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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 content levels of 0.37, 0.56, and 0.73 m<sup>3</sup> water per cubic meter of dry soil) representative of southeastern Canada. Survivorship and development duration of *A. tumida* pupae, as well as sex ratio and life span 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 (69–78 d at 16°C, 47–54 d at 18°C, and 36–39 d at 20°C), but was longer in dryer soil. Optimal soil water content for pupal development was 0.56 m<sup>3</sup> water per cubic meter of soil. We estimated that the minimum development temperature for pupae is between 10.2 and 13.2°C, depending on soil water content. The 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 life span of emerging adults by half. This study contributes to a better understanding of *A. tumida* population dynamics in eastern Canada.

**KEY WORDS** *Aethina tumida*, temperature, soil water content, pupal development

*Aethina tumida* Murray (Coleoptera: Nitidulidae), or the small hive beetle, is a honey bee (*Apis mellifera* L.) pest indigenous to South Africa (Lundie 1940). Adult small hive beetles, known to live several months (Lundie 1940, Murrle and Neumann 2004, Haque and Levot 2005, Meikle and Patt 2011), 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. 2007), 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 first discovered in 1998 in the U.S. state of Florida (Thomas 1998) and then in 2002 in Australia (Somerville 2003). The first occurrences of small hive beetles 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 Quebec), a small hive beetle invasion was discovered during the fall of 2008 (Giovenazzo and Boucher 2010). Presence of small hive beetles in this

region can be attributed to the invasion of beetles from the United States (Giovenazzo and Boucher 2010). More recently, the pest was reported in Ontario (Kozak 2010) and again in Manitoba (2012). The damage caused by small hive beetles in Canadian honey bee colonies is not as significant as that experienced in the southern United States (Florida, Georgia, and South Carolina). The colder Canadian climate may explain why small hive beetle populations have failed to establish to date.

At the pupal stage, the small hive beetle is particularly vulnerable to the impact of both climatic factors and predators. De Guzman and Frake (2007) observed mortality of small hive beetles mainly at this stage when reared at temperatures of 24–28 and 34°C. Thus, many authors, such as Lundie (1940) and Ellis et al. (2004), suggest that environmental factors affect the reproduction potential of small hive beetles. Because small hive beetles pupate in the soil, edaphic factors including moisture and density, field slope, drainage, rainfall, and temperature greatly influence this stage of their development (de Guzman et al. 2009). Soil temperature (de Guzman and Frake 2007, de Guzman et al. 2009, Meikle and Patt 2011) and soil moisture (Lundie 1940, Schmolke 1974, Ellis et al. 2004, Haque and Levot 2005) are the edaphic factors that have the greatest impact on pupal development and survivorship. Finally, soil type does not seem to affect the

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development of small hive beetle pupae (Schmolke 1974, Ellis et al. 2004, de Guzman et al. 2009).

Pupal development has been measured at temperatures of 21–35°C (Neumann et al. 2001, Murrle and Neumann 2004, Ellis et al. 2004, Haque and Levot 2005, de Guzman and Frake 2007, de Guzman et al. 2009, Meikle and Patt 2011, Meikle and Diaz 2012), which are representative of climatic conditions in Africa, the southern United States, and Australia. Moreover, Meikle and Patt (2011) estimated that pupae could not develop at temperatures <10°C. Nonetheless, soil temperatures in Canada during beekeeping season can range between 10 and 21°C. To our knowledge, pupal development has not been tested at these temperatures, and thus should be investigated to gain knowledge on the reproduction of small hive beetles in temperate climates. Furthermore, seasonal rainfall is an important indicator of small hive beetle 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 small hive beetles in moist soils, but did not measure the 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 the pupal development of small hive beetles at various thermo-hygrometric soil conditions similar to those observed in southeastern Canada. We also measured the small hive beetle sex ratio and life span of emerging adults.

