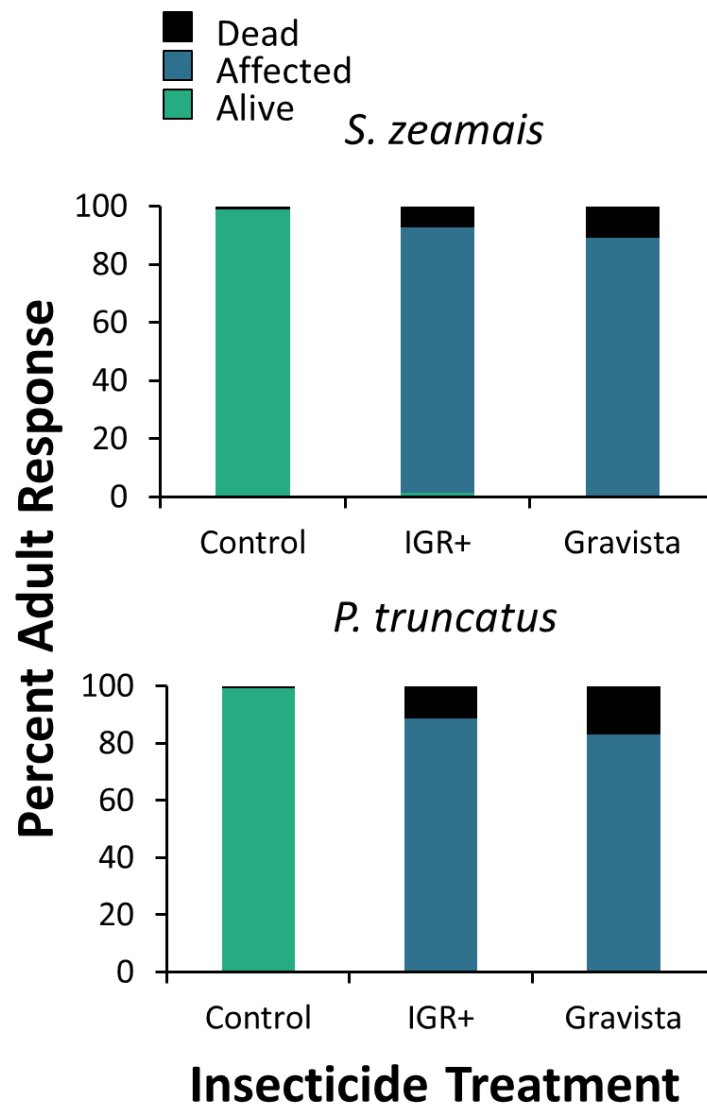
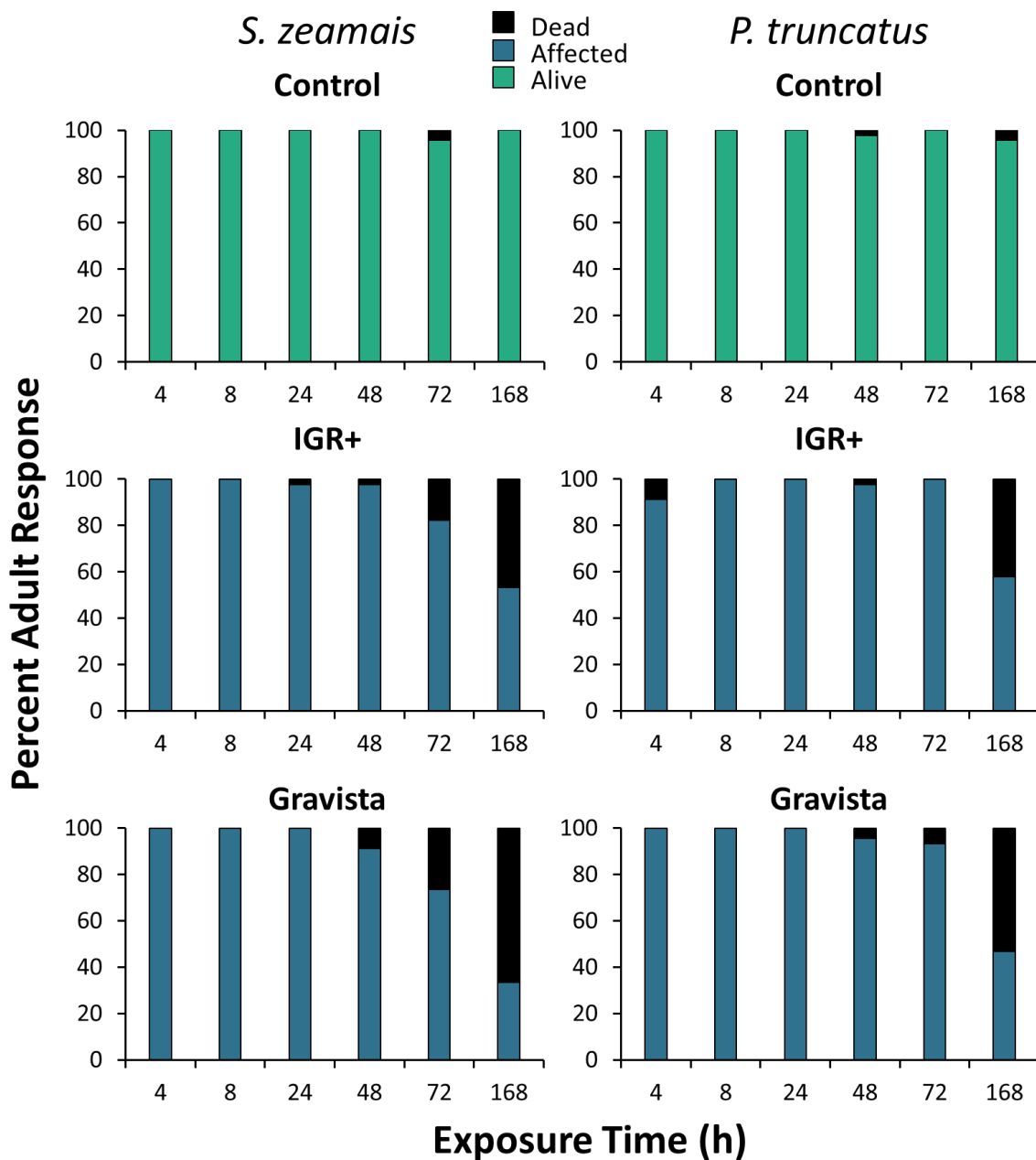


Figure 2-6 Percent mortality induced by Gravista and Diacon IGR+ on maize by exposure time

Percent mortality induced by an existing (methoprene + deltamethrin, IGR+) and novel (methoprene + deltamethrin + synergist, Gravista) commercial formulation of insecticide when applied against *S. zeamais* (left column) and *P. truncatus* (right column) on maize broken down by exposure time, resulting in alive (light purple), affected (dark purple), or dead (black) individuals.

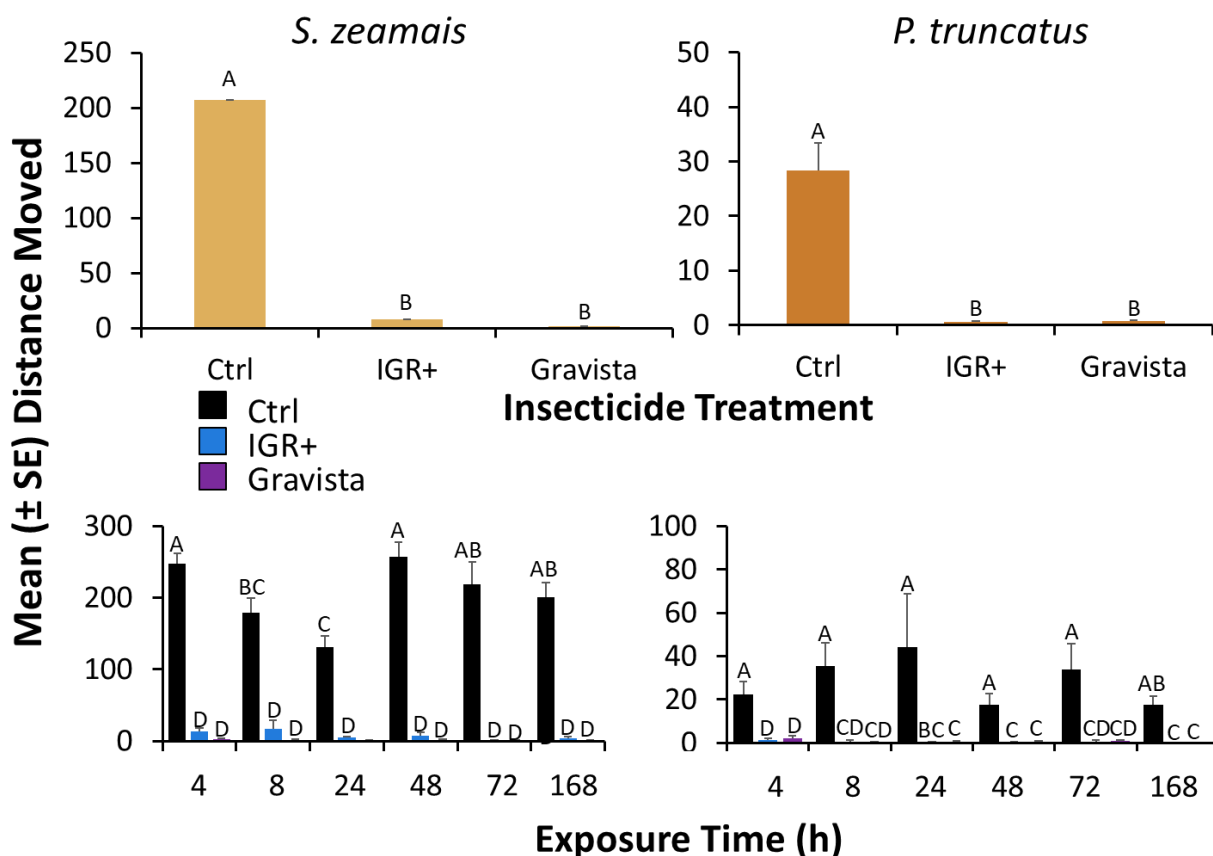


Mobility Effects on Maize

On maize, the distance moved by *S. zeamais* was significantly affected by exposure to IGR+ and Gravista compared to the control (Table 2.3). IGR+-exposed and Gravista-exposed *S. zeamais* showed a 26-fold and 148-fold decrease, respectively, in distance moved compared to the control (Figure 2.7). Exposure time significantly affected the distance moved by *S. zeamais*, with adults 4 h after exposure moving 2-fold more than those 24 h afterwards. There was a significant interaction between insecticide and exposure time on the distance moved by *S. zeamais*, with control adults moving the least 24 h after exposure to the insecticide treatments and the most 4 and 48 h afterwards (Figure 2.7).

Figure 2-7 Distance moved ($\pm$ SE) by *S. zeamais* and *P. truncatus* after exposure to Gravista and Diacon IGR+ on maize

Distance moved ($\pm$ SE) by *S. zeamais* (left column) and *P. truncatus* (right column) after application of an existing (methoprene + deltamethrin, IGR+) and novel (methoprene + deltamethrin + synergist, Gravista) commercial formulation of insecticide to maize as a grain protectant compared to a control overall (top panels) or broken down by exposure time (bottom panels). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).



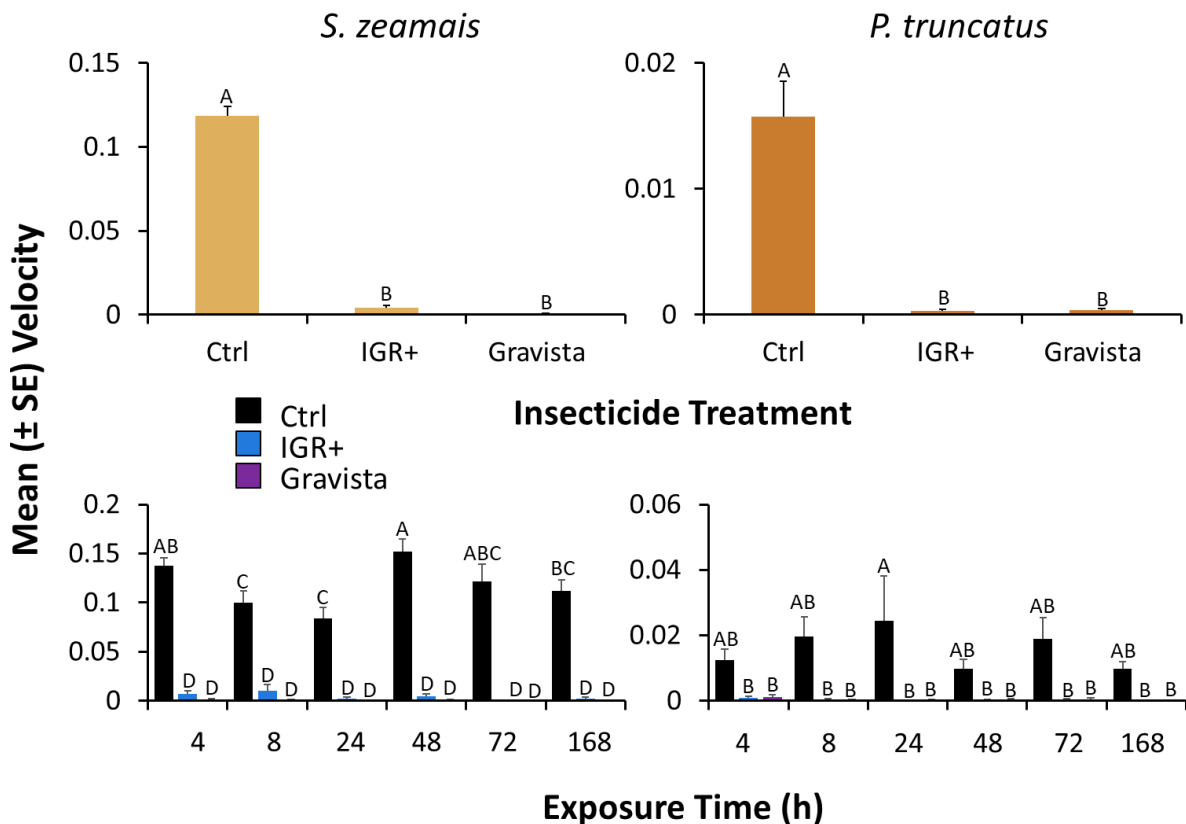
In addition, on maize, the distance moved by *P. truncatus* was significantly affected by exposure to both insecticide formulations (Table 2.4). IGR+-exposed *P. truncatus* saw a 49-fold decrease in movement compared to the control, while Gravista-exposed *S. zeamais* exhibited a 39-fold decrease in movement compared to the control. Exposure time did not significantly affected the distance moved by *P. truncatus*, with adults moving 2.5-fold more after 24 h

exposure compared to 168 h afterwards. There was a significant interaction between insecticide and exposure time on distance moved by *P. truncatus*, with movement less at later exposure times for the control adults (Figure 2.7). It should be noted that both *S. zeamais* and *P. truncatus* were more susceptible to both insecticide formulations when exposed on maize versus concrete, as both species saw nearly zero movement after exposure compared to the controls. When exposed on concrete, *S. zeamais* moved on average as much as 44 cm after exposure and *P. truncatus* 16 cm after exposure.

Similarly, on maize, the velocity by *S. zeamais* was significantly affected by exposure to both insecticide formulations (Table 2.4). IGR+-exposed and Gravista-exposed *S. zeamais* saw a 30-fold and 150-fold decrease, respectively, in velocity compared to the control (Figure 2.8). Exposure time significantly affected the velocity by *S. zeamais*, with adults moving 1.8-fold more 48 h after exposure compared to 24 h afterwards. The interaction between insecticide and exposure time did not have a significant effect on velocity moved by *S. zeamais*.

Figure 2-8 Velocity ($\pm$ SE) by *S. zeamais* and *P. truncatus* after exposure to Gravista and Diacon IGR+ on maize

Velocity ($\pm$ SE) by *S. zeamais* (left column) and *P. truncatus* (right column) after application of an existing (methoprene + deltamethrin, IGR+) and novel (methoprene + deltamethrin + synergist, Gravista) commercial formulation of insecticide to maize as a grain protectant compared to a control overall (top panels) or broken down by exposure time (bottom panels). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).



Finally, the velocity by *P. truncatus* on maize was significantly affected by exposure to both insecticide formulations (IGR+ and Gravista) (Table 2.4). IGR+-exposed *P. truncatus* showed a nearly 67-fold decrease in movement compared to the control, while Gravista-exposed *P. truncatus* exhibited a 50-fold decrease (Figure 2.8). Exposure time did not significantly affect the distance moved by *P. truncatus*. Baseline mobility was greater for *Sitophilus zeamais*, with *S. zeamais* control individuals moving an average of 0.12 cm/s compared to 0.02 cm/s by *P. truncatus*.

Discussion

We examined both mortality and sublethal effects by the novel insecticide (Gravista) compared to IGR+ for two stored products pests. Up to this point, no one has evaluated the baseline mobility or sublethal effects of insecticides on the invasive *P. truncatus*. Importantly, we found that both compounds significantly reduced the movement of *P. truncatus* and *S. zeamais* compared to the controls, even after brief 4-h exposures. This is the first reported experiment using Ethovision coupled with video-tracking to capture sublethal reduction in movement (e.g., distance moved and velocity) related to an insecticide for *P. truncatus*. Baseline movement of *P. truncatus* decreased from 28–52 cm in 30 min in the controls to 0.56–16 cm, on average, while *S. zeamais* distance moved decreased from 199–207 cm to 7–44 cm in the same period. The reduction in movement by *S. zeamais* (5–27-fold) is in alignment with prior results on sublethal exposure of the species to deltamethrin (Morrison et al. 2021). *Prostephanus truncatus* movement was reduced by 3–50-fold compared to control individuals. The use of IGR+ and Gravista has the potential to manage both *P. truncatus* and *S. zeamais* and prevent their further movement. As Guedes et al. 2011 pointed out, understanding the sublethal effects of insecticide exposure allows for a more comprehensive picture of efficacy compared to solely focusing on direct lethality. Our study adds to a growing body of literature with different species of stored products insects that document sublethal exposure to insecticides (Arthur et al. 2020; Wilkins et al., 2020; Morrison et al., 2018; Morrison et al. 2019; Morrison et al., 2021).

Both IGR+ and Gravista provided effective control of both *P. truncatus* and *S. zeamais*, but the latter does it with 4-fold less active ingredient because of the addition of a synergist. Both insecticides caused direct mortality in the two pests, though markedly greater in *P. truncatus* compared to *S. zeamais*. It was clear *P. truncatus* was the more susceptible species, with a

mortality of 40–47% by 168 h on concrete, whereas *S. zeamais* mortality reached 33–37%. Interestingly, mortality was higher when insects were exposed on grain instead of concrete. Mortality reached 47–67% and 42–53% for insecticide-exposed *S. zeamais* and *P. truncatus* by 168 h on maize. By contrast, immediately after treatment with Gravista in a residual study, *S. oryzae* mortality was 91% after 168 h (Arthur and Morrison 2020). The differences in the mortality for the two weevils could be the result of the recent range expansion of *S. zeamais* (Correa et al. 2017), and its greater resistance to insecticides more generally (Ribeiro et al. 2003). Regardless, both IGR+ and Gravista can be considered effective at inducing mortality in both species even over the brief exposure times evaluated in this study.

In our study, we evaluated the relative efficacy of IGR+ and Gravista as a residual treatment on concrete or as a grain protectant on maize. We found efficacy was greater when insects were placed on maize than on concrete. There have been many studies that examine how the efficacy of insecticides varies on different substrates. These studies often compared concrete to varnished wood, metal, tile or laminated paper (Jenson et al. 2009; Williams et al. 1983; White and Leesch 1996; Hagstrum and Subramanyam, 2006; Wijayaratne et al., 2012; Arthur, 1997; Arthur 2008). Generally, insecticides are found to have the greatest efficacy on non-porous substrates such as metal or tile, followed by porous substrates like concrete or wood. In addition, residual efficacy of insecticides was found to be greatest when applied on metal surfaces (Jankov et al. 2013). Concrete generally absorbs much of the formulation applied to it. In a few cases, depending on the insecticide and the insect species, efficacy was actually greater on concrete compared to other substrates. For example, when insecticidal pyrrole chlorfenapyr was applied to concrete, vinyl tile, and plywood surfaces, control of two *Tribolium* species was greater on concrete compared to vinyl tile and plywood (Arthur 2008). As Chadwich (1985) noted,

substrate type and its porosity may impact the absorption of the insecticide into the substrate and subsequent degradation of the insecticide. It would appear that at least in this study, IGR+ and Gravista were more effective on maize kernels that had been sprayed with insecticide compared with concrete, which had likely absorbed more of the insecticide below the surface, sequestering it from contact with the insects. It is possible that only the legs come into contact with the residual insecticides when insects move on the treated but flat concrete surface whereas the rest of the body can also come into contact with the residual insecticides when insects move around the treated maize kernels. However, both IGR+ and Gravista seem useful both as a residual surface treatment and as a grain protectant despite their varying efficacy in those forms. Both residual treatments around a food facilities and grain protectants for bulk storage situations have an role to play in comprehensive integrated pest management programs (Arthur and Subramanyam 2012), with both of the compounds assessed in this study showing promise for incorporation.

Another aspect that may affect efficacy is the development of resistance to deltamethrin by one or both species. The use of deltamethrin in the US is relatively new, and to date, there has been no documented instance of resistance. However, there has been documented deltamethrin resistance in a variety of other countries, including Australia (Daglish and Nayak 2018) and Brazil (Guedes et al. 2009). Incomplete dosing is known to induce resistance, but this is not likely to occur with the lower amounts of active ingredients in Gravista, because there is a much higher amount of synergist, which often acts to break resistance to pyrethroids in a variety of systems (Forrester et al. 1993).

Currently, *S. zeamais* is one of the most important pests of stored maize grains in the US and elsewhere. Despite the significance of the *S. zeamais* as a pest of maize grains globally, there

is relatively little information compiled about the life history and biology of the insect pest. This could be due to the fact that *S. zeamais* is nearly indistinguishable from *S. oryzae* and was often referred to as “*Sitophilus* species” in the literature. *Sitophilus zeamais* is less selective when it comes to the commodities it attacks, feeding on commodities as diverse as apples, rice, wheat, and maize (Longstaff 1981). The damage caused on maize by *S. zeamais* has been estimated by Boxall (2002) to be 10–20% after only 3 months in storage.

While *S. zeamais* is already present in the US, *P. truncatus* may invade under shifting distributions from climate change. Both are important and pernicious pests of maize (Quellhorst et al. 2021). Thus, the use of IGR+ and Gravista as crack-and-crevice treatments in or around food facilities in the US where grains are typically kept and grain protectant treatments of kernels stored for extended periods in bulk storage may be especially beneficial. Most food facilities lose some maize in both quantity and quality over the time if stored for extended periods. Both compounds appear like feasible options. Gravista, a formulation with much less active ingredient, may be an option to delay resistance development or overcome existing resistance problems for food facilities, because of the synergist.

While we have assessed both direct and sublethal effects of two compounds on two economically important species in stored products using two application methods, in the future it will be important to 1) determine whether exposure to these compounds affects dispersal to novel food patches, 2) evaluate changes in flight capacity after exposure to insecticides (e.g., Morrison et al. 2017), and 3) elucidate molecular mechanisms of stress after exposure to these compounds. While prior work has documented that *S. zeamais* and *P. truncatus* prefer different ends of the temperature spectrum (Quellhorst et al. 2020), it is unknown how temperature mediates response especially to the new formulation with synergist (e.g., Gravista). Follow-up studies on these

topics will help more fully characterize important new compounds coming onto the market for stored product protection, while providing information on potential new chemical tools for stakeholders.

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Chapter 3 - Efficacy of deltamethrin and pirimiphos-methyl in layer-treated maize against the larger grain borer and the maize weevil

Submitted as: Hannah Quellhorst^{1*}, Maria Sakka³, Marina Gkourgkouta³, William R. Morrison III³, Kun Yan Zhu¹, and Christos G. Athanassiou³. Efficacy of deltamethrin and pirimiphos-methyl in layer-treated maize against the larger grain borer and the maize weevil. Journal of Economic Entomology 2023. In Press. DOI: <https://doi.org/10.1093/jee/toad072>.

Abstract:

Two grain surface treatment insecticides (deltamethrin and pirimiphos-methyl) were evaluated in laboratory assays as surface treatments for maize to control adult *Prostephanus truncatus* and *Sitophilus zeamais*. Both insecticides were applied to 20 g of maize placed in a vial or to the upper one half, one fourth, or one-eighth layer of the maize. Insects were either added to the vials before or after the maize. Mortality, progeny production, and insect damaged kernels (IDK) were then evaluated for each vial. Insect introduction method (before or after) did not have any impact on any of the variables. Mortality was nearly 100% for all treatments for both insecticides for *P. truncatus*. Subsequently, progeny production and the number of insect damaged kernels were very low or zero for *P. truncatus*. Mortality for *S. zeamais* remained low across layer treatments for deltamethrin. However, *S. zeamais* was easily controlled by pirimiphos-methyl. The results of this laboratory study show that while deltamethrin and pirimiphos-methyl have some effectiveness as a layer treatment on a column of maize, efficacy will be dependent on the target species, and the depth of the treated layer, as well as the location on which the insects are present.

Key Words: larger grain borer, maize weevil, population growth, insecticide, grain protectant

Introduction

The larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae), is an invasive insect pest of maize across much of the world. The insect was thought to have originated from Mexico, Central America, and Northern South America (Hodges et al., 1983). Most of the efforts to control *P. truncatus* have been focused on the use of a wide range of insecticides, with often dissimilar results (Golob, 2002; Rumbos et al., 2013; Quellhorst et al., 2021; Quellhorst et al., 2022). However, there are disproportionally few published data on the use of contact insecticides and fumigants for the management of this species, as compared with the vast majority of major stored product insect species (Quellhorst et al., 2021). After the rapid appearance of *P. truncatus* in Africa, management of this species was based on the use of active ingredients that were effective for other stored product insect species that were prevalent those new locations, including the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), which seems to be the main competitor of *P. zeamais* in this geographical area (Giga and Canhao, 1993; Sakka and Athanassiou, 2018; Quellhorst et al., 2020). Nevertheless, it was soon found that the insecticides that were effective for the control of *S. zeamais* were not effective against *P. truncatus*, probably due to a natural tolerance of this species to certain active ingredients (Golob et al., 1983, Golob et al., 2002). For *S. zeamais*, pyrethroids are considered less effective than organophosphorous (OP) compounds, while the opposite was recorded in the case of *P. truncatus*, leading farmers to use mixtures of two insecticide formulations to attempt to manage both species simultaneously (Golob et al., 2002; Golob et al., 1985; Dales and Golob, 1997). After decades of use as grain protectants, the pyrethroid deltamethrin and the OP pirimiphos-methyl are still extensively used, and are still considered the main grain protectants in the market in several geographical areas, including the European Union (EU).

Insects infesting bulked grains can be found in several locations of the grain mass depending on the type of storage, insect species, and other factors such as the design of the storage structure or even sanitation (Morrison et al., 2019; Gerken and Campbell, 2022). In grain bins where there are false floors, insects can be found infesting the grain from the bottom of the grain mass (Weston and Barney, 1998). Depending on the species, insects may tend to move from the edges of the store to the center, especially when there are differences in the levels of temperature and moisture content between these two zones (Vela-Coiffier et al., 1997). Other studies have shown that densities of insects are typically higher in the upper layers of bulk grains (Hagstrum, 1989; Hagstrum et al., 1998; Athanassiou et al., 2003). Species like the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) have positive geotaxis and do not easily move upward in grain columns, whereas other species, such as the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), exhibit both positive and negative geotaxis (Athanassiou et al., 2009).

There have been a few studies that have focused on the concept of only applying grain protectants on the upper surface or layer of the grain mass, a technique that is also commonly known as “top dressing” (Collins et al., 1988; Athanassiou et al., 2009; Athanassiou et al., 2010; Vassilakos and Athanassiou, 2012a; Tsaganou et al., 2021). This method is logistically practical because when grains receive grain protectant treatments using conveyor belts, typically a subset of the grain being loaded into storage or a specific surface of the grain is sprayed, while other surfaces or layers may be untouched. This results in grain with a mixture of treated and untreated surfaces or a mixture of treated and untreated kernels, thereby making treatment with the grain protectant uneven (Daglish and Nayak, 2010; Subramanyam et al., 2014). Next, if the majority of the insects infesting a grain store or column are found in the top 0.5 meters (Athanassiou et al.,

2003), then this method of spraying only the top layer of the grain mass could reduce the amount of insects present in the grain while simultaneously reducing economic costs of treatment and the presence of insecticide residues throughout the grain (Athanassiou et al., 2009, 2011). To date, top dressings have only been assessed with a few different insecticide chemistries and only in the case of few stored product insect pests. These include the bacterial insecticide spinosad for the control of *R. dominica*, *S. oryzae*, and three species of Liposcelididae (Psocoptera), the insect growth regulator methoprene for the control of *R. dominica*, the neonicotinoid thiamethoxam for the control of *R. dominica* and *S. oryzae*, and the bacterial insecticide spinetoram for the control of *R. dominica*, *S. oryzae*, and the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) (Athanassiou et al., 2009; Athanassiou et al., 2011; Vassilakos and Athanassiou, 2012a; Tsaganou et al., 2021). Moreover, the vast majority of these studies are mostly focused on wheat, while there is still inadequate information in the case of maize, and important and widespread grain commodities. In addition, these types of experiments have yet to be done with *P. truncatus* or to our knowledge, *S. zeamais*. Therefore, the aim of this study was to investigate the efficacy of two grain protectants, deltamethrin and pirimiphos-methyl, which are commercially available for use in the EU, when used as a layer or top dressing treatment for the control of *P. truncatus* and *S. zeamais*.

Methods and Materials

Insecticides Formulations and Spray Rates on Maize

Untreated, clean, and uninfested yellow maize was used in the experiments. Grain was sourced from the 2020 Greek harvest, from the region of Thessaly (Central Greece). The commercial formulations that were used in this study were Seguro 2.5EC (Agrotechnica, Thessaloniki, Greece) for deltamethrin and Actellic 50 EC (Syngenta Crop Protection AG,

Switzerland) for pirimiphos-methyl. The highest label rate for deltamethrin on maize is 1 ppm (i.e., 1 mg of deltamethrin per kg of maize), while the highest label rate for pirimiphos-methyl was 4 ppm (i.e., 4 mg of pirimiphos-methyl per kg of maize). The spray solutions of each insecticide were prepared by diluting the 2 mL of deltamethrin and 4 mL of pirimiphos-methyl in 100 mL of distilled water. Then 1 mL of each insecticide solution was sprayed onto lots of 500g of maize. An additional series of lots were sprayed with distilled water and served as a control. Each of the two insecticides was applied on maize by using a Kyoto BD-183K airbrush (Graphotech, Japan) at an operating pressure of 2.2 bar.

