1 **Title**

2 Geostatistical analysis of adult *Rhyzopertha dominica* (F.) (Coleoptera:

3 Bostrichidae) in wheat stored at constant temperatures.

4

5 **Authors**

6 Roderic Steel[1], David Elmouttie[1], Grant Hamilton[1]

7

8 **Addresses**

9 [1] Earth, Environmental and Biological Sciences, Queensland University of

10 Technology, GPO Box 2434, Brisbane, Queensland, Australia, 4001.

11

12 **Corresponding Author**

13 Grant Hamilton

14 Earth, Environmental and Biological Sciences, Queensland University of

15 Technology, GPO Box 2434, Brisbane, Queensland, Australia, 4001.

16 Ph: +61 7 3138 2318

17 Fax: +61 7 3138 1535

18 Email: g.hamilton@qut.edu.au

1   1.   **Introduction**

2

3   Insect pests of stored products are a major problem worldwide, resulting in both

4   direct loss of production and indirect losses due to secondary infestation and trade

5   restrictions (Oerke, 2005; Adam et al., 2006; Fornal et al., 2007). This can result in

6   considerable economic impact to growers, bulk handlers, and distributors (Hagstrum

7   and Subramanyam, 2006, pp14-21; Adam et al., 2010). Management practices for

8   pests typically involve monitoring and sampling programmes for the detection of pest

9   insects, or for estimation of the density of insects in grain bulks (Flinn and Hagstrum,

10   1990; Flinn et al., 2007). While practices vary worldwide depending upon country,

11   region and producer (Kogan, 1998; Jefferies, 2000; Adam et al., 2006), a common

12   requirement is that infestations be detected at low levels in order to minimise the

13   costs of both insect damage and treatment (Adam et al., 2006).

14

15   The spatial distribution of insects influences detection and abundance estimates

16   (Hagstrum et al., 1985; Trematerra et al., 2007; Elmouttie et al., 2010; Athanassiou

17   et al., 2011), and this can be especially relevant at low infestation rates (Taylor,

18   1984; Hagstrum, 2000). As a result, infestations can remain undetected when

19   abundances are low and sampling effort restricted (Gu and Swihart, 2004). As grain

20   commodities are stored in large quantities, and sampling costs increase with the

21   number of samples taken, generally only a small portion of a grain lot is sampled

22   (Binns and Nyrop, 1992; Adam et al., 2010).

23

24   It is clear that a better understanding of the spatial distribution of pests in stored

25   grain can help to improve both pest detection and treatment methods (Taylor, 1984;

26   Hagstrum et al., 1985). However, the method used to resolve spatial distribution

27 impacts on the inferences that can be drawn (Stejskal et al., 2010). For example,

28 previous research in large grain bulks has found that spatial distribution is influenced

29 by factors such as seasonal variations in moisture and temperature (Hagstrum,

30 1987; Flinn et al., 2004), and interspecies associations (Nansen et al., 2009;

31 Hagstrum et al., 2010). While these studies have provided valuable information,

32 assessing exactly which factors are responsible for particular effects can be

33 challenging, as it is difficult or impossible to control for interactions between these

34 and other environmental factors (Athanassiou et al., 2011).

35

36 Conversely, previous laboratory-based studies have focussed on evaluating the

37 influence of individual environmental factors on insect movement. Largely, these

38 have used smaller 2-D systems (e.g. Flinn and Hagstrum, 1998; Jian et al., 2003;

39 Jian et al., 2005) or low sampling intensities in larger 3-D systems (e.g. Plarre, 1996;

40 Collins and Conyers, 2009; Jian et al., 2011). While these studies have also provided

41 valuable information, the restrictions of a 2-D environment or low sampling intensity

42 limit their ability to accurately define pest distributions in 3 dimensions, restricting the

43 understanding of spatial distribution in representative systems at a fine scale.

44 Combining the use of intensive sampling with a representative 3 dimensional system

45 would improve understanding of pest spatial distributions.

46

47 To fully understand the effect of individual environmental factors on the spatial

48 distribution of grains pests, establishing a 'baseline' spatial distribution for

49 comparison is highly useful. In this study, we develop new methods to create a 'null

50 model' using a geostatistical approach to spatial analysis (Davis, 1994). We then

51 apply this technique to examine the effect of a single environmental parameter (grain

52 temperature) on the spatial distribution of a typical stored product pest insect. This

53 knowledge may then be used as a reference for future studies examining the effect

54 of other factors on spatial distribution. Ultimately it is expected that the improved

55 understanding of these studies can be applied to the improvement of population and

56 detection sampling models, with the aim of enhancing pest management practices

57 (Phillips and Throne, 2010).

58

59 **2.   Materials and Methods**

60

61 **2.1   Insects and Grain**

62 The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), was

63 chosen for this study. Both larvae and adults are internal feeders, causing

64 considerable damage to grain. Due to its wide tolerance of environmental conditions,

65 it is an economically important pest worldwide (Osuji, 1982; Fields et al., 1993).

66

67 Insects for this study were obtained from the Department of Employment, Economic

68 Development and Innovation, Queensland, Australia. Cultures originated from a wild

69 strain collected during May 2010 near Surat in the western Darling Downs region.

70 Cultures were maintained on whole wheat at 30°C and 55% r.h. (Hagstrum and

71 Subramanyam, 2006; Flinn and Friesen, 2010). As adult *R. dominica* are difficult or

72 impossible to accurately sex without negatively affecting survival or reproductive

73 potential (Sinclair, 1981; Edde, 2012), no attempt was made to sex selected adults.

74 To maximise the probability of obtaining an optimum sex ratio while remaining within

75 the confines of typical economically important infestation rates (<2 adults / kg), 30

76 recently emerged (0-7 day old) adults were randomly selected for use in each

77 experimental replicate.

78

79 Certified organic, pesticide-free (CO_2-treated) Australian Prime Hard wheat (*Triticum*

80 *aestivum* (L.)) was used in all experiments. To ensure freedom from live pests and

81 viable eggs, all grain was frozen (-18°C) for 2 weeks, followed by storage at 5 ±1°C

82 until required for use (Fields, 1992). At the commencement of experiments, 80 litres

83 (~ 20%) of grain was randomly selected and inspected for pests using a 2mm

84 stainless steel mesh grain hand-sieve. No adult insects were found.

