Le petit coléoptère de la ruche (*Aethina tumida* Murray, Coleoptera : Nitidulidae) développement, reproduction et survie à l'hivernage au Québec

Rapport final

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Fiche de transfert

Invasion et survie du Petit coléoptère de la ruche au Québec

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Durée : 06/2009 – 12/2012

FAITS SAILLANTS

L'invasion d'*Aethina tumida* (petit coléoptère de la ruche, PC) fut confirmée pour la première fois au Québec, en septembre 2008, dans les colonies d'abeilles mellifères de la région Montérégie-ouest. Cette invasion est le résultat d'une migration de PC à partir des colonies infestées situées dans l'état de New York (États-Unis). Nos travaux démontrent que l'utilisation de pièges mortels, placés dans les ruches, réduit le nombre de PC dans les colonies infestées sans nuire à leur productivité. Les PC survivent à l'hiver dans les ruches du sud du Québec et ils pourraient y réaliser entre deux et trois cycles de développement par année.

OBJECTIFS ET MÉTHODOLOGIE

Les objectifs de ce projet de recherche étaient de: 1) décrire l'invasion du PC dans la région Montérégie-ouest; 2) comparer l'efficacité de différents types de pièges mortels: 3) déterminer l'effet de facteurs édaphiques sur le développement pupal du PC.

En juillet 2009, 40 colonies sentinelles d'abeilles mellifères furent placées dans 7 sites en Montérégie-ouest. Deux de ces sites infestés par les PC ont été utilisés de mai 2011 à octobre 2011 pour tester l'efficacité des pièges mortels suivants: Beetle Barn (Rossmann Apiaries), AJ's Beetle Eater (AJ's Beetle Eater) et Hood trap (Rocky Mountain Bee Farm).

Le développement pupal du PC a été étudié in vitro (en incubateur) dans un sol organique à 16, 18 et 20°C avec un contenu gravimétrique en eau de 0,150, 0,192 et 0,250 gg^{-1}. Ces valeurs représentent l'étendu des conditions édaphiques retrouvées en été dans le sud-est du Québec.

RÉSULTATS SIGNIFICATIFS POUR L'INDUSTRIE

Invasion du Petit coléoptère de la ruche en Montérégie-ouest

Il y a une invasion annuelle (juillet et août) des PC en provenance de colonies d'abeilles infestées de l'état du New York à proximité de la frontière (environ 500 mètres). Les PC adultes survivent à l'hivernage (novembre à avril) dans les colonies infestées situées en Montérégie-ouest.

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Efficacité des pièges mortels
L'utilisation de pièges mortels dans une ruche réduit le nombre de PC sans nuire au développement et à la productivité de la colonie d'abeilles. En Montérégie-Ouest, le piège Beetle Barn (placé sur le plateau inférieur de la ruche) a été le plus efficace durant la première semaine de piégeage en mai. Dans le comté d'Essex (Ontario), le piège AJ's Beetle Eater a été le plus efficace de août à octobre. L'ajout de vinaigre de cidre de pommes n'améliore pas l'efficacité de piégeage.

Développement pupal
La survie de la pupe augmente avec la température, mais diminue avec l'augmentation du contenu en eau du sol. La durée du développement pupal augmente avec la diminution de température (69 à 78 jours à 16°C, 47 à 54 jours à 18°C et 36 à 39 jours à 20°C) et avec la diminution du contenu en eau du sol. Le développement pupal a été optimal dans une hygrométrie du sol intermédiaire de 0,192 gg-1.

APPLICATIONS POSSIBLES POUR L'INDUSTRIE

L'industrie apicole québécoise et canadienne reconnait maintenant la capacité de survie des PC adultes dans notre climat. Les résultats et observations issues de ce projet de recherche confirment sa capacité d'hivernage dans les colonies et suggèrent que ce ravageur peut réaliser entre deux et trois cycles de développement dans les conditions thermo-hygrométriques du sud québécois. La prolifération des populations de PC est fortement influencée par la force des colonies d'abeilles. De plus, les PC envahissent rapidement les colonies faibles et le matériel apicole abandonné. Les apiculteurs doivent donc ajuster leur régie pour ne pas faciliter l'invasion des PC dans leur entreprise. L'invasion des PC qui se produit actuellement au sud du Québec doit être contrôlée et surveillée. L'utilisation de pièges mortels placés dans les ruches est un moyen efficace de contrôle et de surveillance/dépistage de ces coléoptères. Nous recommandons d'utiliser le Beetle Barn en début de saison avant la miellée et le AJ's beetle eater lorsqu'il y a des hausses à miel.

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Activités de diffusion et de transfert d'information

- Symposium Apimondia Québec 2012 : Affiche Martine Bernier
- Colloque 2012 de la Fédération des apiculteurs du Québec, Drummondville : Présentation orale Pierre Giovenazzo
- Colloque 2012 de l'Union des syndicats apicoles du Québec, Victoriaville : Présentation orale Martine Bernier
- Colloque 2012 de l'Association des apiculteurs de l'Ontario, Lindsay : Présentation orale Pierre Giovenazzo
- Journée champêtre 2011 Centre de référence en agroalimentaire du Québec, au CRSAD à Deschambault : Présentation de Martine Bernier
- Congrès annuel 2011 du Conseil canadien du miel et de l'association des apiculteurs professionnels du Canada, Winnipeg Manitoba : Présentation orale Martine Bernier et Madame Bernier reçoit le prix <Étudiante CAPA 2012> pour ses travaux sur le petit coléoptère de la ruche
- Colloque 2011 de la Fédération des apiculteurs du Québec, Drummondville : Présentation orale Pierre Giovenazzo
- Colloque 2011 de l'Union des syndicats apicoles du Québec, Victoriaville : Présentation orale Martine Bernier
- Congrès annuel 2011 de l'Association apiculteurs de l'Alberta, Edmonton : Présentation orale Pierre Giovenazzo
- Congrès annuel 2011 de l'Association des apiculteurs de la Colombie-Britanique, Surrey : Présentation orale Pierre Giovenazzo
- Colloque 2010 de la Fédération des apiculteurs du Québec, Drummondville : Présentation orale Pierre Giovenazzo
- Colloque 2010 de l'Union des syndicats apicoles du Québec, Victoriaville : Présentation orale Martine Bernier
- Congrès annuel 2010 de l'Association des apiculteurs du Manitoba, Winnipeg : Présentation orale Pierre Giovenazzo
- Congrès annuel 2010 du Conseil canadien du miel et de l'association des apiculteurs professionnels du Canada, Markham Ontario : Présentation orale Pierre Giovenazzo
- Colloque 2010 des éleveurs de reines en France ANERCEA, Limoges : Présentation orale Pierre Giovenazzo

