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Do temperature, relative humidity and interspecific competition alter the population size and the damage potential of stored-product insect pests? A hierarchical multilevel modeling approach

Nikos E. Papanikolaou[a,b*], Nickolas G. Kavallieratos[a], Maria C. Boukouvala[a,c], Chrisovalantis Malesios[d]

[a]Laboratory of Agricultural Zoology and Entomology, Department of Crop Science, Agricultural University of Athens, 75 Iera Odos str., 11855, Athens Greece

[b]Directorate of Plant Produce Protection, Greek Ministry of Rural Development and Food, 150 Sygrou Ave., 17671, Athens, Greece

[c]Laboratory of Organic Chemistry, Department of Chemistry, University of Ioannina, Panepistimioupolis, 45110, Ioannina, Greece

[d]Aston Business School, Aston University, Birmingham B4 7ET, UK

[*]Corresponding author. nepapanikolaou@yahoo.gr (N.E. Papanikolaou)

ABSTRACT

The premises of stored agricultural products and food consists of a complex ecosystem in which several pests can seriously affect the quality and quantity of the products. In this study we utilize a 4-level hierarchical linear multilevel model in order to assess the effect of temperature, relative humidity (RH) and interspecific competition on the population size and damage potential of the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae) and the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). As RH was increased, we observed higher percentage of live insects, while increased levels of temperature significantly decreased the percentage of live insects. The combination of *R. dominica* and *P. truncatus* lead to reduction of the percentages of live insects in comparison to single species treatments. However, *P. truncatus* is more damaging than *R. dominica* in maize, based on the proportion of damaged kernels which were infested by each insect species. We expect our results to have bearing in the management of these species.

1. Introduction

The knowledge of abundance of living organisms through time is a central goal in ecology (Hall et al., 1992; Ehrlén and Morris, 2015; Boyce et al., 2016). Birth and death rates, as well as migration, are the main factors that determine the population size of species and therefore their temporal and spatial dynamics (Huddleston, 1983; McCoy and Gillooly, 2008; Taylor and Norris, 2010; Papanikolaou et al., 2014). The population of a species may experience the effect of several abiotic factors during its lifetime. Temperature is a key abiotic factor that affects several biological features of insects, such as development, survival and fecundity (Honěk and Kocourek, 1988; Mironidis and Savopoulou Soultani, 2008; Papanikolaou et al., 2013; Athanassiou et al., 2014, Kavallieratos et al., 2017b; Athanassiou et al., 2017a). This results in a further effect on insects' performance and growth rate, leading to several fluctuations through time (Kontodimas et al., 2007; Papanikolaou et al., 2014). Like temperature, relative humidity (RH) may affect life history traits of insects and consequently their growth rate. For example Pelletier (1995) reported that the larvae, pupae and adults of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) regulate the equilibrium of water in their organisms through different mechanisms according to the environmental conditions they face. Guarneri et al. (2002) found that eggs of the blood-sucking bug, *Triatoma brasiliensis* Neiva (Hemiptera: Reduviidae) substantially reduce hatching at 9.3% RH to avoid desiccation during this process. Similarly, 20% RH reduced the number of hatched eggs of the pine caterpillar *Dendrolimus tabulaeformis* Tsai and Liu (Lepidoptera: Lasiocampidae) and increased mortality or prolonged development of first instar larvae (Han et al. 2008).

Resource competition is a common biotic factor that may affect the population outcome among living organisms (Le Bourlot et al., 2014; Pedruski et al., 2015). It may affect several biological features, such as population size of herbivorous species (Karban, 1986; Athanassiou et al., 2014, 2017a; Kavallieratos et al., 2017b), progeny production, fitness and prey consumption of beneficial insects (Xu et al., 2013; Cusumano et al., 2016; Papanikolaou et al., 2016). In addition, direct or indirect competitive interactions between species may lead to displacement of established species from a habitat (Reitz and Trumble, 2002).

The premises of stored agricultural products and food consists of a complex ecosystem in which several pests (e.g. insects, mites, rodents) can seriously affect the quality and quantity of the products (Athanassiou et al., 2005, 2011; Hubert, 2012; Mason and McDonough, 2012; Kumar and Kalita, 2017). For example, insects, mainly coleopterans, are responsible for huge grain losses of up to 57% in Africa (Kumar and Kalita 2017). Several stored-product coleopterous species co-exist in storage facilities and consequently compete for the same food resources (Smith and Lessells, 1985; Smith, 1990, 1991; Athanassiou et al., 2003, 2005, 2011, 2017a; Kavallieratos et al., 2017b). Although competition is considered a serious issue in storage ecosystem (Smith and Lessells, 1985; Smith, 1990, 1991; Kavallieratos et al., 2017b) there is a gap of knowledge regarding interspecific competition. The significance of interspecific competition in stored-product insect pests has recently been highlighted, demonstrating its effect in community structure (Athanassiou et al. 2014, 2017a; Kavallieratos et al. 2017b).

The larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae), is one of the most damaging insect pests of stored maize and cassava tubers (Muatinte et al., 2014). It is not clear whether *P. truncatus* can develop on commodities other than maize or cassava (Howard, 1983; Hodges et al., 1983; Hodges, 1986). In a recent study, Athanassiou et al. (2017b) reported that triticale, rice, whole barley flour, whole oat flakes, white soft wheat flour, whole soft wheat flour, white hard wheat flour and whole rye flour favored the marginal surviving of *P. truncatus* but most probably did not favor its population growth. *Prostephanus truncatus* was accidentally introduced from Central America into Africa and thereafter it was spread in numerous countries in this continent (Farrel and Schulten, 2002; EPPO, 2018). *Prostephanus truncatus* is also distributed in Asia, North and Central America (EPPO, 2018). Currently, in certain countries of Asia (i.e., Israel, Jordan) and Oceania (i.e., New Zealand) it is of quarantine importance (EPPO, 2018). It is a primary colonizer and can easily infest the intact kernels (Hill et al., 2002; Nansen and Meikle, 2002). Adults of *P. truncantus* live long and can multiply rapidly, only in a few weeks (Nansen and Meikle, 2002; Hill et al., 2002). Furthermore, due to its longevity, this species has a long period to devastate the stored grains (Nansen and Meikle, 2002).