85

2.2   Lot preparation

87   Twenty litre food grade polypropylene containers (26w x 26d x 39h cm) were used

88   for all experiments. A rubber bung, used for the later introduction of CO_2 to euthanize

89   insects, was fitted near the base of each container. Grain was transferred from

90   refrigerated storage and 20 litres measured into each container. Containers were

91   then covered with a tight fitting polyester mesh to prevent insect ingress / egress

92   whilst allowing air circulation. These were then transferred to pre-conditioned

93   temperature controlled cabinets and allowed to acclimatise for 48 hours to ensure

94   even temperature distribution throughout the grain mass. Each container formed one

95   experimental lot.

96

2.3   Experimental design

98   Four experimental treatments were conducted, consisting of three replicates each. A

99   30°C 2 week control treatment was undertaken as a comparison baseline for other

100   treatments, based on a fixed known population and time. The three remaining

101   treatments were undertaken for 1 generation at 25, 30, and 35°C (Table 1).

102   Generation times for these treatments were calculated using existing population and

103   developmental time models (Wagner et al., 1984; Hagstrum and Milliken, 1988;

104   Driscoll et al., 2000) and published data (Hagstrum and Subramanyam, 2006, p. 98).

105

106   Thirty insects were introduced to the top centre of the grain surface, and each lot

107   incubated in temperature controlled (± 0.5°C) cabinets for the duration of each

108   treatment. Humidity in the cabinets was controlled to 55% ± 5% r.h. using a

109   saturated sodium bromide solution (Greenspan, 1977). Temperature and humidity

110   were monitored using environmental dataloggers.

111

2.4   Sample Preparation

113

After incubation, insects were euthanized by the introduction of CO_2 gas and

refrigeration (5 ±1°C) for a minimum of 10 days. Prior to sample preparation, lots

were frozen (-24 ±2°C) for a minimum of 48 hours to assist in preparation.

117

Granulated gelatine from a commercial supplier was used to prepare a liquid solution

(60g/L) according to the two-step process described in Schrieber and Gareis (2007,

pp. 138-141). Individual lots were removed from the freezer immediately prior

sample preparation, and the gelatine solution poured evenly to fill the container using

a mesh spreader to eliminate disturbance of the grain and insects. Once poured, lots

were frozen (-24 ±2°C) for two hours to rapidly set the gel before being held in

refrigerated storage (5°C ±1°C) for a minimum of 72 hours.

125

A wooden form with cutting guide slots spaced at 5cm intervals along opposing sides

was used to prepare samples. Once set, the lot was removed from the container and

placed in the form, aligned so that slice thickness was referenced to the top surface

of the grain, secured in place, and cut into 5cm lateral slices. Each slice was then

placed on a cutting board marked with a 5cm^2 grid, and cut into 5cm cubes. The

location of each sample was recorded in an X-Y-Z co-ordinate system before being

frozen (-24°C ± 2°C) until examination. A total of 200 samples, accounting for >95%

of the total volume, were produced from each lot.

134

2.5   Sample Examination

136   Samples were placed in a large plastic beaker, the gel dissolved with hot water,

137   and the grain transferred to an examination tray for visual inspection. Adult insects

138   recovered outside of grain kernels were counted as 'loose adults'. Individual grain

139   kernels were then examined for signs of internal insects, and kernels identified were

140   dissected and examined under a microscope. Adult insects (with mature abdominal

141   and elytra colouring) were recorded as "in-grain adults". The total count of all adults

142   within each sample was used for all analyses.

143

144   **2.6   Data Analysis**

145   All data analysis was undertaken using the statistical application and programming

146   language R (R Core Development Team, 2011). The standardised Morista index I_p

147   (Smith-Gill, 1975; Krebs, 1999, pp. 216-217) was used to compare dispersion

148   between treatments and replicates. This index ranges from +1 (clumped) to -1

149   (dispersed), with ±0.5 being the 95% confidence limits around random patterns ($I_p =$

150   0). Clusters were defined as spatially-contiguous groups of samples occupied by at

151   least one insect. Two-level mixed-model nested ANOVAs with post-hoc Tukey's

152   HSD tests were used to identify significant variations in mean sample abundance

153   between all experiments (top level, fixed effects) and replicates (level 2, random

154   effects). Similarly, to detect significant differences in insect vertical movement,

155   nested ANOVAs were used to identify significant variations in mean per-layer (Z-

156   axis) abundance between experiments and replicates. Figures were created using

157   R's 'lattice' (Sarkar, 2008) and 'graphics' (R Core Development Team, 2011)

158   packages.

159

160   Spatial correlations between sample abundance and inter-sample distance were

161   calculated using the 'gstat' package (Pebesma, 2004) in R. Correlation coefficients

162 between sample abundances were calculated in one 2-dimensional horizontal (H)

163 plane (X-Y), two 2-dimensional vertical (V) planes (X-Z and Y-Z), and in 3-

164 dimensional (X-Y-Z) space (D). Distances between samples were calculated as the

165 straight line (Euclidean) distance between sample centres; results that were fractions

166 of the sample size were grouped into 5cm categories. Correlation coefficients were

167 calculated between all occupied samples and those located a given distance class

168 away. For example, V(0,[5-10]) represents the correlation coefficient between

169 samples spaced from 5 to 10cm apart in the vertical direction, while D(0,[10-15])

170 represents the correlation coefficient between samples spaced 10 to 15cm apart in

171 any direction. Significance thresholds for correlation coefficients were determined

172 using the method outlined by Anderson (1942), which accounts for differing spatial

173 distances and sample abundances. As the extra dimension used in 3D analysis

174 influences the significance of correlations compared to 2D analysis, significance for

175 geostatistical analyses were determined at p=0.10.

176

177 **3.   Results:**

178

179 A total of 2400 samples were examined across the four treatments. Abundance

180 varied with temperature, and the number of occupied samples increased with insect

181 abundance (Table 1). Between-replicate variance in abundance increased with

182 temperature (Table 1). Irrespective of treatment and replicate, spatial distributions

183 were clumped (*Ip* > 0.5). The number of discrete clusters identified within each

184 replicate was also similar (mean = 2.45, SE = 0.463) across all treatments, with the

185 exception of replicate 1 in the control experiment where 6 distinct clusters were

186 identified.

187

188 Sample abundance differed significantly between treatments ($F_{3,84}$ = 4.8574, p =

189 0.004), but no difference was found between replicates within treatments ($F_{8,84}$ =

190 0.4096, p = 0.912). The 30°C treatment was significantly different in sample

191 abundance compared to both the control (p = 0.004) and 25°C (p = 0.019)

192 treatments (post-hoc Tukey's HSD). Mean sample abundance between treatments

193 varied significantly with sample depth ($F_{28,64}$ = 8.2274, p < 0.001), with post-hoc

194 Tukey's HSD tests showing that the 35°C treatment was significantly different to the

195 control (p = 0.005), 25°C (p = 0.045), and 30°C (p = 0.029) treatments. Sample

196 abundance at 35°C and >15cm from the top differed significantly (p < 0.001) from

197 the control, 25°C and 30°C treatments. In the control, 25°C, and 30°C treatments,

198 abundances were highest at 0-15cm from the grain surface, with insect numbers at

199 these depths accounting for >80% of total insects in each case. At 35°C, abundance

200 peaked at 15-30cm from the grain surface, with > 70% of insects found within this

201 range (Figures 1 & 2).