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Note scientifique sur la présence du petit coléoptère de la ruche (*Aethina tumida* Murray) au Québec

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Article publié :

L'abeille, printemps 2010 (article en annexe)

American Bee Journal, March 2010 (article en annexe)

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Dans leur éditorial, Neumann et Ellis (Neumann and Ellis 2008) font une description des plus récentes découvertes sur la dispersion mondiale du petit coléoptère de la ruche (PCR) *Aethina tumida* (Murray) et soulignent la rapidité avec laquelle ce ravageur des colonies d'abeilles, d'origine africaine, a réussi à envahir la plupart des pays apicoles à partir de son continent d'origine l'Afrique. Le PCR a été observé en Amérique du nord pour la première fois en 1998 dans l'état de la Floride et dès l'an 2000, on le trouve dans les états adjacents au Québec (Vermont, Maine et New York). Cette dispersion rapide du PCR suggère que le transport des colonies (transhumance) en est la principale cause. Le petit coléoptère de la ruche a été trouvé occasionnellement au Canada depuis 2002 mais il n'a pas réussi à s'implanter comme espèce invasive (communications personnelles : Apiculteur provincial du Manitoba, Réal Laférièniére et Apiculteur provincial de l'Alberta, Medhat Nasr). Dans cette note scientifique nous décrivons la récente découverte du PCR en Montérégie près de la frontière Canada-USA.

La découverte des premiers coléoptères au Québec a été faite par un apiculteur de la région de la Montérégie en septembre 2008. Durant le même mois, une inspection par les autorités provinciales a permis de trouver 6 sites (ruchers) infestés et plusieurs stades du développement d'*A. tumida* ont été observés (Figures 1 et 2). Une analyse génomique de quelques-uns de ces coléoptères a confirmé la présence de l'haplotype des USA (Boucher 2009). Ce résultat suggère qu'il y a eu une migration des PCR du sud des USA vers le nord. À la suite de cette découverte, les responsables du MAPAQ en

collaboration avec l'équipe de recherche apicole du Centre de recherche en sciences animales de Deschambault (CRSAD) ont proposé de faire une étude sur la dispersion de ce ravageur et de vérifier s'il sera en mesure de compléter son cycle vital dans le sud du Québec.

Au cours du mois de mai 2009 l'équipe du CRSAD a réalisé une inspection de toutes les colonies d'abeilles présentes dans un rayon de 20 km autour du site de la découverte originale (figure 3).Plus de 250 colonies ont été examinées pour la présence du PCR selon la méthode décrite dans la littérature (Neumann and Hoffmann 2008). À l'issu de cette inspection, aucun PCR n'a été trouvé (même dans les ruchers où l'on avait trouvé des PCR à l'automne 2008). Ce résultat suggérait que les PCR n'avaient pas réussi à survivre à l'hivernage. Par contre, on soupçonnait qu'un faible nombre avait probablement réussi à survivre l'hivernage et que nous n'avions pas réussi à les repérer lors de l'inspection visuelle des colonies d'abeilles.

Le 13 juillet 2009 nous avons placé 40 colonies sentinelles dans 7 sites entre la ville de Dundee et Franklin (Figure 4). Le choix et la recherche des sites sentinelles ont été grandement facilités par la collaboration d'un apiculteur de la région : Miels Laberge, propriétaire Joël Laberge, Saint-Stanislas-de-Kostka. Ces colonies sentinelles ont été fabriquées à partir de deux cadres à couvain avec abeilles et un cadre de miel placés dans une ruche Langstroth à 10 cadres. Nous avons fabriqué des petits pièges à PCR avec un morceau de Coroplast™ ondulé (15 x 15cm avec des ouvertures de 5mm, Figure 3). Ces pièges ont été installés sur le plateau inférieur de la chambre à couvain (Elzen, Baxter et al. 1999). Nous

avons également placé des pièges extérieurs fabriqués à partir de sceaux en plastiques (Figure 4) tel que décrit par Elzen (Elzen et al 2000) et Arbogast (Arbogast, Torto et al. 2009). Ces pièges/sceaux ont été placés dans les arbres autour de chaque site sentinelle et un appât composé de 20 grammes de miel et de pollen a été placé dans chaque sceau. Les colonies et les pièges/sceaux ont été vérifiés à toutes les trois semaines de juillet à novembre 2009.

Les premiers PCR ont été trouvés le 19 août dans deux des sites sentinelles les plus proches de la frontière Canada-USA (Table 1). Les PCR se déplaçaient sur le plateau inférieur des ruches, sur les cadres et certains étaient cachés dans les ondulations des pièges en Coroplast™. Des PCR ont été trouvés dans ces deux site jusqu'en novembre. Des PCR ont également été trouvés dans un troisième site en novembre. Nous n'avons pas observé d'œufs ni de larves durant toutes les inspections 2009. Aucun PCR n'a été trouvé dans les pièges/sceaux.