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), is a serious pest of stored grains that has global distribution causing serious damages in grains (Hagstrum and Subramanyam, 2009). As a primary colonizer, it can easily infest sound kernels (Hill, 2002). Adult females oviposit among grain kernels and the young hatched larvae consume the grain debris or dust and later, complete their development inside the grain seed (Hill, 2003; Mason and McDonough, 2012). Contrary to other primary insect pests of stored products, *R. dominica* can develop and reproduced rapidly in dry grains (Edde, 2012) given that it is able to grow in grains even with 9% moisture content (Hill, 2003), which is an inhibitive level for other primary pests to survive.

It has previously been documented that temperature is an abiotic factor that crucially regulates the overall performance of interspecific competition between stored-product psocids (Psocoptera) (Athanassiou et al. 2014) or among stored-product coleopterans (Giga and Canhao 1993; Kavallieratos et al. 2017b). However, there is a gap of knowledge when another important abiotic factor (RH) of the storage ecosystem participates in this type of competition. Therefore, the aim of this study was to investigate the effects of temperature, RH, and competition between *P. truncantus* and *R. dominica* on their population size and damage potential infesting stored-maize. In this study, we utilize a 4-level hierarchical linear multilevel (HLM) model (Raudenbush and Bryk, 2002) in order to assess the effect of the previously described covariates on the dependent variables. The HLMs belong to the family of Ordinary Least Squares (OLS) models and are preferred when the predictor variables differ at more than one level.

2. Materials and Methods

2.1. Insects

Prostephanus truncatus and the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) were cultured on whole maize in a Sanyo MLR-350T/350HT incubator set at 30 ^{o}C, 65% RH and continuous darkness. The *P.*

truncatus colonies were started in 2003 while the *R. dominica* colonies, initially collected from Greek storage facilities, were established in 2002. All cultures were maintained in the Agricultural Zoology and Entomology laboratory, Agricultural University of Athens. The individuals that were used in the experiments were unsexed *P. truncatus* and *R. dominica* adults < 2 weeks old.

2.2. Commodities

Clean maize, *Zea mays* L. (var. Dias) that was free of previous infestation and pesticides was used in the tests. Prior to experimentation, the moisture content of grains was adjusted to 13.5 ± 0.5% as determined by a moisture meter (mini GAC plus, Dickey-John Europe S.A.S., Colombes, France). Thus, grains were dried inside an oven at 50 ^{o}C or by adding distilled water according to their initial moisture content (Kavallieratos et al., 2012, 2017b).

2.3. Bioassays

Three groups of insects of the two tested species were prepared in various density combinations as follows: 10 adults of *P. truncatus*, 10 adults of *R. dominica*, and 10 adults of *P. truncatus* with 10 adults of *R. dominica*. Each group was prepared three times. The groups were placed separately into cylindrical glass vials (7 cm diameter, 12 cm height) containing 40 g (Precisa XB3200D compact balance, Alpha Analytical Instruments, Gerakas, Greece) of maize. The lids of the vials had a 1.5 cm diameter opening in the middle that was covered by muslin gauze to permit sufficient aeration. To prevent insects from escaping, the internal walls of the vials were covered by polytetrafluoroethylen (60 wt % dispersion in water) (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) with swap, 1 d before the introduction of the insects inside the vials. Afterwards, all vials were put inside incubators (Elvem, Spata, Greece) set at 25 °C and 55% RH. The vials were opened after 65 days and the total numbers of individuals alive and dead per species within each vial were recorded. Identification of *P. truncatus* and *R. dominica* was based on Rees (2004). The numbers of damaged kernels by insects (kernels with holes and/ or chewings) per sample of 30 kernels per vial was recorded and the percentage of damaged kernels in each sample per vial was calculated. The progeny production and the damaged kernels by insects were determined under an Olympus stereomicroscope at 57x total magnification (SZX9, Bacacos S.A., Athens, Greece). The test was repeated three times, by preparing new series of insects and maize in new vials each time. Two new more series of insects, maize and vials were prepared as above, at the same conditions, but the vials were opened after 130 or 195 days. The same procedure was followed for 25 °C and 75% RH, 25 °C and 75% RH, 30 °C and 55% RH, 30 °C and 75% RH, 35 °C and 55% RH, 35 °C and 75% RH by preparing new series of insects, maize and vials.

2.4. Modeling specification

Based on the variables chosen and in order to assess the effect of the explanatory variables on the two dependent variables, i.e., the ratio of insects alive divided by the total number of insects (percentage of insects alive), and the percentage of damaged kernels, we utilize two separate HLM models (Raudenbush and Bryk, 2002). The model for the percentage of insects alive (progeny model) is a four-level hierarchical model in the sense that the model's independent variables are measured on different levels on the dependent variable (in this case four levels). The same structure holds for the model for the percentage of damaged kernels (damage model). The HLM belong to the family of Ordinary Least Squares (OLS) and are used when the predictor variables vary at more than one level. An important advantage of this methodology approach is that it provides the ability to perform a within- and between-group comparison. Multilevel models recognize this hierarchy, allowing for residual components at each level. Furthermore, in can incorporate missing data and multiple continuous and discrete depended variables in the same analysis (Raudenbush and Bryk, 2002). These types of models are most suitable for multilevel research, offering better capacity for handling the model complexity of nested correlated data, in comparison to cross-section or time-series models (Katahira, 2016).

