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Pupal development of *Aethina tumida* (Coleoptera: Nitidulidae) in thermo-hygrometric soil conditions encountered in temperate climates

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Abstract

The pupal development of *Aethina tumida* Murray (Coleoptera: Nitidulidae) was studied at various combinations of thermo-hygrometric soil conditions (temperatures of 16, 18 and 20°C, and soil water contents of 0.37, 0.56 and 0.73 m^3 water/m^3 dry soil) representative of southeastern Canada. Survivorship and development duration of *A. tumida* pupae, as well as sex ratio and lifespan of emerging adults, were assessed. Assays were conducted in growth chambers on an average of 50 third instar larvae per thermo-hygrometric combination. Results show that survivorship of pupae decreased with lower temperature and higher soil water content. Pupal development time shortened as temperature increased (69 to 78 days at 16°C, 47 to 54 days at 18°C and 36 to 39 days at 20°C) but was longer in dryer soil. Optimal soil water content for pupal development was 0.56 m^3 water/m^3 soil. We estimated that the minimum development temperature for pupae is between 10.2 and 13.2°C depending on soil water content. Sex ratio of emerging adults was influenced by soil water content. We measured one female to one male for dry and intermediately wet soils and three females to one male for wet soils. Higher soil water content reduced the lifespan of emerging adults by half. This study contributes to a better understanding of *A. tumida* population dynamics in eastern Canada.

Keyword: *Aethina tumida*, temperature, soil water content, pupal development

1 Introduction

Aethina tumida Murray or the small hive beetle (SHB), is a honey bee (*Apis mellifera* L.) pest indigenous to South Africa (Lundie 1940). Adult SHB, known to live several months (Lundie 1940, Haque and Levot 2005, Meikle and Patt 2011, Murrle and Neumann 2004), infiltrate honey bee colonies to lay their eggs and allow their larvae to feed and develop. The larvae cause significant damage, while their associated yeast, *Kodamaea ohmeri* (NRRL Y-30722) (Torto et al. 2007b), causes the honey to ferment (Lundie 1940, Elzen et al. 1999) and thus lose its nutritional value. High infestation rates will cause the colony to collapse (Elzen et al. 1999).

This pest was discovered in 1998 in the state of Florida, USA (Thomas 1998) and in 2002 in Australia (Somerville 2003). First occurrences of SHB in Canada were observed in 2002 (Manitoba) and 2006 (Alberta and Manitoba) without any sign of population survival after winter (Dixon and Lafrenière 2002, Nasr 2006). In southeastern Canada (southern Québec), a SHB invasion was discovered during the fall of 2008 (Giovenazzo and Boucher 2010). Presence of SHB in this region can be attributed to the invasion of beetles from the USA (Giovenazzo and Boucher 2010). More recently, the pest was reported in Ontario (Kozak 2010) and again in Manitoba (2012). The damage caused by SHB in Canadian honey bee colonies is not as significant as that experienced in the southern USA (Florida, Georgia and South Carolina). The colder Canadian climate may explain why SHB populations have failed to establish to date.

47 At the pupal stage, the SHB is particularly vulnerable to the impact of both
48 climatic factors and predators. De Guzman and Frake (2007) observed SHB
49 mortality mainly at this stage, when reared at temperatures of 24-28 and 34°C.
50 Thus many authors, such as Lundie (1940) and Ellis et al. (2004), suggest that
51 environmental factors affect the reproduction potential of SHB. Because SHB
52 pupate in the soil, edaphic factors including moisture and density, field slope,
53 drainage, rainfall and temperature greatly influence this stage of their development
54 (de Guzman *et al.* 2009). Soil temperature (de Guzman and Frake 2007, de
55 Guzman et al. 2009, Meikle and Patt 2011) and soil moisture (Lundie 1940,
56 Schmolke 1974; Ellis et al. 2004, Haque and Levot 2005) are the edaphic factors
57 that have the greatest impact on pupal development and survivorship. Finally, soil
58 type does not seem to affect the development of SHB pupae (Schmolke 1974, Ellis
59 et al. 2004, de Guzman et al. 2009).

60

61 Pupal development has been measured at 21-35°C (Neumann et al. 2001,
62 Murrle and Neumann 2004, Ellis et al. 2004, Haque and Levot 2005, de Guzman
63 and Frake 2007, de Guzman et al. 2009, Meikle and Patt 2011, Meikle and Diaz
64 2012), which are representative of climatic conditions in Africa, the southern USA
65 and Australia. Moreover, Meikle and Patt (2011) estimated that pupae could not
66 develop below 10°C. Nonetheless, soil temperatures in Canada during beekeeping
67 season can range between 10 and 21°C. To our knowledge, pupal development
68 has not been tested at these temperatures and thus should be investigated to gain
69 knowledge on SHB reproduction in temperate climates. Furthermore, seasonal

rainfall is an important indicator of SHB population growth (Torto et al. 2010). Only a few studies have mentioned the importance of soil water content. Neumann et al. (2001), Murrle and Neumann (2004), de Guzman and Frake (2007) and de Guzman et al. (2009) experimented with SHB in moist soils, but did not measure soil water content. Ellis et al. (2004) compared two soil water levels (0% and 11% water by weight) and concluded that dry soil was unsuitable for pupal development. However, a water content of 0% is not representative of field conditions because soil always retains a certain amount of water (Buckman and Brady 1960).