Au cours de l'été 2009, nous avons visité des colonies aux USA situées à proximité de la frontière et de nos sites sentinelles (Figure 4). Les colonies USA étaient infestées de PCR (larves et adultes) et les ruches abandonnées étaient particulièrement très infestées. Le propriétaire de ces colonies nous a informés qu'elles provenaient de la Floride et de la Virginie et qu'il les avait transportées à l'état de New York au début juillet pour la production de miel (communication personnelle avec David Hackenburg). Un des ruchers USA infestés était situé à environ 1 km de nos sites sentinelles 1 et 2. À cet endroit il y a une ligne de transport d'électricité déboisée qui traverse la frontière.

Ces résultats préliminaires suggèrent que les PCR trouvés au Québec proviennent des colonies d'abeilles situées de l'autre côté de la frontière Canada-USA. Les apiculteurs USA transportent les colonies utilisées pour la pollinisation des fruits dans les états du sud vers les états du nord pour assurer leur développement, la formation de nucléis et la production de miel. Cette opération contribue à la dispersion nordique des PCR jusqu'au Québec. Les travaux de Spiewok (Spiewok, Pettis et al. 2007; Spiewok, Duncan et al. 2008) ont montré que les PCR se propagent facilement entre les colonies d'un rucher et peuvent même voler sur des distances de près de 15 km. Nos observations montrent qu'il y avait des populations de PCR situées tout près de nos ruches sentinelles au Québec (environ 1 km) et que leur migration était facilitée par un corridor déboisé d'une ligne de transport d'électricité.

Nous ne savons pas si les PCR peuvent compléter leur cycle vital dans les conditions environnementales du Québec ou s'ils peuvent survivre avec une colonie d'abeilles hivernée à l'extérieur. Par contre certains travaux réalisés en chambre environnementale contrôlée ont déjà démontré que de petits nombres de PCR réussissent à hiverner avec les colonies d'abeilles (Evans, Pettis et al. 2000; Hood 2000; Pettis and Shimanuki 2000). L'équipe du CRSAD poursuivra son étude du PCR dans cette région au cours de la saison 2010-2011.

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Table 1. Petits coléoptères de la ruche trouvés lors des inspections réalisées au cours de la saison 2009 sur les colonies sentinelles situées près de la frontière Canada-USA. L'emplacement des différents sites est montré dans les Figures 3 et 4.

	20 Juil.	**19 août**	**8 sept.**	**9 oct.**	**2 nov.**
Site 1 Amhurst (8 colonies)	0	9 (5 colonies)	10 (6 colonies)	6 (5 colonies)	2 (2 colonies)
Site 2 Andrew (4 colonies)	0	5 (4 colonies)	6 (4 colonies)	10 (3 colonies)	3 (2 colonies)
Site 3 Leblanc (8 colonies)	0	0	0	0	0
Site 4 Ruby (4 colonies)	0	0	0	0	0
Site 5 Pilon (8 colonies)	0	0	0	0	3 (1 colonie)
Site 6 Lussier (4 colonies)	0	0	0	0	0
Site 7 Fortin (4 colonies)	0	0	0	0	0

	
Figure 1. Plusieurs larves d'*Aethina tumida* et quelques adultes sur un plateau de ruche. (colonie USA 2009, photo PG)	Figure 2. *Aethina tumida* adulte et une abeille ouvrière sur un cadre. (colonie USA 2009, photo PG)

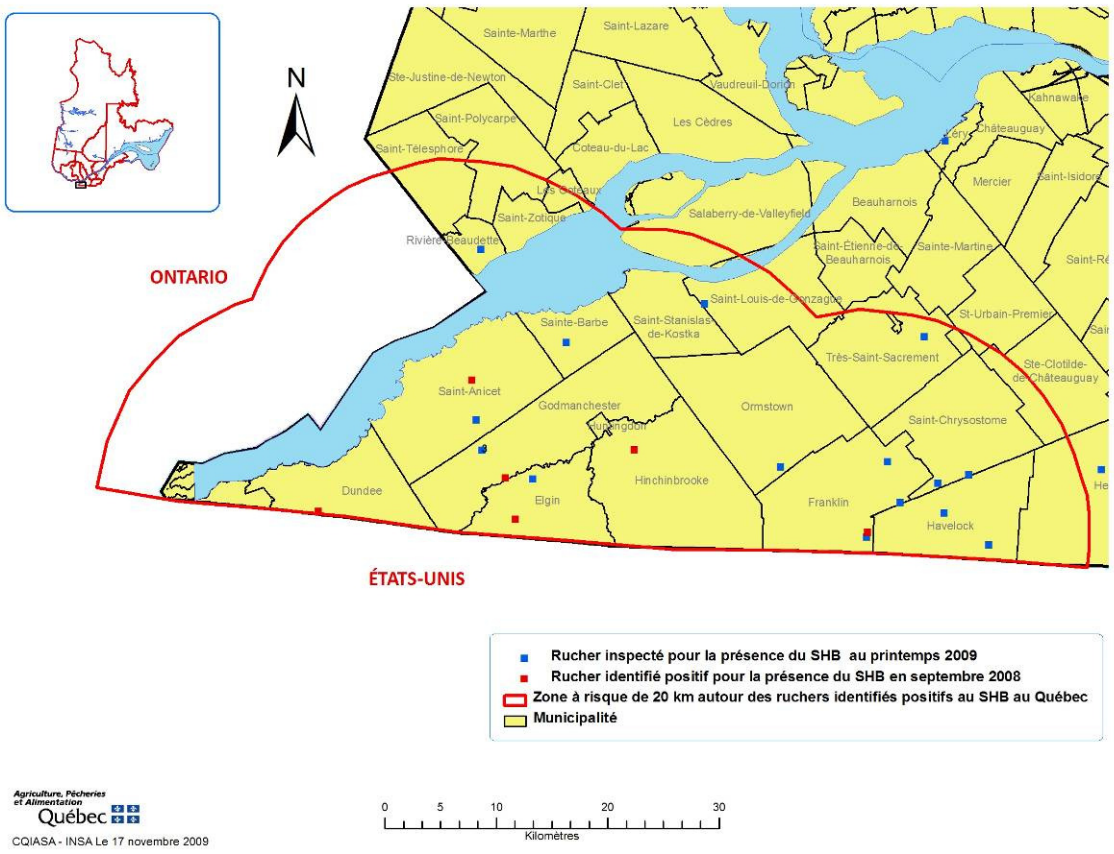

Figure 3. Emplacement des sites/ruchers où les premiers spécimens d'*Aethina tumida* furent trouvés en septembre 2008. La carte montre également les différents ruchers inspectés au printemps 2009 par l'équipe apicole du Centre de recherche en sciences animales de Deschambault.