Since it is rational to assume that the measurements taken from the same insect species are more likely to be correlated when compared with the measurements taken from different insect species, the possible within-insect species correlation in the data should be considered. In addition, nesting of measurements within other categories may also generate correlated observations, such as RH and temperature levels, which should also be taken into account for their modeling.

Specifically, the general framework of the HLM-type modeling approach follows the following format: There are n_{jk} replications ($i = 1,2,3$) responses on the two dependent variables, nested within each of the $j = 1, 2$ RH levels (i.e., 55 and 75%), in turn nested within each of $k = 1,2,3$ categories of temperature levels (i.e., 25 ^{o}C, 30 ^{o}C and 35 ^{o}C). Finally, for the progeny model, the measurements are nested within the variable of insect species combination ($\ell = 1,2,3$) (i.e., *R. dominica, P. truncatus* and their combination). Then, the fitted HLM models are expressed as:

Level - 1:

$$y_{ijk\ell} = \beta_{0ijk\ell} + \beta_{1ijk\ell} \cdot X_{ijk\ell} + e_{ijk\ell}$$

where $y_{ijk\ell}$ denotes the continuous response variable, $X_{ijk\ell}$ are the level-1 predictor variable and $e_{ijk\ell} \sim N\left(0, \sigma_e^2\right)$. With $\beta_{0ijk\ell}$ and $\beta_{1ijk\ell}$ we denote the intercept and slope of level-1 independent variable. As level-1 predictor we hypothesize the covariate of [replication], $X_{ijk\ell}$.

Level - 2:

The β-coefficient of replication slope is subsequently treated as response variable through the following equation:

$$\beta_{1ijk\ell} = b_{10jk\ell} + b_{1jk\ell} \cdot X_{jk\ell} + r_{jk\ell},$$

where $X_{jk\ell}$ is the level-2 predictor of [RH], the $b's$ are the intercept and slope of RH and $r_{jk\ell} \sim n\left(0, \sigma_r^2\right)$.

Next, we define the level-3 equation as follows:

Level - 3:

$$b_{1jk\ell} = \gamma_{0k\ell} + \gamma_{1k\ell} \cdot X_{k\ell} + u_{k\ell}$$

where $X_{k\ell}$ denotes the level-3 predictor of [temperature].

Finally, the level-4 equation is given by:

Level - 4:

$$\gamma_{1k\ell} = \delta_{0\ell} + \delta_{1\ell} \cdot X_{\ell} + v_{\ell},$$

with X_{ℓ} denoting the variable of [insect species].

In the previous model formulation, the outcome variables y_{ijk} measure the percentage of insects alive and percentage of damaged grains for the two models, respectively. The factors of replication, RH, temperature and insect species constitute the fixed-effects incorporated into our models. We have designated the subpopulation category of replication = “replication 1” as a reference category for the replication factor.

Accordingly, RH = “55”, temperature = “25 °C”, insect species = “*R. dominica*” are the reference categories assigned for the rest of the independent factors.

2.5. Assessment of model fit

In order to assess model fit, we obtain a model comparison approach, by starting fitting a null model (denoted by Model 1) including as predictors only the grand mean, i.e.:

$$y_{ijk\ell} = \beta_{0jk\ell} + e_{ijk\ell} \text{ (null Model1)}$$

and subsequently add one new predictor variable each time to perform the comparison between the two models.

In the current analysis, we are performing both model selection as well as covariate selection through a model comparison approach, which includes the comparison of all nested models, starting from the null model (which includes only an intercept as explanatory variable) and adding sequentially all (nested) covariates. The overall significance of each model is then evaluated through the deviance statistic (based on the likelihood of each model), which acts as a stand-alone measure of goodness-of-fit, as well as through the model comparisons. Thus the (non)-significance of each model is assessed through the (non)-significance of the added covariate in comparison to the previous model. Particularly, to test the significance of each model fitted we conduct likelihood ratio tests (LRT) comparing each time the initially fitted model (null model) with the alternative model including an additional parameter (Raudenbush and Bryk, 2002). (Model 2 is tested against the null Model 1 that includes only a grand mean term in the form of an intercept, subsequently Model 3 is tested against the Model 2 etc.).

The rational of the LRT is to compare the likelihood of the two models (Model i with Model i-1) with each other through the following likelihood ratio statistic:

$$D = 2 \times \left(\ln(likelihood_{Mi}) - \ln(likelihood_{Mi-1}) \right),$$

where with M_{i-1} we denote the reduced model and with M_i the model with the additional parameter.

2.6. Sensitivity analysis in the models parameters

A deterministic sensitivity analysis was performed to provide quantitative measurable results regarding the magnitude of impact of the explanatory indicators on the two models' dependent variables, i.e., the percentage of insects alive and the percentage of damaged kernels. Sensitivity analysis methods are suitable for understanding how the explanatory variables, contribute to the response, of statistical models in a simple and efficient way. The main reason is to determine which of the two model's inputs contribute most to the variability of the dependent variables. Conceptually, the common approach for performing sensitivity analysis is to repeatedly vary one parameter of an explanatory variable at a time while holding the others fixed at their medium values. Saltelli et al. (2000) provides an overview of the field of sensitivity analysis. To perform sensitivity analysis for our two models, the R package "pse" is utilized (Chalom and Prado, 2012). In particular, we used as input data for performing sensitivity analysis the estimation results obtained from the fit of the two HLM models, i.e., we utilized regression coefficient estimates along with the use of the uniform distribution to cover the complete range of values taken by the explanatory variables. These issues were formulated as an R function and subsequently the R package "pse" has been used for performing sensitivity analysis.