The objective of this study was to investigate SHB pupal development at various thermo-hygrometric soil conditions similar to those observed in southeastern Canada. We also measured SHB sex ratio and lifespan of emerging adults.

2 Materials and Methods

2.1 Small hive beetle rearing

Adult beetles, both males and females, were collected in May 2010 from infested honey bee colonies located in West-Montérégie, southern Québec, Canada (N45.003983, W74.449317). These SHB were used to establish an

experimental population reared in growth chambers (Conviron, model PGR15 and E15) at Laval University, Québec, Canada. SHB were kept in darkness at 30 ± 0.5°C and 50-60% RH. Adult beetles were placed in 550-ml cylindrical plastic containers (10 cm diameter, 7 cm deep) with perforated screw-top lids fitted with a mesh cloth to prevent escape and provide air circulation. Four moistened cotton balls provided humidity in plastic containers. Adults and larvae were fed *ad libitum* with honey bee pollen collected with pollen traps from colonies of the Centre de recherche en sciences animales de Deschambault, Deschambault, Québec, in the summer of 2010.

2.2 Pupal development

Survival rate and duration of pupation in soil were measured in the growth chambers at 16, 18 and 20°C and at 0.37, 0.56 and 0.73 m^3 water/m^3 dry soil. These values correspond, in an organic soil, to dry, intermediate and wet (near saturation) soil. Organic potting soil (Pro-mix® by Premier Tech, Rivière-du-Loup, Canada, bulk density of 0.293 g/cm^3) was pasteurized (30 min at 60°C) and oven dried (40°C for 48 h). Dry soil (0.12 kg) was put in 3.1-liter plastic containers. These containers had a plain lid that allows little to no moisture or gas exchange, but contained enough air for pupae to breath. Sterilized water (150-ml, 230-ml and 300-ml) was added to the dry soil to obtain the different soil water content levels. Probes were used to record soil temperature (12-Bit Temp Smart Sensor S-TMB-

M006, Onset® HOBO® Data Loggers, Massachusetts, USA) and soil water content (EC-5 Moisture sensor S SMC-M005, Decagon Devices, Pullman, Washington, USA). They were inserted to a depth of 3 cm and recorded data every 15 min. There was no need to add water throughout the trial because water content remained constant. Mature larvae (wandering stage) were placed in plastic containers with specific thermo-hygrometric soil conditions and allowed to burrow naturally into the soil (depth of 5-6 cm). Larvae of the same age were obtained from five sexually mature females and males that mated and laid eggs over a 24-h period. Young larvae that developed afterwards were fed *ad libitum* with pollen and water for 15 d. On day 15, these larvae were distributed equally into the 9 different experimental groups (3 different temperatures X 3 different soil water contents). Further details on the experimental design are provided in the Statistics section.

Plastic containers were examined daily to monitor adult emergence. Pupation duration at each temperature and water content combination was measured, and emerging adults were counted. Soil was then searched for any dead SHB at any life stage.

2.3 Sex ratio and lifespan of emerging adults

Adults that emerged from pupation containers were collected with an aspirator (Schmolke 1974, Ellis et al. 2004). They were then sexed by applying gentle pressure on their abdomen with finger tips to reveal either the female's ovipositor or the male's 8th tergite (de Guzman and Frake 2007). All emerging adults were kept in growth chambers at 30.0 ± 0.5°C They were placed by couples in 50-ml plastic tubes (Starstedt™, Montréal, Canada) containing a moistened cotton ball and pollen *ad libitum*. These tubes were covered with a perforated lid to provide air circulation. If an adult died, the date was recorded and it was replaced with another adult of the same treatment and gender if available (Meikle and Patt 2011).

2.4 Statistics

The experiment was planned as a split-plot design with temperature as main plot and soil water contents as subplots. The temperatures were randomized into a 3x3 Latin square with blocks (date) as rows and growth chambers as columns. There were three replications for the temperatures 16°C and 18°C and only two repetitions for 20°C (one repetition at 12°C as a preliminary test). Each of the three soil water contents (0.37, 0.56 and 0.73 m^3 water/m^3 dry soil) was repeated twice per growth chamber using two groups of about 50 pupae. Because pupae in each group are pseudoreplications, all analyses were done on the average value per group, except for experiment in which the sex effect was also studied. In that case,

155 average values of the response variables were computed for each sex in each
156 group and analysis were done using a split-split plot design in analysis of variance
157 (ANOVA) with sex in the sub-subplots. All analysis were done at $\alpha=0.05$ level of
158 significance and they considered temperatures, soil water contents and sex (when
159 appropriate) as fixed effects, and blocks, chambers and groups as random effects.