Insect Mortality on Treated Maize and Progeny Production

For each replicate, 500 g of maize were treated with each insecticide or H₂O (e.g., control) as described above. Before proceeding with the experiments, the grain moisture content (m.c.) was assessed, using a moisture meter (mini GAC plus, Dickey-John Europe S.A.S., Colombes, France). The standard plastic cylindrical vials of the Laboratory of Entomology and Agricultural Zoology (LEAZ) were used for insect rearing bioassays (3 × 8 cm in diameter by height, Rotilabo Sample tins Snap on lid, Carl Roth, Germany). These were filled with 20 g of maize, lids contained small holes for ventilation, and the upper rings of the vials were also treated with Fluon (Northern Products Inc., Woonsocket, USA) to prevent insects from moving away from the grain and or escaping. In each vial, we treated either all the grain (1/1), 1/2, 1/4 or 1/8 of the maize with one of the two insecticides (deltamethrin or pirimiphos-methyl) at the labeled rate. We also either placed the insects at the bottom of the vial (before the maize has been added) or at the top (after the maize has been added). Sets A, B, and C were treated with insecticide on separate days. Insects were given 14 days before mortality counts were performed. After this interval, the mortality was assessed. It is difficult to estimate the upper 1/8 etc. of

maize, therefore we based our experiments on ratios of 20 g treated, 20 g untreated, 10 g treated with 10 g untreated, 5 g treated with 15 g untreated and 2 g treated with 18 g untreated. The exact quantities of the samples were weighed with a Precisa XB3200D compact balance (Alpha Analytical Instruments, Gerakas, Greece). Each vial then received 10 *P. truncatus* adults of mixed sex and age from the Tanzania strain or 10 *S. zeamais* from Brazil. The vials were placed inside incubators set at 30°C and 65% R.H. After the parental mortality count, all adults were removed, and the vials with maize were returned to the incubator at the conditions indicated above. Sixty days later, the vials were opened again to check progeny production and the number of insect damaged kernels (IDK).

The experiment was replicated over time with sets A, B, and C were treated with insecticide on separate days. For each combination, e.g., insecticide $\times$ insect species, there were three replicates with three subreplicates (total $3 \times 3 = 9$ vials or replicates per combination). There were 2 insecticides $\times$ 2 insect species $\times$ 4 grain treatments (1/1, 1/2, 1/4, 1/8) $\times$ 2 insect introduction methods (before or after) $\times$ 9 replicates/subreplicates = 288 vials total, 5760 g of maize, 10 insects per vial $\times$ 288 = 2880 total (1440 per LAGB and MW). We also had a separate set of vials for the control with no insecticide = 9×2 insect species = 18 vials, 360 g of maize, and 180 insects (90 per species).

Statistical Analysis

The mortality was analyzed with a generalized linear model based on a quasipoisson distribution to account for overdispersion. Treatment (depth of the treated layers – 1/8, 1/4, 1/2, and 1/1) and method of insect introduction into the vials (insects added before or after grain) were used as explanatory, main effects, along with their interaction. A loglikelihood statistic based on a chi-

square distribution was used to calculate significance. Multiple comparisons used Tukey–Kramer HSD test ($\alpha = 0.05$).

The progeny and IDK were analyzed separately with a linear model using treatment (depth of the treated layers – 1/8, 1/4, 1/2, and 1/1) and method of insect introduction into the vials (insects added before or after grain) as explanatory, main effects, along with their interaction.

Assumptions of normality and homogeneity of variance were confirmed by inspecting histograms, residual plots, and Q-Q plots. Models that deviated from assumptions underwent log or square root transformation as applicable, which corrected issues. Multiple comparisons used Tukey–Kramer HSD test ($\alpha = 0.05$). All analyses were conducted using R Software (R Core Team, 2022).

Results

Insect Mortality on Layer-Treated Maize

There was no significant effect of insect position (insects placed in the vial before or after the layer treatments), layer treatment, insecticide or any interaction between, on the mortality of *P. truncatus*. However, for *S. zeamais* there was a significant effect of layer treatment, insecticide, and their interaction of the two on mortality (Table 3.1). In the vials containing maize totally treated with pirimiphos-methyl, all *P. truncatus* adults died whether added before or after the maize (Fig. 3.1). There were some *P. truncatus* individuals who remained alive or affected for the pirimiphos-methyl treated maize; however, mortality remained above 97%. There was also no significant difference between the different layer treatments (1/8, 1/4, 1/2, 1/1). In the vials containing maize completely treated with pirimiphos-methyl, all *S. zeamais* adults died whether they were added before or after the maize (Fig. 3.1). Mortality was 100%

across all the treatments. There was no significant difference between the different layer treatments for pirimiphos-methyl (1/8, 1/4, 1/2, 1/1).

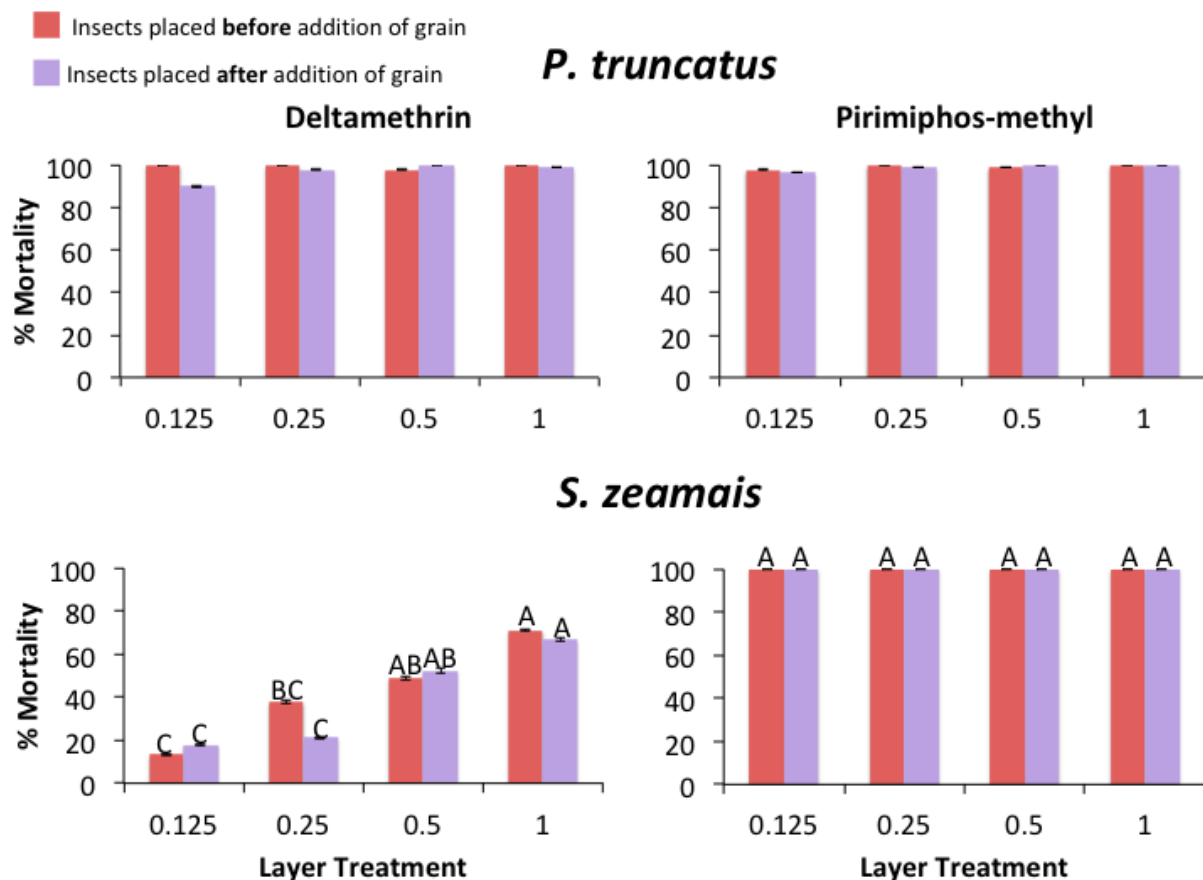
Table 3-1 Summary of statistics for GLM for mortality in the layer treatment study

Summary of statistics for generalized linear model for mortality in the layer treatment study. Variables italicized are significant ($\alpha = 0.05$).

Species	Variable	χ^2	DF	P Value
LAGB	Position (Before/After)	0.91	1	0.34
	Layer Treatment	3.17	3	0.37
	Insecticide	0.37	1	0.54
	Position*Treatment	2.76	3	0.43
	Position*Insecticide	0.63	1	0.43
	Treatment*Insecticide	0.22	3	0.97
	Position*Treatment*Insecticide	1.50	3	0.68
MW	Position (Before/After)	0.23	1	0.63
	<i>Layer Treatment</i>	<i>34.1</i>	3	<i>0.0001</i>
	<i>Insecticide</i>	<i>295</i>	<i>1</i>	<i>0.0001</i>
	Position*Treatment	1.63	3	0.65
	Position*Insecticide	0.59	1	0.44
	<i>Treatment*Insecticide</i>	<i>88.8</i>	3	<i>0.0001</i>
	Position*Treatment*Insecticide	5.85	3	0.12

Figure 3-1 Mean percent mortality of *P. truncatus* and *S. zeamais* after exposure in layer treatment to Deltamethrin and Pirimiphos-methyl.

Mean percent mortality of *P. truncatus* and *S. zeamais* after exposure in layer treatment to Deltamethrin and Pirimiphos-methyl. Letters displayed on graphs only for significant models. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Error bars equal plus or minus standard error.



In the vials containing maize totally treated with deltamethrin, all *P. truncatus* adults died whether added before or after the maize (Fig. 3.1). Some *P. truncatus* remained alive or affected after exposure to deltamethrin-treated maize; however, mortality remained above 90%. There was also no significant difference between the different layer treatments (1/8, 1/4, 1/2, 1/1), however mortality was numerically the lowest when only 1/8th of the maize was treated and the insects had been placed into the grain after the maize was added (Fig. 3.1). In the vials containing maize treated with deltamethrin, mortality for *S. zeamais* remained low whether

added before or after the maize (Fig. 3.1). When *S. zeamais* were placed into maize completely treated with deltamethrin, mortality did not exceed 71%. Mortality was even lower for *S. zeamais* when the insects were added after the grain was added (67%). When *S. zeamais* were placed into maize with only 1/4th of the grain treated with deltamethrin, mortality did not exceed 38%. Mortality was significantly lower for *S. zeamais* when the insects were added after the grain was added (21%). When *S. zeamais* were placed into maize with only 1/8th treated with deltamethrin, mortality did not exceed 18%. However, there was no significant difference between the two methods of introduction (e.g., before or after).

Progeny Production on Layer-Treated Maize

In vials containing grain treated with deltamethrin, progeny production remained below three individuals for *P. truncatus* and was only observed in the 1/4th and 1/8th layer treatments while the controls yielded nearly 30 progeny per vial on average (Fig. 3.2). Interestingly, progeny were only observed in treatments where insects were added after the grain was placed. However, despite this, there was no significant effect of any of the variables or interactions on the progeny production of *P. truncatus* on either deltamethrin- or pirimiphos-methyl-treated grain (Table 3.2).

Table 3-2 Summary of statistical output for ANOVA for progeny production in layer treatment studies.

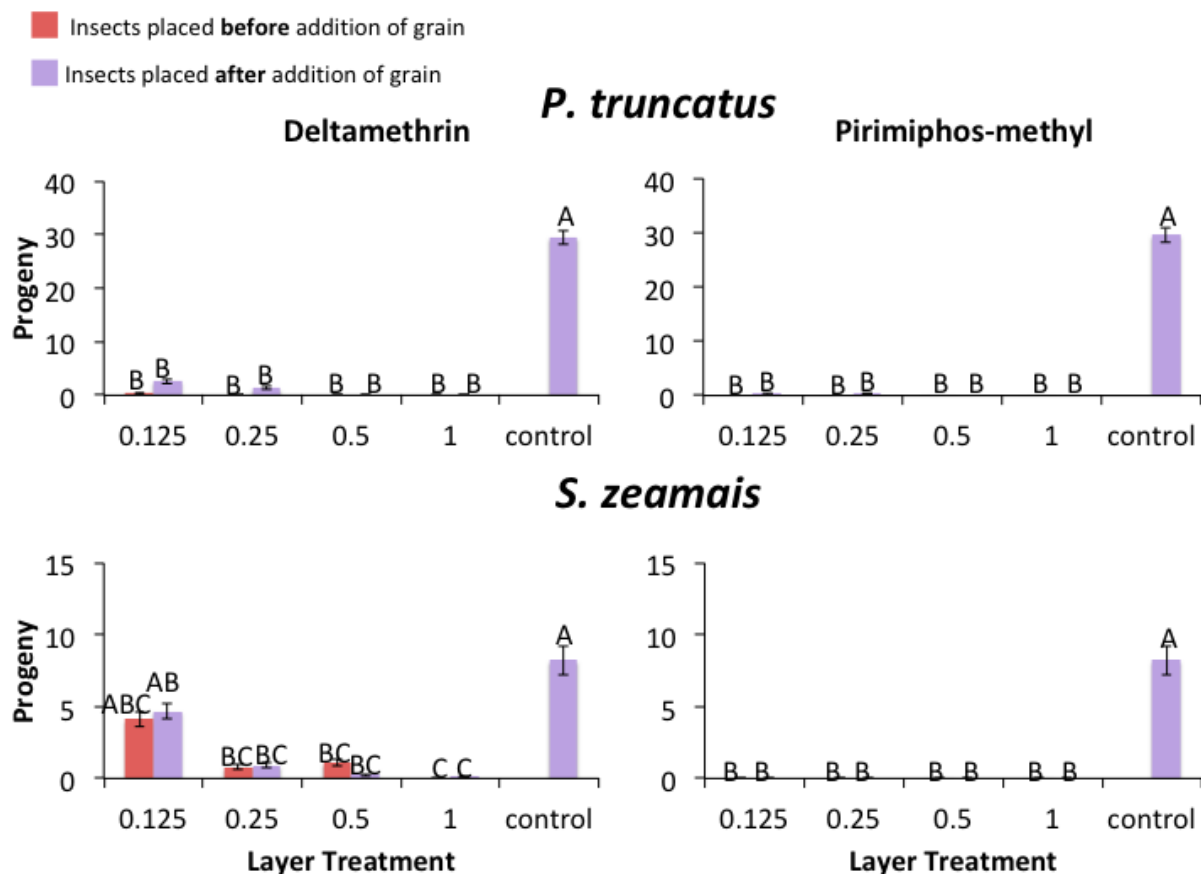
Summary of statistical output for ANOVA for progeny production in layer treatment studies. Variables that are italicized were significant.

Species	Variable	df¹	F	P
LAGB	Position (Before/After)	1	1.19	0.278
	Layer Treatment	3	0.62	0.60
	Insecticide	1	0.76	0.39
	Position*Treatment	3	0.43	0.73
	Position*Insecticide	1	0.50	0.48
	Treatment*Insecticide	3	0.38	0.77
	Position*Treatment*Insecticide	3	0.22	0.88
	Residuals	136		
MW	Position (Before/After)	1	2.14	0.15
	<i>Layer Treatment</i>	3	<i>28.0</i>	<i>0.0001</i>
	<i>Insecticide</i>	<i>1</i>	<i>19.4</i>	<i>0.0001</i>
	Position*Treatment	3	0.16	0.92
	Position*Insecticide	1	2.71	0.10
	<i>Treatment*Insecticide</i>	3	<i>2.69</i>	<i>0.04</i>
	Position*Treatment*Insecticide	3	0.21	0.89

¹Residuals df = 136

Figure 3-2 Mean progeny production of *P. truncatus* and *S. zeamais* after exposure in layer treatment to Deltamethrin and Pirimiphos-methyl.

Mean progeny production of *P. truncatus* and *S. zeamais* after exposure in layer treatment to Deltamethrin and Pirimiphos-methyl. Letters displayed on graphs only for significant models. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Error bars equal plus or minus standard error.



In vials containing grain treated with deltamethrin, progeny production remained below a mean of five individuals for *S. zeamais* and was observed in the 1/4th, 1/8th and, 1/2 layer treatments compared to the control which yielded nearly nine progeny on average (Fig. 3.2). There was no difference in introduction method (e.g., before or after) on progeny production for *S. zeamais*. In vials containing grain treated with pirimiphos-methyl, progeny production was zero across the layer treatments for *S. zeamais*. There was a significant effect of insecticide and

layer treatment, and the interaction of the two on the progeny production of *S. zeamais* (Table 3.2).

Table 3-3 Summary of statistics for ANOVA of insect-damaged kernels (IDK) in the layer experiment study.

Summary of statistics for ANOVA of insect-damaged kernels (IDK) in the layer experiment study. Italicized variables are statistically significant ($\alpha = 0.05$).

Species	Variable	df¹	F	P Value
LAGB	Position (Before/After)	1	1.10	0.30
	Layer Treatment	3	7.88	0.001
	Insecticide	1	0.91	0.03
	Position*Treatment	3	0.62	0.61
	Position*Insecticide	1	2.95	0.01
	Treatment*Insecticide	3	2.16	0.1
	Position*Treatment*Insecticide	3	0.32	0.01
MW	Position (Before/After)	1	3.12	0.08
	Layer Treatment	3	23.6	0.0001
	Insecticide	1	168	0.0001
	Position*Treatment	3	33.3	0.0001
	Position*Insecticide	1	5.99	0.02
	Treatment*Insecticide	3	22.8	0.0001
	Position*Treatment*Insecticide	3	35.5	0.0001

¹ Residuals df = 136

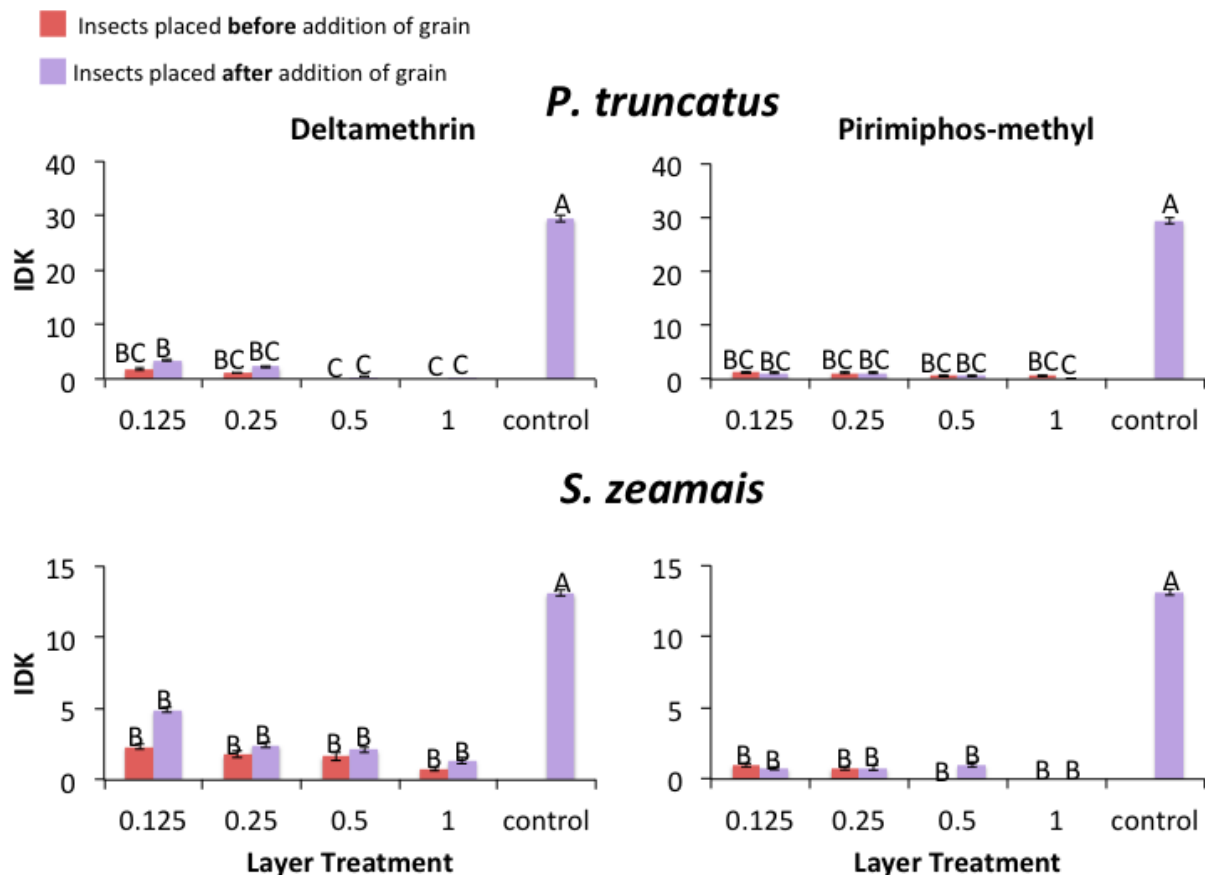
Insect Damaged Kernels on Layer-Treated Maize

In vials containing grain treated with deltamethrin, the number of insect damaged kernels (IDK) remained below a mean of four kernels per vial for *P. truncatus* and was only found in the 1/4th and 1/8th layer treatments compared to the control which yielded nearly 30 IDK (Fig. 3.3). There was a significant effect of treatment, insecticide and all variable interactions on the IDK for *P. truncatus* on either deltamethrin or pirimiphos-methyl (Table 3.3).

In vials containing grain treated with deltamethrin, the IDK remained below a mean of five IDK for *S. zeamais* and across all layer treatments compared to the control which yielded an average of 13 IDK per vial (Fig. 3.2). The majority of the damaged kernels were found in vials containing the 1/8th Deltamethrin layer treatment. There did not seem to be a difference in introduction method (e.g., before or after) on progeny production for *S. zeamais*. There was a significant effect of treatment, insecticide and all variable interactions on the IDK for *S. zeamais* on either deltamethrin or pirimiphos-methyl (Table 3.3).

Figure 3-3 Mean number of insect damaged kernels (IDK) by *P. truncatus* and *S. zeamais* after exposure in layer treatment to Deltamethrin and Pirimiphos-methyl.

Mean number of insect damaged kernels (IDK) by *P. truncatus* and *S. zeamais* after exposure in layer treatment to Deltamethrin and Pirimiphos-methyl. Letters displayed on graphs only for significant models. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Error bars equal plus or minus standard error.



Discussion

Prostephanus truncatus was the more susceptible species compared to *S. zeamais* to both deltamethrin and pirimiphos-methyl, which is also commonly observed in other studies, especially for deltamethrin (Quellhorst et al., 2022). In our study, mortality for *P. truncatus* adults exposed to deltamethrin was 97% or greater regardless of whether the insects were placed in the vials before or after the placement of maize. Also, mortality in response to pirimiphos-methyl was greater than 90% even in the 1/8th layer treatment, meaning that when *P. truncatus*

adults move through a thin layer of insecticide-treated grain to colonize untreated areas of the maize bulk, this species could be vulnerable to deltamethrin and pirimiphos-methyl even during brief exposures. These results suggest that less insecticide could be used to successfully control *P. truncatus* in bulk grain stores. These results are similar to those found for *R. dominica* (Athanassiou et al., 2009).

Sitophilus zeamais was highly susceptible to pirimiphos-methyl but not deltamethrin. In our study, mortality against deltamethrin for *S. zeamais* was no higher than 72% for the completely treated grain. Mortality dropped off to 52% for the 1/2 treated grain and less than 20% for the 1/8th treated grain. These results suggest that in the case of deltamethrin, *S. zeamais* is not effectively controlled by a layer treatment. These same trends can be seen for progeny production and IDK for both *P. truncatus* and *S. zeamais*. Insect damage to grain and progeny production remained low after exposure to both insecticides for *P. truncatus*. By contrast, *S. zeamais* was able to produce viable progeny and inflict damage to grains when exposed to deltamethrin-treated grain. For a similar experimental setup with spinosad, Athanassiou et al. (2009) found that progeny production of *S. oryzae* in wheat layers that were not completely treated with the insecticide could not be totally avoided, possibly due to the fact that this species was moving both upwards and downwards, infesting parts of the grain quantity that were untreated. Moreover, the adults of this species, as well as those of *S. zeamais*, oviposit directly in the internal part of the kernel, where they remain unaffected by insecticides that are applied on the kernel's surface (Arthur and Throne, 2003; Athanassiou et al., 2009; Vassilakos and Athanassiou, 2012a, b).

In practice, even when the entire grain mass is treated with a certain protectant, there might be parts of the grain quantity that are untreated, or have received only a proportion of the

target dose range, since the grains are not sprayed as “monolayers”. This may allow some colonization in the untreated parts of the grain mass by parental adults before death through surviving progeny production. Nevertheless, progeny production has not always been proportionally representative of parental mortality in similar studies (Athanasassiou et al., 2009; Daglish and Nayak, 2010; Vassilakos and Athanasassiou, 2012a; Tsaganou et al., 2021). For instance, in a study with grains that were partially treated with thiamethoxam, it was found that although parental mortality of *R. dominica* was higher than that of *S. oryzae*, the reverse was observed for progeny production (Tsaganou et al., 2021). This seems to be particularly important in the case of grains treated with S-methoprene, which affects only immature development and not the parental adults (Daglish and Nayak, 2010). However, in the case of neurotoxic insecticides, such as the ones tested here, a considerable proportion of any offspring may eventually die, when they emerge as adults and move to treated layers of the grain mass (Vassilakos and Athanasassiou, 2012a; Tsaganou et al., 2021). Moreover, even the short exposures to these insecticides may cause some parental mortality, which will also reduce progeny production capacity (Tsaganou et al., 2014; Vassilakos and Athanasassiou, 2012b). The same effects have been recorded in the case of short exposures of different stored product insects to grains treated with grain protectants (Athanasassiou et al., 2010; Hertlein et al., 2011; Tsaganou et al., 2014; Subramanyam et al., 2014). For instance, Athanasassiou et al. (2010) found that exposure for 4 hours of *R. dominica* adults on spinosad-treated wheat or maize caused 100 % delayed mortality when the exposed individuals were removed from the treated substrate.

In previous studies, *P. truncatus* was found to be less susceptible to OPs, specifically pirimiphos-methyl (Golob et al., 1983, Golob et al., 2002). However, in our study, we found no difference between the two insecticides in their efficacy for controlling *P. truncatus* on layer

treated maize. Additionally, in prior work, *S. zeamais* was found to be less susceptible to pyrethroids, such as deltamethrin. The results of our study, support these reports, as pirimiphos-methyl was highly effective at controlling *S. zeamais* compared to deltamethrin on layer treated maize (Golob et al., 2002; Golob et al., 1985; Dales and Golob, 1997). Considering the overall data, and taking into account the potential withdrawal of most OPs from the EU market, our study identifies a grey area in the future of grain protectants, and the need to evaluate additional active ingredients for this purpose in the very near future.

In conclusion, it may be possible to simultaneously control *P. truncatus* while reducing costs and insecticidal residues, and still provide protection for the entire grain mass. Our laboratory results indicate that even the short contact with a small deltamethrin/pirimiphos-methyl-treated portion of the grain mass might be effective against *P. truncatus*. However, results for *S. zeamais*, show that these species may be able to pass through a treated layer of maize and survive, depending on the depth of this layer, especially for deltamethrin.

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Chapter 4 - The dispersal ability of the invasive larger grain borer and the cosmopolitan maize weevil after brief exposure to a newer insecticide formulation

Submitted as: Hannah Quellhorst^{1*}, Frank H. Arthur^{2,3}, Kun Yan Zhu¹, Alex Bruce⁴, and William R. Morrison III⁵. The dispersal capacity of the invasive larger grain borer and the cosmopolitan maize weevil after brief exposure to a novel insecticide formulation. Journal of Stored Products Entomology, 2023. In review.

Abstract

The invasive larger grain borer (*Prostephanus truncatus*) and the cosmopolitan maize weevil (*Sitophilus zeamais*) are two major pests of stored maize. Given the threat of these insects to our maize supply, the increasing insecticide resistance to currently used chemical control tactics such as phosphine, and the removal of effective chemistries from the market (methylbromide), there is a need for alternative integrated pest management tools. Here, we present a more realistic test of the effectiveness of a novel insecticide formulation (methoprene + deltamethrin + piperonyl butoxide; tradename, Gravista ®) compared to an older formulation (methoprene + deltamethrin; tradename, Diacon IGR+®) at reducing the horizontal dispersal capacity to food patches by both of these stored product species. To do this, we used a dispersal apparatus consisting of 30 or 70 cm PVC pipe modified with a baited pitfall trap at one end where we introduced cohorts of adults after exposure on concrete arenas at labeled rates and gave them 48 h to disperse to a novel food source consisting of 15 g of maize. The dispersal capacity for *P. truncatus*, measured as the percentage of adults dispersing to the novel food source, was significantly reduced by 90-97% after exposure to Gravista or Diacon IGR+ compared to the control. By contrast, the dispersal capacity for *S. zeamais* was only reduced by 5-25% after exposure to either insecticide formulation compared to the control. Both formulations performed equally. Overall, our study highlights the importance of using realistic behavioral assays in comprehensive insecticide testing to provide a full picture of insecticide efficacy.

Key Words: grain protectant, insecticide formulation, maize, larger grain borer, maize weevil, dispersal

Introduction

There are several control strategies that can be employed to mitigate the damages/losses to stored products caused by stored product insect pests, such as mating disruption (Morrison et al., 2021), biocontrol (Hervet and Morrison, 2021), insecticides (Stejskal et al., 2021), and other methods such as heat and cold treatments (Phillips and Throne, 2010) and modified atmospheres (Navarro, 2006). Despite all these options, the use of insecticides continues to be the most common and effective choice by stakeholders. However, we are now facing serious challenges in controlling these insects as our most effective fumigant methyl bromide was phased out of use and now with a stark rise in global insecticide resistance to our second most effective fumigant, phosphine (Nayak et al., 2020). This issue of resistance has made it critical that we find different pest management strategies, such as alternatives and new formulations of insecticides for use against stored products insect pests.

Gravista (Central Life Sciences, Schaumburg, IL, US) is a newer formulation of Diacon IGR+ and contains an insect growth regulator IGR (2.85% methoprene, 0.027kg [AI]/L), a pyrethroid (1.2% deltamethrin, 0.012kg [AI]/L), and a synergist (33.3% piperonyl butoxide 0.32kg [AI]/L). The newer formulation, Gravista contains less active ingredient due to the addition of the synergist. Synergists such as piperonyl butoxide are known to inhibit cytochrome P450 detoxification, and as a result, allow for the use of less active ingredient (Quellhorst et al., 2022). Gravista has been used for reducing progeny production and grain damage from *Sitophilus* spp. and the lesser grain borer, *Rhyzopertha dominica* on grain (Arthur and Morrison, 2020), and has also been found to significantly decrease four stored product species' movement (*Sitophilus oryzae*, *S. zeamais*, *Tribolium castaneum*, *R. dominica*) after exposure over

successive months (Morrison et al., 2021). Prior work found *S. oryzae* was the least susceptible to Gravista, while *R. dominica* was the most susceptible to exposure (Arthur et al., 2020).