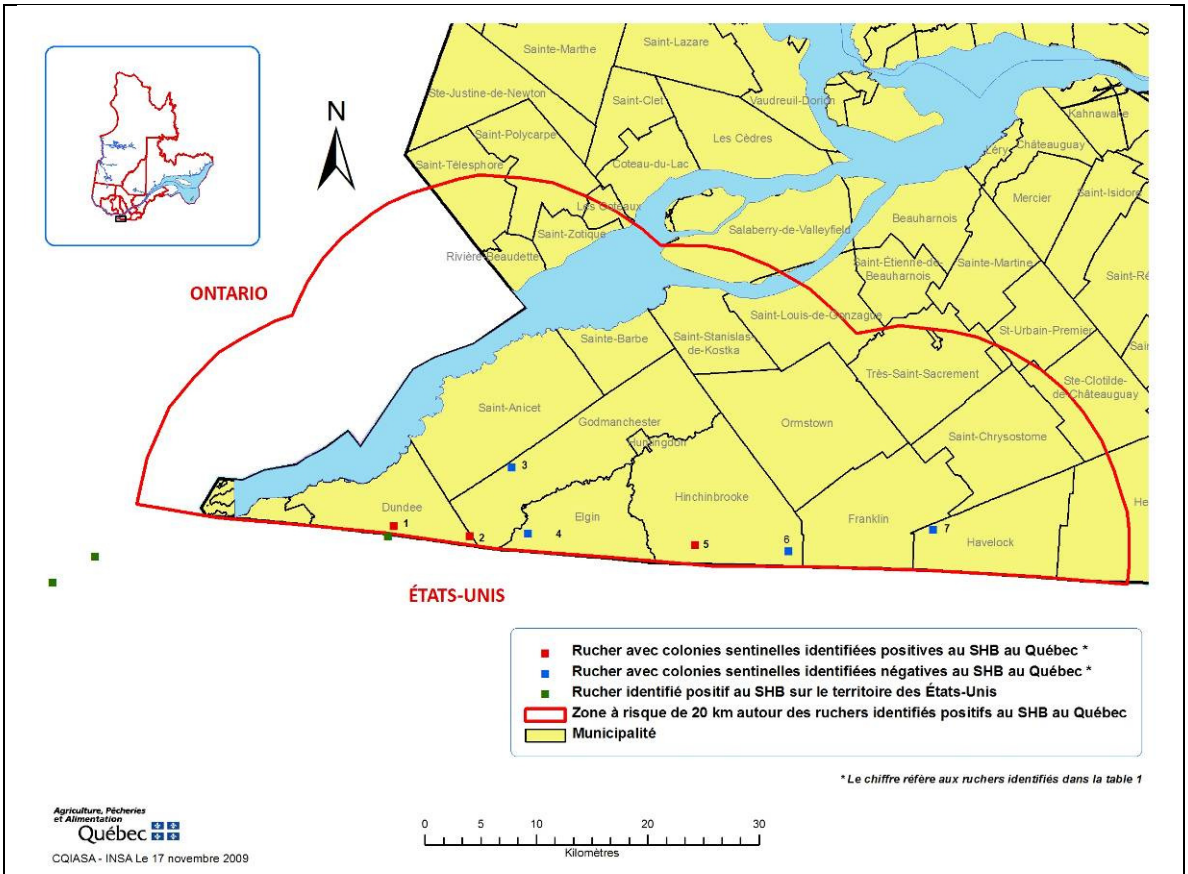

Figure 4. Emplacement des 7 sites/ruchers où ont été installées les colonies sentinelles en 2009. La carte montre également les ruchers USA infestés par *Aethina tumida* visités par l'équipe apicole du Centre de recherche en sciences animales de Deschambault.

	
Figure 5. Pièges en Coroplaste™ placés sur le plateau inférieur de la ruche. Les tiges en métal facilitent la manipulation du piège. (photo PG)	Figure 6. Un piège fabriqué à partir d'un sceau de plastique. Les trous permettent l'entrée des *Aethina tumida* attirés par l'appât de miel et de pollen placé à l'intérieur. (photo PG)

Control of the small hive beetle, *Aethina tumida,* using deadly in-hive traps

Martine Bernier, Valérie Fournier and Pierre Giovenazzo

Ce manuscrit sera soumis au journal ***Canadian Entomologist***.

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Résumé

L'efficacité de trois pièges dans la colonie et d'un piège utilisé pour capturer les larves migrantes d'*Aethina tumida* furent testés au printemps en Montérégie-Ouest (sud du Québec) et à la fin de l'été dans le comté d'Essex (sud de l'Ontario). En Montérégie-Ouest, les pièges furent placés dans la chambre à couvain inférieure de 12 colonies tandis que dans le comté d'Essex, les pièges furent installés dans la hausse à miel supérieure de 48 colonies. Les pièges sélectionnés furent le AJ's Beetle Eater (AJ's Beetle Eater), le Beetle Barn (Rossmann Apiaries), le piège Hood (Rocky Mountain Bee Farm) et le piège Teal. Les pièges utilisés dans la colonie ont significativement réduit la population de Petits coléoptères (PC) de la ruche par rapport aux colonies témoins. Ils n'ont pas eu d'effet sur la population d'abeilles immatures, ni sur la récolte en miel. En Montérégie-Ouest, le Beetle Barn a été le piège le plus efficace, mais seulement à la première date de récolte, lorsque la population de PC était élevée. Cependant, les abeilles ont tendance à boucher les ouvertures de ce piège, qui perd de son efficacité lorsque plus de 3 ouvertures sont scellées. Dans le comté d'Essex, c'est le AJ's Beetle Eater qui fut significativement plus efficace et ce, tout au cours de la durée de l'étude. Dans le piège Hood, nous n'avons pas trouvé de différence de capture selon le type de liquide utilisé, c'est-à-dire soit de l'huile minérale seule ou l'utilisation de l'huile minérale et du vinaigre de cidre de pommes. Enfin, le piège Teal est un bon moyen de briser le cycle de reproduction du PCR, mais il ne permet pas d'estimer le niveau d'infestation des colonies.