2.7. Implementation details

We have used the R software (R Core Team, 2012) and specifically the lme4 library (Bates et al., 2015) to fit the linear mixed effects hierarchical models of the relationship between our dependent variables and the explanatory factors. R's main commands for the fit of multilevel models are included in the lme4 library. We used the lmer() function to fit the linear mixed-effects multilevel models.

3. Results

3.1. Progeny model results

Table 1 shows the results of covariate comparisons for the Progeny HLM model. Specifically, the values of the likelihood ratio statistics (D) and corresponding significances (*P* values) show that, among the examined covariates, only replication effects are non-significant to explain variations of percentage of insects alive (P = 0.905). All remaining factors are significant ($P < 0.01$). Hence, RH, temperature and interspecific competition affect the percentages of insects alive and thus the population size of *P. truncantus* and *R. dominica*. Table 2 shows the best selected model's (Model 5 in Table 1) parameters estimates and corresponding 95% confidence intervals for the Progeny model.

The three replications do not differ significantly to each other, on their effect on the dependent variable of percentage of insects alive. As RH increases, we observed higher percentage of insects alive (b = 0.144; $P < 0.05$). On the other hand, increased levels of temperature significantly decreased the percentage of insects alive (b = - 0.091 and b = - 0.386 for temperatures of 30 ^{o}C and 35 ^{o}C, respectively, in comparison to the reference level of 25 ^{o}C). Finally, the combination of the two insect species *R. dominica* and *P. truncatus* lead to reduction in the percentages of insects alive (b = - 0.066; $P < 0.05$) in comparison to single species treatments.

3.2 Damage model results

The replication effects were not significant for the dependent variable (D = 12,770). On the contrary, RH, temperature and insect species effects are all important predictors for percentage of damaged kernels, since the stepwise inclusion of each one of these covariates improved model fit (Table 3). The corresponding parameter estimate results and corresponding significances are presented in Table 4. The increase in RH from 55 to 75% significantly increased the levels of percentage of damaged kernels (b = 6.745; $P < 0.05$). Variations in temperature levels also differentiated percentage of damaged kernels, with the latter being lower at 35 ^{o}C (b = - 5.783; $P < 0.05$). However no differences were observed between 25 ^{o}C and 30 ^{o}C. When *P. truncatus* was alone or competed with *R. dominica* the percentage of damaged kernels was increased (b = 10.29 and 11.487, respectively) in comparison to *R. dominica* alone.

Finally, Fig. 1 shows the assessment of model fit, for the two HLM models through the percentages of total variance of the dependents explained. The highest percentage of variance is explained by the first model (Progeny model), with the factors included explaining a total of approximately 66.3% of the total variance of the percentage of insects alive.

3.3. Sensitivity analysis in the models parameters

The results of sensitivity analysis of each significant explanatory variable for each of the two fitted models are summarized in Fig. 2 to 5. A scatterplot of the sensitivity analysis result displays the function of each parameter for the progeny model (Fig. 2). It is clear that the higher levels of sensitivity are due to RH and temperature. On the other hand, the dependent variable of percentage of insects alive was found to be less sensitive to changes of the insect species. Fig. 3 presents the partial rank correlation coefficients for each one of the covariates. This coefficient measures how strong the associations are between the dependent variable and each input parameter, after removing the effects of the rest of covariates. Accordingly, Fig. 4 shows the summary of results of sensitivity analysis for the damage model, where the percentage of damaged grains is varying with respect to the significant factors identified from HLM modeling, i.e., RH, temperature, and insect species. The percentage of damaged kernels is more sensitive to the changes in the values of RH and temperature; however the former is more robust to changes in the insect species variable. The sensitivity analysis results regarding the damage model are completed by the partial correlation coefficients shown in Fig. 5. The partial correlation coefficients measure how strong is the association between the damaged grains and each independent variable, after removing the effect of the other independents. Inspection of the graph verifies that all three independent variables are strongly associated with the damaged grains.

4. Discussion

Our results indicate that abiotic factors alter the population size of stored-product insects and the magnitude of damage in stored-products. As temperature increased from 25 to 35 °C, we detected reduced proportion of *R. dominica* and *P. truncatus* adults alive, which is depicted to the reduced percentage of damaged kernels, albeit insignificant between 30 and 35 °C. However, an increase of RH from 55 to 75% resulted in increased proportion of *R. dominica* and *P. truncatus* live adults, as well as in increased percentage of damaged kernels. It has been previously reported that elevated temperatures (34 – 36^{o}C) lead to rapid development of *R. dominica* (Birch, 1945; Mason and McDonough, 2012) and consequently to earlier death. Similarly, 35 ^{o}C caused high mortality (42%) to immature developmental stages (eggs, larvae and pupae) of *P. truncatus* (Bell and Walters, 1982). Relative humidity also plays an important role in the development of stored-product insects as the majority of them are favored by 60 – 80% (Rees, 2004; Mason and McDonough, 2012). Although both *R. dominica* and *P. truncatus* develop in a wide range of RH levels, they respond better to 70% and 70 – 80%, respectively (Bell and Walters, 1982; Driscoll et al., 2000; Edde, 2012).