These insecticide formulations can also be utilized as a grain protectant or surface treatment for management of maize weevil, *Sitophilus zeamais* (Motschulsky), and the larger grain borer, *Prostephanus truncatus* (Horn). These are two important maize (*Zea mays*) pests that are highly damaging and directly compete with each other (Quellhorst et al., 2020; Quellhorst et al., 2023). Prior work has evaluated movement in a grain column, and found both species move to the same areas, but mixed cultures result in shifted damage by *P. truncatus* upwards in the grain mass (Quellhorst et al. 2023). Previously, the direct (e.g., mortality) and sublethal effects (e.g., movement) by *P. truncatus* and *S. zeamais* after exposure to Gravista and Diacon IGR+ were evaluated (Quellhorst et al., 2022). Both species were found to be equally affected by both formulations, and movement in small Petri dishes was reduced after exposure compared to untreated controls. However, no research has been conducted to determine if these formulations affect dispersal at longer distances from one food foraging patch to another after exposure or for longer periods of time. In addition, species may vary in movement capacity according to life history traits. For example, *S. zeamais* is very mobile, and often moves more than other stored product species (Morrison et al., 2021). By contrast, *P. truncatus* has very short legs and a large body, which results in poor mobility (Quellhorst et al., 2022). Finally, while species may vary in movement capacity, they may also vary in their ability to normally forage using semiochemicals after exposure to insecticides at a distance (Quellhorst et al., 2021). Therefore, the aims of this study were to investigate the (i) dispersal capacity after exposure to Gravista and Diacon IGR+ compared to an untreated control at two different distances and (ii) assess the mortality and recovery of the insects after exposure to each insecticide formulation

before and after the walking experiment. We hypothesized that Gravista is as effective or more effective compared to Diacon IGR+ at reducing dispersal capacity for both the maize weevil and the larger grain borer due to the addition of the synergist.

Methods and Materials

Insect Material

The adult *P. truncatus* and *S. zeamais* used in this experiment were reared in insect colonies kept at the United States Department of Agriculture (USDA), Agricultural Research Service, Center for Grain and Animal Health Research facility in Manhattan, Kansas.

Prostephanus truncatus were kept on whole kernels of maize in environmental chambers at $27.2 \pm 0.1^{\circ}\text{C}$, $37 \pm 1\%$ RH, and continuous darkness as mandated by the applicable USDA-APHIS PPQ-approved permit (P526-191021-020). *Sitophilus zeamais* were kept on whole kernels of maize in environmental chambers at $27 \pm 0.1^{\circ}\text{C}$ (mean $\pm$ SE), $64 \pm 1\%$ RH, and 14:10 (L:D) h photoperiod.

Insecticide Rates

The insecticides used in this experiment were both sprayed at the label rate on concrete petri dish arenas. The older formulation, Diacon IGR+® (Central Life Sciences, Schaumburg, IL, USA), had a label rate of 0.12 kg AI/L and 0.05 kg AI/L. When used as a surface treatment on concrete petri dish arenas, a range was given (28.5 mL AI/L–171 mL AI/L H₂O to cover 94 m²). We chose to use the highest labeled rate of 24 mg AI/m² for deltamethrin and 57 mg AI/m² for s-methoprene, or 0.3 ml of the insecticide in 25 ml H₂O, sprayed at the rate of 0.3 ml per 50.3 cm² concrete arena. We sprayed the insecticide using an artist's air brush and a compressor pump (Badger 100 series, Badger Corporation, Franklin Park, IL, US), with a separate clean airbrush reserved for the water control only. The newer formulation, Gravista® had one labeled rate

regardless of use, of 684 ml formulation/L H₂O to cover 92.9 m², or 0.5 ml of insecticide mixed in 10 ml H₂O. Distilled water served as the control and was sprayed on concrete petri dish arenas using the same methods as the insecticide. All concrete petri dish arenas dried for 8 hours before insects were placed into the dishes. Insects (20 of each species per replicate) were exposed on the insecticide-treated petri dishes for either 4 or 24 h. Insects were given a clean/untreated petri dish to rest in while examining condition after insecticide exposure. Insects of both species were evaluated for their condition after exposure to the insecticides by examination under a stereomicroscope (SMZ-18, Nikon Inc., Tokyo, Japan). Insects were classified into three groups: alive, affected, and dead (as previously published in Ranabhat et al. 2022). Insects were scored as alive if there were moving/walking at normal speeds, able to right themselves when flipped over, and observable twitching. Insects were scored as affected if they were seen moving slowly/sluggishly or erratically, unable to right themselves after being flipped over, and observable twitching of appendages such as legs and antennae. Insects were scored as dead if they were completely immobile with no detectable movement after continuous prodding with forceps.

Dispersal and Mortality

To evaluate the dispersal ability of *P. truncatus* and *S. zeamais* after insecticide exposure to new food patches, a dispersal apparatus was employed. Species-specific cohorts of 20 adults (*P. truncatus* or *S. zeamais*) were exposed to Gravista, Diacon IGR+, or an untreated control as above for 4 or 24 h. The insects were then given 48 h to disperse across 30 or 70 cm standardized sections of PVC pipe (3.175 cm ID). After exposure to an insecticide formulation, insects were evaluated for condition after exposure before placing them in the dispersal apparatus. The ends of both sides of the PVC pipe were sealed with mesh (425 µm) to prevent escape. At the far end

of the pipe, a hole (2 cm D) was drilled and centered over a glass jar (5 × 6.5 cm D:H) to create a pitfall trap design. The glass jar contained 20 g of whole maize kernels, representing a novel food patch, to induce insects to disperse with food kairomones. Untreated, clean, and uninfested yellow maize was used in the experiments. Grain was sourced from Heartland Mills (Marienthal, KS, USA), and frozen for 72 h prior to use to ensure no prior insect infestation was present. At the end of the sampling period, the number of insects in the jar and their mortality was scored as alive, affected or dead. In addition, the position of each individual was recorded as residing in zone 1 (at the release point), zone 2 (in first half of tube), zone 3 (in second half of tube), or zone 4 (collection jar with maize). In total, there were $n = 12$ replicate cohorts for each species and combination of distance and treatment. In total, 1,440 *P. truncatus* and 1,440 *S. zeamais* were tested in this experiment.

Statistical analysis

Mortality was analyzed with a generalized linear model using a binomial or quasibinomial distribution, with separate models run for mortality before and after the assay for each species. Treatment (Gravista, Diacon IGR+, or untreated control) and distance (30 or 70 cm) were used as fixed, explanatory variables. Date of testing was used as a random variable. Log-likelihood tests for significance were conducted based on a Chi-squared distribution. Upon a significant result from the overall model, multiple comparisons employed chi-squared tests with a Bonferroni correction. For all statistical analysis, R Software was used (R Core Team, 2023).

Dispersal was evaluated with a multivariate analysis of variance (MANOVA), using number of *P. truncatus* or *S. zeamais* in each zone as a response variable. Treatment (Gravista, Diacon IGR+, or untreated control) and distance (30 or 70 cm) were used as fixed, explanatory variables. Sequential ANOVAs in the same form were run upon a significant result from the

overall test. Upon a significant result from an ANOVA, multiple comparisons employed Tukey HSD. For this test, $\alpha = 0.05$. All figures were made with the package *ggplot2* (Wickham, 2016).

Results

Dispersal

After a 4 h exposure period to the control, over 75% of *P. truncatus* adults ended up in zone 4 (e.g., pitfall jar with novel food source) at the 30 cm dispersal length (Figure 4.1, Table 4.1). This was significant when compared to the less than 25% of *P. truncatus* control adults that made it to the same zone (e.g., zone 4) in the 70 cm apparatus. Exposure to each formulation (Gravista and Diacon IGR+) resulted in many more adult *P. truncatus* recaptured where they were released in the dispersal apparatus (e.g., Zone 1) compared to the control at both 30 and 70 cm length dispersal (Figure 4.1, Table 4.1). There was no significant difference between the two insecticide formulations, with nearly 100% of *P. truncatus* adults remaining in zone 1 for both durations as well (e.g., 4 h and 24 h; Figure 4.1). However, there were a small number of individuals exposed to the insecticides that were able to make it to the pitfall jar (e.g., commodity) in Zone 4.

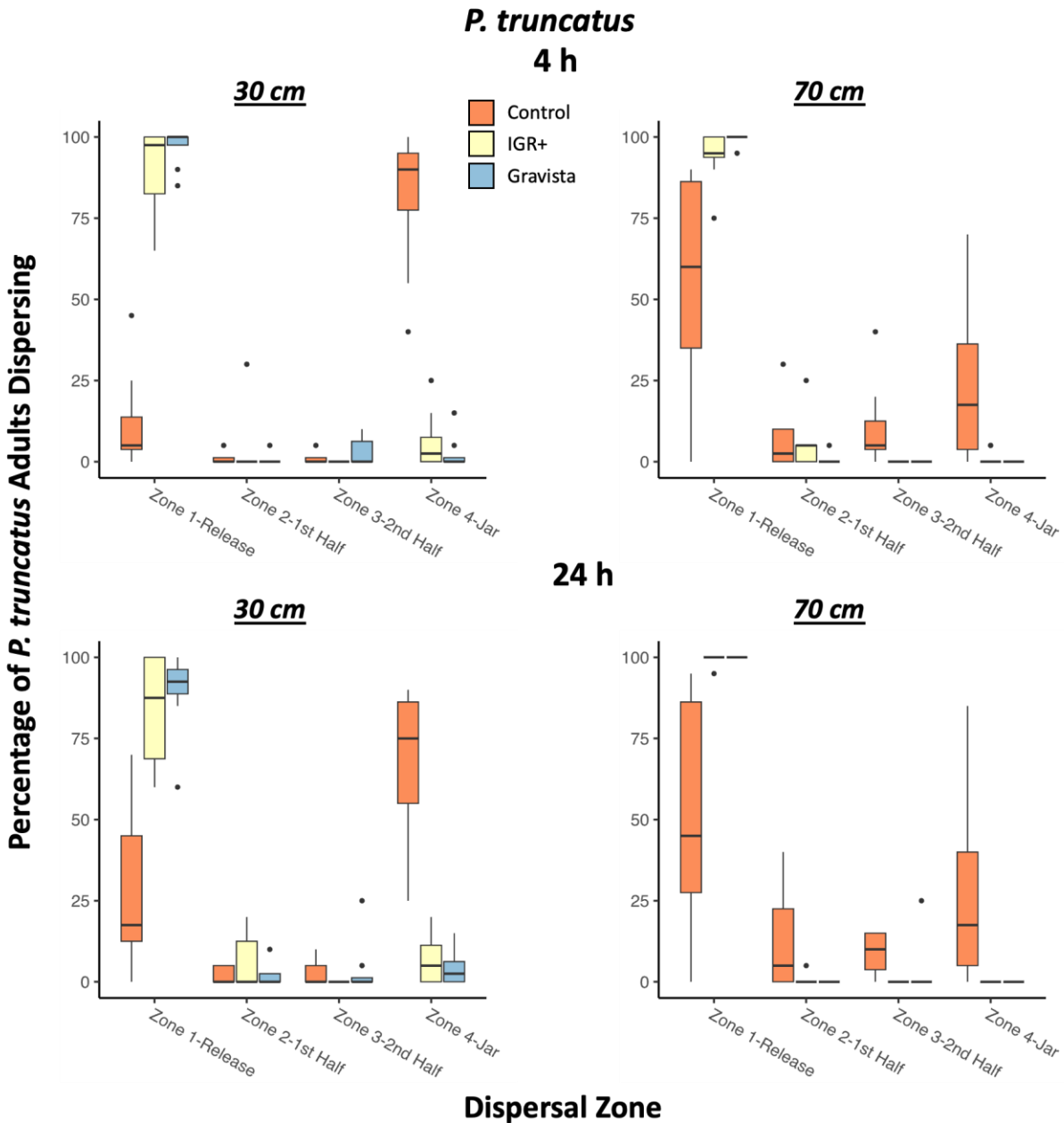
Table 4-1 Summary of dispersal GLM statistical output for *S. zeamais* and *P. truncatus*

Summary of dispersal generalized linear model statistical output based on quasipoisson distributions for number of insects dispersing to each zone for *S. zeamais* and *P. truncatus* in dispersal apparatus.

Variable	<i>P. truncatus</i>			<i>S. zeamais</i>		
	df	χ^2	<i>P</i>	df	χ^2	<i>P</i>
position	3	111	0.0001	3	246	0.0001
treatment	2	87.4	0.0001	2	0.64	0.72
distance	1	16.7	0.0001	1	0.02	0.89
exposure	1	0.95	0.33	1	0.37	0.54
position:treatment	6	133	0.0001	6	11.4	0.08
position:distance	3	35.1	0.0001	3	2.27	0.52
treatment:distance	2	6.47	0.04	2	0.31	0.86
position:exposure	3	6.80	0.08	3	7.77	0.051
treatment:exposure	2	0.91	0.63	2	0.11	0.95
distance:exposure	1	0.73	0.39	1	0.01	0.91
position:treatment:distance	6	14.7	0.02	6	5.42	0.49
position:treatment:exposure	6	5.22	0.52	6	8.41	0.21
position:distance:exposure	3	5.62	0.13	3	2.45	0.48
treatment:distance:exposure	2	0.98	0.61	2	1.07	0.59
position:treatment:distance:exposure	6	8.07	0.23	6	5.10	0.53

Figure 4-1 Percentage of *P. truncatus* dispersing by zone after 4 or 24 h

Percentage of *P. truncatus* adults dispersing to each zone in a dispersal apparatus after exposure for 4 h (top panels) or 24 h (bottom panels) to either the H₂O control (green), Diacon IGR+ (deltamethrin + methoprene; purple), or Gravista (deltamethrin + methoprene + piperonyl butoxide; orange) over 30 (left column) or 70 cm (right column) distances. Points indicate outliers. Zone notation corresponds to the following locations: Zone 1 – release point, Zone 2 – 1st half of dispersal apparatus, Zone 3 – 2nd half of dispersal apparatus, and Zone 4 – pitfall collection jar with maize.

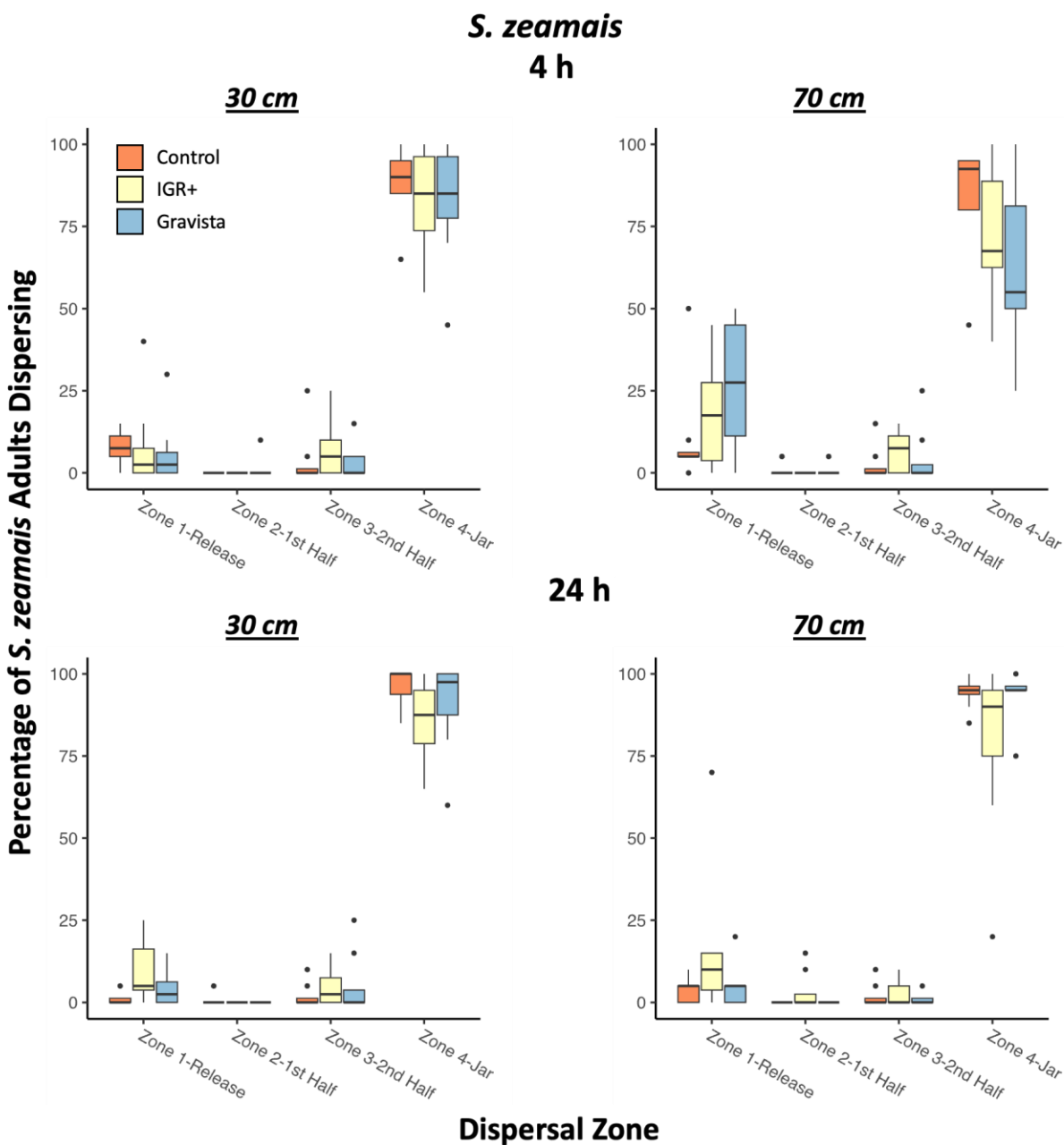


After a 24 h exposure period to the control, 28–69% of *P. truncatus* adults dispersed to Zone 4 (e.g., pitfall jar with novel food source) at 70 or 30 cm length dispersal apparatus, respectively (Figure 4.1). After exposure to Gravista or Diacon IGR+, 84–100% of *P. truncatus* adults remained in Zone 1 (e.g., the release zone) after 24 h regardless of dispersal apparatus length (Figure 4.1, Table 4.1). There were a few individuals exposed to the insecticide that were able to make it to the commodity in Zone 4 at the 30-cm length, but not the 70-cm length. After 24 h exposure, 99–100% of *P. truncatus* adults remained in Zone 1 for the 70 cm dispersal.

In contrast to *P. truncatus*, *S. zeamais* was much less affected and much more mobile. None of the main effects for the variables significantly affected dispersal of *S. zeamais*, except there were significantly different percentages of adults by Zone (Figure 4.2, Table 4.1). There were 84–99% fewer adult *S. zeamais* in Zones 1–3 after 4 h than in Zone 4 (e.g., the collection jar with commodity) regardless of insecticide exposure. Similarly, after 24 h, there were 93–99% fewer adult *S. zeamais* in Zones 1–3 than in Zone 4 (e.g., the collection jar with commodity) regardless of insecticide exposure. There were no significant 2-way, 3-way, or 4-way interactions among the variables in the model on dispersal of *S. zeamais* adults (Table 4.1). For the 30 and 70 cm length dispersal apparatus, 82–83% and 63–73% of the *S. zeamais* adults, respectively, exposed to either formulation reached the Zone 4 commodity after 4 h (Figure 4.2). After 24 h, 86–91% and 79–94% of *S. zeamais* adults exposed to insecticide made it to the commodity in Zone 4 at 30 and 70 cm lengths (Figure 4.2).

Figure 4-2 Percentage of *S. zeamais* dispersing by zone after 4 or 24 h

Percentage of *S. zeamais* adults dispersing to each zone in a dispersal apparatus after exposure for 4 h (top panels) or 24 h (bottom panels) to either the H₂O control (green), Diacon IGR+ (deltamethrin + methoprene; purple), or Gravista (deltamethrin + methoprene + piperonyl butoxide; orange) over 30 (left column) or 70 cm (right column) distances. Points indicate outliers. Zone notation corresponds to the following locations: Zone 1 – release point, Zone 2 – 1st half of dispersal apparatus, Zone 3 – 2nd half of dispersal apparatus, and Zone 4 – pitfall collection jar with maize.



Mortality

Exposure to the control or one of the two formulations significantly affected mortality of *P. truncatus* adults before and after the dispersal experiment (Figure 4.3, Table 4.2). The percentage of *P. truncatus* in the control that remained alive was 100% regardless of dispersal distance or whether assessments occurred before or after dispersal. By contrast, after exposure to either formulation (Diacon IGR+ or Gravista) but before dispersal, *P. truncatus* adult condition was 100% affected, while after dispersal (e.g., 48 h later) adults were 94–100% affected (Figure 4.3). For *P. truncatus*, while there was a statistically significant difference in mortality between the two dispersal distances (30 and 70 cm) after the experiment, it is likely not biologically relevant (63% affected and dead at 30 cm compared to 66% at 70 cm). We observed most of the individuals affected after exposure (70% and greater), with the remaining (~30%) alive, with no dead individuals (Figure 4.3), which did not change with the conclusion of the experiment.

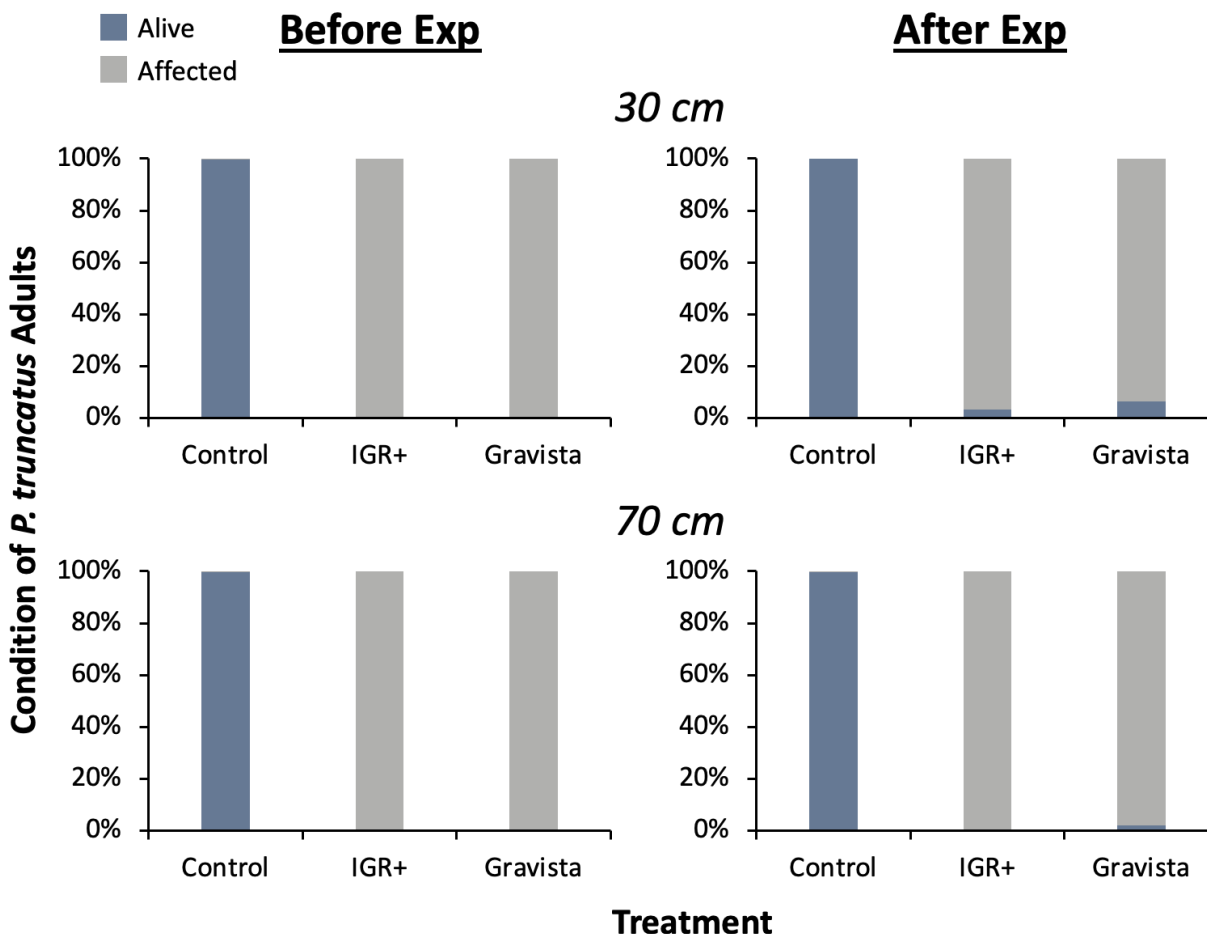
Table 4-2 Summary of mortality statistical output for dispersal of *S. zeamais* and *P. truncatus*

Summary of mortality statistical output from generalized linear models based on binomial distributions for measures taken before and after the dispersal experiment for *S. zeamais* and *P. truncatus*.

Variable	Mortality Before Experiment			Mortality After Experiment		
	df	χ^2	P	df	χ^2	P
<i>P. truncatus</i>						
treatment	2	2417	0.0001	2	2177	0.0001
distance	1	0	1	1	19.1	0.0001
exposure	1	2.78	0.09	1	0.03	0.87
treatment:distance	2	0	1	2	4.20	0.12
treatment:exposure	2	0	1	2	5.86	0.053
distance:exposure	1	0	1	1	7.99	0.01
treatment:distance:exposure	2	0	1	2	0	1
<i>Sitophilus zeamais</i>						
treatment	2	1701	0.0001	2	66.0	0.0001
distance	1	0.28	0.59	1	0.98	0.32
exposure	1	9.05	0.01	1	3.92	0.048
treatment:distance	2	0.03	0.98	2	1.68	0.43
treatment:exposure	2	10.7	0.01	2	1.20	0.55
distance:exposure	1	0.14	0.71	1	0.80	0.37
treatment:distance:exposure	2	0.44	0.80	2	5.83	0.054

Figure 4-3 Percent condition of *P. truncatus* after exposure to Gravista and Diacon IGR+ before and after dispersal

Percent condition of cohorts of 20 adult *P. truncatus* after treatment with H₂O (control), Diacon IGR+ (deltamethrin + methoprene), or Gravista (deltamethrin + methoprene + piperonyl butoxide) but before the addition of insects into the dispersal apparatus (left column) or at the conclusion of the experiment 48 h later (right column) in apparatuses that are 30 (top panels) or 70 cm long (bottom panels). Adult condition was rated individually as alive (blue) or affected (grey) under a stereomicroscope at 60×. *Prostephanus truncatus* did not recover by the end of the experiment. There were no dead individuals.

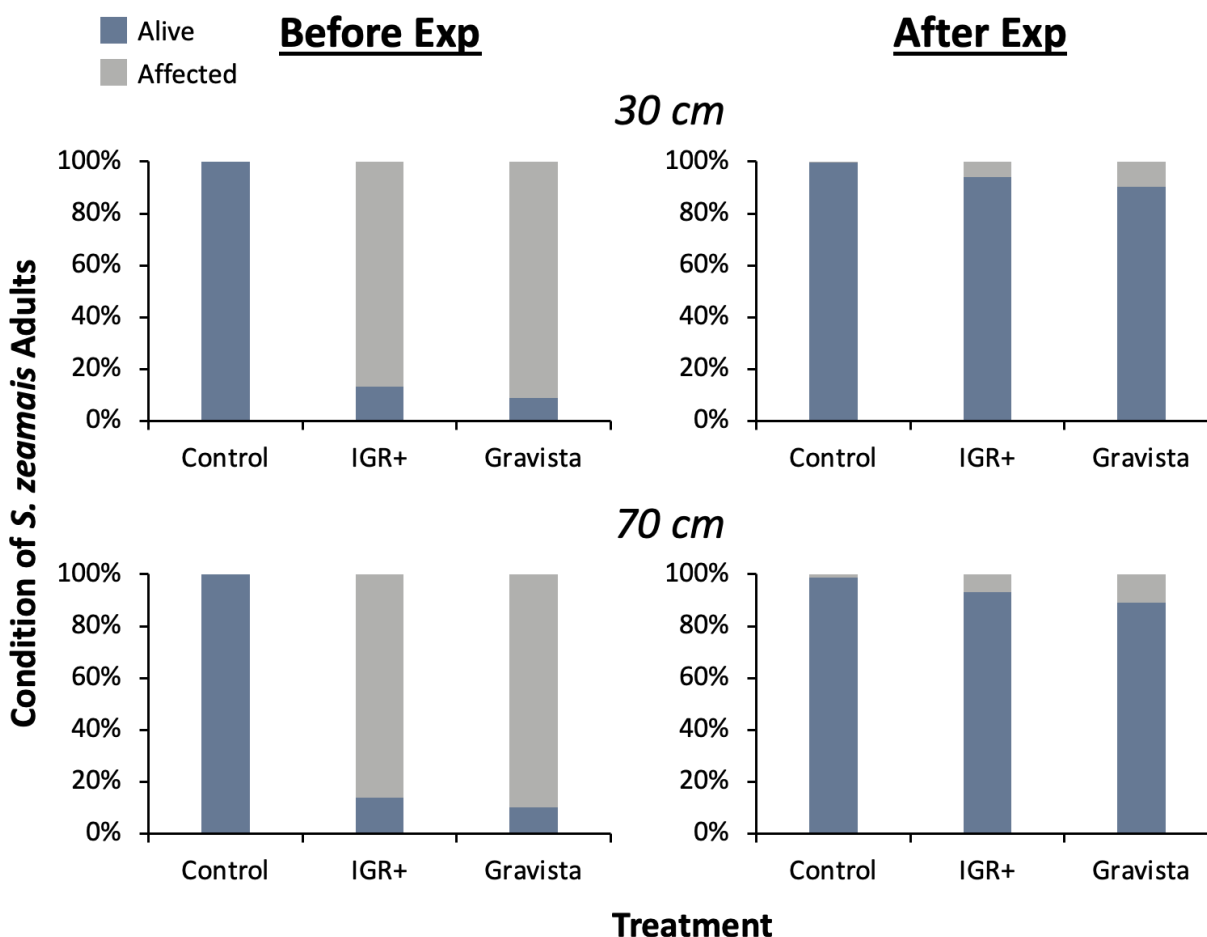


Exposure to either formulation (Diacon IGR+ or Gravista) significantly changed adult *S. zeamais* condition from 100% alive in the controls to 86–91% affected in the insecticide treatments (Figure 4.4, Table 4.2). For *S. zeamais*, there was no significant difference in mortality between the two dispersal distances (30 and 70 cm), which ranged from 58–59% affected and 40–41% alive. There was significant recovery by adult *S. zeamais* by the end of the

dispersal experiment 48 h later, even for insecticide-exposed individuals with only 5–10% of adults affected and the remainder 89–94% alive (Figure 4.4, Table 4.2).

Figure 4-4 Percent condition of *S. zeamais* after exposure to Gravista and Diacon IGR+ before and after dispersal

Percent condition of cohorts of 20 adult *S. zeamais* after treatment with H₂O (control), Diacon IGR+ (deltamethrin + methoprene), or Gravista (deltamethrin + methoprene + piperonyl butoxide) but before the addition of insects into the dispersal apparatus (left column) or at the conclusion of the experiment 48 h later (right column) in apparatuses that are 30 (top panels) or 70 cm long (bottom panels). Adult condition was rated individually as alive (blue) or affected (grey) under a stereomicroscope at 60×. *Sitophilus zeamais* mostly recovered by the end of the experiment. There were no dead individuals.



Discussion

Overall, we have shown the importance of evaluating the behavioral effects of insecticides to provide a comprehensive picture of insecticide efficacy. Our assay here was of longer duration and longer distances than prior work which utilized EthoVision insect tracking software in smaller petri dish arenas over shorter time periods (Quellhorst et al., 2022). In this study we have shown that these two insecticide formulations, Gravista and Diacon IGR+ are more effective at preventing dispersal of *P. truncatus* than *S. zeamais*. It is important to highlight that the newer formulation Gravista, is just as effective as the older formulation Diacon IGR+, when looking at movement, dispersal, and mortality. This was also shown in the prior study (Quellhorst et al., 2022).