Abstract

The effectiveness of three inside-colony traps to capture adult *Aethina tumida* (small hive beetle – SHB) and one trap outside the colony to capture wandering larvae were tested in springtime in Montérégie-Ouest (southern Quebec) and late summer in Essex County (southern Ontario). Traps were placed in the brood chamber of 12 colonies of Montérégie-ouest and on the top of the honey super in 48 colonies of Essex County. These traps were the AJ's Beetle Eater (AJ's Beetle Eater), the Beetle Barn (Rossmann Apiaries), the Hood trap (Rocky Mountain Bee Farm) and the Teal Trap. Inside-hive traps were effective to reduce SHB population without compromising bee population or honey yield. In Montérégie-Ouest, Beetle Barn traps were most effective before honey harvest. These traps lose their efficacy after two weeks as a result of bees covering the holes with propolis. In Essex County, AJ's Beetle Eater traps were most effective throughout the season. Adding apple cider vinegar with the mineral oil in Hood traps did not improve SHB trapping success.

Introduction

The small hive beetle (SHB), *Aethina tumida* Murray, is an indigenous pest of honey bees in South Africa (Lundie, 1940; Schmolke, 1974). It was first reported in the United States (Florida) in 1998 (Thomas, 1998) and is now an invasive species in the USA, Mexico, Australia and, more recently, in Canada (Somerville, 2003; Dixon and Lafrenière, 2002; Giovenazzo and Boucher, 2010; Kozak, 2010). Adults enter hives or honey houses and reproduce. Larvae will then cause the worst damages when associated yeast, *Kodamaea ohmeri* induce honey fermentation (Torto *et al.*, 2007b). The bees may also leave the hive when SHB infestation is important (Ellis *et al.*, 2003b).

Since the introduction of SHB in USA and Australia, various kinds of traps were tested. In-hive traps are useful to slow SHB population build-up (Ellis, 2005) and reduce damages to colonies by reducing the number of larvae produced. Trapping wandering larvae outside the colony will break the reproductive cycle of SHB and thus, reduce damages to the colonies. Efficacy of the different available traps is variable. Modified hive entrances and screened bottom boards are inefficient (Ellis *et al.*, 2003a; Hood and Miller, 2005; Ellis and Delaplane, 2006) while others such as the Hood trap, jar-bottom board traps and modified bottom board traps have been showed to be efficient (Hood, 2006; Torto *et al.*, 2007a)). Other traps seem to be effective and are sold without any available scientific data (AJ's Beetle Eater (Cobey, 2008). These SHB traps have never been tested in Canadian honey bee colonies.

SHB traps are designed on the same principle. Small hive beetles (4.7 to 6.3 mm long x 3.1 to 3.5 mm wide) are much smaller than honeybees (12 to 15 mm long). Beetles search shelter in dark places (Lundie, 1940) and enter in traps by their openings that are too small for honey bees. SHB death occurs inside traps by contact with a pesticide or by drowning in a liquid. Coumaphos is the main pesticide used for killing SHB in honey bee colonies (1 cm^2 piece of Checkmite+™ strip). Baxter *et al.* (1999), Elzen *et al.* (1999) and Neumann and Hoffmann (2008) found between 53.29 and 95% mortality in field trials with in-hive coumaphos traps and recommend its use for the control of SHB. Work by Hood and Miller (2003) testing efficacy of drowning traps showed that mineral oil and alcohol (95% ETOH) resulted in the highest SHB mortality (99%). They also showed that traps filled with apple cider vinegar caught the most adult beetles, probably because it acts as an attractant. Use of alcohol solutions are not recommended because it evaporates quickly and it does not attract SHB. Using both food grade mineral oil and apple cider vinegar in drowning traps have showed good efficacy for attracting and killing adult SHB. Gillard (2008) recommend filling the middle compartment of the Hood trap with apple cider vinegar to attract the beetles and the outer compartments with food grade mineral oil to kill them. Moreover, these substances are natural, with no risks for beetles of developing resistances. It can easily be used in an IPM approach.

Lundie (1940) observed that SHB tend to congregate at the rear section of the bottom board and also under the inner cover. Schmolke (1974) also observed SHB on the outside frames, where honey bee density is low. Torto *et al.* (2007a)

found that there were more SHB captured in bottom board traps than in top traps, mostly when number of SHB was high (Delaware and Pennsylvania, July and August 2004). Finally, Neumann and Hoffmann (2008) recommended using deadly traps on the bottom board to estimate the number of SHB in a hive. Higher numbers of adult beetles were captured near the entrance of the hive. They also recommended placing more traps elsewhere in the colony (e.g. side walls, outer comb and top frames), because they found only 43 ± 27% of the total colony SHB in traps located on the bottom board. Neumann and Hoffmann's study was conducted for a month in Australia during springtime and we do not know if location changes within the colony during the rest of the season.

The aim of this study is to determine the effectiveness of various traps for both adults and wandering larvae of SHB in Canadian honey bee colonies.

Materials and Methods

Study sites

The first field trial took place from May 24th to June 28th, 2011, in Montérégie-Ouest (municipality of Dundee, southern Québec). Experimental colonies were placed in two bee yards infested with SHB the previous year used during Giovenazzo and Boucher (2010). They were located 6.7 km apart. Location of the colonies, number of colonies in each site and the distance with the US border is shown in table 1.