160

161 The survival rates of pupae and sex ratios were compared between levels of
162 temperature and water contents using a split-plot ANOVA with a logit link function
163 for a binomial response distribution (Hosmer and Lemeshow 2000). Test-of-effect
164 slices were used to evaluate significant interaction effects, and the protected LSD
165 multiple comparisons technique was used to identify the treatment differences.
166 Linear and quadratic contrasts were also computed to study the relation of the
167 temperature on the response variable. The model was fitted to the data using the
168 GLIMMIX procedure of SAS software (SAS Inst., NC, 2010, release 9.3).

169

170 The pupal development length and lifespan of emerging adults were
171 compared between levels of soil temperatures and soil water contents using the
172 traditional split-plot Anova model for a Gaussian response variable. The same
173 types of test-of-effect slices, multiple comparisons, and contrasts as those
174 previously mentioned were used to identify the treatment differences. The model
175 was fitted to the data using the MIXED procedure of SAS. The potential effect of
176 sex on these responses variables was also studied using a split-split plot Anova

model. Meikle and Patt (2011) developed a linear mixed model to estimate the minimal temperature for pupae development. We used a similar approach, but chose between linear and quadratic relation based on the Akaike information criterion (AIC) (Burnham and Anderson 2002).

Starting dates of the first, second and third experimental blocks were July 8th 2011, October 11th 2011, and January 18th 2012 respectively. A total of 972, 1224 and 774 larvae were produced for block 1, 2 and 3 respectively. They were split equally on each of the 18 groups of each block; 2 groups for each of the 9 experimental conditions (3 temperatures X 3 soil water content levels). Thus, a total of 54, 68 and 43 larvae were put into each group.

3 Results

3.1 Pupal development

There was a significant interaction between soil temperature and water content on the survival rate of pupae ($F = 15.91$; $df = 4,28$; $p < 0.001$). The contrasts showed a significant effect of temperature for water content of 0.37 and 0.56 m^3 water/m^3 soil, but not in wet soils (0.73 m^3 water/m^3 soil). More precisely, for water content of 0.37 m^3 water/m^3 soil, the survival rates were significantly higher at 20°C and 18°C (97.4% ± 1.7 and 90.3% ± 4.2 respectively). For water

199 content of 0.56 m^3 water/m^3 soil, all temperatures were significantly different from
200 each other, with the highest survival at 20°C (97.8% ± 1.5) (Table 1). There was
201 also a significant effect of water content for each temperature. At both
202 temperatures of 18°C and 20°C, the survival rates were significantly higher in dry
203 (0.37 m^3 water/m^3 soil) and intermediate (0.56 m^3 water/m^3 soil) soil water contents.
204 At temperature of 16°C, the survival rates were low especially for water contents of
205 0.37 m^3 water/m^3 soil (14.7% ± 5.9) and 0.73 m^3 water/m^3 soil (12.5% ± 5.8). High
206 soil water content and temperature of 16°C were limiting factors on the
207 development of *A. tumida* pupae (Table 1 and Fig. 1). The temperature had a
208 significant linear relation with the logit of the survival probability but a different
209 pattern of relation among levels of water contents ($F = 29.93$; $df = 2,28$; $p < 0.001$).
210 The models had a good discrimination rate based on the area under the Roc Curve
211 (Hosmer and Lemeshow 2000), which is a coefficient similar to the R^2, but for
212 binomial regressions, as used in our study. (AUROC = 0.91 for 0.37 m^3 water/m^3
213 soil; AUROC = 0.88 for 0.56 m^3 water/m^3 soil and AUROC = 0.67 for 0.73 m^3
214 water/m^3 soil) (Fig. 1).

215

216 Pupal development time was affected by the interaction between soil
217 temperature and soil water content ($F= 5.23$; $df = 4,28$; $p = 0.003$). Mean
218 development time varied from 69.1d ± 2.1 to 78.1d ± 2.1 at 16°C, from 47.6d ± 2.2
219 to 54.4d ± 2.1 at 18°C and from 36.8d ± 2.2 to 39.0d ± 2.3 at 20°C. At 16 and
220 18°C, development time was longer for pupae in a soil water content of 0.37 m^3
221 water/m^3 soil than at 0.56 m^3 water/m^3 soil and 0.73 m^3 water/m^3 soil. At 20°C,