We found that both formulations reduced dispersal by *P. truncatus* but not *S. zeamais*. This could be due to the fact that weevils are not as susceptible to pyrethroids such as deltamethrin (Golob et al., 2002; Golob et al., 1985; Dales and Golob, 1997), which is one of the main active ingredients targeting adults in both Diacon IGR+ and Gravista. It has commonly been found that for *S. zeamais*, organophosphates are more effective compared to pyrethroids and the opposite is generally true for *P. truncatus* (Golob et al., 2002; Golob et al., 1985; Dales and Golob, 1997). Despite this, grain protectants such as pyrethroids continue to be used for protecting maize.

We found that there was significant recovery after exposure to both formulations for *S. zeamais*, while *P. truncatus* remained largely affected and immobilized after dispersal. Recovery by *S. zeamais* and subsequently higher baseline mobility, which is ≈ 3.6 -fold higher than *P. truncatus* (Quellhorst et al., 2022), probably contributed to differences in the results between the two species. There are many studies that document insect recovery after knockdown from a

pyrethroid, but an important one to highlight is prior work which found that insects could recover with access to an uncontaminated food source within close proximity of exposure (Arthur, 2000; Arthur, 2013). Recovery has also been reported for several species of stored products insects when exposed to spinosad and allowed to recover with a food source for 24 h (Toews et al., 2003). Finally, Morrison et al. (2019) found that distance moved (e.g., recovery) after exposure to a green fumigant (e.g., methyl benzoate) was greater in the presence of food than in its absence.

In prior work on Gravista and Diacon IGR+, *Sitophilus* spp. weevils were found to be relatively less susceptible compared to other stored product insects (Arthur et al., 2020; Morrison et al., 2021; Arthur and Morrison, 2020). On aged residues in commodities, Gravista and Diacon IGR+ generally lost their ability to suppress the movement of *S. oryzae* and *S. zeamais* 3–6 months after application as a grain protectant. Likewise, *S. oryzae* was found to be the least susceptible stored product species among three tested at different rates and exposure times (Arthur et al., 2020). Lastly, Quellhorst et al. (2022) found that distance moved by *P. truncatus* and *S. zeamais* was reduced by ≈ 3.7 -4.4-fold after exposure to Gravista and Diacon IGR+ compared to controls in small Petri dishes. In this study, we found that *S. zeamais* was the less susceptible species, and that over longer periods, and in the presence of food, there was substantial recovery by *S. zeamais* compared to *P. truncatus*.

Overall, Gravista was able to provide the equivalent control for *P. truncatus* and *S. zeamais* as the older formulation Diacon IGR+ while using 4 times less active ingredient (deltamethrin and s-methoprene), resulting in a more cost-effective formulation due to the addition of a synergist. In the case of *P. truncatus*, this control appears sufficient, while it does not appear sufficient for *S. zeamais*. Despite the lack of effectiveness in reducing dispersal for *S.*

zeamais, Gravista was able to significantly reduce dispersal of *P. truncatus* after exposure to prevent the insects from reaching the novel food patch and thus preventing damage to grain. Future work should assess these formulations and their ability effects on flight capacity. It would also be important to understand how the new formulation, Gravista, compares to the older formulation when dealing with stored product insect strains that are resistant to pyrethroids.

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**Chapter 5 - Temperature-mediated competition between the
invasive larger grain borer, *Prostephanus truncatus* (Coleoptera:
Bostrichidae) and the cosmopolitan maize weevil, *Sitophilus zeamais*
(Coleoptera: Curculionidae)**

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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

Introduction

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 ferrugineus* (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 postharvest 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 oryzae* (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 $27 \pm 0.1^{\circ}\text{C}$ (mean $\pm$ SE), $64 \pm 1\%$ RH, and 14:10 (L:D) h

photoperiod for *S. zeamais* and at $27.2 \pm 0.1^{\circ}\text{C}$, $37 \pm 1\%$ 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 $25\text{--}35^{\circ}\text{C}$ (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 ($38 \times 26.5 \times 14.5$ 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 $135\times$ 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 μ l of extracted DNA, 1 μ l of each primer (10 μ M), 9.5 μ l

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

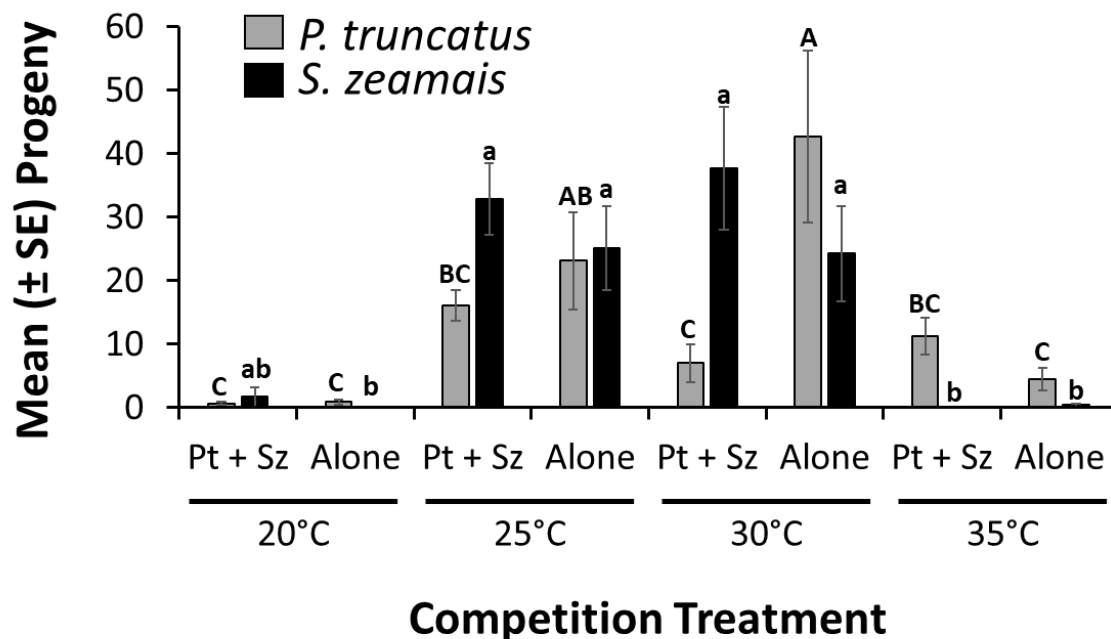
The species present in the container had a significant effect on the overall number of progeny produced (MANOVA: Wilks Approx. $F = 94.1$; $df = 2, 100$; $P < 0.0001$). For the combined species mixtures and *P. truncatus* alone, there were 30–31% more progeny produced, respectively, compared with *S. 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 at 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. 5.1). 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. 5.1, Tukey HSD).

Figure 5-1 The mean (SE) progeny produced after 65 d by *P. truncatus* and *S. zeamais* at different temperatures

The mean (SE) progeny produced after 65 d by combined species mixtures (“Pt + Sz”) of *S. zeamais* (black bars) and *P. truncatus* (grey 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, $\alpha = 0.05$).



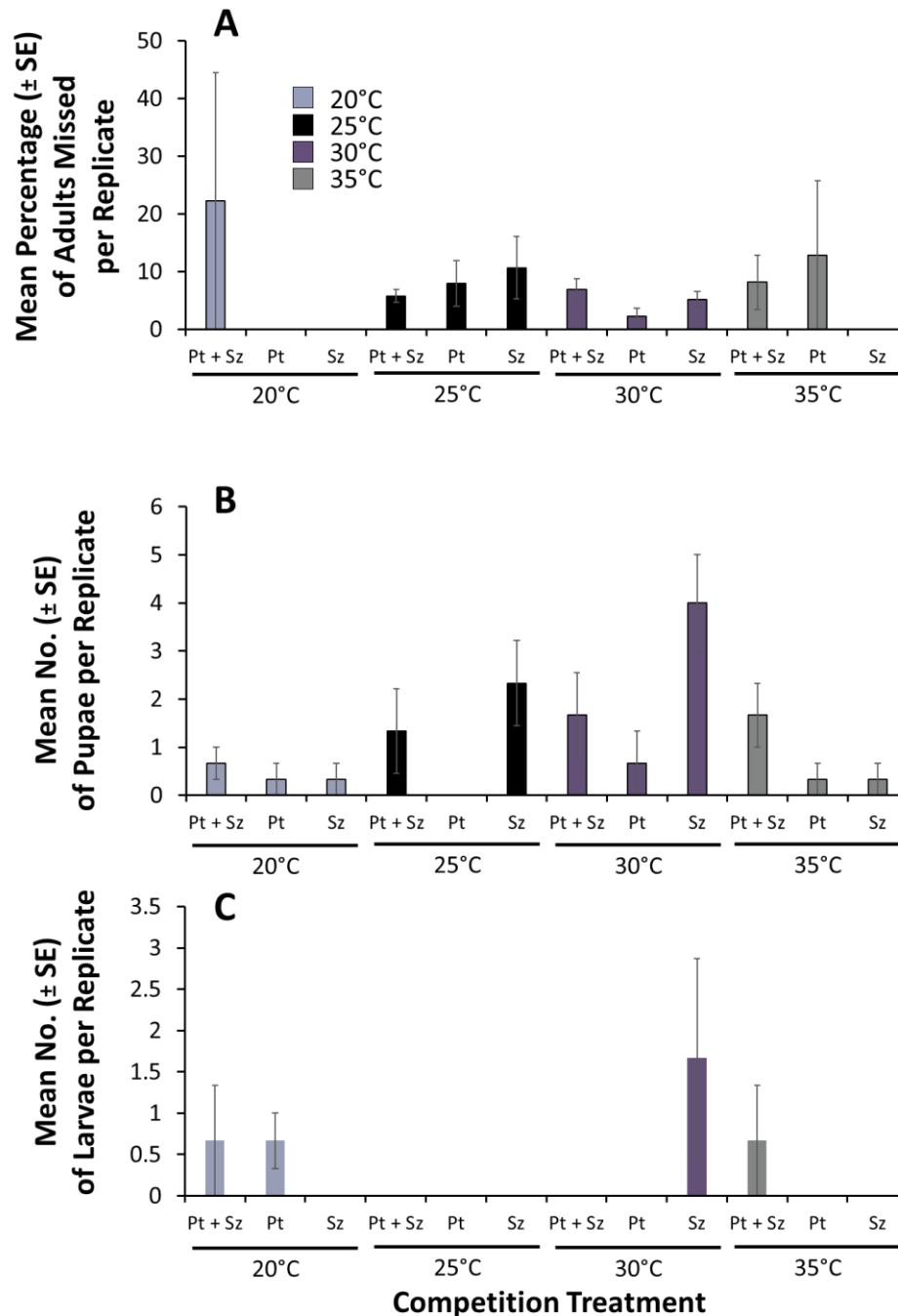
In the same vein, the number of *S. zeamais* progeny produced also varied, depending on whether it was alone or combined with *P. truncatus* ($F = 249$; $df = 2, 100$; $P < 0.0001$; Fig. 5.1). For instance, when *S. zeamais* was alone, 31% more progeny were produced at the end of 65 d than when it was 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. 5.1, 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. 5.2) 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. 5.2A), 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. 5.2A).

Figure 5-2 Confirmation of life stage abundance through the use of soft X-rays in the laboratory after 65 d

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, $\alpha = 0.05$).



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

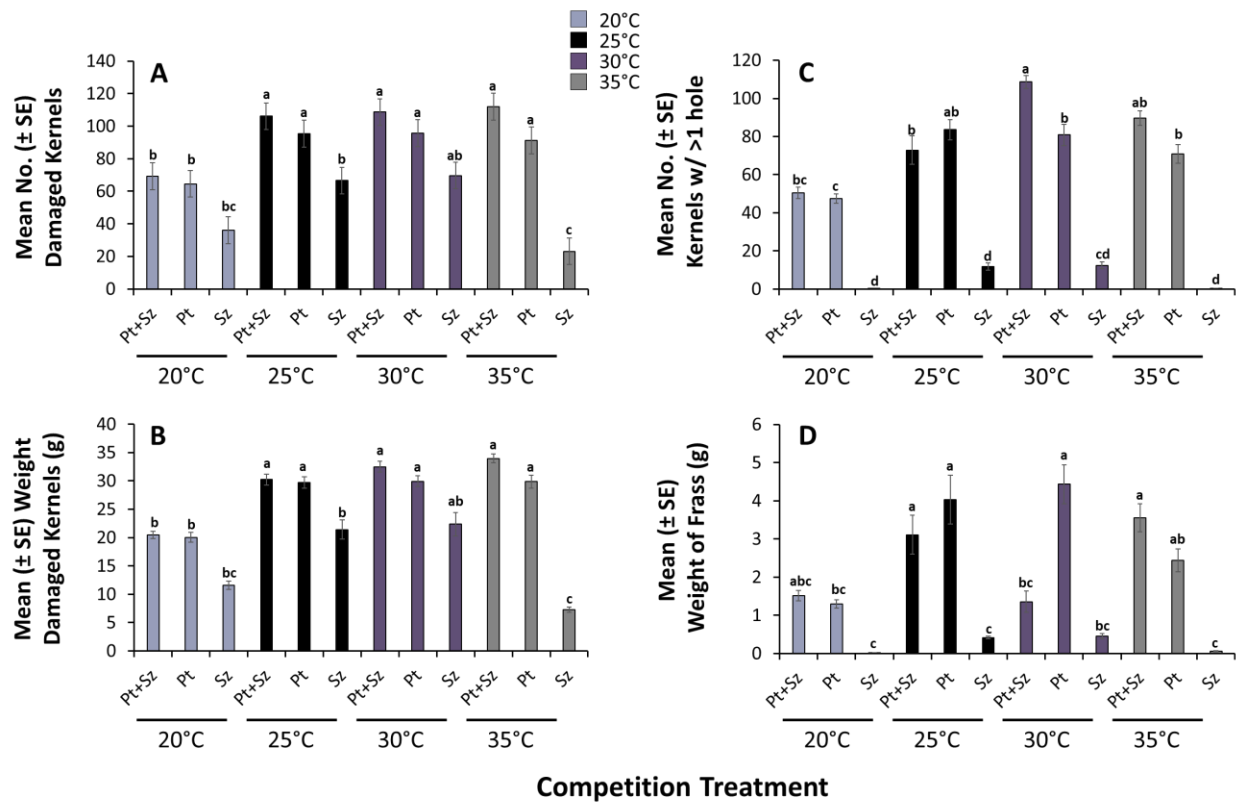
Prior to the beginning of the trials, there was an average of 23 ± 6 kernels with defects (insect or otherwise) amounting to an average weight of 5.6 ± 1 g, with zero kernels containing more than one defect, and no frass present. Importantly, grain damage measures were significantly affected by the interspecific 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. 5.3A; Supp Fig. 1A). 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.

Figure 5-3 The effect of *S. zeamais* and *P. truncatus* on maize grain quality at different temperatures

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 (grey bars) after 65 d, with particular reference to A) the mean number of kernels with any signs of insect damage, 2) 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, $\alpha = 0.05$).



There were similar results for the weight of damaged kernels (Fig. 5.3B; Supp Fig. 1B). 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. 5.3B; Supp Fig. 1B). 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. 5.3C; Supp Fig. 1C). 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 $\times$ 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. 5.3C, Tukey HSD; Supp. Fig. 1C), while at lower temperatures (20–25°C) there were fewer or the same number of kernels.

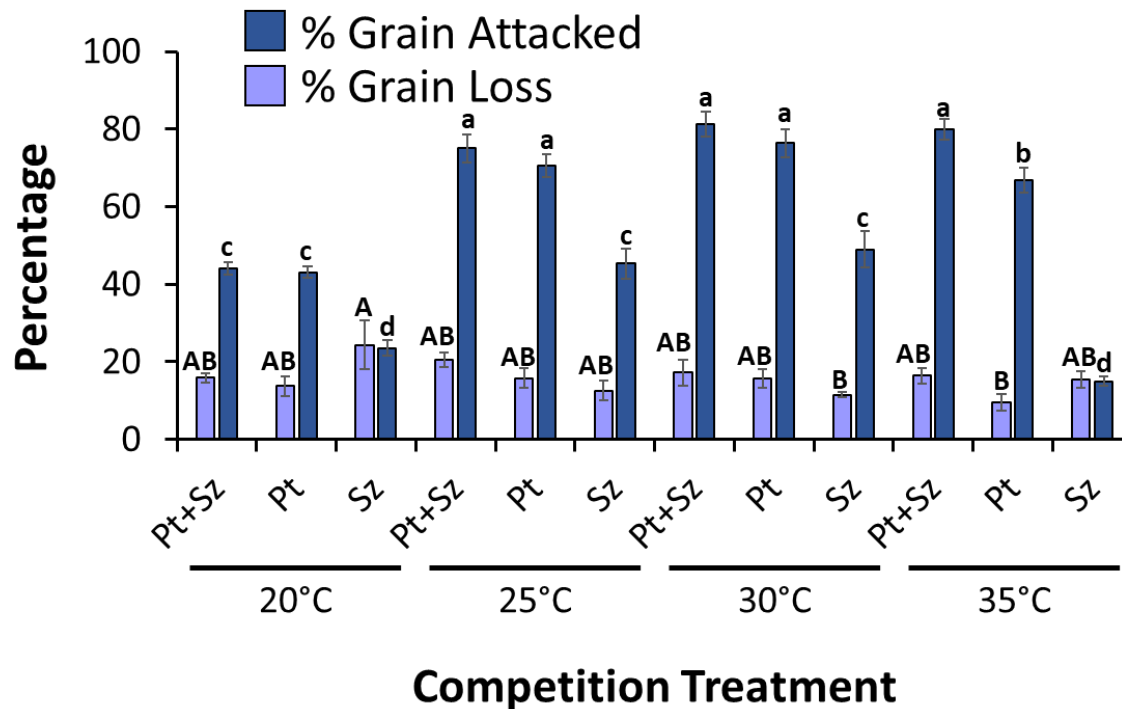
The weight of frass was also significantly affected by the species present ($F = 23.1$; $df = 2, 100$; $P < 0.0001$; Fig. 5.3D; Supp Fig. 1D). For instance, there was 9- to 13-fold more frass in the colonies containing *P. truncatus* alone and combined species mixtures, respectively, compared to when *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. 5.3D, Tukey HSD; Supp Fig. 1D).

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. 5.4), with *P. truncatus* attacking much more of the grain, especially at temperatures above 20°C.

Figure 5-4 The effect of *S. zeamais* and *P. truncatus* on percentage of grain attacked and grain loss at different

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 (grey bars) on A) percentage of grain attacked in each competition treatment, and B) percentage grain loss in each treatment after 65 d. Case of letters denotes comparisons within a response variable. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).



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, $\alpha = 0.05$).

Finally, the percentage grain loss did not differ by species present in colonies ($F = 2.70$; $df = 2, 100$; $P = 0.07$; Fig. 5.4) 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.

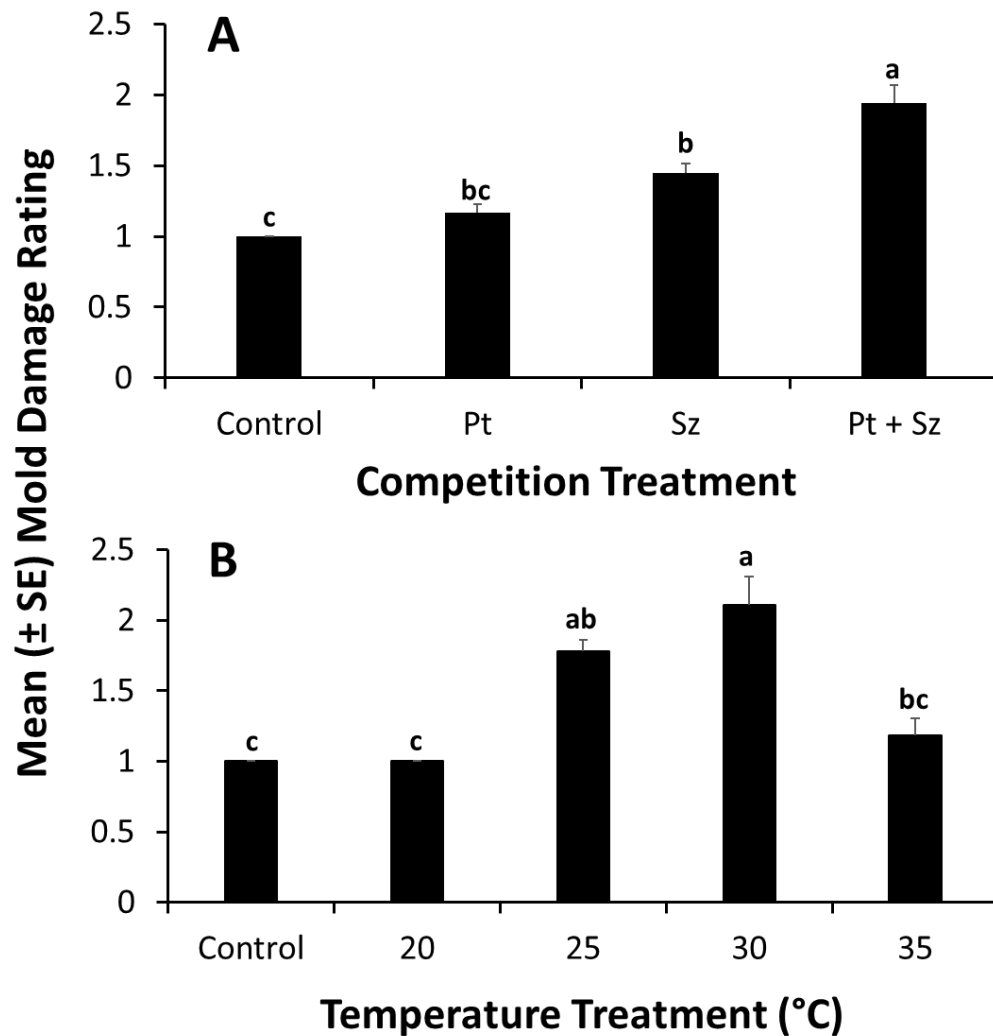
Mold Identity and Damage

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*. 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. 5.5A). 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. 5.5B, Tukey HSD).

Figure 5-5 The average effect of *S. zeamais* and *P. truncatus* by varying temperature on mold growth in maize over 65 d

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).



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). 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 F₁ 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.

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Chapter 6 - Short-term spatial dispersion patterns between the larger grain borer and the maize weevil in grain columns with implications for management of stored maize

Submitted as: Hannah Quellhorst^{1*}, Tania N. Kim¹, Kun Yan Zhu¹, and William R.

Morrison III². Short-term spatial dispersion patterns between the larger grain borer and the maize weevil in grain columns with implications for management of stored maize. *Ecological Entomology* 2023. Submitted.

Abstract

The invasive larger grain borer (*Prostephanus truncatus*), and the maize weevil (*Sitophilus zeamais*) co-occur in many regions of the world. While competition between these two species has been studied extensively, there is little information on spatial dispersion patterns in bulk storage of grain. To evaluate potential overlap in realized niche, we evaluated the short-term spatial dispersion behavior of *P. truncatus* and *S. zeamais* in vertical monolayers of maize (*Zea mays*) alone or together for 1 d compared to 7 d. We evaluated competition under three different densities, namely 10-20, 75-150, and 150-300 insects/kg for *P. truncatus* and *S. zeamais*. The monolayers were equally divided into 24 zones to track the location and the abundance of insects and their damage on maize. We found that both species generally aggregated together and were correlated to the same location. After 1 d, most of the insects for both species were near the top of the monolayer, but by 7 d, most individuals were in the bottom of the monolayers. In monolayers, when alone, *P. truncatus* created a clear path of destruction to the bottom of the monolayer, but when *S. zeamais* was present, damage was lessened and shifted upwards in the grain column. In an olfactometer assay, *P. truncatus* preferred maize odors, while *S. zeamais* exhibited no preference among maize, conspecifics, or heterospecifics. In evaluating relative volatile emissions, each of these treatments emitted unique volatile compounds but with significant overlap. These data may improve targeting of pest control tactics by identifying the position of these insects in the grain mass.

Key Words: *Prostephanus truncatus*, *Sitophilus zeamais*, competition, spatial dispersion

Introduction

Competition between insect species that share a common resource, such as a food or space, has the potential to impact the distribution and abundance of the community of insects (Diamond, 1975; Stone & Roberts, 1990; Taylor & Woiwod, 1975; Kunte, 2008). Interspecific competition is predicted to be stronger within feeding guilds compared to between different feeding guilds and stronger between morphologically similar species (Gilbert & Owen, 1990). Competition is also likely to be more apparent in species from tropical regions compared to temperate regions (Horgan & Fuentes 2005). A recent meta-analysis importantly found that competition in insects also increased with population density and niche overlap, using physical proximity and phylogenetic relatedness, which was used as a proxy (Bird et al., 2019).

Stored product insects represent a good model for evaluating interactions between species, such as interspecific competition and intraspecific competition (Gerken & Morrison, 2023; Park 1948, 1954, 1957; Park and Mertz, 1964; Park et al., 1970). The postharvest system has advantages for these types of studies due to the fact that commodities are often stored separately and the storage environment can be controlled and or replicated at smaller scales. For any one commodity like maize, there are multiple species that use the commodity as a food source. In addition, the basic biology of stored product insects is generally well-understood, including for the invasive larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) (Quellhorst et al., 2021), red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Campbell et al., 2022), and grain weevils, *Sitophilus* spp. (Coleoptera: Curculionidae) (Longstaff, 1981). For example, *T. castaneum* emits both aggregation and anti-aggregation pheromones, depending on density (Duehl et al., 2011), and

females are able to assess both current and future competition during oviposition (Halliday et al., 2019).

Prostephanus truncatus is endemic to southern North America and Central America, but invaded Africa with severe economic consequences for maize and cassava production (Golob & Hodges 1982). Its main competitor is the more cosmopolitan maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae)(Quellhorst et al 2021). These insects co-occur in many regions of the world and are both threats to maize production. Interspecific competition between *P. truncatus* and *S. zeamais* has been studied for several decades. Most competition studies have come to the same general conclusion that *P. truncatus* performs better (higher population growth) and may outcompete *S. zeamais* at higher temperatures (30–35°C), while *S. zeamais* performs better at cooler temperatures (20–25°C) (Giga & Canhao, 1993; Quellhorst et al., 2020). Founder effects were determined to be important in prior work in the competition between *P. truncatus* and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), because when *P. truncatus* was present 7 d prior to *S. oryzae*, *P. truncatus* was able to outcompete the other species (Baliota et al., 2022). It has been suggested that the presence of *S. zeamais* in grains suppresses activity by and repels *P. truncatus* (Giga & Canhao, 1993). Prior work reported that *S. zeamais* was attracted to kernels of corn infested by *P. truncatus*, while *P. truncatus* avoided *S. zeamais*-infested kernels. This was confirmed by an observation in the field, where kernels infested by *S. zeamais* had no *P. truncatus* (Ngom et al 2020). This could be attributed to the ability of *S. zeamais* larvae to cannibalize other larvae of their own species, as well as those of other species, when inside the same kernel of grain.

However, the majority of the prior work has focused on competition experiments with small arena sizes (small vials filled with < 80 g of grain) confined to the laboratory. Generally,

the two species are placed together in a vial filled with a small portion of grain, are allowed to colonize for 60–90 d, and then the damage and progeny production is assessed (Giga & Canhao, 1993; Sakka & Athanassiou, 2018; Quellhorst et al., 2020). It is unknown whether *P. truncatus* and *S. zeamais* aggregate in a larger column of grain. In the field, there is little if any information on spatial partitioning of either species in bulk storages of grain. However, there have been studies on other stored product insect pests in spatial distribution and movement inside grain storage bins, other storage facilities, and grain processing facilities (Abdelghany & Fields 2017; Arbogast et al., 1998; Cordeiro et al., 2016, 2019; Jian et al., 2006, 2009; Arbogast et al., 2000; Danso et al., 2019).

As insects move in a grain mass, some cues may be more important than others. Jian et al. (2006, 2009) suggests that stored product insect pests do not exhibit geotactic behavior and suggest that drifting of adult insects may simply cause downward movement. While the insects may move randomly, they are moving towards an optimal area induced by temperature, humidity, food, or mates (Jian et al., 2009). Also, movement or niche-finding within a grain mass may be dependent on the type of feeder the insect is: secondary versus primary pests. Secondary pests will be looking for dockage, broken grains, and flour. By contrast, primary pests will be attacking any whole kernels. Finer sediments/flours and broken pieces may be found towards the bottom of the grain mass, along the edges, or even on the top if the grain has been sifted and the smaller/lighter particles land on top.

Semiochemicals may also play a role in movement by insects in a grain mass. For example, some of the commodity in a grain mass may be hotspots for the production for microbial volatiles (Ponce et al., 2021), which may affect the behavior of stored product insects generally (Morrison et al., 2021). Specifically, *S. zeamais* was repelled by individual microbial

volatiles in 74% of cases (Ponce et al., 2021). Moreover, insect infestation may present reliably detectable cues emitted by insects, consisting of aggregation or sex pheromones and other cues (Maille et al., 2023). *Prostephanus truncatus* produces a two-component male-produced aggregation pheromone, consisting of (2*E*)-2-methyl-2-pentenoate and 1-methylethyl- (*E, E*)-2,4-dimethyl-2,4-heptadienoate (Hodges et al., 1984; Dendy et al., 1989; Hodges, 1998). Similarly, male *S. zeamais* produce an aggregation pheromone consisting of (*R, S*)-5-hydroxy-4-methyl-3-heptanone (Walgenbach et al., 1986). When these species inhabit the same grain column, these semiochemicals may be diffusing simultaneously and modifying the behavior and movement of both *P. truncatus* and *S. zeamais*. For example, research on *T. confusum* found conspecifics were able to distinguish among the volatile cues emitted by other secondary pests, and these consisted of emissions of unique compounds (Giunti et al., 2018). Thus, detection of the heterospecific pheromone by, say, one species may provide impetus to partition a grain mass to minimize niche overlap.

Therefore, the aims of this study were to (i) investigate the short-term spatial dispersion patterns of *P. truncatus* and *S. zeamais* in a monolayer column of grain (ii) determine if population density (10, 75, or 150), species community treatment (single or mixed) and time in the mass (1 or 7 d) had an effect on the spatial distribution, abundance of, and damage by species and (iii) investigate whether volatiles provide a mechanism for spatial dispersion patterns by *P. truncatus* and *S. zeamais* using 4-way olfactometer and headspace volatile collection experiments. We hypothesized that when *P. truncatus* and *S. zeamais* are sharing resources, they would partition the grain column vertically, this would be more pronounced after longer periods, and be mediated by volatile stimuli.

Methods and Materials

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 insect species were reared on whole kernels of maize and held in incubators under constant conditions at $27 \pm 0.1^{\circ}\text{C}$ (mean $\pm$ SE), $64 \pm 1\%$ RH, and 14:10 (L:D) h photoperiod for *S. zeamais* and at $27.2 \pm 0.1^{\circ}\text{C}$, $37 \pm 1\%$ RH, and continuous darkness for *P. truncatus* as mandated by the applicable USDA-APHIS PPQ-approved permit (P526-191021-020).