Infested colonies were placed on wooden pallets (two to four colonies per pallet), approximately 2 metres apart in each bee yard. Colonies consisted of one

brood chamber and one honey super separated by a queen excluder. Colonies were checked weekly and queen cells were destroyed. No supers were added or withdrawn during the experiment. The colonies were destroyed a few days after the end of the experiment in order to limit SHB invasion.

The second field trial took place from August 8th to October 5th, 2011, in Essex County, southern Ontario. Experimental colonies were in two bee yards infested with SHB the previous year (Les Eccles, Ontario technical transfer team, personal communication). Bee yard location and number of colonies are shown in table 2.

Each SHB infested colony was placed on a wooden base and grouped by two or four colonies. These groups were randomly dispersed in the bee yard. Colony consisted of one brood chamber and two to four honey supers, separated by a queen excluder. Colonies belonged to a beekeeper of the area and were managed according to his regular method. Whenever honey supers were added or removed, traps were relocated.

Small hive beetle traps

Four different deadly traps were tested. Three were placed inside the colony to catch adult beetles: AJ's Beetle Eater™ (AJ's Beetle Eater), Beetle Barn™ (Rossmann Apiaries) and Hood trap™ (Rocky Mountain Bee Farm). They were ordered through F.W. Jones & Son Ltd. (Bedford, Québec). The other trap, Teal trap™ was placed outside the colony to catch wandering larvae. Below is a brief description of these traps:

- *AJ's Beetle Eater :* This rectangular plastic trap (20.0 cm long x 1.1 cm wide x 2.0 cm deep) trap was designed by Tom Kennedy, an Australian beekeeper (Cobey, 2008). It can contain 30 mL of food grade mineral oil. The comb shaped lid of the container has spacing's of 0.3 cm that allow adults SHB to enter the trap and eventually drown in oil (fig. 1A). This trap is placed on top of the brood chamber or the honey super, in between the first and the second frame (Cobey, 2008) (fig. 1B).
- *Beetle Barn™ :* This is a flat rectangular trap made of black plastic (9 cm long x 7.5 cm wide x 0.7 cm deep, fig. 2). It has small openings on each side (1.3 cm x 0.3 cm) that allows adults SHB to enter. A square piece (2 cm^2) of CHECK MITE+™ strip (Coumaphos 10% - Bayer Health Care Animal Health Canada) is placed in the middle section of the trap. Adults SHB tend to hide from the bees by entering the trap and die at contact with the insecticide strip. The trap openings are too small for the honey bees to enter. The Beetle Barn is placed on the floor of the hive or on top just over the frames.
- *Hood™ trap*: This trap was developed by Dr Mike Hood, Clemson University, South Carolina (Hood, 2006). It is a see-through plastic container (15 cm long x 2.5 cm wide x 8 cm deep, fig. 3A) divided in three compartments (fig. 3B) that can hold up to 210 mL of food grade mineral oil or apple cider vinegar. The lid has a 12.8 x 0.3 cm opening (fig. 3A) which allows adults SHB to enter, but not the honey bees. The hood trap

is fixed on the bottom part of an empty wood frame (fig 3A) and placed next to the wall of a brood chamber or a honey super, at position 1 or 10.

- *Teal™ trap :* This is a see-through plastic container placed at the entrance of the hive (fig. 4). Wandering larvae fall into the trap, which is filled with food grade mineral oil (Vetoquinol) and die from asphyxiation.

Brood population, honey yield and SHB population

In the Montérégie-Ouest field trial, impact of traps on honey bee population was evaluated by comparing initial (May 23rd, 2011) and final (June 27th, 2011) number of immature bees in each colony. The method used is described in Giovenazzo and Dubreuil (2011). After initial colony evaluation, colonies of similar strength were divided in two groups: 1) without traps (control) and 2) colonies with traps. Every week, colonies were weighted (in kg) and SHB in traps were counted and removed. Detection of SHB in hives was done according to the methodology described by Neumann and Hoffmann (2008). The lid, the inner cover, the bottom board, the top of frames, each frame and the side walls of each hive were carefully inspected. We tried to estimate SHB population in the Essex County field trial, but there were too many SHB and they were difficult to count with the method previously described. Most of the beetles we observed were on the top super at that moment.

In-hive trap positioning

For each experiment, the colonies with traps contained all the traps selected for each field trial. In Montérégie-Ouest, selected traps were: 1) AJ's Beetle Eater with mineral oil, 2) Beetle Barn and coumaphos 10% and 3) Hood trap with mineral oil, and, in Essex County: 1) AJ's Beetle Eater with mineral oil, 2) Beetle Barn and coumaphos 10%, 3) Hood trap with mineral oil, 4) Hood trap with mineral oil and apple cider vinegar and 5) Teal trap with mineral oil (table 2). In Montérégie-Ouest, traps were placed in the brood chamber and in Essex County, they were placed on top of the honey super. Traps were positioned to avoid any interaction between them. The AJ's Beetle Eater and Hood trap was placed either on left (L) or on right side (R) of the honey super, but never on the same side. The Beetle Barn was placed either at the front (F, 20 cm behind the hive entrance) or rear (Re, 1 cm away from the back of the bottom board). In the Essex County field trial, the Beetle Barn™ was placed on top of the last honey super, just underneath the lid. The four placement possibilities (table 3) were randomly distributed among the group "with traps". In Essex County, there were eight possible combinations because the Hood trap was filled either with mineral oil or with mineral oil and apple cider vinegar. The Teal trap was placed at the entrance of each hive in Essex County.

Traps were inspected every week and SHB were counted and removed. The two last inspections were done at a two week interval in Essex County. When necessary, the piece of coumaphos was replaced and fresh mineral oil and apple cider vinegar were added. A summary of all measured variables is given in table 4.