202

203 Examination of between-sample covariance was performed in both 2 and 3

204 dimensions. Across all treatments, in the horizontal [X-Y] plane there was a trend of

205 decreasing correlations with increasing distance, with results significant ($p \leq 0.10$) at

206 25°C (10-15cm) and 35°C (0-5cm and 10-15cm) (Table 2). This same trend was also

207 evident in the vertical [X-Z] and [Y-Z] planes (Tables 3 & 4). At 30°C and 35°C,

208 significant ($p \leq 0.10$) positive correlations were found at distances of 0-10cm, while

209 negative correlations were significant at distances $\geq$ 10cm in the 25°C, 30°C, and

210 35°C treatments. This pattern of decreasing correlations with increasing distance

211 was also evident when examined in 3 dimensions (Table 5). At 30°C and 35°C,

212 significant ($p \leq 0.10$) positive correlations were found at distances of 0-10cm.

213   Significant ($p \leq 0.10$) negative correlations were found at distances $\geq$ 10cm in the

214   25°C, 30°C, and 35°C treatments.

215

216   **4.   Discussion:**

217

218   Field based studies of large grain lots are useful to examine the spatial distribution of

219   grain pests in real systems (Hagstrum et al., 1985; Lippert and Hagstrum, 1987).

220   Nonetheless, the spatial resolution of data in such studies are typically limited by

221   factors such as available sampling methods, difficulty in accessing all parts of the

222   grain bulk, and relatively low sampling intensity. Additionally, the effect of individual

223   factors such as temperature and moisture gradients or inter- and intra-species

224   competition on pest spatial distribution has been difficult to isolate. For these

225   reasons, high resolution laboratory studies allow for the collection of data by isolating

226   single factors and gathering data at an appropriate resolution for the particular

227   question at hand. In a recent study, Jian et al. (2011) examined insect movement

228   and spatial structure in a large (1.5 tonne) laboratory volume. The high sampling

229   intensity (~15% of the total volume) employed in that study was sufficient to show

230   spatial structuring of the pest population occurred, but large sample sizes (~15kg)

231   restricted the ability to examine the spatial structure in detail. Again, while this design

232   was appropriate for the questions Jian et al. (2011) were examining, fine-scale

233   spatial structuring of populations could not be assessed.

234

235   In the current study, adult *R. dominica* were found to establish a spatially

236   heterogeneous distribution pattern in grain within 2 weeks of introduction. The

237   observed pattern of horizontal dispersion was similar across all treatments, but

238   vertical dispersion was found to differ considerably at 35°C (Figures 1 and 2), with

239   the majority of insects being found further down in the grain mass. Such spatial

240   structuring, found consistently across replicates in each experiment, is unlikely to

241   occur due to random insect movement. This suggests that behavioural variations

242   due to environmental conditions are an important influence on spatial distribution.

243   Flinn et al. (2011) showed that *R. dominica* tends to avoid temperatures above 35°C,

244   favouring areas where the temperature was below 32°C. In the current study, such

245   avoidance was not possible as grain temperature was constant throughout the

246   volume. Our results suggest that where avoidance is impossible vertical dispersion is

247   increased, with insects moving further into the grain mass. *R. dominica* is known to

248   move deeper into bulk-stored grain than other grain pest species (Flinn et al., 2010).

249   This behaviour appears to be enhanced at higher temperatures, potentially

250   increasing the difficulty of detection and estimation of infestations. While higher

251   temperatures such as these are close to the limit of *R. dominica*'s environmental

252   tolerance (Longstaff, 1999; Hagstrum and Subramanyam, 2006), such temperatures

253   can be found inside bulk grain storages in warmer grain producing regions (Flinn et

254   al., 2004).

255

256   Insect abundance had little effect on patterns of spatial distribution. Observed

257   dispersion patterns were consistent within each treatment and at insect densities

258   ranging from approximately 1.5 insects / L to more than 8 insects / L. Abundance in

259   each treatment was found to be lower than predicted by the population model used

260   (Driscoll et al., 2000). This model assumes a stable age structure, which would not

261   be the case within one generation of initial pest introduction, and hence is likely to

262   over-estimate populations in this scenario. However, the low abundance found at

263   25°C indicated an unexpectedly low population growth rate of only ~50% per

264   generation. Conversely at 35°C there was higher variation in abundance between

265   replicates. While the model used does not predict population variance, it is

266   occasionally accounted for in other models (e.g. Hagstrum, 1996). In cases where

267   accurate estimation of population after one generation is required, a population

268   growth model accounting for variable age structure would be required.

269

270   It was found that analysis in 3 dimensions allowed for the easy identification of

271   strong correlations between sample abundances at varying distances. However, in

272   the absence of a directional component to individual correlations, it was not able to

273   describe the variations from a basic spherical diffusion pattern found. It was also

274   found that correlations in one plane tended to oppose those in other planes, reducing

275   the significance of the overall result. For example, negative correlations at 10-15cm

276   in the vertical direction of the 30°C treatment affected the positive correlations found

277   at this distance in the horizontal direction, reducing the significance of both.

278   Conversely, performing individual 2 dimensional analyses in the X-Y, X-Z, and Y-Z

279   planes, while slightly more complex to undertake and interpret, allowed for the

280   identification and evaluation of the wide horizontal but limited vertical dispersion

281   pattern found. In cases where the direction of dispersion or shape of aggregations is

282   unknown, performing 2 dimensional correlations in multiple planes may provide a

283   more accurate description of the observed spatial pattern when compared to 3

284   dimensions.

285

286   There is little available data on the movement rates of R. dominica in stored grain.

287   Field-validated modelling studies (e.g. Flinn et al., 2004) have suggested a dispersal

288   rate of approximately 1.2 meters per week at 29°C in a 3-dimensional storage.

289   Laboratory studies under controlled conditions (Surtees, 1964a; Surtees, 1964b) in

290   a 3D volume have suggested a rate of more than 15cm per week (at 25°C), and a

291   spatial distribution approaching homogeneity. In contrast, this study shows

292   considerable difference in insect movement rates in the vertical and horizontal

293   directions, with variations in the resultant spatial structure occurring over distances

294   as short as 10-15cm. This results in a significantly non-random spatial distribution,

295   which in turn can influence the results of sampling and predictions based on an

296   underlying assumption of random spatial distribution.