The percentage of live adults of both tested species is negatively affected by the presence of a competitor. The reduced population sizes of *R. dominica* and *P. truncatus* in the presence of a competitor could be the result of either interference or exploitative competition. Interference competition occurs via direct interactions between foraging species (Begon et al., 1996). On the other hand, exploitative competition refers to situations where individuals are affected by the quantity of the remaining resources, which have been previously exploited and therefore have been depleted (Begon et al., 1996). We can not exclude any of these situations that take place between *R. dominica* and *P. truncatus*, since they have coexisted for a long time. Competition could start from the very early stages of their lives. Although, *R. dominica* and *P. truncatus* are internal feeders, newly hatched larvae of both species feed externally on the kernel and later bore inside (Hodges, 1986) and consequently, they easily compared with each other. In a previous competition study between *P. truncatus* and another internal feeder, the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), Giga and Canhao (1993) reported that *S.*

oryzae outcompeted *P. truncatus* at 25 ^{o}C. Contrary to *P. truncatus, S. oryzae* follows a different strategy to oviposit by laying the eggs directly inside the kernel and covers the hole with a secretion (Mason and McDonough, 2012). Thus, the advantage of insusceptible development falls towards *S. oryzae*. However, the competition continues when larvae of both species enter maize kernel given that only a certain number of adults can maximally emerge per single kernel, i.e., 5 in the case of *R. dominica* (Adams, 1976), and 6 in the case of *P. truncatus* (Vowotor et al., 1998). Further experimentation is needed to clarify these issues.

The presence of *P. truncatus* is more damaging than *R. dominica* on maize, based on the proportion of damaged kernels which are infested by each insect species. The size of *P. truncatus* differs from that of *R. dominica*. Adults and larvae of the former are 3.5 – 4 mm and maximally 10 mm, respectively vs. 2 – 3 mm and maximally 3.9 mm respectively of the latter (Potter, 1935; Farrell and Haines, 2002; Suma and Ruso, 2005). Therefore, *P. truncatus*, as a bigger species, which bears powerful mandibles (Farrell and Haines, 2002), would cause more elevated damage in the maize kernels than the smaller *R. dominica*. It should also be noted that the biomass of *P. truncatus* is about 4 times more than that of *R. dominica* (Demianyk and Sinha, 1988). The big body size of *P. truncatus* is linked with the food consumption that is needed to cover its developmental requirements and consequently with the losses of the infested commodities. Demianyk and Sinha (1988) reported that larval and adult consumption of *P. truncatus* reached 252 and 4210 J upon maize while the respective consumption of *R. dominica* upon wheat was 86 and 2637 J. Furthermore, Nansen and Meikle (2002) and Athanassiou et al. (2017b) have reported high population growth of *P. truncatus* in whole maize kernels, given that this commodity, along with dried cassava, is mostly preferred (Hodges, 1986).

Concluding, we expect our results to have bearing on the management of *R. dominica* and *P. truncatus*. The findings of the conducted statistical analysis showed that fluctuations in RH and temperature are crucial predictors of the insects' population size and damage potential. Insect species is also a major factor for the variability of the data. Therefore, the application of insecticides or other management strategies against *R. dominica* and *P. truncatus* should be adapted according to the presence of each species, alone or in competition, in conjunction with the prevailing conditions of RH and temperature of the storage environment. The fact that competition negatively affects the population growth of both species tested should not be overlooked since *P. truncatus* exhibits strong ravenous activity on preferred commodities (Demianyk and Sinha, 1988; Athanassiou et al. 2017b). Competition could be considered as a natural vehicle of moderation of the activity of both species but also working as a reservoir of potential elevated damage if the participating species are splitted and start developing without competitor. This is a realistic scenario since both species are able to bore packaging material and easily escape (Kavallieratos et al., 2017a). Given that *P. truncatus* is under quarantine inspection in several countries, further competition studies involving it with other major stored-product insects will shed light on the complexity of associations among species that regulate damages during storage.

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Fig. 1. Percentage of variance of dependent variables explained by the two fitted HLM models.

Fig. 2. Sensitivity analysis plots for the progeny model.

Fig. 3. Partial correlation coefficients (PRCC) for the progeny model.

Fig. 4. Sensitivity analysis plots for the damage model.

Fig. 5. Partial correlation coefficients (PRCC) for the damage model.