Statistical analysis

The experimental design used was a generalized randomized block design with repeated measures. Statistical analysis was performed with SAS software (SAS Institute 2000, Version 9.2) using proc MIXED. The number of adult SHB captured per trap and the initial and final number of SHB in colonies were transformed (square-root) to reach normality. A two-way ANCOVA (site and traps) was used to analyse the final number of adult SHB and immature bee population. In both cases, the initial number was corrected because of variation of data. An ANCOVA with repeated measures was used for the efficacy of traps.

Results

Montérégie-Ouest trial

There was no significant effect of traps on honey bee brood population. ($F = 0.37$; $df = 1,14$; $P = 0.5524$). On June 27th, 2011, colonies without traps had 6189 ± 2751 immature bees (mean ± SE) and colonies with traps had 8345 ± 2232 immature bees. There was no significant effect of traps on colony honey yield ($F = 0.01$; $df = 1,52$; $P = 0.9091$). On June 27th, 2011, the colonies without traps weighted 43 ± 3 kg (mean ± SE) and the colonies with traps weighted 42 ± 2 (kg).

The traps significantly reduced the SHB population in honey bee colonies ($F = 11.86$; $df = 1,14$; $P = 0.0040$) during the trials. On May 24th, 2011, the initial average of adult SHB was 11 per colony (varied from 0 to 88 adult SHB per

colony). On June 27th, 2001, the final average of adult SHB was 4 for colonies without traps and 0.3 for colonies with traps (table 12). This is a reduction of 91.8% compared to the colonies without traps

The interaction between traps and time was marginally significant ($F = 1.87$; $df = 8,139$; $P = 0.0691$). However, on May 30th, 2011, first trap sampling, the Beetle Barn caught significantly more adult SHB than the Hood trap and the AJ's Beetle Eater ($F = 4.72$; $df = 2,139$; $P = 0.0104$) (fig. 11). Variance of captured SHB was high every week (from 0.24 to 0.80).

The location of traps in colonies, i.e. left or right for the AJ's Beetle and the Hood trap and front or rear for Beetle Barn, had no influence on the number of SHB caught ($F = 0.90$; $df = 3,109$; $P = 0.4456$).

Essex county trial

The use of mineral oil with or without apple cider vinegar in Hood traps had no significant effect on the number of SHB captured in the ($F = 0.01$; $df = 1,2$; $P = 0.9476$). Traps with mineral oil captured an average of 1.4 adults SHB per week while in traps with mineral oil and apple cider vinegar the average was of 1.5 adults SHB per week (table 13).

The interaction between traps and time was significant ($F = 4.81$; $df = 10,798$; $P < 0.0001$, fig. 12). There is no correlation between the number of wandering larvae that are caught in the Teal trap and the number of adult SHB captured inside the colony ($F = 1.61$; $df = 5,214$; $P = 0.1583$). Honeybees tend to seal the openings of the trap Beetle Barn with propolis, which reduces its

effectiveness if it is not cleaned regularly. The interaction between the number of sealed holes and time is significant ($F = 2.82$; $df = 5,229$; $P = 0.0173$), specially for week 1 ($P < 0.0001$) and week 2 ($P = 0.0158$). The more holes are sealed, the lower the Beetle Barn capture adult SHB (fig. 13).

Discussion

In the spring in Montérégie-Ouest, the use of in-hive deadly traps was an effective way to reduce beetle population. Hood (2006) reached the same conclusion with Hood traps and jar-bottom board traps. In control colonies (no traps, Montérégie-Ouest), the final number of adult SHB was lower than the initial number. We suggest that this is a result of SHB movement within colonies and that many were caught in colonies that had traps. Adult SHB are active flyers and they are known to frequently move from one colony to another (Ellis, 2005). In Essex County, we did not count the initial and final number of SHB. SHB are not easily visible in July and August in strong colonies with two brood boxes and two honey supers. They move quickly (Schmolke, 1974) and will hide from light. There is an important bias when trying to count them. There was also a risk of SHB reintroduction because of the high infestation rates in nearby apiaries. In Canada, SHB invasion is recent and there is no report on the seasonal dynamic of SHB or on the location of adult beetles in colonies depending on the moment of the season and the amount of food in the colony. These parameters should be verified in a future study.

In this study, we assessed the trap efficacy when placed in the same colony. This protocol allowed us to remove the effect of differences between colonies. It would be interesting to compare trap efficacy with one kind of trap per colony. However, this must be done with a higher number of replicates to reduce the effect of variation between colonies. In Montérégie-Ouest trial, there were significant differences between traps on May 30th 2011, after one week of positioning traps in colonies. This could be the result of the low population of adult beetles in colonies. Ellis (2005) and Torto *et al.* (2007a) proposed that sampling devices or traps for SHB might be not sensitive enough when beetle population is low. This seems to be confirmed in our Essex County trial where SHB populations were higher that the Montérégie-Ouest trial and where we measured significant differences every week. Our traps could have been more effective by adding an attractant in them, such as pollen dough inoculated by the yeast *Kodamaea ohmeri*, as suggested by Arbogast *et al.* (2007). This should be tested in another experiment.

Traps had no effect on brood population or honey yield during the Montérégie-Ouest trial. Adult SHB densities were low and they might have not been enough to cause any damages in the colonies. Moreover, colonies were strong and quite aggressive and thus able to control beetles. No larvae were seen during this trial.

The Teal trap is an effective method to capture wandering larvae (Arbogast *et al.*, 2012), even if it cannot be used to predict beetle infestation. In our study, there was only a few larvae caught in Teal traps (0 to 12 larvae),

except for one colony, where we found up to 3920 wandering larvae. This colony was defective; it had laying workers and no queen.