Monolayers

Untreated, clean, and uninfested yellow maize was used in the experiments. Grain was sourced from a local farmer in northeast Kansas, US and frozen at -20°C for 72 h prior to use to ensure no prior insect infestation was present. Monolayers were created with two sheets of glass cut to 75×27 cm (H:W). A spacer was then created using acrylic to form a barrier at the edges of the monolayer and to create a sufficient gap for a single kernel layer of grain between the two sheets of glass. The sheets of glass were taped with packing tape to ensure all gaps were adequately sealed to prevent insects from escaping and then held firmly in place along the edges of the glass sheets by binder clips (5.08 cm wide with 1.59 cm capacity). Before assembly, uninfested maize was uniformly and tightly laid out on one of the sheets of glass before the other one was added with spacers. Each assembled monolayer, consisting of two sheets of glass and spacers, was inserted into a wooden frame block to hold it vertically upright ($7.5 \times 14 \times 38$ cm, H:W:L) with a gap in the wood just wide enough to accommodate the monolayer (~ 1.75 cm) and tightened with screws and wingnuts for foundational support. In addition, binder clips (similarly

sized to above) were used to firmly hold the monolayer together. Each sheet of glass was marked with 24 zones from top to bottom (8 rows and 3 columns of intersecting zones). Zones were numbered from 1 to 24 starting in the top left corner of the monolayer. Once the insects were placed into the central top zone, the top of the monolayer was sealed with wire mesh to prevent insects from escaping at the top while also allowing air exchange.

Treatments in Monolayer Assay

There were three insect densities and three species treatments. The three insect densities were 10, 75, or 150 insects of either species. The species treatments were either pure colonies of *P. truncatus* or *S. zeamais* or mixed species communities (both *P. truncatus* and *S. zeamais* together). The experimental design for this assay was a response surface model, which has shown utility in the past in evaluating intraspecific competition from interspecific competition (Inouye, 2001 and Kim et al., 2013). Insects were released simultaneously into the central top square of the monolayer and allowed to disperse for either one or seven days. Assays were run in an environmental chamber with constant temperature $27.5 \pm 0.1^{\circ}\text{C}$ and $65 \pm 0.3\%$ RH at 14:10 L:D photoperiod. After the time period, each monolayer was taken apart carefully and the grain and insects from each zone were placed into separate bags and frozen to stop insect damage, movement, and growth. There were three replicates for each treatment combination. After all the samples were collected and frozen, each zone was carefully assessed for the abundance of each species and weight of grain damage by each species.

Spatial Analysis of Monolayers

Kriging is an interpolation method that is based on the surrounding measured values and the statistical relationships among the measured points (Bivand et al., 2013). We utilized an exponential semivariogram model to estimate the value of points at unsampled locations. The

distance between points in the monolayer and the variation between points within each neighboring zone of the monolayer was used to estimate these values. Ordinary kriging was used where the mean is assumed to be constant in the local neighborhood of each estimation point. Interpolation was conducted to estimate the abundance of and grain damage caused by *S. zeamais* and *P. truncatus* at each density (10, 75, and 150), in single or mixed cultures, and after 1 or 7 d. This analysis was conducted in R Software using the *ksline* function of the *geoR* package and the *krige* command of the *gstat* package (Ribeiro & Diggle, 2001).

Spatial autocorrelation measures the similarity of a set of spatially distributed points and their associated values (Koenig, 1999; Legendre, 1993). Compared to randomly associated points, positively autocorrelated points are more similar and occur near one another while negatively autocorrelated points are less similar than expected. The Moran's I statistic has been used in ecological and agricultural studies to measure spatial autocorrelation (Hahn et al., 2017), and was used as a response variable to test for spatial autocorrelation of abundance of or damage by *S. zeamais* and *P. truncatus* in the monolayers. This test explains to what degree the occurrence of an event in space is likely to affect the occurrence of an event in a neighboring or nearby space. Moran's I varies on a scale of -1 to +1, with -1 indicating a high negative spatial autocorrelation, or a highly dispersed pattern (e.g., taken here as evidence for spatial niche partitioning), 0 indicating no spatial autocorrelation, or a random pattern, and +1 indicating high positive spatial autocorrelation, or a clustered pattern (e.g., lack of spatial niche partitioning). The Euclidean distances between the zones in the monolayers were used as an *a priori* weighted matrix to assess spatial autocorrelation. A Z-score and corresponding p-value were calculated to indicate whether or not the null hypothesis can be rejected and to conclude that the abundance or grain damage values for a species are randomly distributed across the monolayer. The function

dist from the base package *stats* and *Moran.I* from the *ape* package was implemented in R Software using $\alpha = 0.05$ (Paradis & Schliep 2019; R Core Team, 2020).

In order to evaluate whether the position of *S. zeamais* and *P. truncatus* in the monolayers was antipodal or similar, we correlated the occurrence of each species using Mantel tests. First, Bray-Curtis Dissimilarity indices were created between the mean abundance or damage values for *S. zeamais* or *P. truncatus* using the function *bcdist* from the *vegan* package (Oksanen et al., 2013). This was done separately for each density (10, 75, or 150), time interval (1 or 7 d), and community type (single or mixed). Then for each density, the distance matrix for *S. zeamais* was tested against *P. truncatus* using the values for either abundance or grain damage and using a Mantel test implemented with the function *mantel.rtest* using $n = 999$ permutations and the package *ade4* in R Software (Thioulouse et al., 2018). In the case of single species communities, *S. zeamais* was tested against *P. truncatus* under equivalent density and time interval to understand if the species move to the same position when they are without their competitor, compared to when they are with them in mixed communities.

Response Surface Analysis of Monolayers

In order to assess the contribution of *P. truncatus* or *S. zeamais* to total populations or damage in the monolayers, response surface regression was implemented with the *rsm* package in R. Densities of *P. truncatus* and *S. zeamais* were coded so that the computer read them as whole integers (e.g., -1, 0, 1) using the function *coded.data*. Both densities were used to predict either total abundance per zone or percent damage in zone using a second-order function. Diagnostic lack of fit was examined to ensure there were no significant deviations in the final model using the summary output function. Contour maps were generated to visualize results. Given a priori hypotheses, if *S. zeamais* densities were more important in determining outcome

of abundance or damage (e.g., competition), then contour lines would be horizontal, whereas they would be vertical if *P. truncatus* densities drives patterns, or diagonal if it is a mixture of both.

Olfactometer Experiment

To evaluate the possible mechanisms for spatial dispersion, we conducted an olfactometer experiment. A total of 385 g of clean grain was placed in mason jars and the three treatments from the monolayer experiments were replicated. For the first, 200 *P. truncatus* were placed into a jar, for the second, 200 *S. zeamais* were placed into a jar, and for the third 100 of each species were placed into the same jar. The jars remained untouched for two weeks. After two weeks the insects were sifted out (1.41×1.41 mm sieve placed under a 2×2 mm mesh sieve) and frozen. The grain treatments were then used in a 4-way, still-air olfactometer (described in Ponce et al. 2022). Briefly, the apparatus consisted of a central release chamber of glass ($12.1 \text{ cm} \times 9 \text{ cm}$ H:D) with the bottom etched by acid, to improve the footing of the insects, connected by four passageways ($3.5 \text{ cm} \times 2.5 \text{ cm}$ L:D) spaced at 90° angles to the odor chambers ($11.5 \text{ cm} \times 7 \text{ cm}$ H:D). Passageways ended in a porous glass barrier that allowed diffusion of volatiles but prevented entry by insects in the first round of testing, and which had two equally spaced small holes. The odor chamber and release chamber were connected with a glass tube ($7.5 \text{ cm} \times 2.2$ L:D) attached on both sides with a PTFE (polytetrafluoroethylene) screw cap and septum. A total of 10 g of each of the three treatments were placed into the olfactometer odor chambers, while one was left empty as a blank control. Individual insects were then placed in the center of the release chamber of the olfactometer and given 5 min to make a decision. A decision was recorded when the insect moved 2.54 cm up the passageway leading to one of the treatments. Individuals that did not respond within 5 min were classified as non-responders and excluded

from the final data analysis. The olfactometer was rotated by 90° after testing each insect to prevent any positional bias. After 10 replicate insects, the olfactometer was cleaned with odor-free detergent and water, followed by methanol and hexane, then completely allowed to dry before use. There were a total of $n = 200$ replicate individuals tested per insect species.

Volatile Collection

Headspace collection was performed to determine relative differences in emissions among competitive treatments between *S. zeamais* and *P. truncatus*. Volatiles were collected for 3 h from each sample held in autoclaved 250-mL media bottles (Corning Inc., Corning, NY, USA) with PTFE-lined septa, using 100 μm polydimethylsiloxane (PDMS) solid phase microextraction (SPME) fibers (Supelco, Bellefonte, PA, USA), onto which sample volatiles were absorbed from the following treatments: 20 g of maize infested with *P. truncatus* for two weeks, 20 g of maize infested with *S. zeamais* for two weeks, 20 g of maize infested with both species for two weeks, or an empty volatile collection container as a negative control. In each case, the volatiles from previously used SPME fiber was removed by heat desorption in the GC (Agilent 7890B gas chromatograph) inlet for 5 min at 250°C prior to collecting samples. Media bottles were laid on their side, but grain never came into contact with SPME fiber. Directly after collection of samples, volatiles were desorbed on the GC-MS as below. An external standard curve of tetradecane was created by adding dilutions of tetradecane with dichloromethane in 1 μL aliquots added to 250-mL media bottles with a 2- μL syringe (Hamilton, Reno, NV, USA) corresponding to 10, 75, 190.5, and 381 ng of tetradecane. Headspace volatiles were collected in triplicate as above using identical SPME-GC-MS procedures. Glassware was wash with soap and water, and subsequently a combination of methanol, then hexane and placed in the drying oven

(Heratherm, Thermofisher, Waltham, MA, USA). A total of $n = 8$ replicate collections were done for each species and treatment combination.

Gas Chromatography Coupled with Mass Spectrometry

All headspace samples were manually injected into the inlet at 250°C for 2.5 min and allowed to desorb from the SPME. Samples were run on an Agilent 7890B gas chromatograph (GC) equipped with an Agilent Durabond HP-5 column (30 m length, 0.250 mm diameter and 0.25 μm film thickness) with He as the carrier gas at a constant 5 mL/min flow and 39 cm/s velocity, which was coupled with an Agilent 5997B mass spectrometer (MS) single quadrupole detector. The compounds were run under splitless mode. The oven program began at 60°C for 1 min followed by 10°C/min ramping to 300°C over 26.5 min, and subsequently held for 4 min at 300°C. Preliminary identification of compounds from representative chromatograms were obtained by comparing sample spectral data with the NIST 17 library.

Statistical Analysis

To analyze the data from the four-way olfactometer, a generalized linear model based on a binomial distribution was used. The explanatory variable included the grain conditioning treatments. Overdispersion was checked and found not to be a problem. Upon a significant result from the model, multiple comparisons in a generalized linear model framework were implemented using the function *ghlt* (e.g., Tukey HSD) from the package *multcomp* in R Software (Hothorn et al., 2008). R software (R Core Team, 2019) was used for this and all other tests, with $\alpha = 0.05$ except where noted.

To characterize the presence of headspace volatiles from the extracts, raw peak areas, retention times, molecular masses, and match factors were extracted from the gas chromatograms using Unknowns Analysis v7.1.524 software (Agilent Technologies, Inc., Santa Clara, CA,

USA) into a single CSV file. The R package *uafR* was used to streamline chemical analysis by taking the outputted CSV file, spreading out compounds (e.g., *spreadOut*), aligning compounds, eliminating redundancy (e.g., *mzExacto*), and transforming into units of external standard (e.g., *standardifyIt*) with a series of functions that access underlying *m/z* information and pull from public databases like PubChem/NIST (Stratton et al., 2023). We used the function *categorate* to pull information about our compounds from five databases, including: LOTUS (natural products database), FEMA (Flavor and Extract Manufacturers Association), KEGG (Kyoto Encyclopedia of Genes and Genomes), FDA (Food and Drug Administration), and Reactive Groups from PubChem. All compounds were retained that exceeded a 75% match to the reference library, and which occurred in more than one sample. Because we were not interested in chemical identity in this study, only relative emissions among our treatments, we used a combination of ordination to visualize data and analysis of similarity of similarity (ANOSIM) to test for statistical significance. From the aligned table of compounds with emissions in ng per gram of grain weight and per hour basis pairwise Bray-Curtis similarities were calculated between each headspace sample, and non-metric multi- dimensional scaling (NMDS) was used to visualize the differences in volatile emissions among treatments. A total of $n = 1,000$ permutations were used for the ordination procedure. Stress values for the NMDS procedure were < 0.1 , indicating that good interpretation was possible.

An analysis of similarity (ANOSIM) was used to determine significant differences for headspace volatiles among odor treatment categories. ANOSIM calculates an R statistic that can vary from 1 to -1, with values above zero interpreted as greater dissimilarity between treatments than within and values below zero interpreted as greater dissimilarity within treatments than between treatments. A total of $n = 1,000$ permutations were performed for the test. For all

Multivariate statistics, the R Package *vegan* was used (Oksanen et al., 2013). To assess differences in the complexity of the headspace blends (e.g., number of compounds per treatment), pairwise chi-square tests were performed among treatments with the null hypothesis of an equal number of compounds identified from each treatment. To determine differences in overall quantitative emissions of volatiles, pairwise t-tests among means were performed using a Bonferroni-correction to the P-value.

Results

Abundance

Abundance for *P. truncatus* and *S. zeamais* after 1 day of dispersal in the monolayer resulted in individuals with a uniform distribution in the monolayer at the 10-insect density for the pure culture (e.g., only one species per monolayer) (Tables 6.1, 6.2 and Figure 6.1). As the density increased to 75 insects, *P. truncatus* and *S. zeamais* both inhabited the top and middle of the monolayer. At the 150-insect density, these patterns were more pronounced, with a greater abundance of individuals of both species at the top and middle of the monolayer. However, *P. truncatus* appeared to group more closely together and more densely in the top portion of the monolayer, while *S. zeamais* remained more evenly dispersed.

Table 6-1 Summary of calculated Moran's I statistics for spatial autocorrelation based on insect abundance in grain column monolayers in the laboratory.

Culture	Species	Day	Density	I	P
Pure	LAGB	1	10	0.02	0.04
Pure	LAGB	1	75	0.01	0.13
Pure	LAGB	1	150	0.06	0.007
Mixed	LAGB	1	10	0.12	0.0001
Mixed	LAGB	1	75	-0.01	0.43
Mixed	LAGB	1	150	0.03	0.047
Pure	MW	1	10	-0.08	0.35
Pure	MW	1	75	-0.05	0.94
Pure	MW	1	150	0.06	0.007

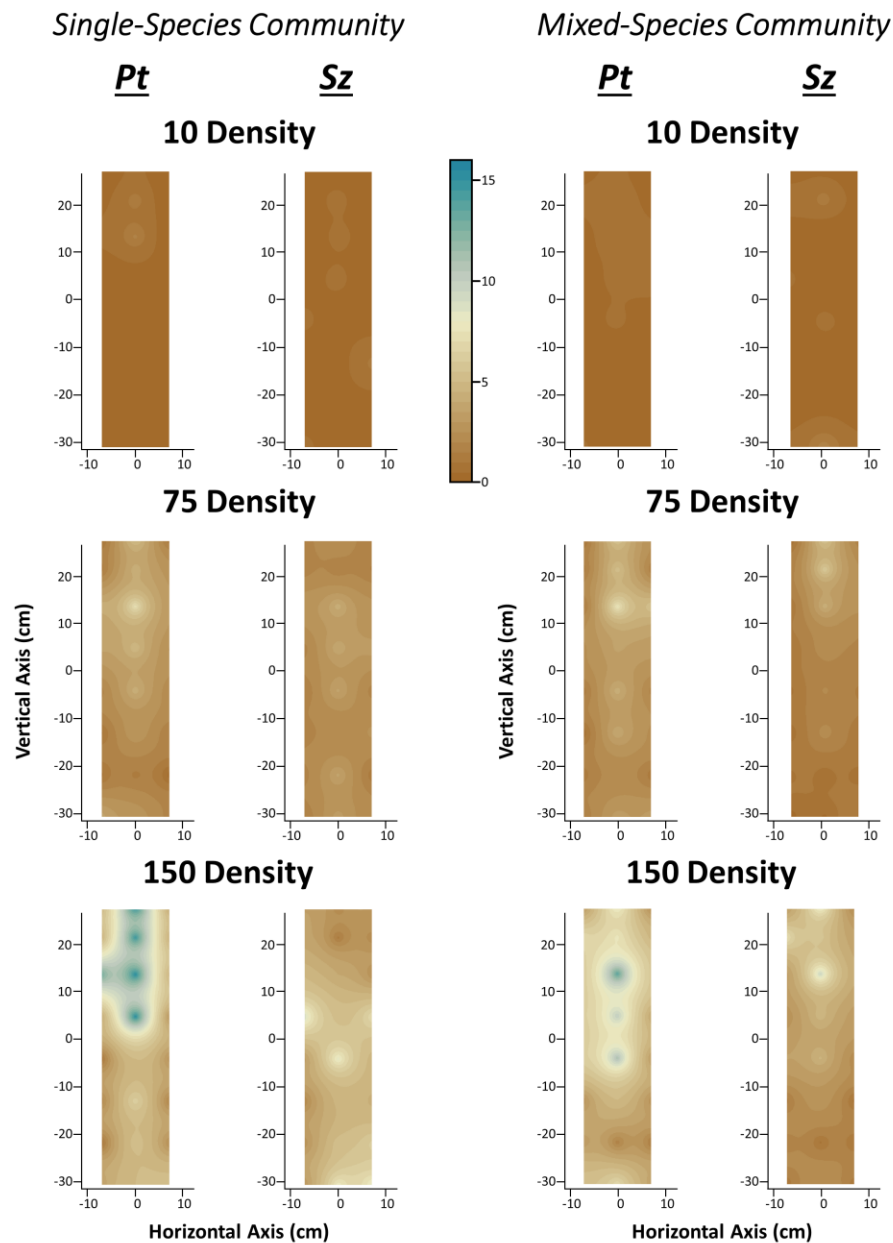
Mixed	MW	1	10	-0.04	0.99
Mixed	MW	1	75	0.10	0.0001
Mixed	MW	1	150	0.16	0.0001
Pure	LAGB	7	10	-0.04	0.80
Pure	LAGB	7	75	0.12	0.0001
Pure	LAGB	7	150	-0.04	0.0001
Mixed	LAGB	7	10	0.00	0.20
Mixed	LAGB	7	75	0.13	0.0001
Mixed	LAGB	7	150	0.13	0.0001
Pure	MW	7	10	0.05	0.01
Pure	MW	7	75	0.20	0.0001
Pure	MW	7	150	0.19	0.0001
Mixed	MW	7	10	0.11	0.0001
Mixed	MW	7	75	0.20	0.0001
Mixed	MW	7	150	0.27	0.0001

Table 6-2 Summary of Mantel test statistics correlation between the abundance of *P. truncatus* and *S. zeamais* in grain column monolayers in the laboratory.

Culture	Day	Density	r	Simulated P
Pure	1	10	0.01	0.34
Pure	1	75	0.19	0.02
Pure	1	150	-0.03	0.62
Mixed	1	10	-0.03	0.58
Mixed	1	75	0.38	0.004
Mixed	1	150	0.46	0.0001
Pure	7	10	0.05	0.16
Pure	7	75	0.19	0.02
Pure	7	150	0.09	0.11
Mixed	7	10	0.04	0.15
Mixed	7	75	0.05	0.21
Mixed	7	150	0.26	0.01

Figure 6-1 Spatially interpolated abundance of *P. truncatus* and *S. zeamais* at 1 d in pure cultures and mixed cultures in monolayers of maize

Spatially interpolated abundance of *P. truncatus* (Pt) and *S. zeamais* (Sz) at 1 d in pure cultures (left column) and mixed cultures (right column) on monolayers (75 × 27 cm, H:W) of maize when placed at a starting density of 10 (top row), 75 (middle row) , or 150 individuals (bottom row). Y and X axes in graphs correspond to cartesian coordinates on monolayers, and light to dark teal represents a greater abundance of individuals. Interpolation was performed on three experimental replicates using local kriging in R Software.

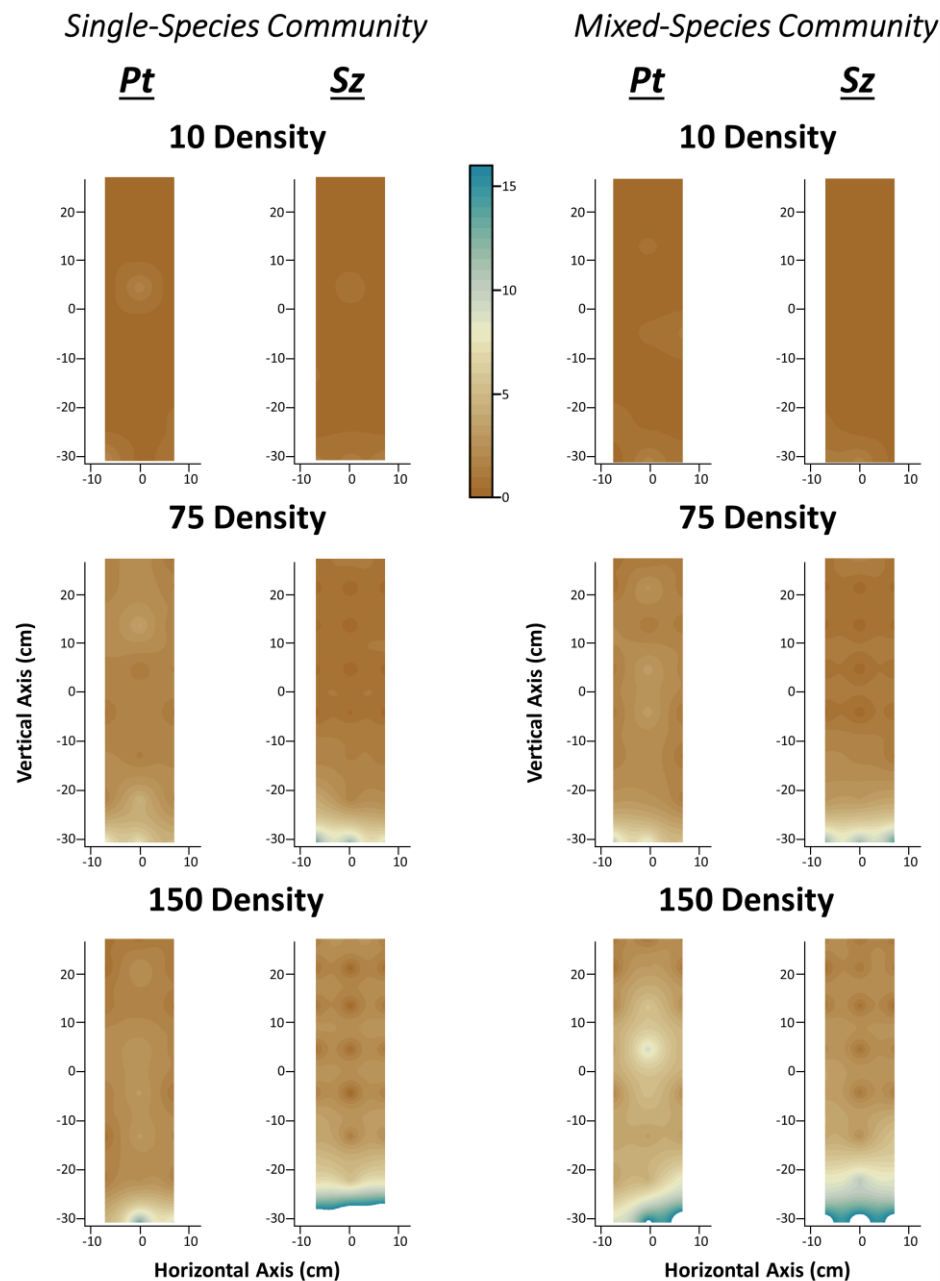


In mixed cultures (e.g., both species present in a single monolayer), there was surprisingly a similar pattern of distribution in the monolayers as in the pure cultures. Final abundance of *P. truncatus* and *S. zeamais* resulted in individuals that were relatively uniform in the monolayer of maize at the 10-insect density after dispersing for 1 day (Table 6.1, 6.2 and Figure 6.1). As the density increased to 75 insects in the mixed culture, *P. truncatus* and *S. zeamais* both aggregated in the top and middle areas of the monolayer (Figure 6.1). At the 75-insect density, the abundance of both species was correlated with each (Mantel Test, Table 6.2). However, it was apparent that *S. zeamais* grouped more closely with conspecifics and closer to the top, while *P. truncatus* was relatively more spread out. At the 150-insect density, the patterns present at the 75-insect density came into sharper relief, with a greater number of individuals of both species that were spread throughout the monolayer for *P. truncatus*, while *S. zeamais* primarily clustered with conspecifics in the top half of the monolayer. It appeared *P. truncatus* formed more dense clusters along the mid-line of the monolayer. Nonetheless, the population abundances of both species were strongly and positively correlated with each other (Mantel test, Table 6.2).

In pure cultures after 7 days, abundance of *P. truncatus* and *S. zeamais* in the monolayer after dispersal generally resulted in uniform distributions of individuals in the monolayer for the 10-insect density (Table 6.1, 6.2 and Figure 6.2). As the density increased to 75 insects, *P. truncatus* and *S. zeamais* were both primarily in the bottom areas of the monolayer. Their populations were independently correlated with each other at the 75-insect density (Table 6.2). At the 150 density, the majority of both species dispersed to the bottom of the monolayer (Figure 6.2). Both *P. truncatus* and *S. zeamais* grouped closely and densely at the midline of the bottom of the monolayer in the pure culture.

Figure 6-2 Spatially interpolated abundance of *P. truncatus* and *S. zeamais* at 7 d in pure cultures and mixed cultures in monolayers of maize

Spatially interpolated abundance of *P. truncatus* (Pt) and *S. zeamais* (Sz) at 7 d in pure cultures (left column) and mixed cultures (right column) on monolayers (75 × 27 cm, H:W) of maize when placed at a starting density of 10 (top row), 75 (middle row) , or 150 individuals (bottom row). Y and X axes in graphs correspond to cartesian coordinates on monolayers, and light to dark teal represents a greater abundance of individuals. Interpolation was performed on three experimental replicates using local kriging in R Software.

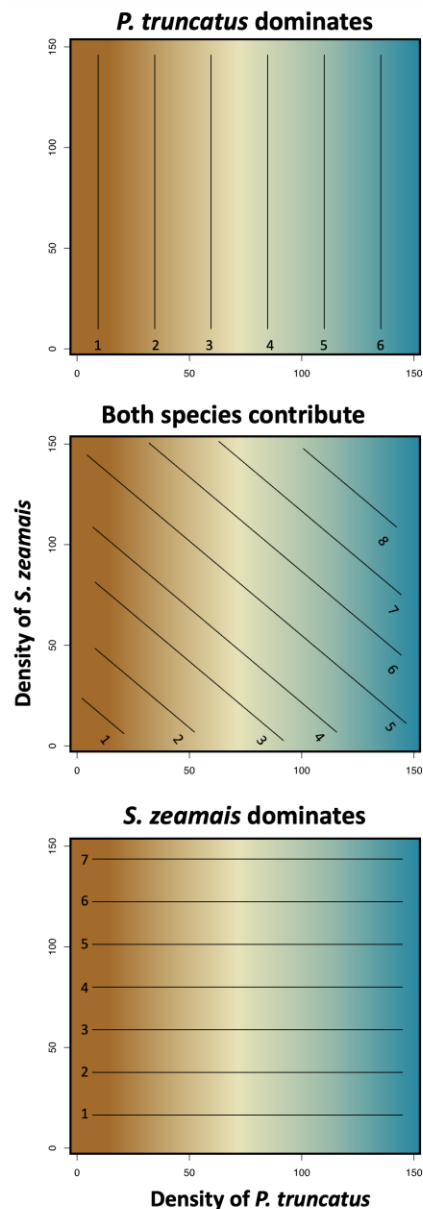


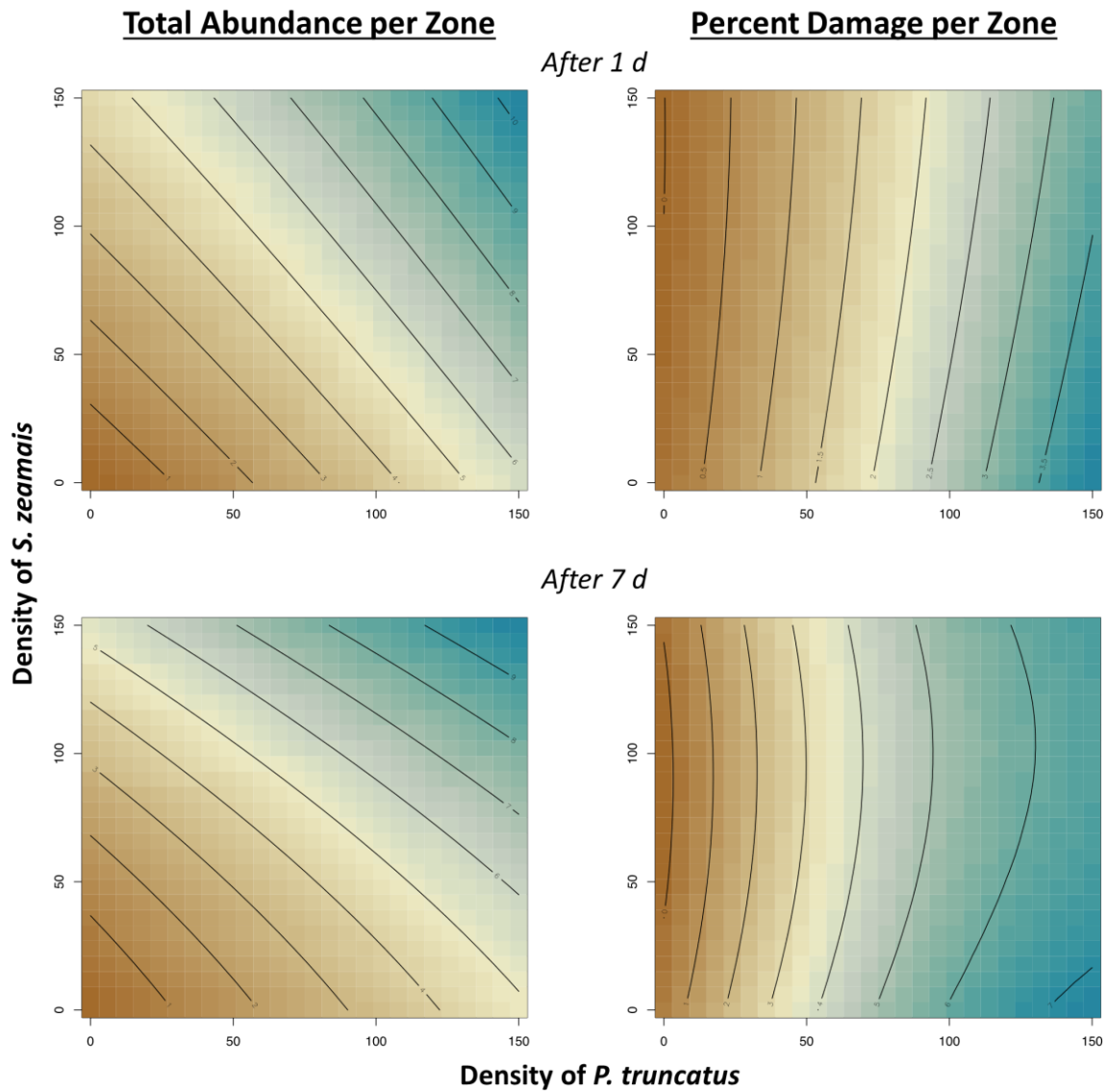
In mixed cultures after 7 d, abundance of *P. truncatus* and *S. zeamais* in the monolayer was generally uniform throughout the monolayer for the 10-insect density (Table 6.1, 6.2 and Figure 6.2). As the density increased to 75 insects, *P. truncatus* and *S. zeamais* inhabited the bottom areas of the monolayer. At the 150-insect density, the majority of both species were at the bottom of the monolayer, and were significantly correlated with each other (Table 6.2). However, compared to the pure culture, individuals of both species in the mixed culture were somewhat more dispersed in the bottom half of the monolayer. Interestingly, a group of 10 or more *P. truncatus* remained behind in the central area of the monolayer. This was not observed in either pure culture or the mixed culture for the 1-day dispersal.