297

298   Several previous studies have examined the spatial structure of insects in grain

299   storages (Flinn et al., 2010; e.g. Athanassiou et al., 2011). While results from these

300   studies indicated similar patterns of insect spatial distribution at larger scales, the

301   relatively large sample sizes and low sampling intensity used did not allow for

302   analysis of variations in population structure over relatively short distances. As our

303   results show, these short distance variations in structure are an important feature of

304   insect clustering. The insect densities used in the current study (1.5 – 8 pests / L)

305   were similar to those required by phytosanitary regulations, commercial

306   requirements, or used in similar studies (Food and Environment Research Agency,

307   2009; Grain Trade Australia, 2011; Jian et al., 2011). The use of appropriately-sized

308   and regularly spaced samples to examine almost 100% of the grain volume

309   (ensuring an accurate population count) both enhances the ability to detect pest

310   aggregations and minimises the influence of sample edge effect (Stenseth and

311   Hansson, 1979; Davis, 1994).

312

313   An improved understanding of the factors affecting pest spatial distribution can be

314   used to inform not only spatially-explicit population models (Thorpe, 1997), but also

315   abundance and detection sampling models (Hagstrum et al., 1985; Flinn et al., 1992;

316   Elmouttie et al., 2010). The observed variation in spatial pattern with temperature, in

317 particular the differences in mean abundance versus depth found at higher

318 temperatures, potentially increases the difficulty of detection and estimation of

319 infestations. These results highlight the fact that temperature and other

320 environmental factors need to be explicitly considered when developing and

321 choosing methods and protocols for detection and abundance sampling of pests. As

322 such small-scale spatial structuring of populations was previously unknown in *R.*

323 *dominica*, this suggests that other pest species may also exhibit spatial structure at

324 similar scales. Further study of this aspect of behaviour in other grain pests is

325 required to determine if this may affect detection and abundance sampling for those

326 species.

327 **References:**

328 Adam, B.D., Phillips, T.W., Flinn, P.W., 2006. The economics of IPM in stored grain: Why

329   don't more grain handlers use IPM? In: Lorini, I., Bacaltchuk, B., Beckel, H.,

330   Deckers, D., Sundfeld, E., dos Santos, J.P., Biagi, J.D., Celaro, J.C., Faroni, L.R.D'A.,

331   Bortolini, L.De O.F., Sartori, M.R., Elias, M.C., Guedes, R.N.C., da Fonseca, R.G.,

332   Scussel, V.M. (Eds.), Proceedings of the 9th International Working Conference on

333   Stored Product Protection, 15-18 October 2006, Campinas, Sao Paulo, Brazil. pp

334   3-12.

335 Adam, B.D., Siaplay, M., Flinn, P.W., Brorsen, B.W., Phillips, T.W., 2010. Factors

336   influencing economic profitability of sampling-based integrated pest

337   management of wheat in country elevators. Journal of Stored Products Research.

338   46, 186-196.

339 Anderson, R.L., 1942. Distribution of the serial correlation coefficient. The Annals of

340   Mathematical Statistics. 13, 1-13.

341 Athanassiou, C.G., Kavallieratos, N.G., Sciarretta, A., Palyvos, N.E., Trematerra, P., 2011.

342   Spatial Associations of Insects and Mites in Stored Wheat. Journal of Economic

343   Entomology. 104, 1752-1764.

344 Binns, M.R., Nyrop, J.P., 1992. Sampling insect populations for the purpose of IPM

345   decision making. Annual Review of Entomology. 37, 427-453.

346 Collins, L.E., Conyers, S.T., 2009. Moisture content gradient and ventilation in stored

347   wheat affect movement and distribution of Oryzaephilus surinamensis and have

348   implications for pest monitoring. Journal of Stored Products Research. 45, 32-39.

349 Davis, P.M., 1994. Statistics for describing populations. In: Pegigo L.P., Buntin E.D. (Eds.),

350   Handbook of sampling methods for Arthropods in agriculture. CRC Press, Boca

351   Raton, FL.

352   Driscoll, R., Longstaff, B.C., Beckett, S., 2000. Prediction of insect populations in grain

353   storage. Journal of Stored Products Research. 36, 131 - 151.

354   Edde, P., 2012. A review of the biology and control of Rhyzopertha dominica (F.) the

355   lesser grain borer. Journal of Stored Products Research. 48, 1 - 18.

356   Elmouttie, D., Kiermeier, A., Hamilton, G., 2010. Improving detection probabilities for

357   pests in stored grain. Pest Management Science. 66, 1280-1286.

358   Fields, P.G., 1992. The control of stored-product insects and mites with extreme

359   temperatures. Journal of Stored Products Research. 28, 89-118.

360   Fields, P.G., Van Loon, J., Dolinski, M.G., Harris, J.L., Burkholder, W.E., 1993. The

361   distribution of Rhyzopertha dominica (F.) in western Canada. Canadian

362   Entomologist. 125, 317-328.

363   Flinn P.W., Friesen K., 2010. Host and Parasitoid Rearing. USDA Agricultural Research

364   Service, Manhattan, KA, USA. URL =

365   http://ars.usda.gov/Research/docs.htm?docid=12885. Last accessed 10 Oct.

366   2010.

367   Flinn, P.W., Hagstrum, D.W., 1990. Stored Grain Advisor: a knowledge-based system for

368   management of insect pests of stored grain. AI Applications in Natural Resource

369   Management. 4, 44-52.

370   Flinn, P.W., Hagstrum, D.W., 1998. Distribution of Cryptolestes ferrugineus (Coleoptera:

371   Cucujidae) in response to temperature gradients in stored wheat. Journal of

372   Stored Products Research. 34, 107 - 112.

373   Flinn, P.W., Hagstrum, D.W., 2011. Movement of Rhyzopertha dominica in response to

374   temperature gradients in stored wheat. Journal of Stored Products Research. 47,

375   407-410.

376 Flinn, P.W., Hagstrum, D.W., Muir, W.E., Sudayappa, K., 1992. Spatial model for

377   simulating changes in temperature and insect population dynamics in stored

378   grain. Environmental Entomology. 21, 1351-1356.