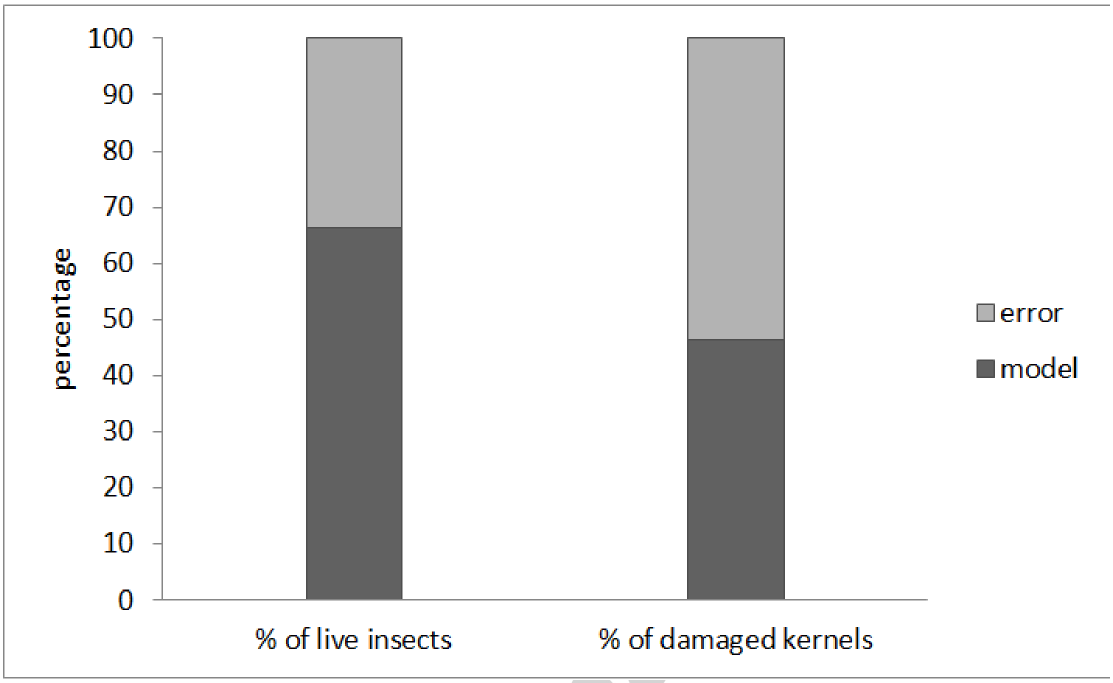

Fig. 1

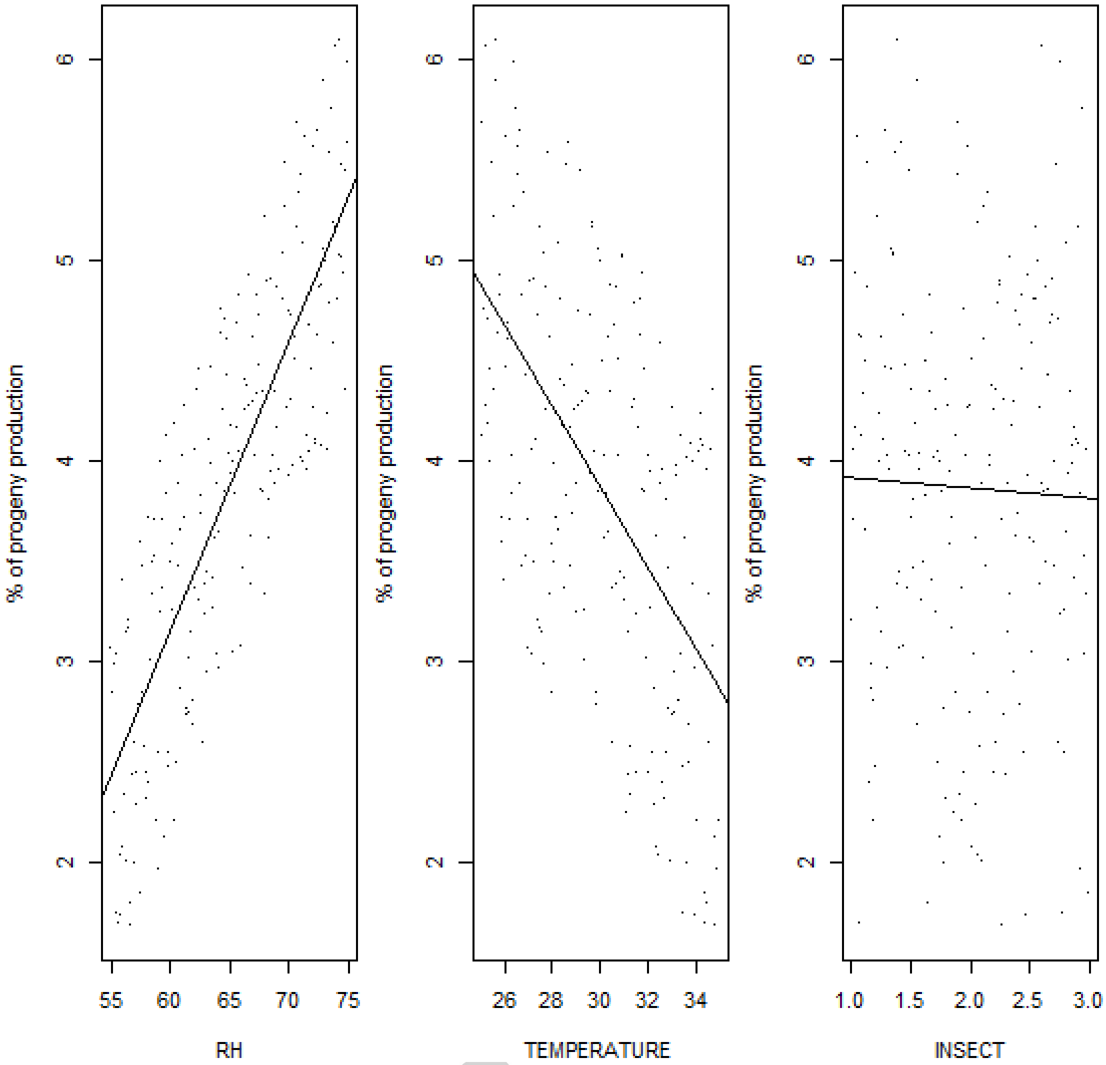
% of progeny production
6
5
4
3
2
55
60
65
70
75
RH
% of progeny production
26
28
30
32
34
TEMPERATURE
% of progeny production
1.0
1.5
2.0
2.5
3.0
INSECT