Using response surface regression, both *S. zeamais* and *P. truncatus* contributed to the total abundance of insects in each at 1 d (second-order: Adj. $R^2 = 0.348$; $F_{5,642} = 69.9$; $P < 0.0001$) and 7 d (second-order: Adj. $R^2 = 0.141$; $F_{5,642} = 22.3$; $P < 0.0001$; Figure 6.3). Higher initial densities of each species resulted in a higher total abundance of insects in each zone, though it was not directly proportional (Figure 6.3).

Figure 6-3 Contour maps from the response surface analysis predicting total abundance of insects per zone (left column) and percent grain damage (right column)

A priori hypotheses and contour maps from the response surface analysis predicting total abundance of insects per zone (left column) and percent grain damage (right column) with different initial starting densities of *S. zeamais* and *P. truncatus* after 1 d (top panels) or 7 d (bottom panels) in monolayers with maize. Teal colors represent higher values of modelled response variables. When *S. zeamais* dominates the response, contour lines are expected to be fully horizontal, whereas when *P. truncatus* dominates, contour lines are expected to be fully vertical. When both species contribute, contour lines are expected to be diagonally drawn.





Grain Damage

Damage to maize in the monolayer was only attributed to one of the two species, *P. truncatus*. After 1 day in the monolayer, *P. truncatus* causing damage in the top half of the monolayer, which corresponds to the abundance and position of the insects at 1 day (Table 6.3, 6.4 and Figure 6.4). There was no difference in the location of damage between the densities, though the 10-insect density remained more uniform in the monolayer. When *P. truncatus* was

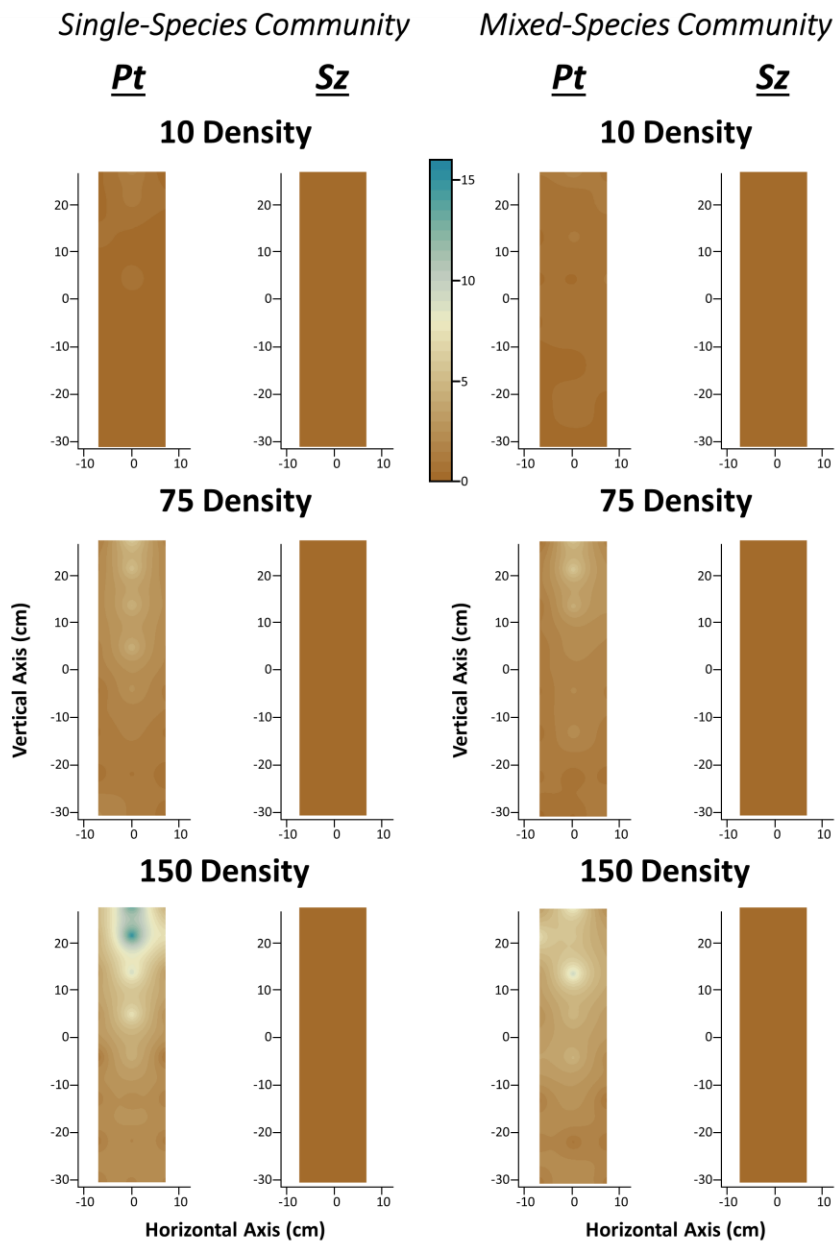
present with *S. zeamais* (pure vs. mixed culture) the damage decreased, especially for the 150-insect density (Figure 6.4).

Table 6-3 Summary of calculated Moran's I statistics for spatial autocorrelation based on insect damage to maize in grain column monolayers in the laboratory.

Culture	Species	Day	Density	I	P
Pure	LAGB	1	10	0.08	0.0003
Pure	LAGB	1	75	0.10	0.0001
Pure	LAGB	1	150	0.17	0.0001
Mixed	LAGB	1	10	0.12	0.0001
Mixed	LAGB	1	75	0.10	0.0001
Mixed	LAGB	1	150	0.16	0.0001
Pure	MW	1	10	0	0
Pure	MW	1	75	0	0
Pure	MW	1	150	0	0
Mixed	MW	1	10	-	-
Mixed	MW	1	75	-	-
Mixed	MW	1	150	-	-
Pure	LAGB	7	10	-0.03	0.69
Pure	LAGB	7	75	0.02	0.09
Pure	LAGB	7	150	-0.03	0.77
Mixed	LAGB	7	10	-0.03	0.69
Mixed	LAGB	7	75	-0.05	0.82
Mixed	LAGB	7	150	-0.03	0.66
Pure	MW	7	10	-0.04	0.94
Pure	MW	7	75	0	0
Pure	MW	7	150	-0.04	0.81
Mixed	MW	7	10	-	-
Mixed	MW	7	75	-	-
Mixed	MW	7	150	-	-

Figure 6-4 Spatially interpolated damage produced by *P. truncatus* and *S. zeamais* at 1 d in pure cultures or mixed cultures in monolayers of maize

Spatially interpolated damage produced by *P. truncatus* (Pt) and *S. zeamais* (Sz) at 1 d in pure cultures (left column) or mixed cultures (right column) on monolayers (75 × 27 cm, H:W) of maize when placed at a starting density of 10 (top row), 75 (middle row), or 150 individuals (bottom row). Y and X axes in graphs correspond to cartesian coordinates on monolayers, and light to dark teal represents a greater abundance of individuals. Interpolation was performed on three experimental replicates using local kriging in R Software.



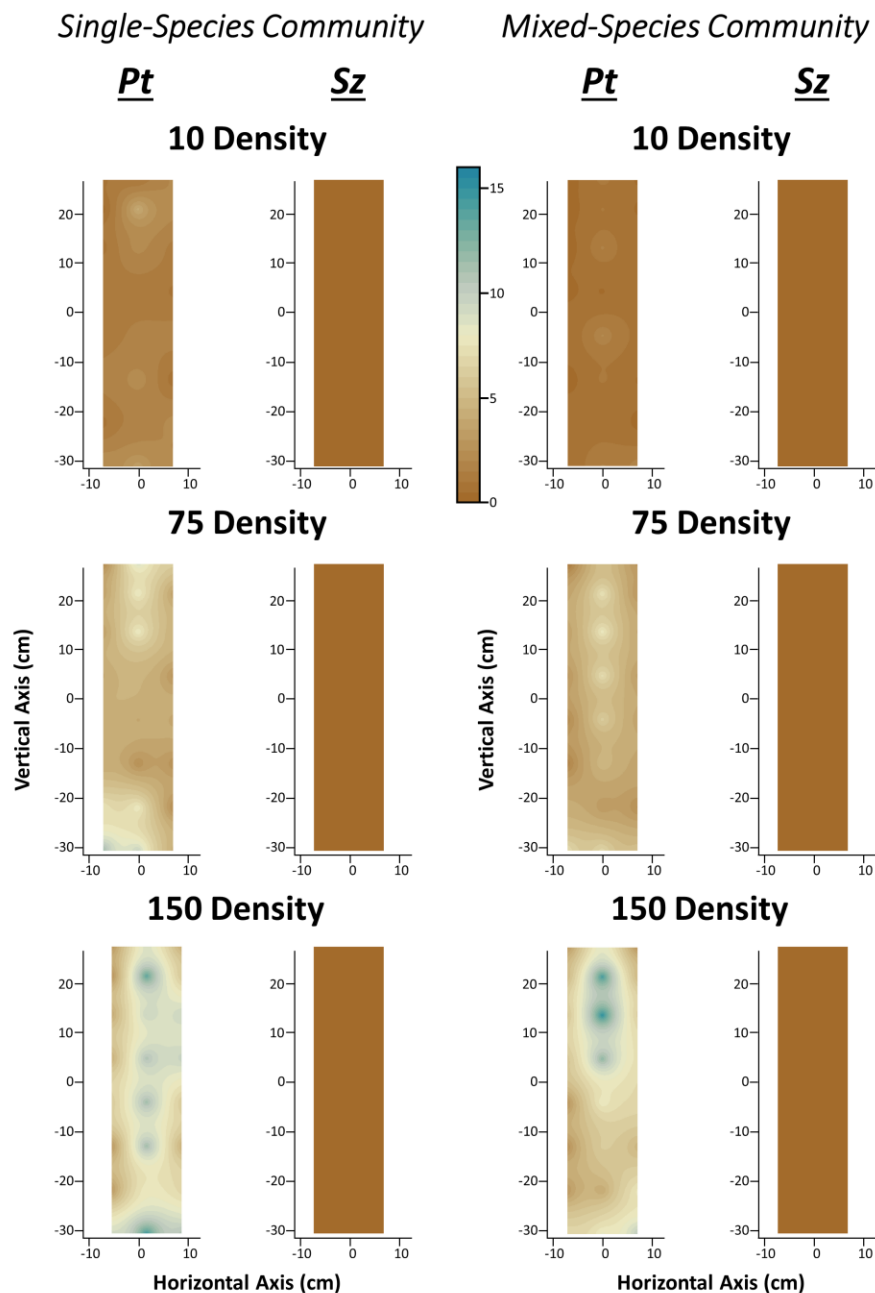
After 7 days, damage to maize in the monolayer was again only attributable to *P. truncatus*. After 7 days of dispersal, *P. truncatus* caused damage throughout the entire grain mass from top to bottom along the midline in pure cultures (Figure 6.5), which corresponds to the abundance and position of the insects at 1 day (Table 6.3 and 6.4). Similar patterns of damage were observed even at the 75-insect density, with a path moving down to the bottom of the monolayer. When *P. truncatus* was present with *S. zeamais* in mixed cultures, by contrast, the damage decreased and shifted upward in the monolayer, which was especially apparent for the 150-insect density (Figure 6.5).

Table 6-4 Summary of Mantel test statistics correlation between the damage of *P. truncatus* and *S. zeamais* in grain column monolayers in the laboratory.

Culture	Day	Density	r	Simulated
				P
Pure	1	10	0	0
Pure	1	75	0	0
Pure	1	150	0	0
Mixed	1	10	0	0
Mixed	1	75	0	0
Mixed	1	150	0	0
Pure	7	10	-0.11	0.87
Pure	7	75	0	0
Pure	7	150	-0.08	0.71
Mixed	7	10	0	0
Mixed	7	75	0	0
Mixed	7	150	0	0

Figure 6-5 Spatially interpolated damage produced by *P. truncatus* and *S. zeamais* at 7 d in pure cultures and mixed cultures in monolayers of maize

Spatially interpolated damage produced by *P. truncatus* (Pt) and *S. zeamais* (Sz) at 7 d in pure cultures (left column) and mixed cultures (right column) on monolayers (75×27 cm, H:W) of maize when placed at a starting density of 10 (top row), 75 (middle row), or 150 individuals (bottom row). Y and X axes in graphs correspond to cartesian coordinates on monolayers, and light to dark teal represents a greater abundance of individuals. Interpolation was performed on three experimental replicates using local kriging in R Software.



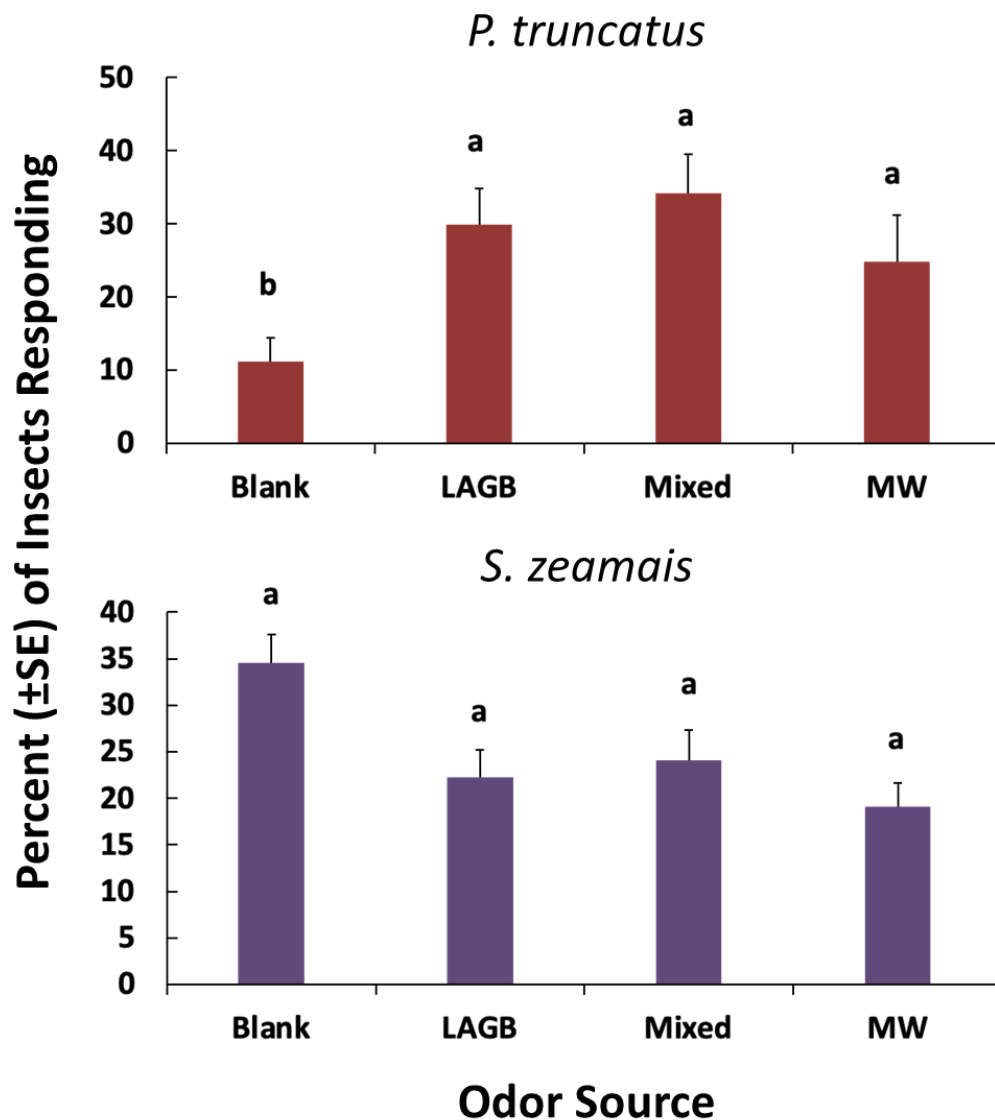
Using response surface regression, both *P. truncatus* densities drove damaged grain in each zone at 1 d (second-order: Adj. $R^2 = 0.304$; $F_{5,642} = 57.4$; $P < 0.0001$) and 7 d (second-order: Adj. $R^2 = 0.426$; $F_{5,642} = 97.2$; $P < 0.0001$; Figure 6.3). Higher initial densities of each species resulted in a higher total abundance of insects in each zone, though it was not directly proportional (Figure 6.3).

Olfactometer Assay

In the 4-way olfactometer, we found that there was a significant difference in the response by *P. truncatus* ($\chi^2 = 12.1$, $df = 3$, $P < 0.01$) to the odor treatments (grain pre-conditioned with *P. truncatus* only, *S. zeamais* only, or mixed culture) and the control (blank chamber) (Figure 6.6). However, there was no significant difference in the response by *P. truncatus* among the three treatments containing maize, meaning that *P. truncatus* responded to grain pre-conditioned with pure and mixed cultures of both species equally. It took *P. truncatus* an average range of 91 ± 15 – 126 ± 98 s to response to the odor sources, with the numerically quickest time to *P. truncatus*-conditioned grain, and the longest time to maize conditioned by both species.

Figure 6-6 Mean percent ($\pm$ SE) of *P. truncatus* or *S. zeamais* responding to odor sources

Mean percent ($\pm$ SE) of *P. truncatus* (top panel) or *S. zeamais* (bottom panel) responding to odor sources, including a negative control, maize with *P. truncatus* cultured on it, maize with *S. zeamais* cultured on it, or maize with both species cultured on it, in a 4-way, still-air olfactometer in the laboratory under constant light and temperature conditions at the Center for Grain and Animal Health Research. Bars with shared letters within a species are not significantly different from each other (χ^2 -test, Bonferroni Correction). N= 200 per species.



For *S. zeamais*, we found that there was no significant difference in the behavioral response to odor treatments in the 4-way olfactometer ($\chi^2 = 5.84$, $df = 3$, $P = 0.11$) (Figure 6.6). There was also no significant difference in the response by *S. zeamais* between the three grain treatments, meaning that *S. zeamais* responded to grain pre-conditioned with pure and mixed cultures of both species equally. It took *S. zeamais* an average of between 25 ± 27 – 38 ± 68 s to respond to the odor sources, with numerically the fastest response to mixed cultures and *S. zeamais*-conditioned maize alone, and the slowest response to *P. truncatus*-conditioned maize.

Headspace Volatiles

There was a small, but significant amount of differentiation in the headspace volatiles among the treatments (ANOSIM: $R = 0.105$; $Perm = 1000$; $P < 0.03$; Figure 6.7). There were a total of 20, 26, and 30 compounds tentatively identified from headspace in the NMDS analysis for the maize conditioned by *P. truncatus*, *S. zeamais*, or both, respectively, compared to just 18 in the control (Supplementary Table 1). Quantitatively, there were significantly greater total volatile emissions in pure *P. truncatus* compared to pure *S. zeamais* cultures ($t = 36.7$; $df = 44$; $P < 0.0001$; Figure 6.8). Likewise, there were significantly greater total volatile emissions in mixed colonies of *P. truncatus* and *S. zeamais* compared to pure cultures of *P. truncatus* ($t = 21.8$; $df = 54$; $P < 0.0001$) or *S. zeamais* ($t = 36.7$; $df = 44$; $P < 0.0001$). were in mixed cultures, followed by pure *P. truncatus* and then pure *S. zeamais* cultures (Figure 6.8). For example, the mixed cultured-conditioned maize emitted 6-fold more volatiles than the *S. zeamais*-conditioned maize. The most frequent chemical motif was carboxylic acid esters (RCOOR') followed by amides (RNH₂), and alcohols (ROH) (Figure 6.9).

Figure 6-7 Non-metric multidimensional scaling (NMDS) ordination of headspace volatiles from different treatments of maize

Non-metric multidimensional scaling (NMDS) ordination of headspace volatiles from maize that was free of insects (black, ctrl), or cultured with *P. truncatus* (teal), *S. zeamais* (pink), or both species (lavender) for 2 weeks prior to collection. Convex hulls of the same color as each treatment have been drawn around the appropriate samples. Ordination is based on pairwise Bray–Curtis similarities calculated among samples. Stress for the NMDS was less than 0.11 after $n = 1000$ permutations, indicating interpretation is possible.

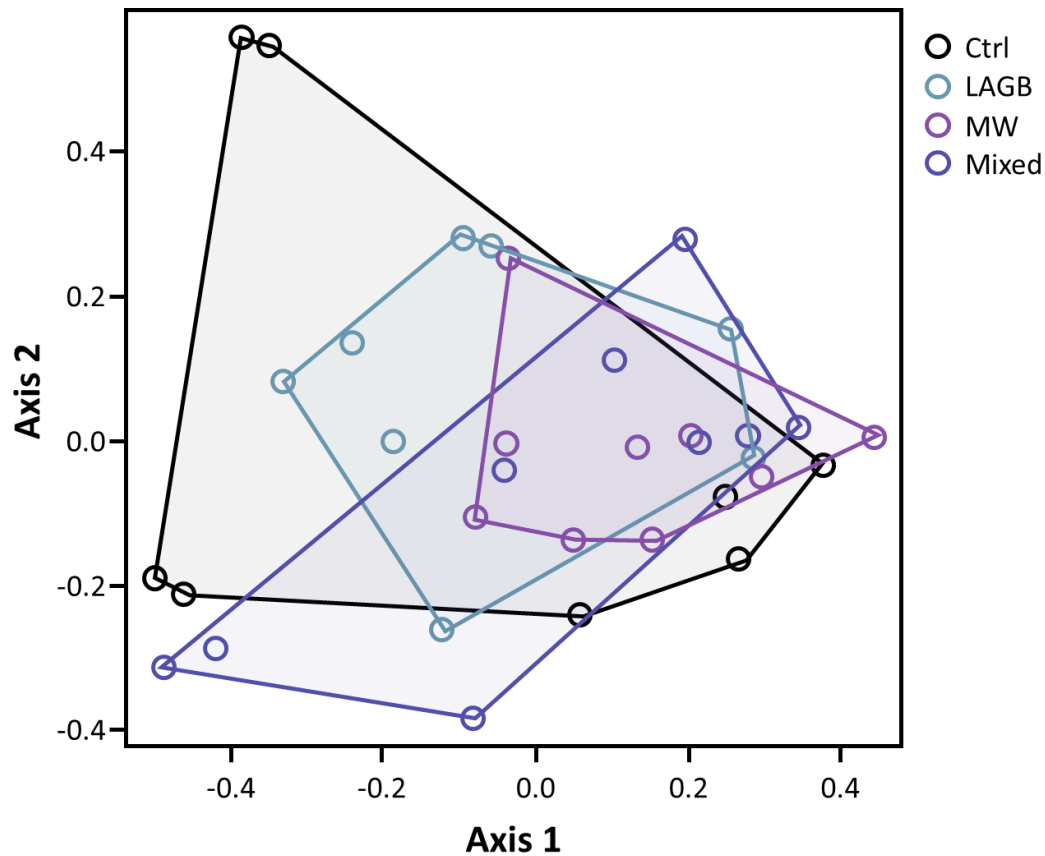


Figure 6-8 Mean (SE) total emissions of volatiles from cultures with *P. truncatus* and *S. zeamais*

Mean (SE) total emissions of volatiles from cultures with *P. truncatus* alone, *S. zeamais* alone, or both together. Bars with shared letters are not significantly different from each other (t-test, Bonferroni correction, $\alpha = 0.017$).

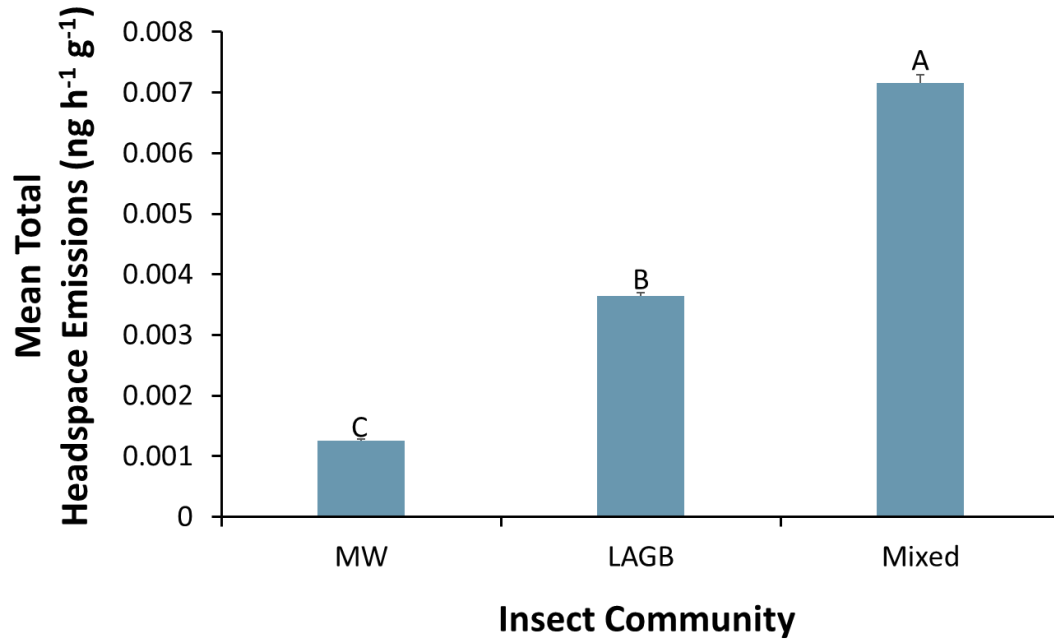
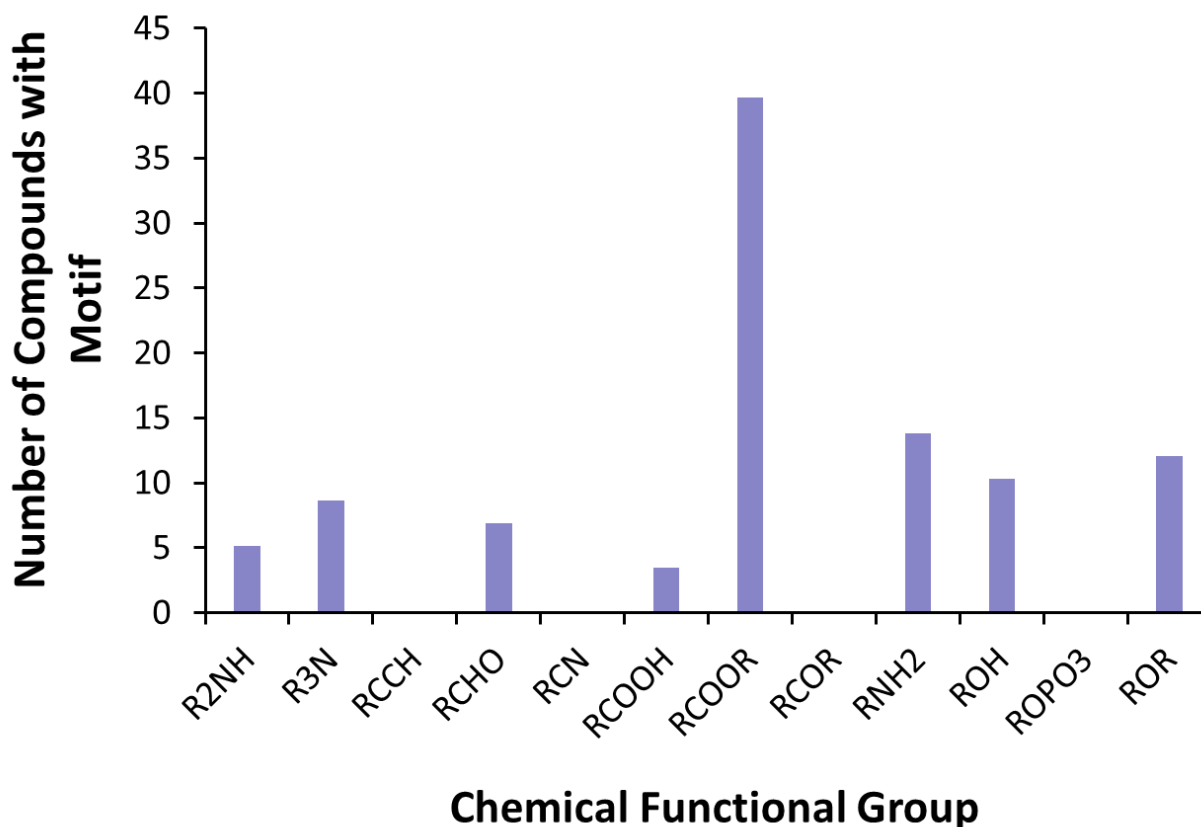


Figure 6-9 Count of functional group motifs among compounds in headspace volatiles produced by *P. truncatus* and *S. zeamais* on maize

Count of functional group motifs among compounds in headspace volatiles produced by *P. truncatus* and *S. zeamais* on maize. “R” represents side chain group. Data obtained from the R package uafR to process chemical ecology data (Stratton et al. 2023).



Discussion

In this study, we evaluated the short-term spatial dispersion patterns between *P. truncatus* and *S. zeamais*. This is the first time that this was performed with these species using monolayer grain columns consisting of a homogenous resource. Importantly, we found that over the time periods tested and size of grain columns here, both of species tend to occupy the same areas, and their populations were largely correlated with each other. However, in the presence of *S. zeamais*, damage produced by *P. truncatus* tends to shift upwards in a grain mass and also tended

to lessen. Nonetheless, at both 1 and 7 d, the occurrence of each species was correlated with the other and at the end of the study period, usually ended up near the bottom of the grain mass. In the case of *P. truncatus*, there is a clear path of destruction from where it enters the grain mass at the top to the bottom. These results are the first report of the spatiotemporal dynamics on this meso-scale compared to the more typical small plastic container for competition studies (e.g., Giga & Canhao, 1993; Papanikolaou et al., 2018; Sakka & Athanassiou, 2018; Quellhorst et al., 2020; Baliota et al., 2022) or observational field studies in grain studies that are limited in manipulating variables (e.g., Nyabako et al., 2020, Muatinte, 2017; Makundi et al., 2010).