there was no significant difference between the three different soil water content levels (38.3d ± 2.2 at 0.37 m^3 water/m^3 soil, 36.8d ± 2.2 at 0.56 m^3 water/m^3 soil and 39.0d ± 2.3 at 0.73 m^3 water/m^3 soil). Lower temperature increased the duration of pupal development (Table 2 and Fig. 2). Temperature had a significant quadratic relation with the pupal development time at all water contents, but the relation at 0.37 m^3 water/m^3 soil was different from the relation at 0.56 and 0.73 m^3 water/m^3 soil ($F=22.42$, $df=3,28$, $p<0.001$ and $F=12.64$, $df=3,28$ $p<0.001$ respectively). The models explained a high percent of variance ($R^2 = 0.95$ for 0.37 m^3 water/m^3 soil; $R^2 = 0.97$ for 0.56 m^3 water/m^3 soil and $R^2 = 0.96$ for 0.73 m^3 water/m^3 soil) (Fig. 2). The development time of females did not differ from that of males ($F = 0.170$; $df = 1,28$; $p = 0.681$). Not all unemerged adults could be recovered. Moreover, some of the dead larvae were colonized by an unidentified fungus.

There was a significant relation between the temperature and the proportion of pupal development per day for all soil water contents, but the relation was quadratic for dry and intermediate soils, and the relation was linear for wet soils The models explained a high percent of variance ($R^2 = 0.98$ for 0.37 m^3 water/m^3 soil; $R^2 = 0.98$ for 0.56 m^3 water/m^3 soil and $R^2 = 0.93$ for 0.73 m^3 water/m^3 soil) (Fig. 3). Extrapolating the curves showed a minimum temperature for pupal development of 13.2°C at 0.37 m^3 water/m^3 soil, 10.2°C at 0.56 m^3 water/m^3 soil, and 11.4°C at 0.73 m^3 water/m^3 soil.

3.2 Sex ratio and lifespan of emerging adults

Sex determination of *A. tumida* was not altered by temperature (F=3.19, df=2,1, p=0.368), but a soil water content effect was marginally significant (F=3.00, df=2,28, p=0.066). Post hoc contrasts were significant when comparing soil water contents of 0.73 vs 0.37 and 0.56 (F = 5.97; df =1,28; p = 0.021) after a Bonferroni adjustment (α = 0.025) (Hochberg and Tamhane 1987). The proportion of females was 0.51 ± 0.03 at 0.37 m^3 water/m^3 soil, 0.52 ± 0.03 at 0.56 m^3 water/m^3 soil and 0.73 ± 0.07 at 0.73 m^3 water/m^3 soil (Table 3). In dry or intermediate soils, the sex ratio was 1♀:1♂, while in wet soils, the ratio was 3♀:1♂.

Lifespan of emerging adults was significantly altered by soil water content (F = 8.34; df = 2,33; p = 0.001). Adults that developed at 0.37 and 0.56 m^3 water/m^3 soil lived twice as long as adults at 0.73 m^3 water/m^3 soil (Table 4). However, lifespan of emerging adults was not affected by temperature (F = 12.06; df = 2,1; p = 0.200) or sex (F = 2.88; df = 1,28; p = 0.101).

4 Discussion

4.1 Survival rate of pupae

Pupal survivorship was influenced by both temperature and soil water content. Survivorship was over 89% at 18 and 20°C with low (0.37 m^3 water/m^3 soil) and intermediate (0.56 m^3 water/m^3 soil) soil water content. Neumann et al. (2001) reported lower emergence (42.6%) at a similar temperature range (17-24°C) in a moist soil (unknown water content). However, they explained high mortality by the limited space available to larvae (2.3 cm^3/larva). In our experiment, each larva had between 6.0 and 9.5 cm^3 of available soil. Soil depth of 5-6 cm was sufficient to provide a successful pupation (Meikle and Diaz 2012). At 20°C, we measured the highest survivorship in dry and intermediate soil water content (97.4% and 97.8%, respectively), which is similar to findings in previous experiments. Ellis et al. (2004) found an emergence level of 91.5% in a mineral soil at 24.6 ± 1.3°C and 10% water by weight, and de Guzman and Frake (2007) measured 93% survival in moist potting soil at 24-28°C (unknown water content). Meikle and Patt (2011) measured 92% pupal emergence at 21°C (soil water content of 5-8% by weight in sandy soil). However, the impact of soil water content should be compared among studies with caution since the amount of available water varies with soil texture (Villani and Wright 1990) and organic matter content (Buckman and Brady 1960). Ellis et al. (2004) recommend that honey bee colonies be placed away from agricultural soils, which are moist, tilled and suitable for SHB pupation.

Meikle and Patt (2011) have suggested that the minimal temperature for SHB development is near 10°C in mineral soil. At 16°C, we found a survival rate between 12.5 and 22.9% in organic soil and we estimated the minimal temperature of development between 10.2 and 13.2°C depending on the soil water content. These findings suggest that the minimal temperature required for SHB development is higher than the previous estimate. However, as mentioned above, comparisons between different soil types might be inaccurate because the values of soil water content do not have the same signification in mineral and organic soils. SHB development may thus be limited by the cold soil temperatures that prevail in southern Canada in spring, winter and fall.