Fig. 2

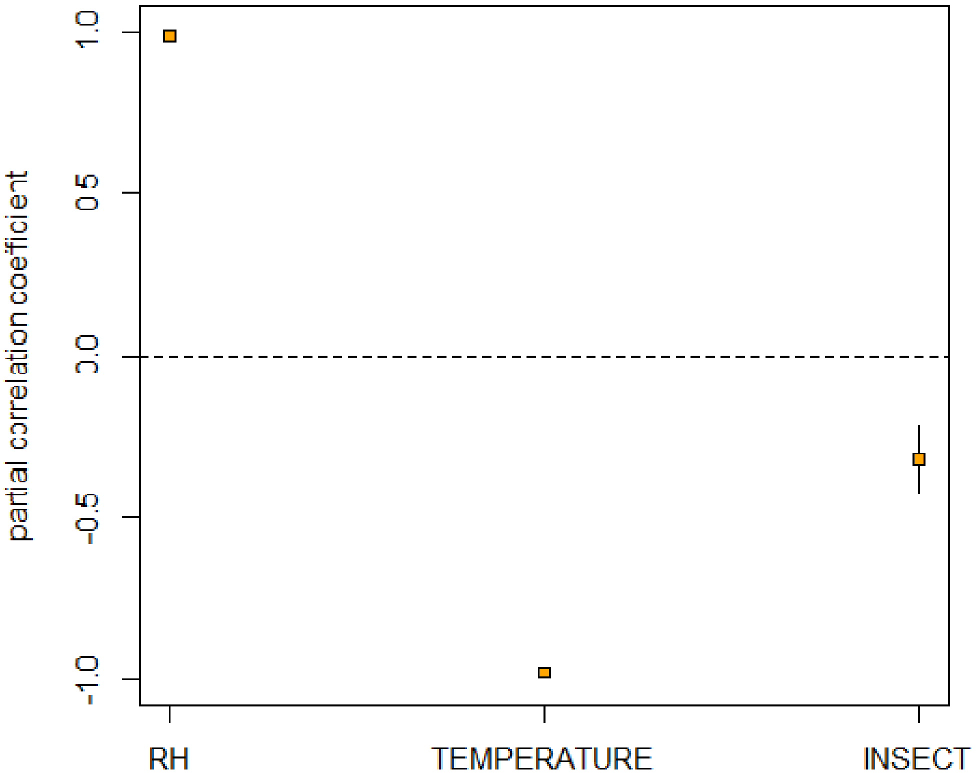
partial correlation coefficient
1.0
0.5
0.0
-0.5
-1.0
RH
TEMPERATURE
INSECT

Fig. 3

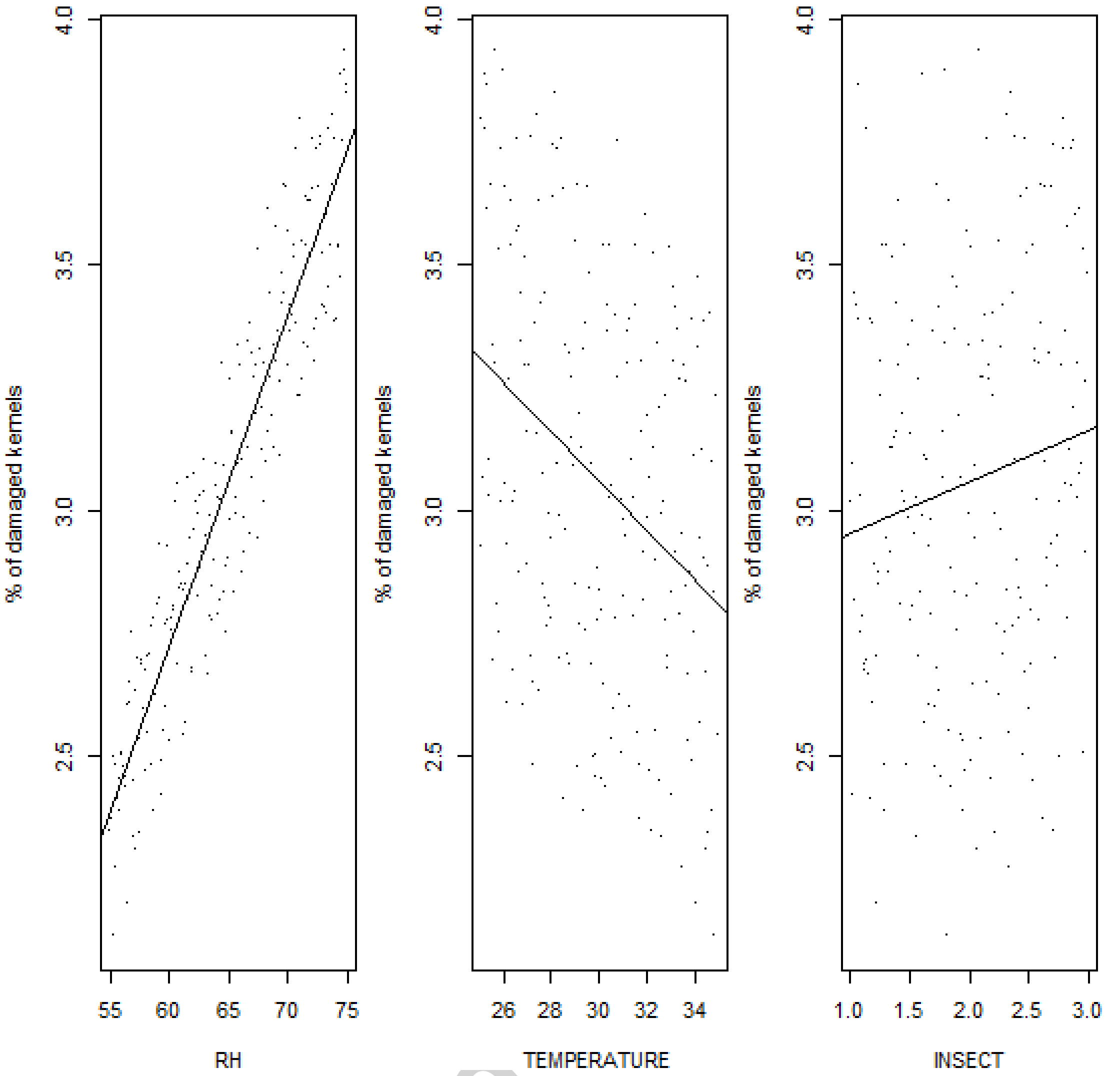
% of damaged kernels
4.0
3.5
3.0
2.5
55
60
65
70
75
RH
% of damaged kernels
4.0
3.5
3.0
2.5
26
28
30
32
34
TEMPERATURE
% of damaged kernels
4.0
3.5
3.0
2.5
1.0
1.5
2.0
2.5
3.0
INSECT