While monolayer experiments have not been performed for *P. truncatus*, they have been extensively used to evaluate the movement and orientation of the related bostrichid, lesser grain borer, *Rhyzopertha dominica* (F.) (Vardeman et al., 2007; Cordeiro et al., 2016; Cordeiro et al., 2018; Cordeiro et al., 2019). For *R. dominica*, most movement occurred within 3 d of introduction to monolayers of grain, and was oriented towards the bottom of the grain mass (Vardeman et al., 2007), which aligned with prior knowledge about the biology of the species, validating monolayers as useful and practical experimental assays. Likewise, in the current study, we found *P. truncatus* typically ended up at the bottom of the monolayer by 7 d, which aligns with prior work, which has shown that this species often occurs at the bottom of the grain mass (Wright, 1993; Wekesa, 1994; Helbig, 1999) and at the bottom of grain bags (Hodges, 2002; Hodges et al., 2003). This can make developing predictions for machine-learning models of *P. truncatus* populations potentially difficult when those are based on probe traps that leave the bottom of the commodity unsampled (Nyabako et al., 2020).

Similarly, we found that *S. zeamais* was found most often near the bottom of the grain column after 7 d regardless of whether its competitor was present. It is well-known that

Sitophilus spp. are negatively geotactic after grain is disturbed (Plague et al., 2010), and we anecdotally observed *S. zeamais* climbing throughout the monolayer after addition to grain during experimental setup. Thus, it makes it even more interesting that they also end up at the bottom of the grain mass. However, prior work supports our finding, because the bottom layers of a bulk storage of grain (0.65-0.75 m deep) had 23-fold more *S. zeamais* than the top layers (Mvumi et al., 2006). When other species, such as *Cryptolestes ferrugineus* and *T. castaneum*, were tested in grain columns, they initially showed a negatively geotactic response (1–3 h after release), but over longer periods (>24 h), ended up at the bottom of the grain column (Jian et al., 2005; Jian et al., 2006), supposedly seeking out grains with more moisture. For its part, naïve male *R. dominica* showed an upward orientation bias during the first 24 h after introduction to a monolayer, but not thereafter (Cordeiro et al., 2019).

In the response surface analysis, we found that total insect abundance in each zone was the result of each species (*S. zeamais* and *P. truncatus*) contributing. As initial densities for both insects increased, so too did the resulting abundance in each insect zone. Overall, competition in monolayers consisting of mixtures of *P. truncatus* and *S. zeamais* was more subtle than hypothesized. Certainly, it appears that damage from *P. truncatus* is shifted upwards in the grain column and lessened overall in mixed cultures with *S. zeamais* compared to pure cultures with conspecifics. However, the abundance of the species was correlated with each other in the monolayers in both the pure cultures and mixed cultures. Thus, one hypothesis is that *P. truncatus* sexes may split up, with females moving upwards to cause damage in mixed cultures, while males proceed down to the bottom of the grain mass. Another hypothesis is that there may be a diurnal pattern of movement between feeding and oviposition sites at the top of the grain

mass and the refuge provided at bottom of the grain mass, but given the low general mobility of *P. truncatus*, this seems unlikely (Quellhorst et al., 2022).

In several cases, there was little or no damage produced by *S. zeamais*, while damage produced by *P. truncatus* was marked for each treatment, even over these short intervals (1–7 d). The response surface analysis indicated that the grain damage in the monolayers was primarily driven by the densities of *P. truncatus*. This does align with prior work showing *Sitophilus* spp. produced far fewer insect-damaged kernels (IDK) in terms of count and weight alone compared to *P. truncatus* alone (Baliota et al., 2022). While prior work found *P. truncatus* is the superior competitor at higher temperatures (Quellhorst et al., 2020; Baliota et al., 2022), the moderate temperature employed here (e.g., 27.5°C) is partway between the optimum temperature for *S. zeamais* and *P. truncatus*. In our study, we added each species simultaneously, but an interesting follow-up experiment is to determine if founder effects would affect spatial niche partitioning in grain columns. Prior work determined that when *S. oryzae* colonized environments with *P. truncatus* already present, there was a marked reduction in progeny production, and this could have been related to changes in grain quality (Baliota et al., 2022). Competition between *P. truncatus* and *S. oryzae* in prior work reduced the level of kernel damage in maize (Papanikolaou et al., 2018), which is similar to what we found here with *S. zeamais*.

The headspace treatments showed differentiation from the negative control compared to the other odor treatments, but significant overlap among cultures of *S. zeamais*, *P. truncatus*, and both on maize. In the olfactometer, we found *P. truncatus* and *S. zeamais* responded behaviorally to the three treatments with insects and maize similarly, though their responses were species-specific. For example, *P. truncatus* was attracted to headspace containing maize volatiles (e.g., all the treatments except the negative control). This corresponds with prior work showing

increased response to maize odors by *P. truncatus* raised on maize or emigrating from maize compared to cowpea or other maize-store-associated volatiles (Scholz et al., 1997). In the homogenous resource of the monolayer, the volatile gradient provided by maize was likely uniform upon first introduction. However, it is known that *P. truncatus* prefer volatiles from maize cultures with low numbers of males, whereas cultures at high densities elicited no attraction (Scholz et al., 1998) or even repellency. Especially in the high density monolayers, feeding and activity by *P. truncatus* at the top of the monolayer where beetles were introduced may have prompted movement deeper in the grain column.

By contrast, *S. zeamais* was slightly repelled from headspace containing conspecifics, heterospecifics, or both compared to the negative control in the olfactometer. Both sexes of *S. zeamais* generally prefer uninfested maize volatiles (Ukeh et al., 2012), and may also show a preference for conspecific-damaged and conditioned maize kernels at 14 d, but not at 7 d (Trematerra et al., 2013). However, we used damaged maize kernels directly, which may be a more realistic test, because there is less chance of losing minor components in the blend to volatilization. Practically in the monolayers of grain, this did not translate to avoidance by *S. zeamais* of conspecifics or heterospecifics.

Overall, this study contributes novel information about the behavioral ecology, competition, and spatial dispersion when *P. truncatus* and *S. zeamais* occupy a single grain column. For the first time, we have employed a response surface analysis in stored products, which has been used elsewhere (Kim et al. 2013; Inouye 2001) and is a powerful technique that may be able to yield future insights with other species after harvest. While much of the current tropics and subtropics are suitable for *P. truncatus* (Arthur et al., 2019), it is projected to shift its distribution poleward significantly by 2050 and 2070 under continued climate change (Harman

et al., 2023). The species has successfully invaded Africa, and continues to be documented in new countries on that continent (Quellhorst et al., 2021). In the US, *P. truncatus* is already an APHIS Species of Concern, and has been documented along the southern border, especially in Texas (Harman et al., 2023). As it invades new areas, it will encounter the already-established and cosmopolitan *S. zeamais*, so studies such as ours to understand its competition in a more realistic and mechanistic manner will be important going forward. In the future, competition studies should be conducted in larger pilot-scale sections of grain to evaluate distribution, and follow-up chemical ecology work should employ GC-EAD to determine which compounds in complex blends are being perceived by each species. Finally, knowledge from this study and others like it will help highlight the risk of *P. truncatus* as its range expands to new areas of the world.

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Chapter 7 - The capacity of the larger grain borer and the maize weevil to vector microbes after foraging at novel food patches

Planned submission: Hannah Quellhorst^{1*}, Marco A. Ponce¹, Jacqueline M. Maille¹,

Christos G. Athanassiou², Erin D. Scully³, Kun Yan Zhu¹, and William

R. Morrison III³. Short-term spatial dispersion patterns between the larger grain borer and the maize weevil in grain columns with implications for management of stored maize.

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Abstract

There has been no work assessing the capacity of the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), to vector microbes after harvest. While there has been some work assessing the microbial ecology of the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Cucurlionidae), none has directly assessed whether they transfer microbes to new food patches. However, this has important ramifications for food safety. Thus, we evaluated the ability of both *P. truncatus* and *S. zeamais* to vector microbes when given the opportunity to forage on potato dextrose agar dishes (e.g., novel food patch) after a 0, 24, or 72 h dispersal period in a sterilized container. We subsequently photographed the dishes at 3 and 5 d after introduction and quantified the microbial growth using ImageJ. In addition, we isolated unique morphotypes of fungi, extracted and amplified ITS4/5 ribosomal subunit DNA, then sequenced to determine fungal identity. We found that both species readily vectored several plant pathogenic microbes, including 21 taxa from 11 genera, notably *Aspergillus*, *Penicillium*, and *Fusarium spp.* Increasing dispersal period (0, 24, 72 h) resulted in a third less microbial growth by *S. zeamais* after 72 h, while for *P. truncatus* it resulted in a 2.7-fold increase in microbial growth. Dispersal by *S. zeamais* resulted in 6.6-fold more microbial growth than dispersal by *P. truncatus*. There was 1.5–3.7-fold more microbial growth after 5 d spent in a novel food patch than 3 d by each species. This research has important implications for food safety in the postharvest environment, especially for maize production.

Key Words: microbes, vectoring, food safety, larger grain borer, maize weevil

Introduction

Together insects and microbes are responsible for postharvest loss of important cereal grains, such as maize (Ricker-Gilbert et al. 2022). The central stored products pests of maize are the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), and the invasive larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), (Subramanyam and Hagstrum 2006; Badu-Apraku and Fakorede 2017). Together, losses from microbes and insects exceed \$100 billion annually worldwide, and are thus important to manage together to help ensure global food security.

There has been a lot of work investigating the interactions of *P. truncatus* or *S. zeamais* with other organisms. This has included investigating interspecific between *P. truncatus* and/or *S. zeamais* with other insects, which has included competition (Quellhorst et al. 2021; Quellhorst et al. 2023; Giga and Canhao 1993; Vowotor et al. 2005), predation (Holst and Meikle 2003), parasitism (Smith 1993), symbiosis (Anselme et al. 2006) and movement ecology after insecticide exposure (Quellhorst et al. 2022). There has also been significant work investigating intraspecific interactions for each species, including population growth (e.g., Altunc et al. 2023), chemical ecology (Scholz et al. 1997), trapping (Dendy et al. 1989), and management (Quellhorst et al. 2023). However, there have been few if any studies on their ability to vector microbes including those that produce mycotoxins (Ponce et al., 2021).

Studies involving *P. truncatus* and *S. zeamais* and microbes have mostly focused on control, such as the use of entomopathogenic fungi and microbial biopesticides (e.g., Kassa et al. 2002; Wakefield 2018). However, there are a few other examples. There was a microbiological study on the digestive system of *P. truncatus*, which found primarily nine bacteria but no fungi (Vazquez-Arista et al. 1997). For their part, Osipitan et al. (2011) found that the frass of *P.*

truncatus from maize consisted of multiple bacteria species, four species of *Aspergillus*, including *A. niger*, and two species of *Fusarium*. *Sitophilus zeamais* has been more of a focus for microbial studies as it is commonly associated with microbes in the stored products environment, such as mycotoxin producing fungi, *Aspergillus flavus* (Bhusal and Khanal 2019). Prior work has found that maize kernels conditioned with fungal volatile organic compounds were less attractive to *S. zeamais* and experienced less damage (Usseglio et al. 2017). In follow-up work, Usseglio et al. (2020) reported that *Fusarium verticillioides* could not pierce the cuticle of and its mycotoxin fumonisin had no negative effect on *S. zeamais*, but in the presence of *F. verticillioides*, the progeny of *S. zeamais* increased. Finally, in four maize ear genotypes inoculated with *F. verticillioides*, *A. flavus*, or uninoculated in Africa, there were significantly more borers and beetles in ears inoculated with *F. verticillioides* (Cardwell et al. 2007). However, much like *P. truncatus*, most of the studies for *S. zeamais* focus on the same interactions such as characterizing gut microbiology and targeting the insect with the help of microbial biopesticides (e.g., Campbell et al. 1992; Adane et al. 1996; Mantzoukas et al. 2023).

Finally, a last component to consider is the movement ecology within a food patch and how this may affect microbial vectoring by insects. For example, some stored product species have higher baseline mobility, such as *S. zeamais* (Quellhorst et al. 2022), and may be expected to spread microbes more quickly in their environment if they are present on their cuticle. By contrast, other species may be relatively poor walkers with low baseline mobility, such as *P. truncatus*, and may be expected to result in less microbial transmission. Thus, length of time foraging in a food patch may be expected to affect vectoring capacity of microbials although to varying extents.

Overall, it remains unknown how frequently *P. truncatus* and *S. zeamais* vector microbes when they reach novel food patches, and thus what the implications of their dispersal may mean for the safety of our food supply. Prior work has found spores on the cuticle of stored product insects, such as the cigarette beetle (Nakagawa et al. 2008). *Sitophilus zeamais* was found to interact with *Fusarium spp.* And the volatiles they produce in maize (Usseglio et al. 2017, 2020). *Sitophilus zeamais* was found to vector aflatoxin B₁ when given the opportunity in maize (Beti et al. 1995). Therefore, the aim of this study was to investigate the capacity of *P. truncatus* and *S. zeamais* to vector microbes after given a 0, 24, or 72 h dispersal period in a sterilized container, and then allowed to forage in a novel food patch for 3 or 5 d. We hypothesized that *P. truncatus* and *S. zeamais* will vector fewer microbes after longer dispersal periods between food patches.

Methods and Materials

Insect Colonies

Adult *P. truncatus* and *S. zeamais* were reared on whole kernels of maize and retained in incubators under constant abiotic conditions at $27 \pm 0.1^{\circ}\text{C}$ (mean $\pm$ SE), $64 \pm 1\%$ RH, and 14:10 (L:D) h photoperiod for *S. zeamais* and at $27.2 \pm 0.1^{\circ}\text{C}$, $37 \pm 1\%$ RH, and continuous darkness for *P. truncatus*. This was mandated by the applicable USDA-APHIS PPQ-approved permit (P526-191021-020). Colonies were maintained at the United States Department of Agriculture (USDA) Center for Grain and Animal Health Research facility in Manhattan, KS. Colony individuals were used for all tests described below. Colonies of the same age were used across the treatments for a species.

Insect Dispersal and Agar

Agar was prepared using 1000-ml capacity glass jars. A total of 900 ml of deionized H₂O was mixed with 32 g of potato dextrose agar (PDA, NEOGEN, Lansing, Michigan, USA) with a

magnetic stirring rod added and autoclaved (533LS, Getinge, Rochester, NY, USA) the media for 30 min and then integrated on a stir plate until the media had cooled to 45–50°C. A biosafety cabinet (75 x 73 x 95 cm L:H:W, #302381101, Labconco, Kansas City, MO, USA) space was prepared by sanitizing with 70% ethanol and exposure to UV light for 10 min. Media was poured in petri dishes (100 x 15mm) then allowed to harden overnight. Fresh agar petri dishes were made for each new replicate.

Insects (colony *P. truncatus* and *S. zeamais*) were given a dispersal period in autoclaved 4-L capacity glass containers and held under the following conditions: 25°C, 60% RH, and 14:10 L:D for 0, 24, or 72 hours. For the 0 h dispersal period, adults were immediately placed into the agar dish instead of holding in the autoclaved jars. After the respective dispersal period in the sterilized jar, the insects were transferred onto Petri dishes according to previously stated methods above. The dispersal period served to emulate insects walking or flying between food patches, without exposure to microbes. Dishes were sealed with parafilm and transferred to an environmental chamber under constant conditions: $30 \pm 0.1^\circ\text{C}$ (mean $\pm$ SE), $64 \pm 1\%$ RH, and 14:10 (L:D) h. The insects were given 3 and 5 days to forage on the agar as a “novel food patch.” At 3 and 5 days, petri dishes were briefly opened to photograph fungal growth and then promptly resealed and placed back into the incubator. There were a total of $n = 15\text{--}30$ replicate individuals per treatment combination (insect species by dispersal period by time in patch) (e.g., methodology adapted from Ponce et al. 2023). It is important to note that Ponce et al. (2023) demonstrated that the brief opening of Petri dishes for photography did not significantly contaminate Petri dishes with microbes in the laboratory environment.

Photography and Image Processing

Pictures of the Petri dish were taken using a DSLR camera (EOS 7D Mark II, Canon, Tokyo, Japan) equipped with a zoom lens (EF 17-40 mm 1:4 L USM, Canon, Tokyo, Japan). The camera was pointed facing downward on a 3D imaging StackShot rail (CogniSys, Inc., Traverse City, MI, USA) to take a single photo of each Petri dish. Each photo was labeled according to treatment parameters.

The images were processed using ImageJ v.1.53k (Schneider et al. 2012) to quantify the microbial growth. The grey background of the images was first subtracted from the rest of the image, then the images were processed using find edges. Afterwards, images were converted to binary, and adjusted with erode or dilate as appropriate based on the original parameters of the microbial growth on the agar medium. By selecting a circle encompassing only the petri dish with microbial growth, the mean greyscale value, were recorded in a separate spreadsheet with explanatory variables. Greyscale value is a measure of microbial growth in the Petri dish. This value is calculated by averaging the number of pixels in the photo that are represented by the fungus which will appear black after the photo has been converted into greyscale (black and white). This allowed a quantitative measure of microbial growth by creating an average of pixels in a given picture. The mean greyscale value could range from 0 (full white), indicating no microbial growth, to 255 (full black), indicating full microbial growth on the entire dish.

DNA Extraction, Sequencing, and Fungal Identification

Fungal morphotypes were isolated and cultured for the purpose of DNA sequencing to identify microbes associated with the cuticle of *P. truncatus* and *S. zeamais*. Pure isolations from new morphotypes were made by excising a 1 × 1 cm agar plug of fungi and subculturing it onto a Petri dish with PDA. In cases where there were multiple morphotypes growing closely together,

this process was repeated iteratively in order to obtain an uncontaminated culture. DNA was extracted from pure cultures using up to 7 d of growth using the Quick-DNA Fecal/Soil Microbe Miniprep Kit (D6010, Zymo Research Corp, Irvine, CA, USA). Photographs of each isolated morphotype were taken for reference. The concentration of DNA and quality were assessed using the Take 3 Assay on a microplate reader (Gen5™, BioTek Instruments, Winooski, VT, USA) prior to performing Polymerase Chain Reaction (PCR). PCR was used to amplify the internal transcribed spacer (ITS) region using primer sets: ITS4 5'-TCCTCCGCTTATTGATATGC-3' and ITS5 5'-GGAAGTAAAAGTCGTAACAAGG-3' (White et al. 1990). Each reaction contained 1.0 µL extracted DNA whose concentration varied by sample, but was quantified and standardized with Take 3 Assay prior to PCR, 1.0 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 master mix (Hot Start Taq 2X Master Mix, Promega, Madison, WI, USA). 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, and a final extension at 72°C for 5 min (S1000 Thermal Cycler, Bio-Rad, Hercules, CA, USA). PCR products were treated with ExoSAP-it for clean-up prior to sequencing following the manufacturer's protocol (ThermoFisher Scientific, Waltham, MA, USA). Amplicons were sequenced bidirectionally on an ABI 3730XL instrument (Eurofins Scientific, Brussels, Belgium), and the resulting sequences were quality-filtered and aligned using the program, Geneious Prime 2021.0.3 (Biomatters Ltd, Auckland, New Zealand). The consensus sequences were searched against NCBI's nucleotide database (nt) using the BLASTn algorithm (Altschul et al. 1997). The ITS consensus sequences were also checked against the UNITE Database using

the Ribosomal Database Project Classifier algorithm (Wang et al. 2007) to prevent taxonomic misassignments.

Statistics

The microbial growth was analyzed with a generalized linear model based on a poisson or quasipoisson distribution (where overdispersion was a problem) using the mean greyscale value derived from ImageJ for each Petri dish. Explanatory variables included dispersal period (0, 24, 72 h) and time in food patch (3 or 5 d). Separate models were run for each stored product species (*P. truncatus*, and *S. zeamais*). Multiple comparisons employed Tukey HSD implemented with *glht* using the *multcomp* package in R (R Core Team, 2023). Unless otherwise noted, α equals 0.05. Figures were generated with *ggplot2* (Wickham 2016).

To characterize differences in the microbial community between *P. truncatus* and *S. zeamais*, pairwise Bray-Curtis similarities were calculated between each microbial community sample (combining by strain + 0 h, strain + 24/72 h) based on *tblastn* searches, and non-metric multi-dimensional scaling (NMDS) was used to visualize the differences in microbial communities between species. A total of $n = 1,000$ permutations were used for the ordination procedure. Stress values for the NMDS procedure were <0.12 , indicating that good interpretation was possible. An analysis of similarity (ANOSIM) was used to determine significant differences in microbial communities between species. ANOSIM calculates an R statistic that can vary from 1 to -1, with values above zero interpreted as greater dissimilarity between treatments than within and values below zero interpreted as greater dissimilarity within treatments than between treatments. A total of $n = 1,000$ permutations were performed for the test. For all multivariate statistics, the R Package *vegan* was used (Oksanen et al. 2022).

Results

Microbial Growth

Dispersal period significantly affected the microbial growth initiated by *S. zeamais* in novel food patches (Figure 7.1, Table 7.1). There was a 29-36% reduction in microbial growth in novel food patches after *S. zeamais* were given the opportunity to disperse 24 or 72 h compared to immediately being introduced to a new area (Figure 7.2). In addition, time spent in foraging patch had a significant effect on microbial growth created by *S. zeamais* (Table 7.1), with 1.5-fold more growth after 5 than 3 d (Figure 7.1 and 7.2). Finally, there was also a significant effect of the interaction between time in patch and dispersal period on microbial growth created by *S. zeamais*, with a larger jump in microbial growth by 5 d in patch after a dispersal period (Figure 7.2), suggesting that small inoculations can still have large effects.

Table 7-1 Summary of GLM statistical output describing the microbial growth (mean greyscale value) for *S. zeamais* and *P. truncatus* after varying dispersal periods.

Variable	df	χ^2	P
<i>Sitophilus zeamais</i>			
Dispersal Period	2	32.9	0.0001
Time in Patch	1	30.8	0.0001
Dispersal Period:Time in Patch	2	7.88	0.02
<i>Prostephanus truncatus</i>			
Dispersal Period	2	19.3	0.0001
Time in Patch	1	32.0	0.0001
Dispersal Period:Time in Patch	2	0.34	0.85

Figure 7-1 Representative habitus images of the fungal growth produced by the foraging of *P. truncatus* or *S. zeamais* after 0, 24, or 72 h dispersal

Representative habitus images of the fungal growth produced by the foraging of *P. truncatus* (left) or *S. zeamais* (right) after given a 0, 24, or 72 h dispersal opportunity in a sterilized, autoclaved container, and the time spent in the novel food patch consisting of a potato dextrose agar dish after 3 or 5 d. All individuals are derived from colony.

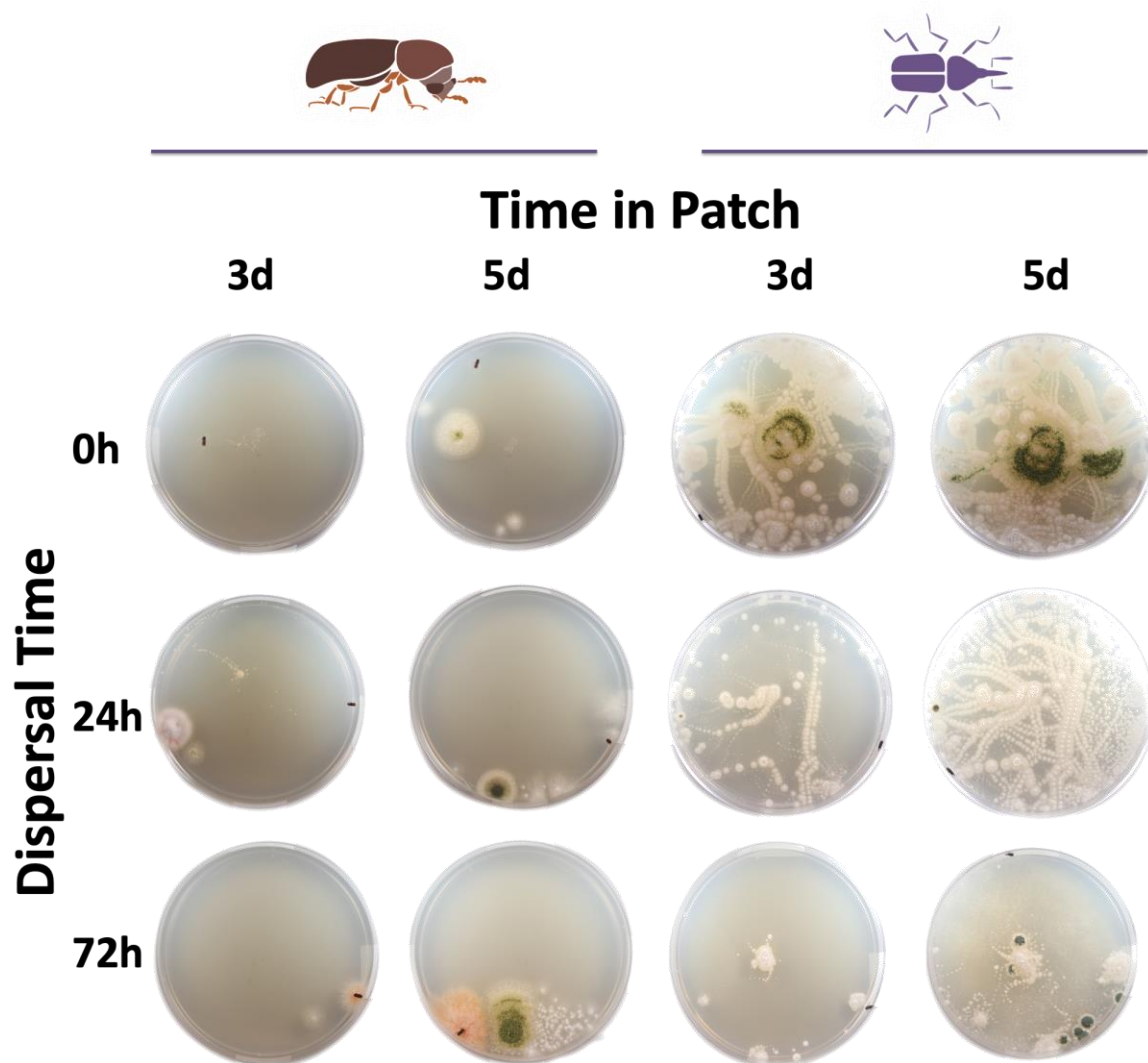
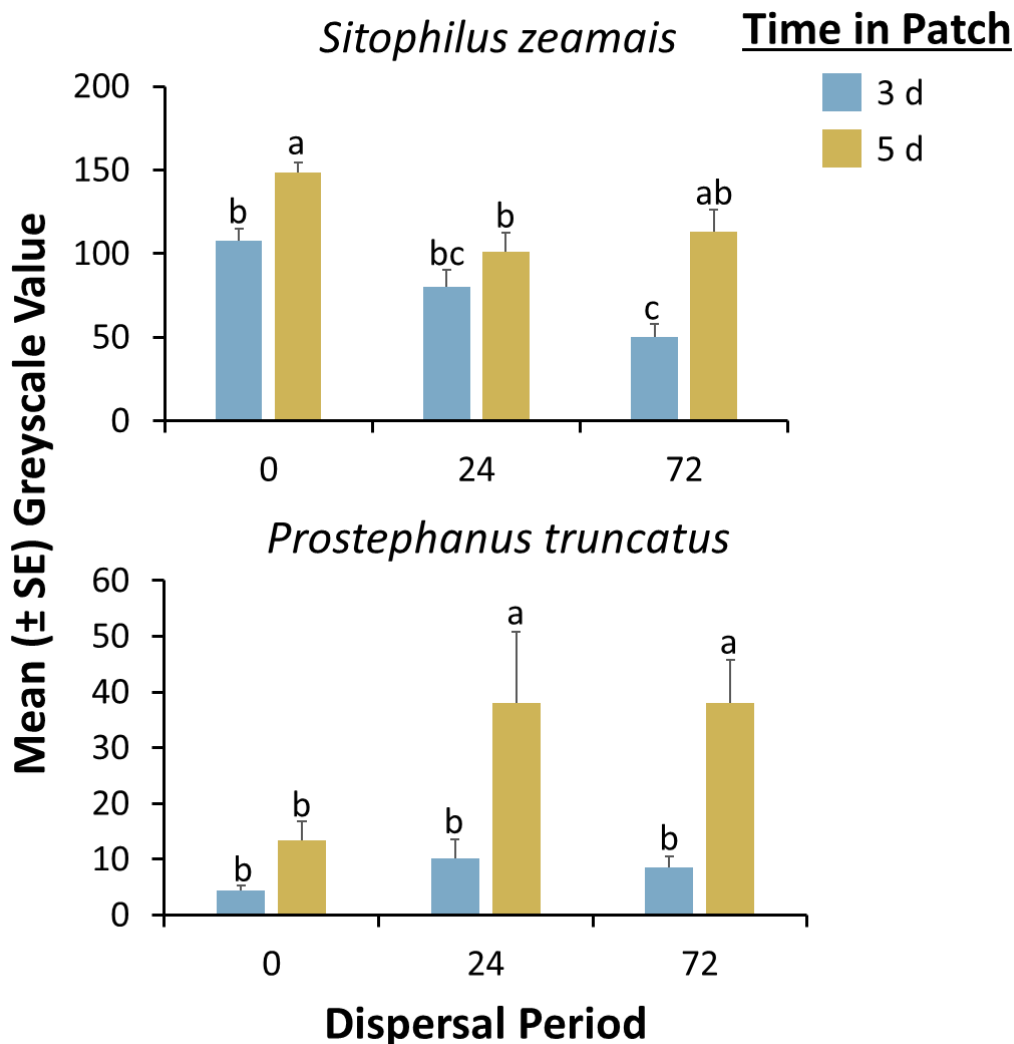


Figure 7-2 The mean microbial growth 3 d or 5 d after introduction of *S. zeamais* (top panel) or *P. truncatus* (bottom panel) into a novel foraging patch given 0, 24, or 72 h dispersal period

The mean microbial growth (e.g. mean greyscale value $\pm$ SE) 3 d (blue bars) or 5 d (yellow bars) after introduction of *S. zeamais* (top panel) or *P. truncatus* (bottom panel) into a novel foraging patch once given the opportunity for a 0, 24, or 72 h dispersal period. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). The mean greyscale value can range from 0 (no microbial growth) to 255 (maximum microbial growth on dish).