### Materials and Methods

**Small Hive Beetle Rearing.** Adult beetles, both male and female, were collected in May 2010 from infested honey bee colonies located in West Montérégie, southern Quebec, Canada (N 45.003983, W 74.449317). These small hive beetles were used to establish an experimental population reared in growth chambers (model PGR15 and EI5, Conviron, Winnipeg, Canada) at Université Laval, Quebec, Canada. Small hive beetles were kept in darkness at  $30 \pm 0.5^\circ\text{C}$  and 50–60% relative humidity. Adult beetles were placed in 550-ml cylindrical plastic containers (10 cm in diameter, 7 cm in depth) 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, Quebec, in the summer of 2010.

**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<sup>3</sup> water per cubic meter of dry soil. These values cor-

respond, in an organic soil, to dry, intermediate, and wet (near saturation) soil. Organic potting soil (Promix by Premier Tech, Rivière-du-Loup, Canada; bulk density of 0.293 g/cm<sup>3</sup>) 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 allowed little to no moisture or gas exchange but contained enough air for pupae to breath. Sterilized water (150, 230, 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; Onset Computer, Bourne, MA) and soil water content (EC-5 Moisture sensor S SMC-M005, Decagon Devices, Pullman, WA). They were inserted to a depth of 3 cm and data were recorded every 15 min. There was no need to add water throughout the trial because the 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 afterward were fed ad libitum with pollen and water for 15 d. On Day 15, these larvae were equally distributed into nine different experimental groups (three different temperatures  $\times$  three different soil water content levels). 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 small hive beetles at any life stage.

**Sex Ratio and Life Span 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 eighth tergite (de Guzman and Frake 2007). All emerging adults were kept in growth chambers at  $30.0 \pm 0.5^\circ\text{C}$ . They were placed in couples in 50-ml plastic tubes (Starstedt, Nümbrecht, Germany) 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).

**Statistics.** The experiment was planned as a split-plot design with temperature as the main plot and soil water content levels as subplots. The temperatures were randomized into a 3 by 3 Latin square with blocks (date) as rows and growth chambers as columns. There were three replications for the temperatures 16 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 content levels (0.37, 0.56, and 0.73 m<sup>3</sup> water per cubic meter of dry soil) was repeated twice per

growth chamber using two groups of  $\approx 50$  pupae. Because pupae in each group are pseudoreplications, all analyses were done on the average value per group, except for the experiment in which the sex effect was also studied. In that case, average values of the response variables were computed for each sex in each group, and analyses were done using a split-split-plot design in analysis of variance (ANOVA) with sex in the sub-subplots. All analyses were done at  $\alpha = 0.05$  level of significance, and they considered temperatures, soil water content levels, and sex (when appropriate) as fixed effects, and blocks, chambers, and groups as random effects.

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

The pupal development length and life span of emerging adults were compared between levels of soil temperatures and soil water contents using the traditional split-plot ANOVA model for a Gaussian response variable. The same types of test-of-effect slices, multiple comparisons, and contrasts as those previously mentioned were used to identify the treatment differences. The model was fitted to the data using the MIXED procedure of SAS. The potential effect of sex on these response 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 the linear and quadratic relation based on the Akaike information criterion (Burnham and Anderson 2002).

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

## Results

**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 per cubic meter of soil, but not in wet soils (0.73  $m^3$  water per cubic meter of soil). More precisely, for water content of 0.37  $m^3$  water per cubic meter of soil,

**Table 1.** Mean percent survival rate  $\pm$  SE for pupae of *A. tumida* at 16, 18, and 20°C and soil water content levels of 0.37, 0.56, and 0.73  $m^3$  water per cubic meter of dry soil

Temperature (°C)	Soil water content ( $m^3$ water per cubic meter of dry soil)	Survival rate (%)
16	0.37	14.7 $\pm$ 5.9Bb
	0.56	22.9 $\pm$ 8.1Ac
	0.73	12.5 $\pm$ 5.8Ba
18	0.37	90.3 $\pm$ 4.2Aa
	0.56	89.0 $\pm$ 4.6Ab
	0.73	41.6 $\pm$ 11.2Ba
20	0.37	97.4 $\pm$ 1.7Aa
	0.56	97.8 $\pm$ 1.5Aa
	0.73	38.3 $\pm$ 13.3Ba