4.2 Pupal development time

As has been observed in other insects (Samara et al. 2011), the development time of SHB pupae decreased as temperature increased. The range of development timesis also narrower as temperature increases. At similar temperatures (17-24°C) and in moistened soil (unknown water content), Neumann et al. (2001) found that pupae took 36 to 53 days to complete metamorphosis, which is a wide range of emergence time. Murrle and Neumann (2004) measured a pupation period of 24.68 ± 1.75 days at room temperature (18-25°C) and in moistened mineral soil (unknown water content). At 21°C, Meikle and Patt (2011) found a pupation period of 32.7 days in a moist (5-8% by weight) sandy soil. They

also estimated a development time of 70 days at 15°C and 174 days at 12°C. At 16°C (lowest temperature tested), pupal development time was between 69.1 and 78.1 days. Meikle and Patt (2011) made a very similar prediction, but at 15°C, we estimate that development time would be between 82 and 93 days. Finally, knowledge of SHB development time at soil temperatures similar to those measured in southern Canada allows us to estimate the generation potential of this pest at up to two generations per year.

4.3 Sex ratio of emerging adults

In our study, sex ratio did not depend on temperature as observed by de Guzman and Frake (2007). Only soil water content was significant. We found an unbiased sex ratio of one female to one male in dry and intermediate soil, as observed by de Guzman and Frake (2007). However, we found a biased sex ratio of three females to one male in wet soils, which differs from reports by Neumann et al. (2001), Ellis et al. (2002a), Ellis et al. (2002b), Ellis et al. (2004) and Murrle and Neumann (2004). It is the first known report of a 3:1 sex ratio affected by soil water content for SHB. We hypothesize that males may be negatively affected by soil water content and die more readily than females. They may also be more affected by soil fungi.

4.4 Lifespan of emerging adults

In our study, the lifespan of emerging adult beetles was significantly affected by the water content of the soil where they pupate. However, the highest average lifespan we observed in emerging adults was 12.3 ± 1.2 days in dry soils, which is much lower than lifespan reported by other authors with similar diets and rearing temperatures. Adult beetles reared by Ellis et al (2002b) lived 123.4 ± 17.5 days at room temperature on a diet of bee pollen. Meikle and Patt (2011) found longevity of 34.7 ± 7.4 days for males and of 43.8 ± 7.0 days for females reared at 32°C on bee pollen. Arbogast *et al*. (2010) found longevity of 81.3 ± 30.0 °C for females reared at 27.5 ± 0.5 °C on a diet of pollen dough inoculated with *K. ohmeri*. Our rearing methods for emerging adults (in plastic tubes) may have reduced their lifespan. We noticed that cotton balls were occasionally soaked instead of moistened, and dead beetles were found in the liquid that accumulated underneath them. Sometimes larvae were not removed quickly enough and clogged the perforations in the lid with their feces. The fermentation produced in the tube may also have caused the adults to asphyxiate. However, to our knowledge, no authors studied the impact of soil pupation conditions on the lifespan of emerging adults. They all used constant temperature and humidity for pupation and then, had different parameters for adult longevity, which is the opposite of what we did. Pupation conditions might limit or enhance fitness of adults.

Despite these low lifespans, our findings on temperature and soil water content levels requirement for pupal development constitute new knowledge on SHB in southeastern Canadian climate. Under these conditions, SHB pupal development appears to be limited when soil temperatures drop below 16°C. Canadian honey bee colonies may thus benefit from a certain climatic protection from this invasive pest.

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Table 1 Mean percent survival rate ± SE for pupae of *Aethina tumida* at 16, 18 and 20°C and soil water contents of 0.37, 0.56 and 0.73 m^3 water/m^3 of dry soil

Temperature (°C)	Soil water content (m^3 water/m^3 dry soil)	Survival rate (%)				
16	0.37	14.7	±	5.9	B	b
	0.56	22.9	±	8.1	A	c
	0.73	12.5	±	5.8	B	a
18	0.37	90.3	±	4.2	A	a
	0.56	89.0	±	4.6	A	b
	0.73	41.6	±	11.2	B	a
20	0.37	97.4	±	1.7	A	a
	0.56	97.8	±	1.5	A	a
	0.73	38.3	±	13.3	B	a

Note: Means followed by the same letter are not significantly different at p = 0.05 (LSD test). Capital letters are for comparisons among water contents within one temperature. Lower case letters are for comparisons among temperatures within one water content level.