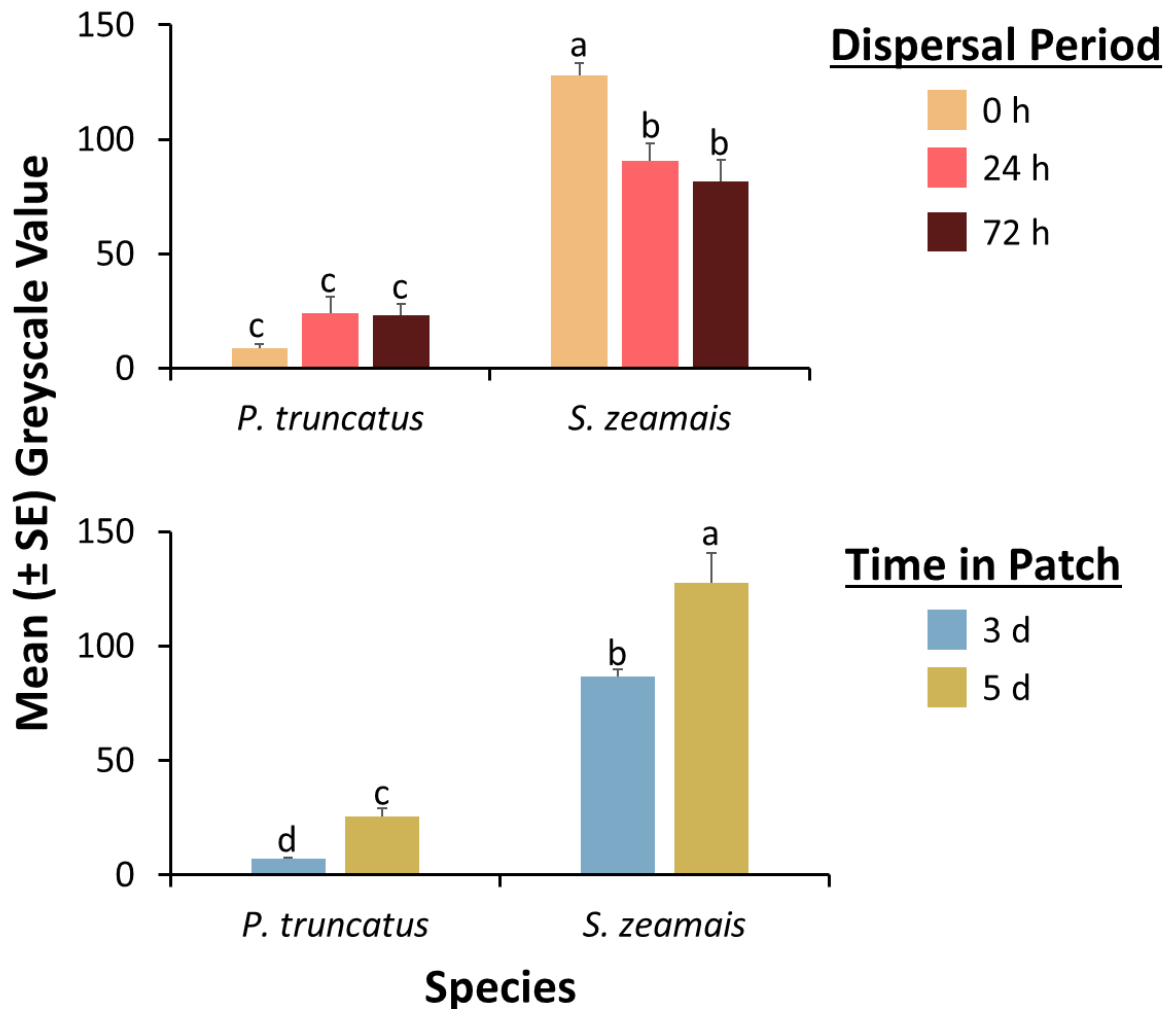
Likewise, dispersal period significantly affected microbial growth by *P. truncatus* (Table 7.1). However, in this case, microbial growth actually increased by 2.6–2.7-fold after a 24 or 72 h dispersal compared to when adult *P. truncatus* were immediately placed in novel food patches

(Figure 7.1, Figure 7.2). Moreover, time spent in the foraging patch significantly affected microbial growth by adult *P. truncatus* (Table 7.1), with 3.7-fold more microbial growth after 5 d compared to 3 d (Figure 7.1 and 7.2). The two-way interaction between these variables did not significantly affect microbial growth in dishes (Table 7.1).

There were significant species-specific differences in microbial growth. For example, it is notable that on average, *S. zeamais* created 6.6-fold greater microbial growth than *P. truncatus*. Regardless of dispersal period or time in patch, *S. zeamais* consistently initiated greater microbial growth than *P. truncatus* (Figure 7.3), ranging from 3.5–14.4-fold greater.

Figure 7-3 Species-specific differences in the mean microbial growth by *S. zeamais* and *P. truncatus* depending on dispersal period

Species-specific differences in the mean microbial growth (e.g. mean greyscale value $\pm$ SE) by *S. zeamais* and *P. truncatus* depending on dispersal period (0 h – salmon, 24 h – pink, 72 h – crimson; top panel) or time in foraging patch 3 d – blue bars, 5 d – yellow bars; bottom panel). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). The mean greyscale value can range from 0 (no microbial growth) to 255 (maximum microbial growth on dish).



Fungal Identification

In total, 45 samples were sequenced and we identified as 21 taxa from 11 genera (Figure 7.4, Table 7.2). The habitus images for the four most commonly identified fungal species for both *P. truncatus* and *S. zeamais* are given in Figure 7.5. Almost half of the samples were identified as *Aspergillus* spp., and 36% were *A. flavus*. *Aspergillus* was the most species-rich

taxon, followed by *Fusarium*, *Penicillium*, and *Talaromyces*. After *Aspergillus*, the most abundant taxon was *Penicillium*, comprising 11% of the samples (Figure 7.4). *Prostephanus truncatus* had a more speciose microbial community with 15 identified taxa, compared to *S. zeamais* which only had 11 identified taxa. The single two most abundant taxa found vectored by *P. truncatus* were *A. flavus* and *Penicillium cinnamopurpureum*, whereas the two most abundant fungi vector by *S. zeamais* were *A. flavus* and *Hypopichia burtonii* (Figure 7.4). There appeared to be more differentiation within species among microbial communities than between species, but there was not sufficient replication for it to be significant (ANOSIM: $R = -0.210$; Perm = 1000; $P = 0.81$; Figure 7.6).

Table 7-2 Summary of top blast hits from isolated fungi from *P. truncatus* and *S. zeamais* and their corresponding accession numbers. Different accession numbers for the same species, indicate the possibility of different strains.

Sample	Cultured from	Dispersal Period	Identified as	Query Cover	Percent Identity	Accession
F11R1_ITS4_CCWD05_33.ab1	<i>P. truncatus</i>	0	<i>Aspergillus flavus</i>	97	99.83	KX462773.1
F12R1_ITS4_CCWD06_35.ab1	<i>P. truncatus</i>	0	<i>Aspergillus flavus</i>	97	99.83	KX462773.1
F21R1_ITS4_CCWD10_43.ab1	<i>P. truncatus</i>	0	<i>Aspergillus flavus</i>	98	99.83	MN069570.1
F22R1_ITS4_CCWD11_45.ab1	<i>P. truncatus</i>	0	<i>Aspergillus flavus</i>	56	99.8	ON619465.1
F5R1_ITS4_CCWD03_29.ab1	<i>P. truncatus</i>	0	<i>Aspergillus flavus</i>	95	100	OP596235.1
M4R1_ITS4_CCWC89_3.ab1	<i>P. truncatus</i>	0	<i>Aspergillus flavus</i>	98	99.83	OP596231.1
LA24-1_IT4_CCWD44_29.ab1	<i>P. truncatus</i>	24	<i>Aspergillus flavus</i>	97	100	OQ422937.1
LA72-3_IT4_CCWD41_23.ab1	<i>P. truncatus</i>	72	<i>Aspergillus flavus</i>	95	100	MT141140.1
DM2R1_ITS4_CCWC94_13.ab1	<i>S. zeamais</i>	0	<i>Aspergillus flavus</i>	97	100	KX462773.1
DM7R1_ITS4_CCWC97_19.ab1	<i>S. zeamais</i>	0	<i>Aspergillus flavus</i>	97	99.83	OP596253.1
DM8R1_ITS4_CCWC98_21.ab1	<i>S. zeamais</i>	0	<i>Aspergillus flavus</i>	97	100	OP596253.1
DMR1_ITS4_CCWC93_11.ab1	<i>S. zeamais</i>	0	<i>Aspergillus flavus</i>	98	100	OP596228.1
MC24-1_IT4_CCWD35_11.ab1	<i>S. zeamais</i>	24	<i>Aspergillus flavus</i>	99	100	MZ158678.1
MC7-1_IT4_CCWD34_9.ab1	<i>S. zeamais</i>	72	<i>Aspergillus flavus</i>	98	100	MZ158678.1
F10R1_ITS4_CCWD04_31.ab1	<i>P. truncatus</i>	0	<i>Aspergillus neotritici</i>	97	99.83	OW984446.1
F3R1_ITS4_CCWD02_27.ab1	<i>P. truncatus</i>	0	<i>Aspergillus niger</i>	96	99.83	MT530263.1
DM5R1_ITS4_CCWC96_17.ab1	<i>S. zeamais</i>	0	<i>Aspergillus niger</i>	98	99.83	MN069568.1
F1R1_ITS4_CCWD00_23.ab1	<i>P. truncatus</i>	0	<i>Aspergillus terreus</i>	98	99.83	MF465863.1
LA24-3_IT4_CCWD46_33.ab1	<i>P. truncatus</i>	24	<i>Epicoccum sorghinum</i>	96	99.43	KX89695.1
M3R1_ITS4_CCWC88_1.ab1	<i>P. truncatus</i>	0	<i>Fomitopsis meliae</i>	96	99.84	OP749085.1
LA72-2_IT4_CCWD40_21.ab1	<i>P. truncatus</i>	72	<i>Fomitopsis meliae</i>	98	99.09	KC585351.1
F20R1_ITS4_CCWD09_41.ab1	<i>P. truncatus</i>	0	<i>Fusarium oxysporum</i>	97	99.82	MH607612.1
F2R1_ITS4_CCWD01_25.ab1	<i>P. truncatus</i>	0	<i>Fusarium proliferatum</i>	97	100	EU821492.1
LA72-1_IT4_CCWD39_19.ab1	<i>P. truncatus</i>	72	<i>Fusarium verticillioides</i>	96	100	KP132239.1
MC24-5_IT4_CCWD38_17.ab1	<i>S. zeamais</i>	24	<i>Fusarium verticillioides</i>	98	99.81	OM460744.1

M5R1_ITS4_CCWC90_5.ab1	<i>P. truncatus</i>	0	<i>Hyphopichia burtonii</i>	95	99.77	MG554339.1
DM4R1_ITS4_CCWC95_15.ab1	<i>S. zeamais</i>	0	<i>Hyphopichia burtonii</i>	92	100	KY103602.1
MC24-3_IT4_CCWD37_15.ab1	<i>S. zeamais</i>	24	<i>Hyphopichia burtonii</i>	98	100	KY103602.1
			<i>Penicillium</i>			
F13R1_ITS4_CCWD07_37.ab1	<i>P. truncatus</i>	0	<i>cinnamopurpureum</i>	95	99.82	KU847973.1
			<i>Penicillium</i>			
F14R1_ITS4_CCWD08_39.ab1	<i>P. truncatus</i>	0	<i>cinnamopurpureum</i>	95	99.82	OW987793.1
			<i>Penicillium</i>			
M7R1_ITS4_CCWC91_7.ab1	<i>P. truncatus</i>	0	<i>cinnamopurpureum</i>	73	99.77	MF442393.1
M9R1_ITS4_CCWC92_9.ab1	<i>P. truncatus</i>	0	<i>Rhizopus arrhizus</i>	96	99.83	MH707054.1
LA24-2_IT4_CCWD45_31.ab1	<i>P. truncatus</i>	24	<i>Talaromyces islandicus</i>	98	99.32	KJ783270.1
MC24-2_IT4_CCWD36_13.ab1	<i>S. zeamais</i>	24	<i>Talaromyces islandicus</i>	98	100	KF031335.1
LA24-4_IT4_CCWD47_35.ab1	<i>P. truncatus</i>	24	<i>Talaromyces purpurogenus</i>	97	99.48	AB872825.1
LA72-4_IT4_CCWD42_25.ab1	<i>P. truncatus</i>	72	<i>Talaromyces stollii</i>	98	99.82	OW988095.1
LA72-5_IT4_CCWD43_27.ab1	<i>P. truncatus</i>	72	<i>Talaromyces stollii</i>	98	99.28	OW988095.1

Figure 7-4 Species composition of preliminary sequencing results from Kansas for microbial community of *S. zeamais* and *P. truncatus*

Species composition of preliminary sequencing results from Kansas for microbial community of *S. zeamais* and *P. truncatus*. There was a total of n = 27 samples identified as 13 taxa from eight genera.

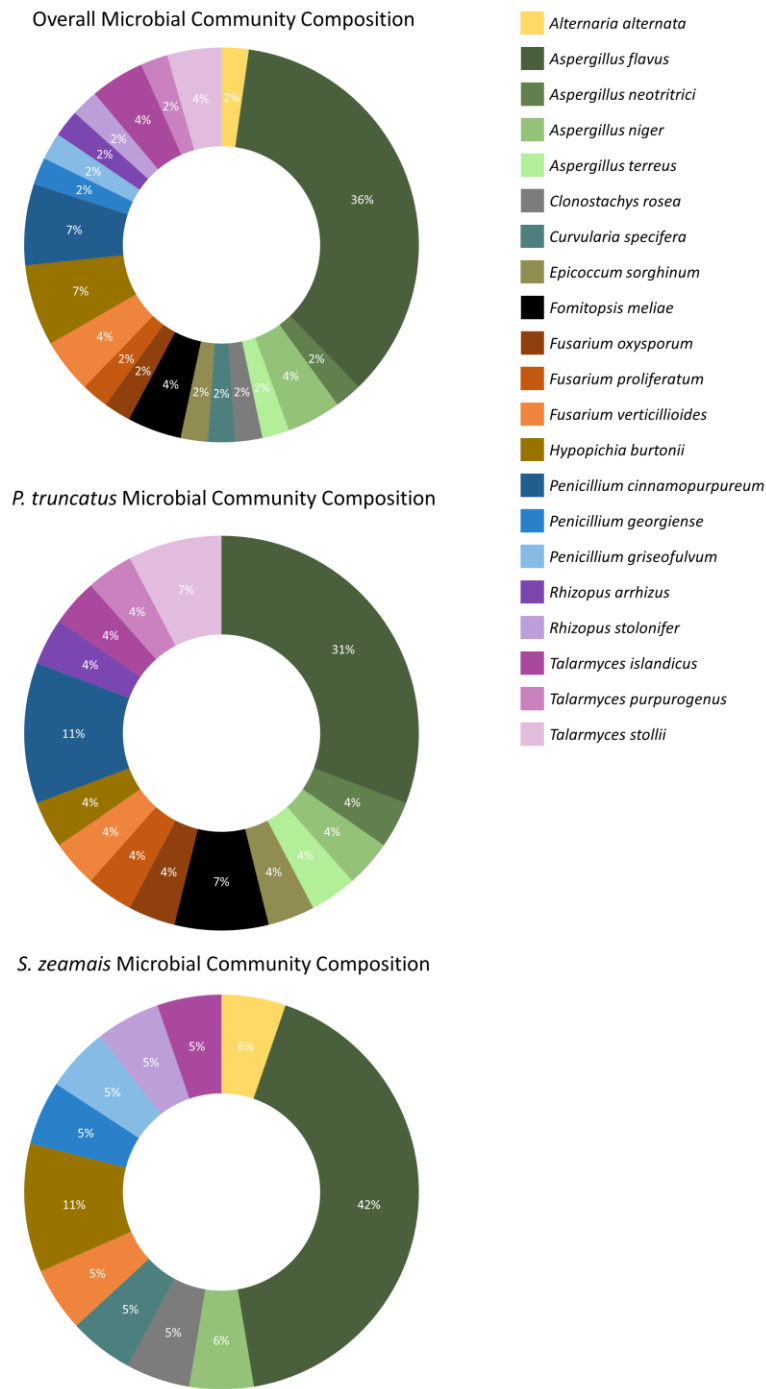
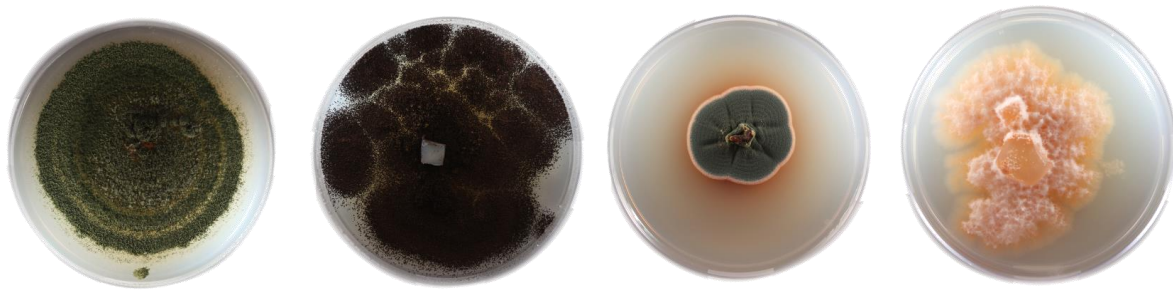


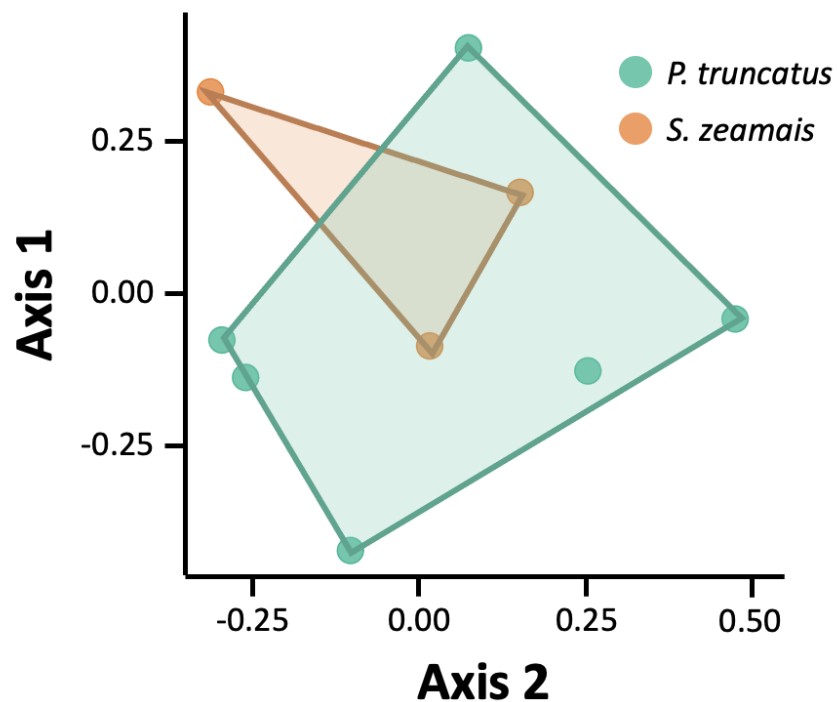
Figure 7-5 Habitus images of the four most commonly found fungal species for both *P. truncatus* and *S. zeamais*.



Aspergillus flavus* *Aspergillus niger* *Penicillium sp.* *Fusarium sp.

Figure 7-6 Non-metric multi-dimensional scaling ordination of microbial community vectored by *P. truncatus* and *S. zeamais* when dispersing to novel food patches

Non-metric multi-dimensional scaling ordination of microbial community vectored by *P. truncatus* (teal) and *S. zeamais* (orange) when dispersing to novel food patches. Stress was less than 0.11. Convex hulls have been drawn for each species grouping. Additional points for *P. truncatus* are the result of additional samples from Greek collaborators.



Discussion

Overall, we have shown that two major stored products pests have the capacity to vector microbes and spread potentially harmful organisms in our food supply when dispersing to new habitats. Of the 13 fungal taxa found, two hold particular importance for human health.

Aspergillus niger was one of these, and is a common mold found throughout the environment (soil, water, air, fecal matter, and decomposing matter). *Aspergillus niger* holds the classification of Generally Recognized as Safe (GRAS) by the US Food and Drug Administration (FDA) (Schuster et al. 2002). However, *A. niger* is capable of producing mycotoxins such as ochratoxin A (Abarca et al. 1994). Ochratoxin A can accumulate over time in humans and cause kidney damage, kidney failure, and certain cancers (Magan et al. 2003). *Aspergillus niger* is also capable of causing aspergillosis, a fungal infection that occurs from inhaling spores of *Aspergillus* spp. (CDC, 2022).

Aspergillus flavus was another common fungus found vectored by both *P. truncatus* and *S. zeamais*. *Aspergillus flavus* is well-known for its association with cereal grains and legumes, especially its ability to cause “ear rot” in maize (Amaike and Keller 2011). *Aspergillus flavus* is also widely known as a human and animal pathogen capable of causing aspergillosis (CDC 2022). Some strains of *A. flavus* produce aflatoxins which are carcinogenic compounds (Magan et al. 2003). Exposure to high levels of aflatoxin can lead to liver damage and liver cancer, as well as stunting and delayed development in children (Williams et al. 2004). *Aspergillus flavus* produces aflatoxin B₁ (AFB) which is considered the most toxic aflatoxin (Marchese et al. 2018). In response to metabolizing aflatoxin B₁, a complication can arise because affected livers may then produce a secondary byproduct, aflatoxin M₁ (Marchese et al. 2018). This partly highlights

the importance of protecting the food supply from proliferation of microbes and their spread by insects, as we have documented here.

Dispersal period significantly affected both species but in opposite ways. For example, we observed a linear decrease in the microbial infestation with increasing dispersal time in a sterilized container. This is concordant with the hypothesis that spores are lodged on the cuticle of *S. zeamais*, and become dislodged during movement while dispersing. By contrast, longer dispersal times resulted in greater microbial growth by 5 d for *P. truncatus*, but not necessarily at 3 d. This suggests that perhaps frass deposition may play a stronger role in vectoring behavior by *P. truncatus*. Prior work has documented that *P. truncatus* frass contains both *Aspergillus* spp. (including *A. niger*) and *Fusarium* spp. (including *F. oxysporum*) (Osipitan et al. 2009). From the sequencing work, we found most of the taxa belonged to *Aspergillus*, and some to *Fusarium*, so this would at least partially support this hypothesis. What is not understood yet is how a flight opportunity may affect vectoring capacity, which may be an especially salient question for *P. truncatus* in future work. Knowing, how flight impacts the vectoring of microbes, would be important as we see *P. truncatus* moving into new areas, potentially longer distances and whether they are bringing microbes with them. Could vector capacity decrease with increasing flight times?

Time spent in a new foraging patch uniformly increased microbial growth for both *S. zeamais* and *P. truncatus*. In both cases, increased time was associated with increased microbial growth. Prior work found +70% of 900 *Lasioderma serricorne* had fungi isolated from their bodies, with *Aspergillus* spp. spores detected on the pronotum, forewing, labrum, mandible, scutellum, abdomen, and femur (Nakagawa et al. 2008). At the shaft of the wing, spores were even found starting to germinate, suggesting that once insects make it to a novel food patch,

microbial growth may proceed quickly. Time has rarely been a factor taken into account in assessments of microbial growth after movement by insects in stored products. Ponce et al. (2023) found similar patterns of increasing microbial growth with time spent in patch for *Lasioderma serricorne* and *Sitophilus oryzae*. Thus, this is in alignment with prior work on this topic.

It is important to consider the vectoring of microbes from insects such as *P. truncatus* and *S. zeamais* and its impact on the quality and condition of the stored grain mass as well. As individuals immigrate into a grain mass, especially around major grain harvests during the height of summer, a grain mass is expected to be very warm. This presents an ideal environment for microbial growth. Prior work has documented that the peak of microbial vectoring by insects corresponds to the major wheat harvest during summer (Ponce and Morrison, unpublished). As insects invade the grain mass, they may bring microbes from other areas, and deposit them (Wallace and Sinha 1962; Ponce et al. 2021). A follow-up investigation might determine how insect immigration affects grain quality, as it is possible the deposition of microbes could result in hotspots of microbial and insect activity. It is quite possible that each of these pests could positively feedback on each other's dynamics (Ponce et al. 2023, but see Duarte et al. 2021), with preferred microclimates formed by microbes, then subsequent colonization and further degradation of the grain by stored product insects. For example, Hubert et al. (2021) found stored product mite abundance was positively associated with bacterial abundance in spent growth medium and negatively correlated with bacterial abundance in mite bodies. That study found host-microbe interactions were critical for affecting all stages of mite growth and led to alteration of the environmental microbiome. However, this needs to be specifically evaluated for stored product insects in future work.

We found that individual microbial trails were apparent in novel food patches for both *P. truncatus* and *S. zeamais*. However, microbial trails were more apparent for *S. zeamais*. Generally, *S. zeamais* vectored more microbes than *P. truncatus*. There could be several reasons for this phenomenon. First, it is known that *S. zeamais* is a much more stable and able cursorial species with the ability to walk longer distances and move more rapidly compared to *P. truncatus* (Quellhorst et al. 2022). *Prostephanus truncatus* is “top-heavy” with legs that are much smaller in proportion to the body. *Prostephanus truncatus* is better equipped for flight followed by walking over much smaller distances, and a grip intended for boring into wood or other food material. These differences in anatomy and physiology could partly explain why *P. truncatus* moved over fewer areas in the novel food patch and thus vectored fewer microbes. It is also possible that there are some differences in anatomy of the insects that could result in fewer microbial spores sticking to the surface of the body of *P. truncatus* compared to *S. zeamais*. This is a point that needs further study. An important caveat is that we only looked at colony populations in this study and it could be that field populations might have more diverse microbial communities compared to colony. This is also a point that needs further study. Overall, these results hold important implications for sanitation and food safety in the postharvest environment, especially for maize production. As we see *P. truncatus* invade new areas under climate change (Arthur et al. 2019), it will be important to consider its vector capacity along with *S. zeamais*.

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Chapter 8 - Research summary and future directions to improve management of stored products insect pests

The invasive larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), originated from Meso-America, and is a devastating stored product pest of both maize (*Zea mays* L.) and cassava (*Manihot esculenta*). This species can destroy up to 40% of stored maize within four months. Despite four decades of intensive research, *P. truncatus* has spread throughout Africa, while decimating maize and cassava production. Given the continuing problems with *P. truncatus*, the likelihood for its continued range expansion under climate change, and its status as a species of concern in many countries including in the U.S., the objective of this work was to improve management for this pest, and its main competitor, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae). By comparison, *S. zeamais* is a more cosmopolitan species and is also a long-standing, major pest of maize throughout the world. I pursued this by exploring several aspects of integrated pest management such as chemical, physical, and cultural control. In Chapter One, I provide an updated literature review for the focal species *P. truncatus*. Chapters two through four focused on improved chemical control of *P. truncatus* and *S. zeamais*. In Chapter Two, I investigated a new formulation for an insecticide from Central Life Sciences, Gravista®, containing methoprene combined with deltamethrin and the synergist piperonyl butoxide. In particular, I evaluated its ability to induce mortality and sublethal changes in mobility for adult *P. truncatus* and *S. zeamais* compared to an older formulation (e.g. Diacon IGR+) without the synergist and compared to a H₂O-treated control. Mortality and sublethal effects were assessed by exposing adults of each species to treated concrete arenas or treated maize for differing amounts of time ranging from 1–168 h. For the sublethal component, I assessed horizontal movement using video-tracking coupled with

Ethovision and tracked distance moved and velocity after exposure to the new or old formulation of the insecticide or control. Chapter Four followed up with the same treatments by testing the dispersal capacity of *P. truncatus* and *S. zeamais* between food patches over longer distances by the adults after exposure to the residual insecticides. Overall, this research is providing important information on a new insecticide formulation with just a fraction of the active ingredient compared to the commercial standard (e.g. Diacon IGR+), and suggests that the new formulation holds promise for management of these two insects, as the new formulation is equally as effective as the old. This novel insecticide showed excellent control for the invasive *P. truncatus*. Ultimately, this tool may be incorporated in integrated pest management programs to protect our global maize supply.

In Chapter Five and Six, I investigated competition, species interactions, and insect behavior and movement between *P. truncatus* and *S. zeamais*. In this portion of my work, I examined how *P. truncatus* and *S. zeamais* colonize, compete, and arrange themselves in bulk storage-mimicking conditions. I determined whether the invasive *P. truncatus* competes with *S. zeamais* and which is the more damaging insect under a range of temperatures, including 20–35°C. I found that while *S. zeamais* is the superior competitor at low temperatures, *P. truncatus* is better at higher temperatures, and most of the damage in combined colonies is from *P. truncatus*. In a separate experiment, I evaluated the distribution of each species in a monolayer of grain, which allows for tracking of the insects visually after pre-determined time intervals. I created 75 cm tall glass sheets with spacers that only allowed a single layer of maize. The monolayers were filled with grain and sealed after the addition of one of three treatments (*P. truncatus* only, *S. zeamais* only, or both species together). Three population densities were included, namely 10, 75, and 150 individuals/species per monolayer. I tracked the insects in one

of 24 equally-sized zones after 1 d or 7 d. Using a response surface analysis, I found that given longer dispersal times, the insects did not partition the grain mass and were found in close proximity at the bottom of the monolayer. However, in mixed colonies, damage produced by *P. truncatus* was shifted upwards in the grain mass and was lessened overall. To determine possible mechanisms for spatial dispersion patterns of the insects, I used a 4-way olfactometer to evaluate the response of each species to grains with or without conditioning by con- or heterospecifics. In addition, I collected headspace from the same treatments. I found overlap in the volatile composition of treatments, but significant partitioning in cues. In the olfactometer, *P. truncatus* positively responded to maize volatiles, while semiochemical stimuli did not affect the behavior of *S. zeamais*. Ultimately, this research will help us to better understand the impact of species interactions and behavior on the success of this invasive species in bulk storage. If we can predict the spatial niche of these insects, we can better target control tactics. Overall, my research is helping to greatly expand our foundational and applied knowledge of the integrated pest managements options available to stakeholders.

Finally, Chapter Seven focuses on examining the interactions between *P. truncatus* and *S. zeamais* and the microbial community in maize, specifically their ability to vector microbial communities in stored maize in the laboratory. I compared the vectoring ability of *P. truncatus* and *S. zeamais* on agar Petri dishes as surrogate novel food patches for lab colony strains. Vectoring capacity was assessed after giving the insects the opportunity to disperse for 0, 24, or 72 h in a sterilized jar. Fungi were then isolated and identified after sequencing based on the different morphotypes of fungus that the insects vectored on agar. Microbial growth was calculated using ImageJ to convert images to binary values and use mean greyscale value as a surrogate measure. This information will help us to understand how often and what types of

fungi these insects are potentially vectoring to our maize supply. Ultimately this research will help us to improve sanitation and food safety and help us to create better trapping and monitoring programs for invasive pests like *P. truncatus*, which threaten the biosecurity of the U.S.

In the future, it may be important to assess recovery of *P. truncatus* and *S. zeamais* after exposure to insecticides, given the chance on a clean grain source. Based on the results in my study, especially from the more realistic assay, there may be more chances of insect recovery than previously thought. It will also be important to understand how insecticide exposure impacts flight capacity for both *P. truncatus* and *S. zeamais*, as well as assessing their flight capacity without insecticides. Flight capacity of stored products insects using flight mills has not been studied and could be due to the size and weight of the insects and the challenges associated with building a flight mill to handle this issue. In addition, it would be useful to assess the new formulation of insecticide with synergist against pyrethroid-resistant strains of *P. truncatus* and *S. zeamais* in order to understand whether its inclusion confers an added benefit. In terms of insect dispersion and competition in grain columns, it would be key to understand how these insects are behaving in even larger or pilot-scale sections of grain compared. While my study was able to improve upon past methods for evaluating competition and dispersion of insects via the monolayer, there is still more to learn from more realistic and larger columns of grain. Finally, while my study has built a foundation for future research on the vectoring capacity of *P. truncatus* and *S. zeamais* and the identity of microbes they are transporting, it would be important to continue to investigate the microbiome of both insects and how the structure and insects and physiology contribute.