Means followed by the same letter are not significantly different at  $P = 0.05$  (LSD test). Capital letters are for comparisons among water content levels within one temperature. Lowercase letters are for comparisons among temperatures within one water content level.

the survival rates were significantly higher at 20 and 18°C (97.4  $\pm$  1.7% and 90.3  $\pm$  4.2%, respectively). For water content of 0.56  $m^3$  water per cubic meter of soil, all temperatures were significantly different from each other, with the highest survival at 20°C (97.8  $\pm$  1.5%; Table 1). There was also a significant effect of water content for each temperature. At both temperatures of 18 and 20°C, the survival rates were significantly higher in dry (0.37  $m^3$  water per cubic meter of soil) and intermediate (0.56  $m^3$  water per cubic meter of soil) soil water content levels. At a temperature of 16°C, the survival rates were low especially for water content levels of 0.37  $m^3$  water per cubic meter of soil (14.7  $\pm$  5.9%) and 0.73  $m^3$  water per cubic meter of soil (12.5  $\pm$  5.8%). High soil water content and temperature of 16°C were limiting factors on the development of *A. tumida* pupae (Table 1; Fig. 1). The temperature had a significant linear relation with the logit of the survival probability but a different pattern of relation among levels of water content ( $F = 29.93$ ;  $df = 2,28$ ;  $P < 0.001$ ). The models had a good discrimination rate based on the area under the ROC curve (Hosmer and Lemeshow 2000), which is a coefficient similar to the  $R^2$ , but for binomial regressions, as used in our study (AUROC = 0.91 for 0.37  $m^3$  water per cubic meter of soil; AUROC = 0.88 for 0.56  $m^3$  water per cubic meter of soil, and AUROC = 0.67 for 0.73  $m^3$  water per cubic meter of soil; Fig. 1).

Pupal development time was affected by the interaction between soil temperature and soil water content ( $F = 5.23$ ;  $df = 4,28$ ;  $P = 0.003$ ). Mean development time varied from 69.1  $\pm$  2.1 d to 78.1  $\pm$  2.1 d at 16°C, from 47.6  $\pm$  2.2 d to 54.4  $\pm$  2.1 d at 18°C, and from 36.8  $\pm$  2.2 d to 39.0  $\pm$  2.3 d at 20°C. At 16 and 18°C, development time was longer for pupae in a soil water content of 0.37  $m^3$  water per cubic meter of soil than at 0.56  $m^3$  water per cubic meter of soil and at 0.73  $m^3$  water per cubic meter of soil. At 20°C, there was no significant difference between the three different soil water content levels (38.3  $\pm$  2.2 d at 0.37  $m^3$  water per cubic meter of soil, 36.8  $\pm$  2.2 d at 0.56  $m^3$  water per cubic meter of soil, and 39.0  $\pm$  2.3 d at 0.73  $m^3$  water per cubic meter of soil). Lower temperature increased