Table 2 Mean development time of *Aethina tumida* pupae ± SE at 16, 18 and 20°C and soil water contents of 0.37, 0.56 and 0.73 m^3 water/m^3 of dry soil

Temperature (°C)	Soil water content (m^3 water/m^3 dry soil)	Development time (d)				
16	0.37	78.1	±	2.1	A	a
	0.56	69.1	±	2.1	B	a
	0.73	71.6	±	2.3	B	a
18	0.37	54.4	±	2.1	A	b
	0.56	48.9	±	2.1	B	b
	0.73	47.6	±	2.2	B	b
20	0.37	38.3	±	2.2	A	c
	0.56	36.8	±	2.2	A	c
	0.73	39.0	±	2.3	A	c

Note: Means followed by the same letter are not significantly different at p = 0.05 (LSD test). Capital letters are for comparisons among water contents within one temperature. Lower case letters are for comparisons among temperatures within one water content level.

Table 3 Proportion of *Aethina tumida* females in soil water contents of 0.37, 0.56 and 0.73 m^3 water/m^3 of dry soil

Soil water content (m^3 water/m^3 dry soil)	Proportion of females			
0.37	0.51	±	0.03	A
0.56	0.52	±	0.03	A
0.73	0.73	±	0.07	B

Note: Means followed by the same letter are not significantly different at p = 0.025 (Bonferroni test).

393 **Table 4. Lifespan of emerging *Aethina tumida* adults in water contents of**
394 **0.37, 0.56 and 0.73 m^3 water/m^3 of dry soil**

Soil water content (m^3 water/m^3 dry soil)	Lifespan (days)			
0.37	12.3	±	1.2	A
0.56	11.9	±	1.2	A
0.73	6.0	±	1.2	B

395 Note: Means followed by the same letter are not significantly different at p = 0.05
396 (LSD test).
397

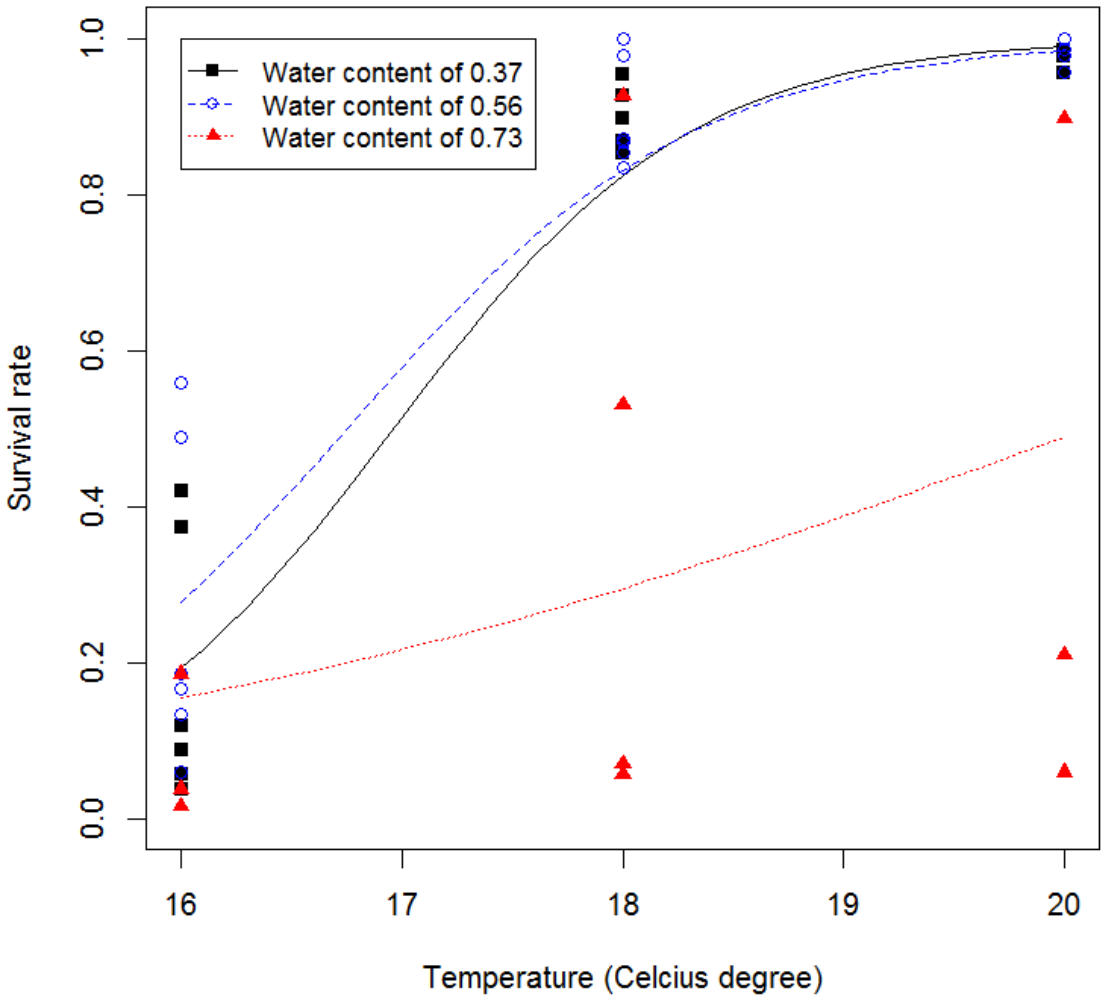


Figure 1 Survival rate of *Aethina tumida* pupae at temperatures of 16, 18 and 20°C and water contents of 0.37, 0.56 and 0.73 m^3 water/m^3 of dry soil (Linear equations in logit model for regression).