379 Flinn, P.W., Hagstrum, D.W., Reed, C., Phillips, T.W., 2004. Simulation model of

380   Rhyzopertha dominica population dynamics in concrete grain bins. Journal of

381   Stored Products Research. 40, 39-45.

382 Flinn, P.W., Hagstrum, D.W., Reed, C.R., Phillips, T.W., 2007. Stored Grain Advisor Pro:

383   Decision support system for insect management in commercial grain elevators.

384   Journal of Stored Products Research. 43, 375-383.

385 Flinn, P.W., Hagstrum, D.W., Reed, C., Phillips, T.W., 2010. Insect population dynamics in

386   commercial grain elevators. Journal of Stored Products Research. 46, 43 - 47.

387 Flinn, P.W., Subramanyam, B., Arthur, F.H., 2004. Comparison of aeration and spinosad

388   for suppressing insects in stored wheat. Journal of Economic Entomology. 97,

389   1465-1473.

390 Food and Environment Research Agency, 2009. Phytosanitary Certification of Grain for

391   Export. Food and Environment Research Agency, United Kingdom.

392 Fornal, J., Jeliński, T., Sadowska, J., Grundas, S., Nawrot, J., Niewiada, A., Warchalewski,

393   J.R., Błaszczak, W., 2007. Detection of granary weevil Sitophilus granarius (L.)

394   eggs and internal stages in wheat grain using soft X-ray and image analysis.

395   Journal of Stored Products Research. 43, 142 - 148.

396 Grain Trade Australia, 2011. GTA Wheat Standards 2011/2012. Grain Trade Australia,

397   Australia.

398 Greenspan, L., 1977. Humidity fixed points of binary saturated aqueous solutions.

399   Journal of Research of the National Bureau of Standards. 81A, 89-96.

400 Gu, W., Swihart, R.K., 2004. Absent or undetected? Effects of non-detection of species

401   occurrence on wildlife–habitat models. Biological Conservation. 116, 195 - 203.

402   Hagstrum, D.W., 1987. Seasonal variation of stored wheat environment and insect

403   populations. Environmental Entomology. 16, 77-83.

404   Hagstrum, D.W., 1996. Monitoring and Predicting Population Growth of Rhyzopertha

405   dominica (Coleoptera: Bostrichidae) Over a Range of Enviromnental Conditions.

406   Environmental Entomology. 25, 1354-1359.

407   Hagstrum, D.W., 2000. Using five sampling methods to measure insect distribution and

408   abundance in bins storing wheat. Journal of Stored Products Research. 36, 253 -

409   262.

410   Hagstrum, D.W., Flinn, P.W., Reed, C.R., Phillips, T.W., 2010. Ecology and IPM of insects

411   at grain elevators and flat storages. Biopesticides International. 6, 1-20.

412   Hagstrum, D.W., Milliken, G.A., 1988. Quantitative analysis of temperature, moisture,

413   and diet factors affecting insect development. Annals of the Entomological

414   Society of America. 81, 539-546.

415   Hagstrum, D.W., Milliken, G.A., Waddel, M.S., 1985. Insect distribution in bulk-stored

416   wheat in relation to detection or estimation of abundance. Environmental

417   Entomology. 14, 655-661.

418   Hagstrum, D.W., Subramanyam, B., 2006. Fundamentals of Stored-Product Entomology.

419   ACCC International, St. Paul, Minnesota, USA.

420   Jefferies, M.G., 2000. Review of grain sampling and inspection methodology. Department

421   of Agriculture, Fisheries and Forestry, Australia.

422   Jian, F., Jayas, D.S., White, N.D.G., 2003. Movement of adult rusty grain beetles,

423   Cryptolestes ferrugineus (Coleoptera: Cucujidae), in wheat in response to 5°C/m

424   temperature gradients at cool temperatures. Journal of Stored Products

425   Research. 39, 87 - 101.

426   Jian, F., Jayas, D.S., White, N.D., 2005. Movement and distribution of adult Cryptolestes

427   ferrugineus (Coleoptera: Laemophloeidae) in stored wheat in response to

428   temperature gradients, dockage, and moisture differences. Journal of Stored

429   Products Research. 41, 401 - 422.

430   Jian, F., Larson, R., Jayas, D.S., White, N.D., 2011. Three-dimensional spatial distribution

431   of adults of Cryptolestes ferrugineus (Coleoptera: Laemophloeidae) in stored

432   wheat under different temperatures, moisture contents, and adult densities.

433   Journal of Stored Products Research. 47, 293 - 305.

434   Kogan, M., 1998. Integrated pest management: historical perspectives and

435   contemporary developments. Annual Review of Entomology. 43, 243-270.

436   Krebs, C.J., 1999. Ecological methodology. 2nd ed. Benjamin/Cummings, Menlo Park.

437   Lippert, G.E., Hagstrum, D.W., 1987. Detection or estimation of insect populations in

438   bulk-stored wheat with probe traps. Journal of Economic Entomology. 80, 601-

439   604.

440   Longstaff, B.C., 1999. An experimental and modelling study of the demographic

441   performance of Rhyzopertha dominica (F.). I. development rate. Journal of Stored

442   Products Research. 35, 89 - 98.

443   Nansen, C., Flinn, P., Hagstrum, D., Toews, M.D., Meikle, W.G., 2009. Interspecific

444   associations among stored-grain beetles. Journal of Stored Products Research.

445   45, 254-260.

446   Oerke, E.C., 2005. Crop losses to pests. The Journal of Agricultural Science. 144, 31-43.

447   Osuji, F.N.C., 1982. Development of the lesser grain borer, Rhyzopertha dominica, in

448   maize kernels as affected by site of larval entry. Entomologia Experimentalis et

449   Applicata. 31, 391-394.

450   Pebesma, E.J., 2004. Multivariable geostatistics in S: the gstat package. Computers &

451   Geosciences. 30, 683-691.

452   Phillips, T.W., Throne, J.E., 2010. Biorational approaches to managing stored-product

453   insects. Annual Review of Entomology. 55, 375-397.

454   Plarre, R., 1996. Three-dimensional distribution of Sitophilus granarius

455   (L.)(Coleoptera: Curculionidae) in wheat influenced by the synthetic aggregation

456   pheromone. Journal of Stored Products Research. 32, 275-283.

457   R Core Development Team, 2011. R: A Language and Environment for Statistical

458   Computing. Vienna, Austria. URL = http://www.R-project.org/

459   Sarkar, D., 2008. Lattice: Multivariate Data Visualization with R. Springer, New York.

460   Schrieber, R., Gareis, H., 2007. Gelatine handbook: theory and industrial practice. Wiley-

461   VCH Weinheim, Germany,.

462   Sinclair, E.R., 1981. Sexing live adult Rhyzopertha dominica (F.) (Coleoptera:

463   Bostrychidae). Journal of Stored Products Research. 17, 143 - 145.

464   Smith-Gill, S.J., 1975. Cytophysiological basis of disruptive pigmentary patterns in the

465   leopard frog Rana pipiens. II. Wild type and mutant cell-specific patterns,.