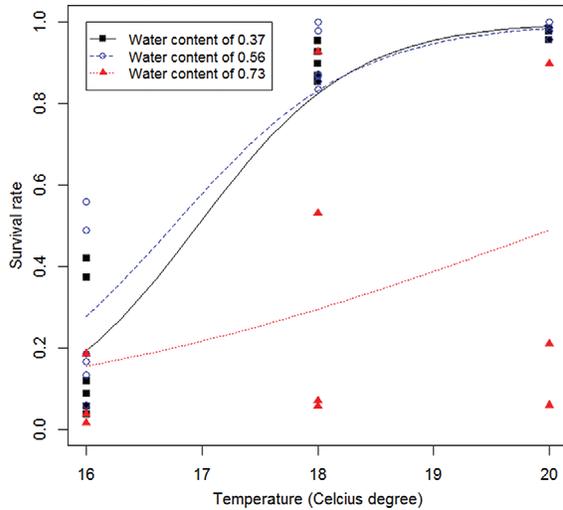


Fig. 1. Survival rate of *A. tumida* pupae at temperatures of 16, 18, and 20°C and water content levels of 0.37, 0.56, and 0.73 m<sup>3</sup> water per cubic meter of dry soil (linear equations in logit model for regression). Equations: Logit (S) = log (S / (1 - S)). Survival rate in soil of 0.37 m<sup>3</sup> water per cubic meter of soil: logit (S) = 1.5420 + 1.4877 (t-18). Survival rate in soil at 0.56 m<sup>3</sup> water per cubic meter of soil: logit (S) = 1.5891 + 1.2736 (t-18). Survival rate in soil at 0.73 m<sup>3</sup> water per cubic meter of soil: logit (S) = -0.8723 + 0.4142 (t-18). (Online figure in color.)

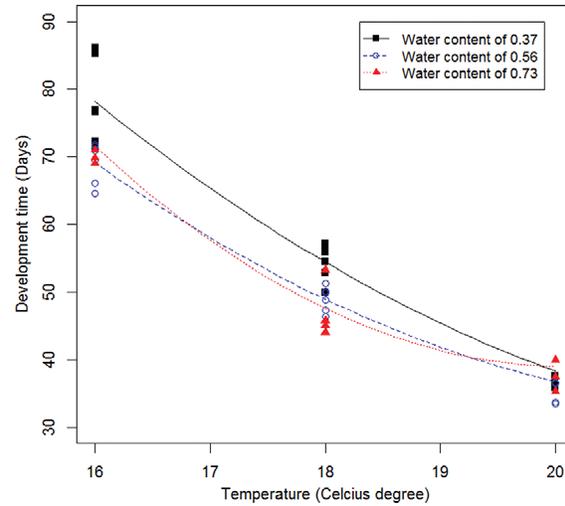


Fig. 2. Development time of *A. tumida* pupae at temperatures of 16, 18, and 20°C and water content levels of 0.37, 0.56, and 0.73 m<sup>3</sup> water per cubic meter of dry soil (quadratic equations for regression). Equations: Development time in soil of 0.37 m<sup>3</sup> water per cubic meter of soil: 54.4041 - 9.9536 (t-18) + 0.9576 (t-18)<sup>2</sup>. Development time in soil at 0.56 m<sup>3</sup> water per cubic meter of soil: 48.9409 - 8.0786 (t-18) + 0.9911 (t-18)<sup>2</sup>. Development time in soil at 0.73 m<sup>3</sup> water per cubic meter of soil: 47.5765 - 8.1446 (t-18) + 1.9216 (t-18)<sup>2</sup>. (Online figure in color.)

the duration of pupal development (Table 2; Fig. 2). Temperature had a significant quadratic relation with the pupal development time at all water content levels, but the relation at 0.37 m<sup>3</sup> water per cubic meter of soil was different from the relation at 0.56 and 0.73 m<sup>3</sup> water per cubic meter of 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 percentage of variance ( $R^2 = 0.95$  for 0.37 m<sup>3</sup> water per cubic meter of soil;  $R^2 = 0.97$  for 0.56 m<sup>3</sup> water per cubic meter of soil, and  $R^2 = 0.96$  for 0.73 m<sup>3</sup> water per cubic meter of soil; Fig. 2). The development time of females did not differ from that of males ( $F = 0.170$ ;  $df = 1,28$ ;  $P =$

Table 2. Mean development time of *A. tumida* pupae  $\pm$ SE at 16, 18, and 20°C and soil water content levels of 0.37, 0.56, and 0.73 m<sup>3</sup> water per cubic meter of dry soil

Temperature (°C)	Soil water content (m <sup>3</sup> water per cubic meter of dry soil)	Development time (d)
16	0.37	78.1 $\pm$ 2.1Aa
	0.56	69.1 $\pm$ 2.1Ba
	0.73	71.6 $\pm$ 2.3Ba
18	0.37	54.4 $\pm$ 2.1Ab
	0.56	48.9 $\pm$ 2.1Bb
	0.73	47.6 $\pm$ 2.2Bb
20	0.37	38.3 $\pm$ 2.2Ac
	0.56	36.8 $\pm$ 2.2Ac
	0.73	39.0 $\pm$ 2.3Ac