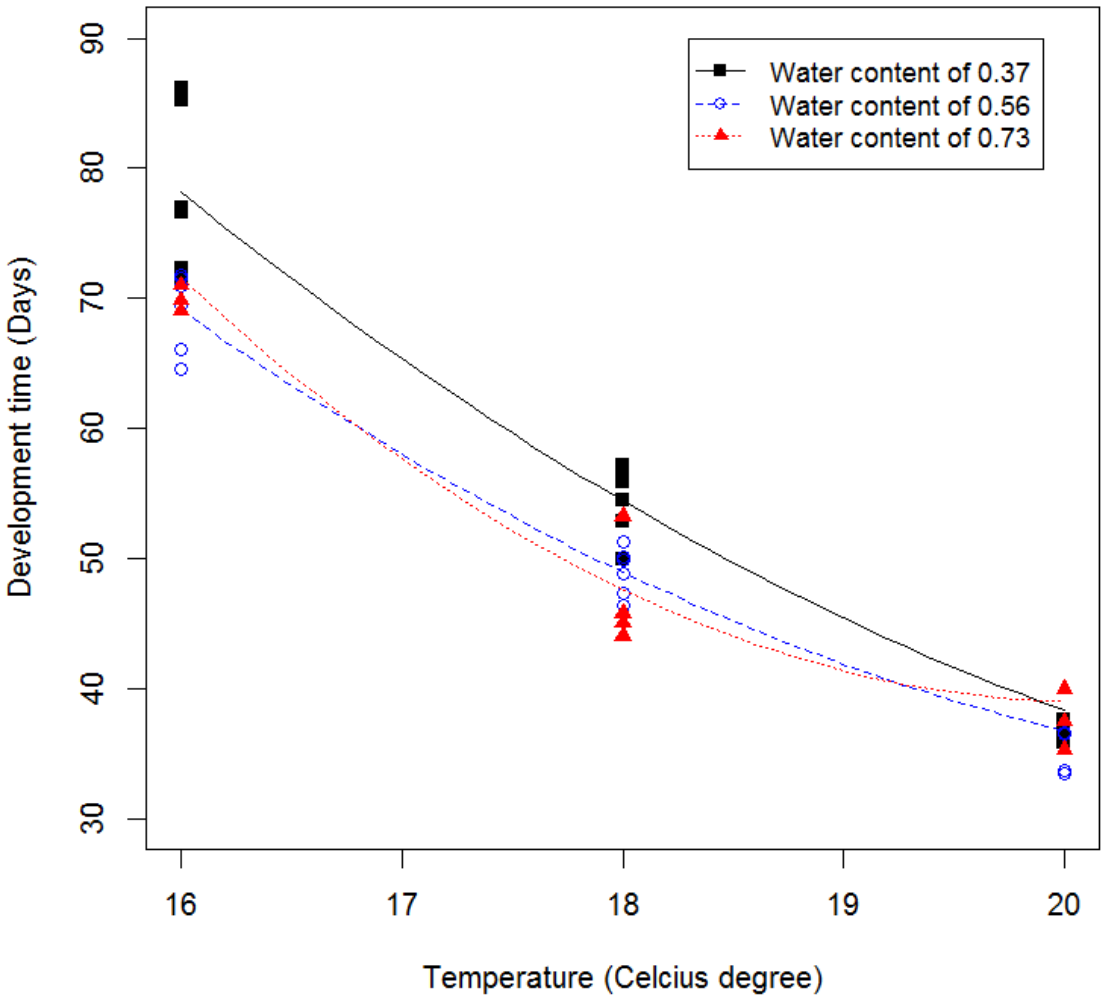
Equations:

Logit (S) = log (S/ (1-S))

Survival rate in soil of 0.37 m^3 water/m^3 soil: logit (S) = 1.5420 + 1.4877 (t-18)

Survival rate in soil at 0.56 m^3 water/m^3 soil: logit (S) = 1.5891 + 1.2736 (t-18)

Survival rate in soil at 0.73 m^3 water/m^3 soil: logit (S) = -0.8723 + 0.4142 (t-18)



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411 Figure 2 Development time of *Aethina tumida* pupae at temperatures of 16, 18 and
412 20°C and water contents of 0.37, 0.56 and 0.73 m^3 water/m^3 of dry soil (Quadratic
413 equations for regression).