Fig. 4

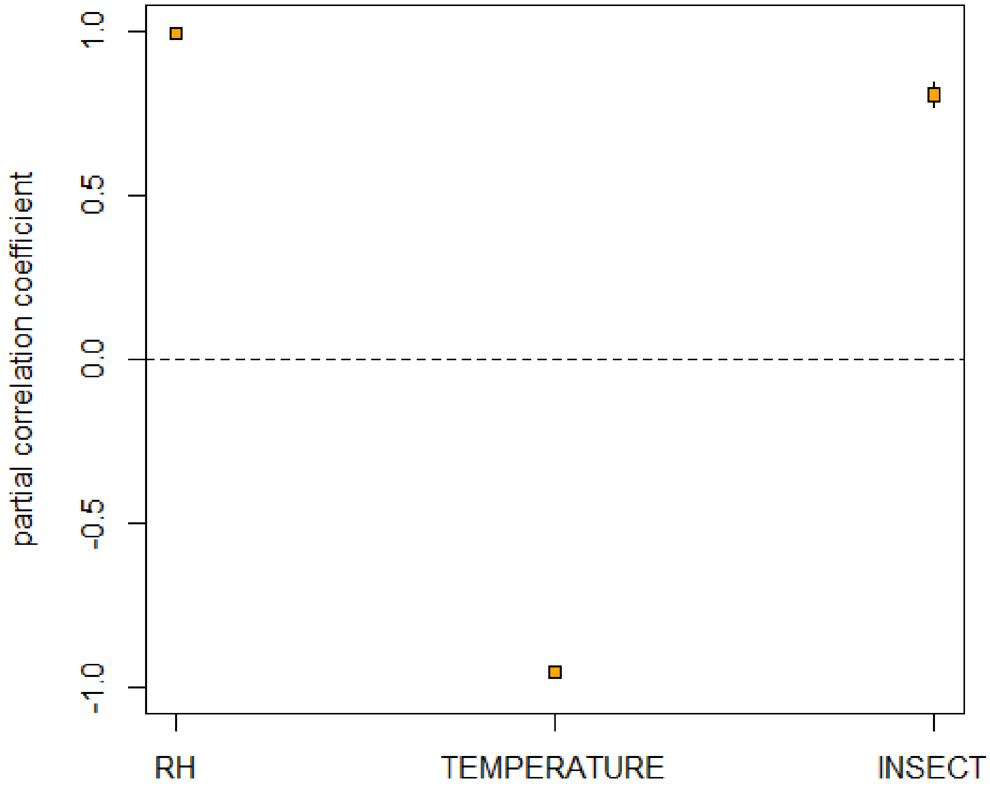
partial correlation coefficient
1.0
0.5
0.0
-0.5
-1.0
RH
TEMPERATURE
INSECT

Fig. 5

Table 1 **Model comparisons of the HLM models via likelihood ratio test (LRT) (D: likelihood ratio statistic; *P*: p-value of the statistical significance of LRT).**

	Dependent variable	
	D	***P***
Model 1 (Null Model)	341.32	----
Model 2 (Replication effects)	341.12	n.s.
Model 3 (Model 2 + RH effects)	305.59	**
Model 4 (Model 3 + temperature effects)	80.67	**
Model 5 (Model 4 + insect species effects)	67.60	**

*Significant at a 5% level of significance

**Significant at a 1% level of significance

n.s.: non-significant

Table 2 Parameter estimates for the best selected model [MODEL 5] (5% level of significance) along with the 95% confidence intervals.

Covariate	**Estimate**	**95% confidence intervals**
Intercept	0.608	(0.421, 0.789)
Replication (ref. category: replication 1)		
Replication 2	- 0.005	(- 0.053, 0.042)
Replication 3	0.007	(- 0.040, 0.055)
RH (Ref. category: RH 55%)		
RH 75%	0.144	(0.105, 0.184)
Temperature (Ref. category: 25 °C)		
Temperature 30 °C	- 0.091	(- 0.139, - 0.042)
Temperature 35 °C	- 0.386	(- 0.434, - 0.338)
Insect species (Ref. category: *R. dominica*)		
P. truncatus	0.011	(- 0.062, 0.096)
R. dominica - *P. truncatus*	- 0.066	(- 0.119, - 0.007)

Table 3 Model comparisons of the HLM models via likelihood ratio test (LRT) (D: likelihood ratio statistic; *P*: value of the statistical significance of LRT).

	Dependent variable	
	D	***P***
Model 1 (Null Model)	12,770	----
Model 2 (Replication effects)	12,770	n.s.
Model 3 (Model 2 + RH effects)	12,724	**
Model 4 (Model 3 + temperature effects)	12,691	**
Model 5 (Model 4 + insect species effects)	12,575	**

[*] Significant at a 5% level of significance

[**] Significant at a 1% level of significance

n.s.: non-significant

Table 4 Parameter estimates for the best selected model [MODEL 5] (5% level of significance) along with the 95% confidence intervals.

Covariate	**Estimate**	**95% confidence intervals**
Intercept	- 3.647	(- 6.558, - 0.736)
Replication (ref. category: replication 1)		
Replication 2	- 0.169	(- 2.424, 2.087)
Replication 3	- 0.189	(- 2.445, 2.065)
RH (Ref. category: 55%)		
RH 75	6.745	(4.903, 8.586)
Temperature (Ref. category: 25 °C)		
Temperature 30 °C	0.444	(- 1.811, 2.699)
Temperature 35 °C	- 5.783	(- 8.038, - 3.527)
Insect species (Ref. category: *R. dominica*)		
P. truncatus	10.290	(8.035, 12.546)
R. dominica - P. truncatus	11.487	(9.231, 13.743)

Highlights

- Temperature and RH alter the population size of stored-product insects
- Temperature and RH alter the magnitude of damage in stored-products
- Temperature and RH affected the % survival and level of kernel damage (%)
- Competition reduced the % survival and level of kernel damage (%)