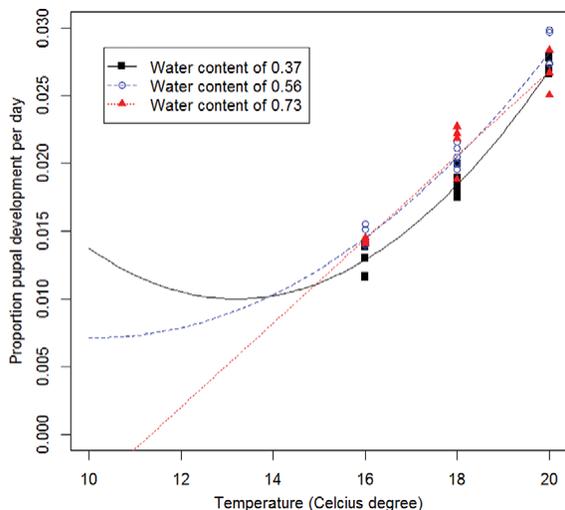
Means followed by the same letter are not significantly different at  $P = 0.05$  (LSD test). Capital letters are for comparisons among water content levels within one temperature. Lowercase letters are for comparisons among temperatures within one water content level.

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 content levels, but the relation was quadratic for dry and intermediate soils, and the relation was linear for wet soils. The models explained a high percentage of variance ( $R^2 = 0.98$  for 0.37 m<sup>3</sup> water per cubic meter of soil;  $R^2 = 0.98$  for 0.56 m<sup>3</sup> water per cubic meter of soil, and  $R^2 = 0.93$  for 0.73 m<sup>3</sup> water per cubic meter of soil; Fig. 3). Extrapolating the curves showed a minimum temperature for pupal development of 13.2°C at 0.37 m<sup>3</sup> water per cubic meter of soil, 10.2°C at 0.56 m<sup>3</sup> water per cubic meter of soil, and 11.4°C at 0.73 m<sup>3</sup> water per cubic meter of soil.

**Sex Ratio and Life Span 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 content levels of 0.73 versus 0.37 and 0.56 ( $F = 5.97$ ;  $df = 1,28$ ;  $P = 0.021$ ) after a Bonferroni adjustment ( $\alpha = 0.025$ ; Hochberg and Tamhane 1987). The proportion of females was 0.51  $\pm$  0.03 at 0.37 m<sup>3</sup> water per cubic meter of soil, 0.52  $\pm$  0.03 at 0.56 m<sup>3</sup> water per cubic meter of soil, and 0.73  $\pm$  0.07 at 0.73 m<sup>3</sup> water per cubic meter of soil (Table 3). In dry or intermediate soils, the sex ratio was 1 ♀:1 ♂, while in wet soils, the ratio was 3 ♀:1 ♂.

Life span of emerging adults was significantly altered by soil water content ( $F = 8.34$ ;  $df = 2,33$ ;  $P =$



**Fig. 3.** Development rate of *A. tumida* pupae at temperatures of 16, 18, and 20°C and water content levels of 0.37, 0.56, and 0.73 m<sup>3</sup> water per cubic meter of dry soil (quadratic equations for 0.37 and 0.56 m<sup>3</sup> water per cubic meter of soil and linear equation for 0.73 m<sup>3</sup> water per cubic meter of soil). Equations: Development rate at 0.37 m<sup>3</sup> water per cubic meter of soil: 0.0184 + 0.0035 (t-18) + 0.0004 (t-18)<sup>2</sup>. Development rate at 0.56 m<sup>3</sup> water per cubic meter of soil: 0.0205 + 0.0034 (t-18) + 0.0002 (t-18)<sup>2</sup>. Development rate at 0.73 m<sup>3</sup> water per cubic meter of soil: 0.0206 + 0.0031 (t-18). (Online figure in color.)