466   Journal of Morphology. 146, 35-54.

467   Stejskal, V., Aulický, R., Kučerová, Z., Lukáš, J., 2010. Method of sampling and laboratory

468   extraction affects interpretation of grain infestation by storage pests. Journal for

469   Plant Diseases and Plant Protection. 3, 129-133.

470   Stenseth, N.C., Hansson, L., 1979. Correcting for the Edge Effect in Density Estimation:

471   Explorations around a New Method. Oikos. 32, 337-348.

472   Surtees, G., 1964a. Laboratory studies on dispersion behaviour of adult beetles in grain.

473   IV.—The lesser grain borer, Rhyzopertha dominica (F.)(Coleoptera,

474   Bostrychidae). Bulletin of Entomological Research. 54, 715-722.

475   Surtees, G., 1964b. Laboratory studies on dispersion behaviour of adult beetles in grain.

476   VI.—Three-dimensional analysis of dispersion of five species in a uniform bulk.

477   Bulletin of Entomological Research. 55, 161-171.

478   Taylor, L.R., 1984. Assessing and interpreting the spatial distributons of insect

479   populations. Annual Review of Entomology. 29, 321-357.

480   Thorpe, G.R., 1997. Modelling ecosystems in ventilated conical bottomed farm grain

481   silos. Ecological Modelling. 94, 255 - 286.

482   Trematerra, P., Gentile, P., Brunetti, A., Collins, E., Chambers, J., 2007. Spatio-temporal

483   analysis of trap catches of Tribolium confusum du Val in a semolina-mill, with a

484   comparison of female and male distributions. Journal of Stored Products

485   Research. 43, 315-322.

486   Wagner, T.L., Wu, H.-I., Sharpe, P.J.H., Schoolfield, R.M., Coulson, R.N., 1984. Modeling

487   insect development rates: a literature review and application of a biophysical

488   model. Annals of the Entomological Society of America. 77, 208-225.

Table 1: Summary of experimental treatments and non-dimensional results. R1-3 are replicates within each treatment. *Ip* is the scaled Morisita's index of dispersion, *Imor* is the unscaled Morisita's index, with associated chi-sq and *p* values.

Treatment	Temp (°C)	Duration (days)	No. of adults recovered	Total Insect density (adults / L)	Population Mean & SE	No. of Clusters	No. of occupied samples	*Ip*	*Imor*	Chi-sq (df = 199)	*p*
Control R1	30	14	26	1.3		6	19	0.5105	6.7692	343.2308	<0.0001
Control R2	30	14	29	1.45	μ = 28 ± 1	4	22	0.5037	3.9409	281.3448	<0.0001
Control R3	30	14	29	1.45		1	21	0.5087	5.9113	336.5172	<0.0001
25°C R1	25	59	43	2.15		3	27	0.5129	7.0875	454.6744	<0.0001
25°C R2	25	59	46	2.3	μ = 44 ± 1	2	22	0.5220	10.6280	632.2609	<0.0001
25°C R3	25	59	43	2.15		1	34	0.5012	2.4363	259.3256	0.0026
30°C R1	30	43	161	8.05		4	63	0.5172	8.0901	1333.41	<0.0001
30°C R2	30	43	163	8.15	μ = 143.67 ± 18.342	3	60	0.5135	6.6349	1111.847	<0.0001
30°C R3	30	43	107	5.35		3	50	0.5127	6.4186	773.3738	<0.0001
35°C R1	35	31	105	5.25		1	51	0.5065	3.9560	506.4286	<0.0001
35°C R2	35	31	45	2.25	μ = 91.7 ± 24.037	4	29	0.5089	5.4545	395	<0.0001
35°C R3	35	31	125	6.25		1	57	0.5071	4.1548	590.2	<0.0001

Table 2: Correlation coefficient of the insect densities at different locations in the 2-D horizontal X-Y plane.

Treatment	N_{adult}	H(0,[0-5])	H(0,[5-10])	H(0,[10-15])	H(0,[15-20])	H(0,[20-25])	H(0,[25-30])
Control R1	26	0.4132 *	-0.1679	-0.3246 *	-0.038	-0.2161	N/A
Control R2	29	-0.1936	0.0199	0.0131	0.2187	0.075	N/A
Control R3	29	0.1896	-0.2237	-0.0666	0.1081	-0.1941	N/A
25°C R1	43	0.2397	-0.153	-0.4156 *	-0.2769	-0.1884	N/A
25°C R2	46	0.2567	-0.3101 *	-0.3997 *	-0.0607	0.1786	N/A
25°C R3	43	0.0873	-0.0074	-0.2227 *	-0.1603	-0.2729	N/A
30°C R1	161	0.126	-0.1589	-0.0519	-0.0555	-0.1779	N/A
30°C R2	163	0.1	0.109	-0.0157	-0.0486	-0.0755	N/A
30°C R3	107	0.2233	-0.0512	-0.0121	-0.1259	-0.2751 *	N/A
35°C R1	105	0.2897 *	-0.1355	-0.3302 *	-0.3041 *	0.032	N/A
35°C R2	45	0.3873 *	-0.0846	-0.0399	-0.2448	-0.4438 *	N/A
35°C R3	125	0.3662 *	-0.0394	-0.27 *	-0.1994	0.0069	N/A

* significant at $p = 0.10$
N/A = no value calculated due to insufficient observations at this distance.

Table 3: Correlation coefficient of the insect densities at different locations in the 2-D vertical X-Z plane.