414

415 **Equations:**
416 Development time in soil of 0.37 m^3 water/m^3 soil: 54.4041 - 9.9536 (t-18) + 0.9576
417 $(t-18)^2$
418 Development time in soil at 0.56 m^3 water/m^3 soil: 48.9409 - 8.0786 (t-18) + 0.9911
419 $(t-18)^2$

Development time in soil at 0.73 m^3 water/m^3 soil: 47.5765 - 8.1446 (t-18) + 1.9216 $(t-18)^2$

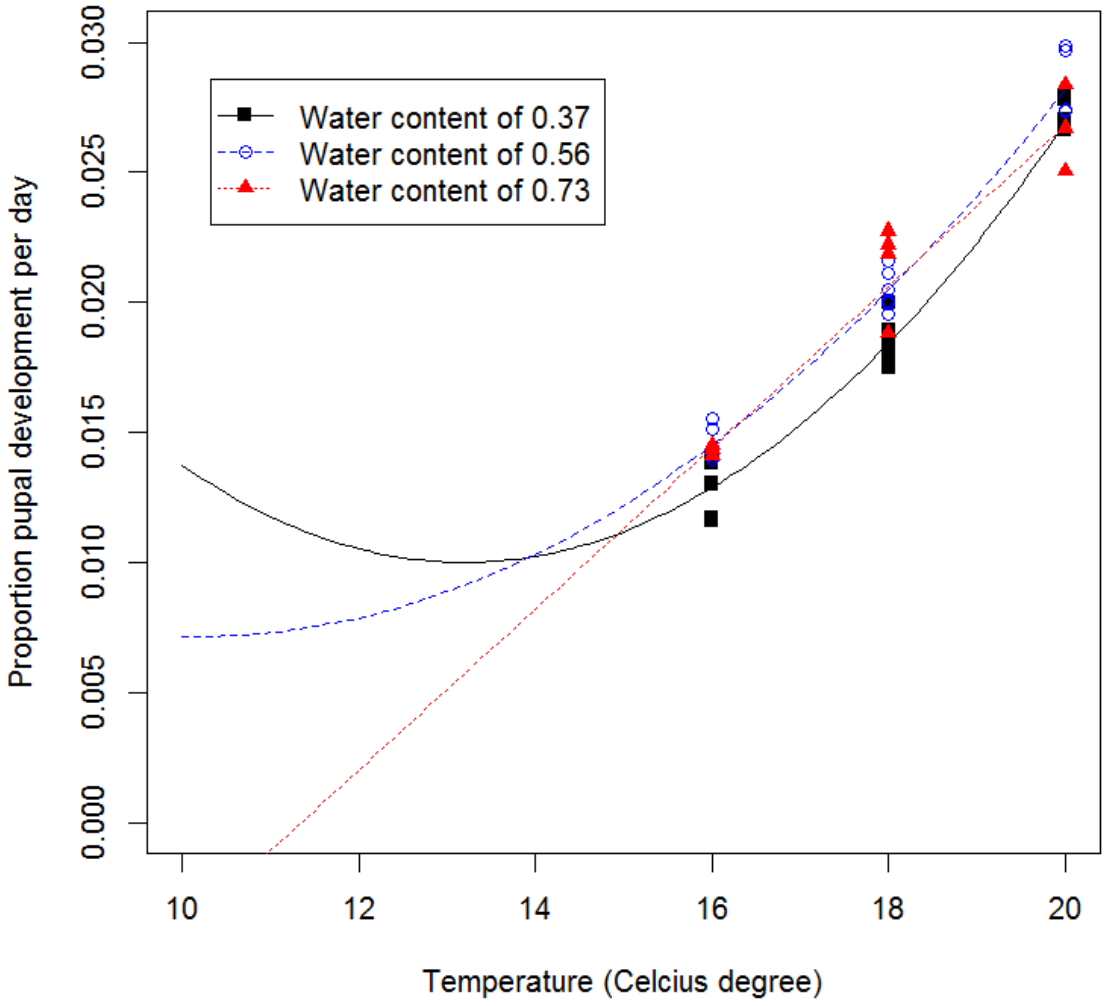

Figure 3 Development rate of *Aethina tumida* pupae at temperatures of 16, 18 and 20°C and water contents of 0.37, 0.56 and 0.73 m^3 water/m^3 of dry soil (Quadratic equations for 0.37 and 0.56 m^3 water/m^3 soil and linear equation for 0.73 m^3 water/m^3 soil).

Equations:

Development rate at 0.37 m^3 water/m^3 soil: $0.0184 + 0.0035\,(t-18) + 0.0004\,(t-18)^2$

Development rate at 0.56 m^3 water/m^3 soil: $0.0205 + 0.0034\,(t-18) + 0.0002\,(t-18)^2$

Development rate at 0.73 m^3 water/m^3 soil: $0.0206 + 0.0031\,(t-18)$

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