0.001). Adults that developed at 0.37 and 0.56 m<sup>3</sup> water per cubic meter of soil lived twice as long as adults at 0.73 m<sup>3</sup> water per cubic meter of soil (Table 4). However, the life span of emerging adults was not affected by temperature ( $F = 12.06$ ;  $df = 2,1$ ;  $P = 0.200$ ) or sex ( $F = 2.88$ ;  $df = 1,28$ ;  $P = 0.101$ ).

**Discussion**

**Survival Rate of Pupae.** Pupal survivorship was influenced by both temperature and soil water content. Survivorship was >89% at 18 and 20°C with low (0.37 m<sup>3</sup> water per cubic meter of soil) and intermediate (0.56 m<sup>3</sup> water per cubic meter of 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<sup>3</sup> per larva). In our experiment, each larva had between 6.0 and 9.5 cm<sup>3</sup> of

**Table 3.** Proportion of *A. tumida* females in soil water content levels of 0.37, 0.56, and 0.73 m<sup>3</sup> water per cubic meter of dry soil

Soil water content (m <sup>3</sup> water per cubic meter of dry soil)	Proportion of females
0.37	0.51 ± 0.03A
0.56	0.52 ± 0.03A
0.73	0.73 ± 0.07B

Means followed by the same letter are not significantly different at  $P = 0.025$  (Bonferroni test).

**Table 4.** Life span of emerging *A. tumida* adults in water content levels of 0.37, 0.56, and 0.73 m<sup>3</sup> water per cubic meter of dry soil

Soil water content (m <sup>3</sup> water per cubic meter of dry soil)	Life span (d)
0.37	12.3 ± 1.2A
0.56	11.9 ± 1.2A
0.73	6.0 ± 1.2B

Means followed by the same letter are not significantly different at  $P = 0.05$  (LSD test).

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, as 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 small hive beetle pupation.

Meikle and Patt (2011) have suggested that the minimal temperature for small hive beetle development is ≈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 small hive beetle 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. Small hive beetle development may thus be limited by the cold soil temperatures that prevail in southern Canada in spring, winter, and fall.

**Pupal Development Time.** As has been observed in other insects (Samara et al. 2011), the development time of small hive beetle pupae decreased as temperature increased. The range of development times is 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 36–53 d to complete metamorphosis, which is a wide range of emergence time. Murrel and Neumann (2004) measured a pupation period of 24.68 ± 1.75 d 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 d in a moist (5–8% by weight) sandy soil. They

also estimated a development time of 70 d at 15°C and 174 d at 12°C. At 16°C (lowest temperature tested), pupal development time was between 69.1 and 78.1 d. Meikle and Patt (2011) made a very similar prediction, but at 15°C, we estimate that development time would be between 82 and 93 d. Finally, knowledge of small hive beetle 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.

**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,b, 2004), and Murrle and Neumann (2004). It is the first known report of a 3:1 sex ratio affected by soil water content for small hive beetle. 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.

**Life Span of Emerging Adults.** In our study, the life span of emerging adult beetles was significantly affected by the water content of the soil where they pupate. However, the highest average life span we observed in emerging adults was  $12.3 \pm 1.2$  d in dry soils, which is much lower than the life span reported by other authors who used similar diets and rearing temperatures. Adult beetles reared by Ellis et al. (2002b) lived  $123.4 \pm 17.5$  d at room temperature on a diet of bee pollen. Meikle and Patt (2011) found longevity of  $34.7 \pm 7.4$  d for males and of  $43.8 \pm 7.0$  d for females reared at 32°C on bee pollen. Arbogast et al. (2010) found longevity of  $81.3 \pm 30.0$  d for females reared at  $27.5 \pm 0.5$ °C on a diet of pollen dough inoculated with *K. ohmeri*. Our rearing methods for emerging adults (in plastic tubes) may have reduced their life span. 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 life span 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 short life spans, our findings on temperature and soil water content levels required for pupal development constitute new knowledge on small hive beetle in a southeastern Canadian climate. Under these conditions, small hive beetle pupal development appears to be limited when soil temperatures drop to <16°C. Canadian honey bee colonies

may thus benefit from a certain climatic protection from this invasive pest.

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