Treatment	N_{adult}	V(0,[0-5])	V(0,[5-10])	V(0,[10-15])	V(0,[15-20])	V(0,[20-25])	V(0,[25-30])	V(0,[30-35])	V(0,[35-40])
Control R1	26	-0.2719	0.0325	-0.0225	-0.1587	0.1153	N/A	N/A	N/A
Control R2	29	0.0287	-0.2894	-0.114	0.2239	0.071	N/A	-0.405 *	N/A
Control R3	29	0.073	-0.2418	-0.1837	-0.1015	0.3714	N/A	N/A	N/A
25°C R1	43	0.2596	-0.1175	-0.4312 *	0.0064	N/A	N/A	N/A	N/A
25°C R2	46	0.3361 *	-0.1375	-0.2497	-0.1845	-0.2223	N/A	N/A	N/A
25°C R3	43	0.0513	0.0098	-0.306 **	-0.2881 *	-0.2908	N/A	N/A	N/A
30°C R1	161	0.3256 *	-0.0391	0.0564	-0.0676	-0.2679 *	-0.2577	-0.3203	N/A
30°C R2	163	0.5075 *	0.2654 *	0.1016	-0.1298	-0.2193 *	-0.4179 *	-0.5473 *	N/A
30°C R3	107	0.2283	0.1633	-0.0924	-0.0771	-0.3247 *	-0.3204	-0.4483 *	N/A
35°C R1	105	0.4275 *	0.224 *	-0.215 *	-0.2934 *	-0.3413 *	-0.3294 *	N/A	N/A
35°C R2	45	0.2651	-0.0148	-0.042	-0.2716 *	-0.1791	-0.1112	N/A	N/A
35°C R3	125	0.2613 *	0.1426	-0.1885 *	-0.1862 *	-0.1498	-0.031	N/A	N/A

* significant at p= 0.10

N/A = no value calculated due to insufficient observations at this distance.

Table 4: Correlation coefficient of the insect densities at different locations in the 2-D vertical Y-Z plane.

Treatment	N_{adult}	V(0,[0-5])	V(0,[5-10])	V(0,[10-15])	V(0,[15-20])	V(0,[20-25])	V(0,[25-30])	V(0,[30-35])	V(0,[35-40])
Control R1	26	0.0819	-0.166	-0.1245	-0.1906	0.2923	N/A	N/A	N/A
Control R2	29	0.006	0.0363	-0.1663	-0.3511	N/A	-0.3472	-0.4777	N/A
Control R3	29	-0.0056	-0.2273	-0.1528	0.0261	-0.1905	N/A	N/A	N/A
25°C R1	43	-0.1017	-0.0381	-0.2015	-0.2981	-0.1902	-0.4359	-0.4324	N/A
25°C R2	46	0.2618	-0.2076	-0.2615	-0.1818	N/A	N/A	N/A	N/A
25°C R3	43	0.2574	0.1104	-0.2305 *	-0.3981 *	-0.3458 *	N/A	N/A	N/A
30°C R1	161	0.4218 *	0.3162 *	0.0691	-0.0571	-0.2749 *	-0.4603 *	-0.4742 *	-0.5913 *
30°C R2	163	0.3784 *	0.0481	-0.0513	-0.1508	-0.2741 *	-0.3997 *	-0.4517 *	N/A
30°C R3	107	0.4762 *	0.2162	-0.0039	-0.2369 *	-0.297 *	-0.3113	-0.3401 *	N/A
35°C R1	105	0.4404 *	0.1011	-0.2621 *	-0.3051 *	-0.2923 *	-0.4217 *	-0.3962	N/A
35°C R2	45	0.3733	0.1203	-0.0821	0.2412	-0.0507	0.2825	-0.0857	N/A
35°C R3	125	0.3342 *	0.0818	-0.067	-0.2863 *	-0.1339	-0.0461	0.1725	N/A

* significant at p= 0.10
N/A = no value calculated due to insufficient observations at this distance.

Table 5: Correlation coefficient of the insect densities at different locations in the 3-D X-Y-Z plane.

Treatment	N_{adult}	D(0,[0-5])	D(0,[5-10])	D(0,[10-15])	D(0,[15-20])	D(0,[20-25])	D(0,[25-30])	D(0,[30-35])	D(0,[35-40])
Control R1	26	0.0905	0.4404	-0.1606	-0.205	-0.1745	0.01874	N/A	N/A
Control R2	29	-0.3948 *	-0.0898	0.0142	-0.0068	0.1251	-0.1749	-0.2549	-0.189
Control R3	29	0.0942	-0.2804 *	0.0282	-0.013	0.0759	N/A	N/A	N/A
25°C R1	43	0.0152	-0.1131	-0.1169	-0.1182	-0.1208	N/A	N/A	N/A
25°C R2	46	0.3027	-0.0861	-0.2556 *	-0.22	-0.2977 *	-0.1348	N/A	N/A
25°C R3	43	0.1305	-0.0112	-0.1569 *	-0.1259	-0.076	-0.0857	N/A	N/A
30°C R1	161	0.2357 *	-0.005	-0.0495	-0.0471	-0.0718	-0.0234	N/A	N/A
30°C R2	163	0.1864 *	0.0256	-0.0193	-0.0211	-0.0658	-0.1493 *	N/A	N/A
30°C R3	107	0.101	-0.0714	-0.0091	-0.0595	-0.0732	-0.132	N/A	N/A
35°C R1	105	0.3297 *	0.0944	-0.1342 *	-0.2423 *	-0.2488 *	-0.2294 *	N/A	N/A
35°C R2	45	-0.1984	0.2743 *	-0.1006	-0.0719	-0.1422	-0.1015	N/A	N/A
35°C R3	125	0.2012*	0.0265	-0.0459	-0.0756	-0.1002 *	0.0213	N/A	N/A

* significant at $p= 0.10$
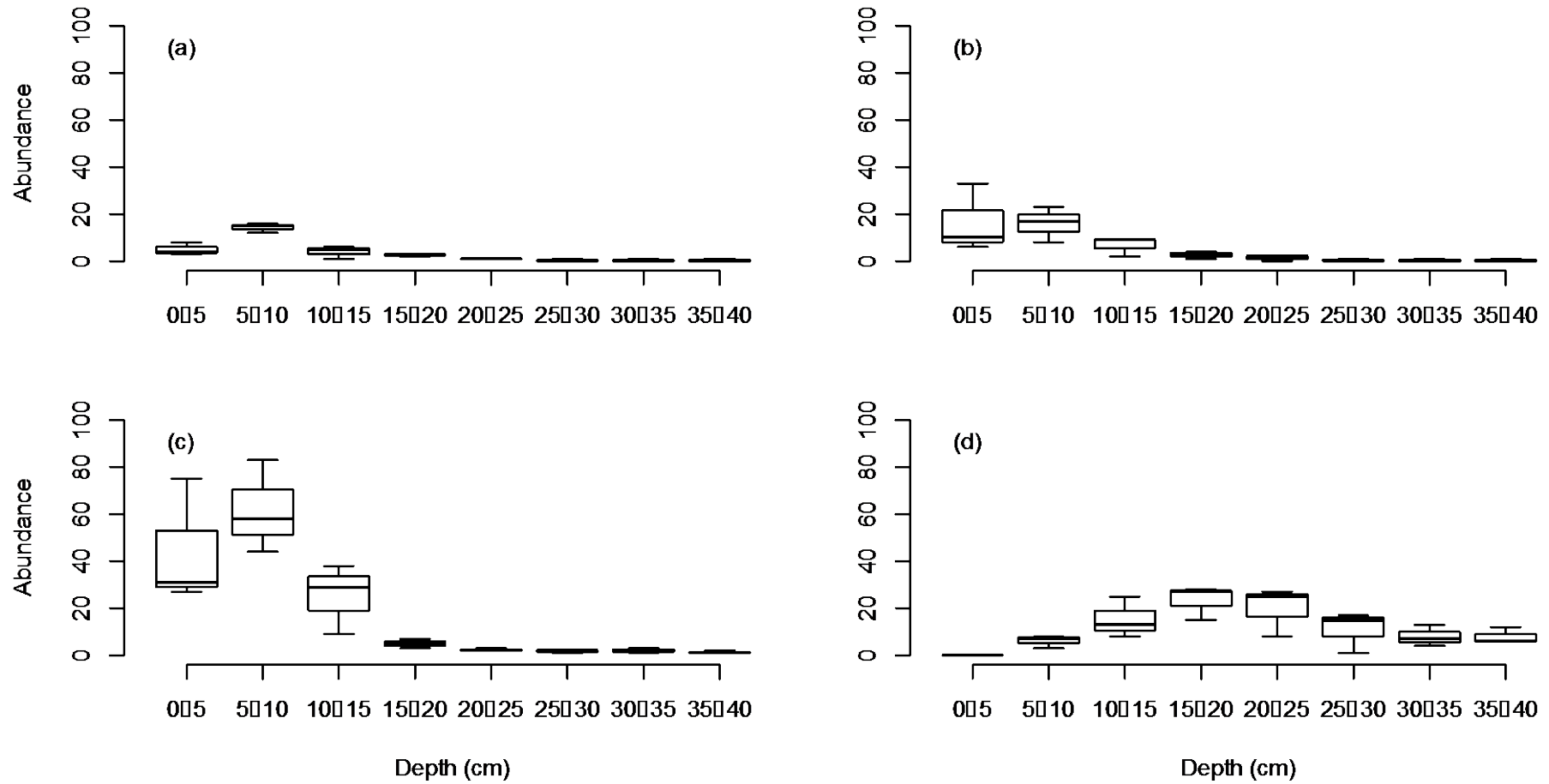N/A = no value calculated due to insufficient observations at this distance.

Fig. 1. Mean abundance of adult *Rhyzopertha dominica* in each 5cm layer, for (a) 30°C control, (b) 25°C, (c) 30°C, and (d) 35 °C treatments.
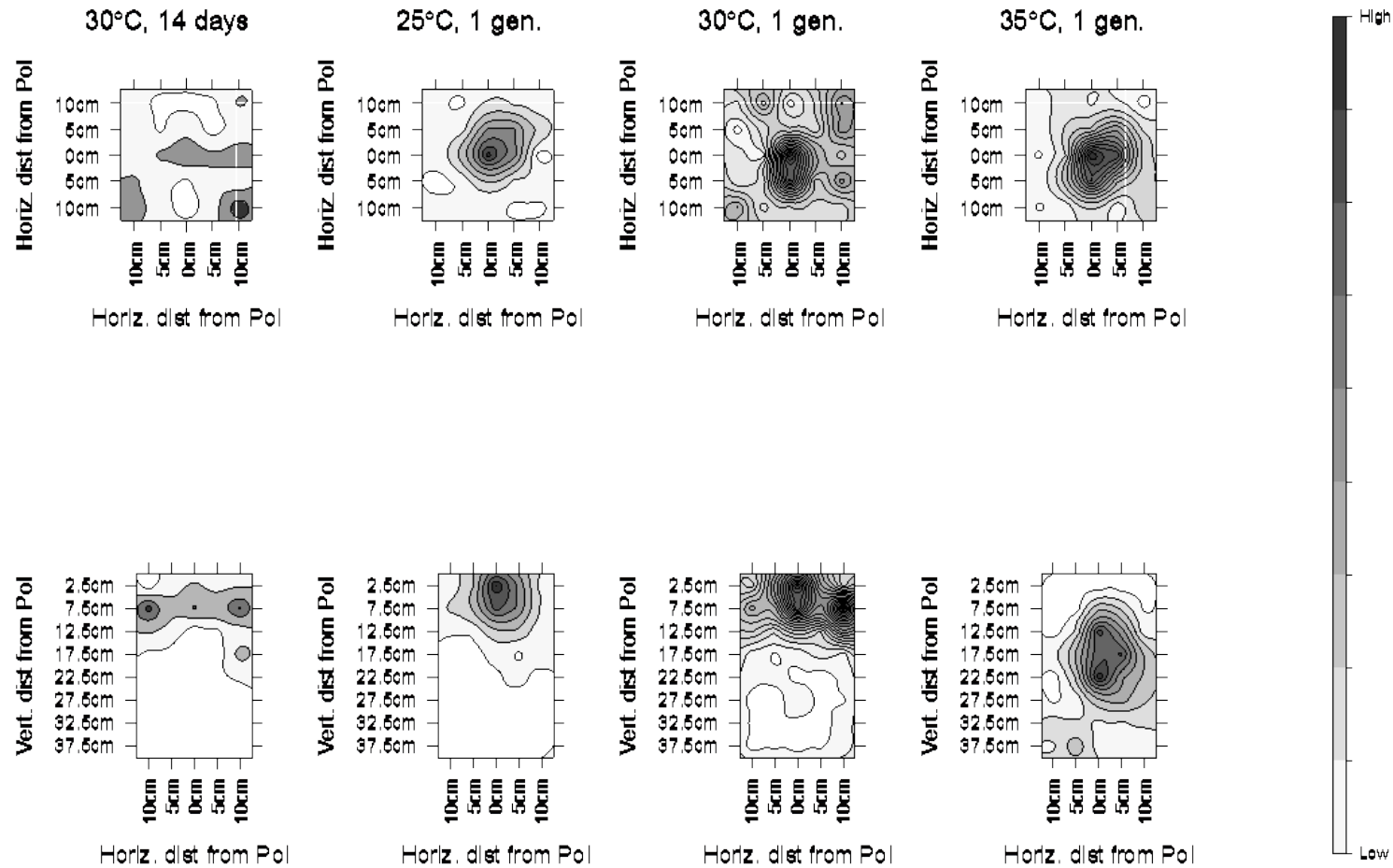
Fig. 2. Representative horizontal (top) and vertical (bottom) spatial distributions, based on the average of 3 replicates. Indicated

pest density is relative to the mean pest density of each treatment; darker areas indicate higher densities.