There are both advantages and disadvantages associated with the traps we tested. All traps are easily obtained and have a relative low cost for purchase (Hood, 2006; Gillard, 2008; Hood and Miller, 2003; Cobey, 2008). They can be placed anywhere in the colony and the location of traps within one super does not influence the number of captured beetles, as we showed in this study. This simplifies the work of the beekeeper that does not have to worry about the trap positioning in the colony. However, further studies should be conducted to determine the most effective location of traps in the colony depending on the moment in the beekeeping season. Bees also tend to propolize or wax in the small openings of in-hive trap (Gillard, 2008; Hood, 2006). For example, Hood and Miller (2003) had 30% of their traps openings sealed with propolis, especially when there was apple cider vinegar inside. According to Cobey (2008), this disadvantage can be avoided with the AJ's Beetle Eater by placing a mat over the trap. The other option is to regularly clean the traps. The use of AJ's Beetle Eater requires opening of the hive. This is time demanding and a draw back from using this type of deadly trap. The container is also small and the liquid used in the trap is subject to evaporation. Moreover, we found the trap difficult to manipulate because of its small size and the way the lid is clipped on the container. The Beetle Barn is the most convenient trap we used. In our study, position of trap did not have an effect on numbers of captured beetles. It can easily be inserted inside the hive through the bottom board entrance with a

standard hive tool. Moreover, a wire can be slipped into the two side openings of the trap to form a large loop that will hang outside of the hive. The trap can be withdrawn by pulling the wire at the same time as taking it off the floor with the hive tool. Hive opening is not required for inspection, which is a great time-saver. However, this trap has to be cleaned frequently because bees tend to seal the openings of the trap with propolis. As showed in this study, the trap is less effective when more than two openings are sealed. The SHB could also develop resistance to coumaphos, as the varroa did (Sammataro *et al*., 2005). Moreover, we do not know if this can lead to the accumulation of coumaphos residues in honey. Kanga and Somorin (2012) showed that chlorpyrifos (LC_{50}=0.53), fenitrothion (LC_{50}=0.53) and parathion (LC_{50}=0.68) were more effective in killing adults SHB than coumaphos (LC_{50}=1.61). However, they all belong to the chemical family organophosphates, the same than coumaphos, which can increase risks of resistance development. Risk for honey bees and human consumption have also to be assessed.

Unlike Hood and Miller (2003), we did not find differences between the utilization of mineral oil and a mix of mineral oil and apple cider vinegar in our Hood traps. Beekeepers could use either of them in their colonies. This trap does not require the opening of the hive as in the AJ's Beetle Eater. Mineral oil or apple cider vinegar have to be occasionally replaced (Gillard, 2008). Bees might fill the container with wax particles (Gillard, 2008). A major disadvantage of the Hood trap is that it uses a frame space. Honey bees can store less honey and pollen, which could lead to a lack of food in dearth periods. Bees will also build

drone cells in this empty frame, which can increase the number of varroa mites if it is not managed properly (Hood, 2006).

The aim of this study was also to propose an IPM approach, in which the use of various means of control is highly recommended (Ellis, 2005). The use of deadly traps can also be combined with good management practices such as keeping colonies strong and healthy, breeding resistant bee stock (Ellis, 2005).

In conclusion, the use of in-hive deadly traps to capture and kill adult SHB in honeybee colonies is an effective way to reduce their infestation levels. Further research should be done on trap efficacy and trap impact on colonies when used at different times during the season.

Acknowledgements

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Table 1 Sites information in the Montérégie-Ouest trial

Site name	**Amhurst**	**Andrew**
Site location	N 45.003983 W 74.449317	N 45.000783 W 74.362683
Total N of colonies	11	8
N of colonies without trap	8	4
N of colonies with traps	3	4
Distance with US border	770 m	805 m

Table 2 Sites information in the Essex County trial

Site name	**Sheply**	**Garnet**	**Smith**
Site location	N 42.120819 W 82.839746	N 42.114615 W 82.868688	N 42.105379 W 82.946616
N of colonies	13	17	18

Table 3 Trap position possibilities for the AJ's Beetle Eater, Beetle Barn and Hood trap in the Montérégie-Ouest field trial.

Position/Trapu	**AJ's Beetle Eater**	**Beetle Barn**	**Hood trapu**
I	R	F	L
II	R	Re	L
III	L	F	R
IV	L	Re	R

Table 4 Measured variables in Montérégie-Ouest (Québec) and Essex County (Ontario).

	Variables	**Montérégie-Ouest (Qc)**	**Essex County (Ont)**
	Trap location	Lower brood chamber	Top honey super
Traps	**AJ's Beetle Eater™**	Mineral oil	Mineral oil
	Beetle Barn™	Coumaphos 10%	Coumaphos 10%
	Hood trap™	Mineral oil	Mineral oil vs mineral oil + apple cider vinegar
	Teal trap™	no	Mineral oil
	Effect of traps on initial and final SHB population	yes	no
	Effect of traps on immature honeybee population	yes	no
	Effect of traps on honey yield	yes	no

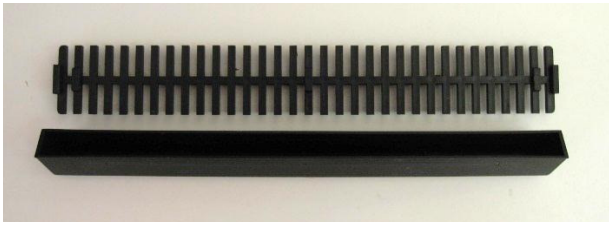
Table 5. Final number of SHB in colonies of Montérégie-Ouest trial on June 27th, 2011.

Group	**N**	**Least square means**	**Standard Error**
Control (without traps)	7	1.95	0.30
With traps	12	0.55	0.25

Table 6. Hood trap: mineral oil vs mineral oil + apple cider vinegar. Essex County trial, from August 8th to October 5th, 2011.

Liquid	**N of colonies**	**Least square means**	**Standard Error**
Mineral oil	23	1.19	0.24
Mineral oil + apple cider vinegar	25	1.21	0.24

A.



B.

Figure 1 AJ's Beetle Eater trap™ (AJ's Beetle Eater) A. Container and lid apart. B. Trap placed in a brood chamber, on top of the first and the second frame. Photo credits: Martine Bernier.

A.

B.

Figure 2 Beetle Barn™ (Rossmann Apiaries). A. Opened. B. Closed. Photo credits: Martine Bernier.

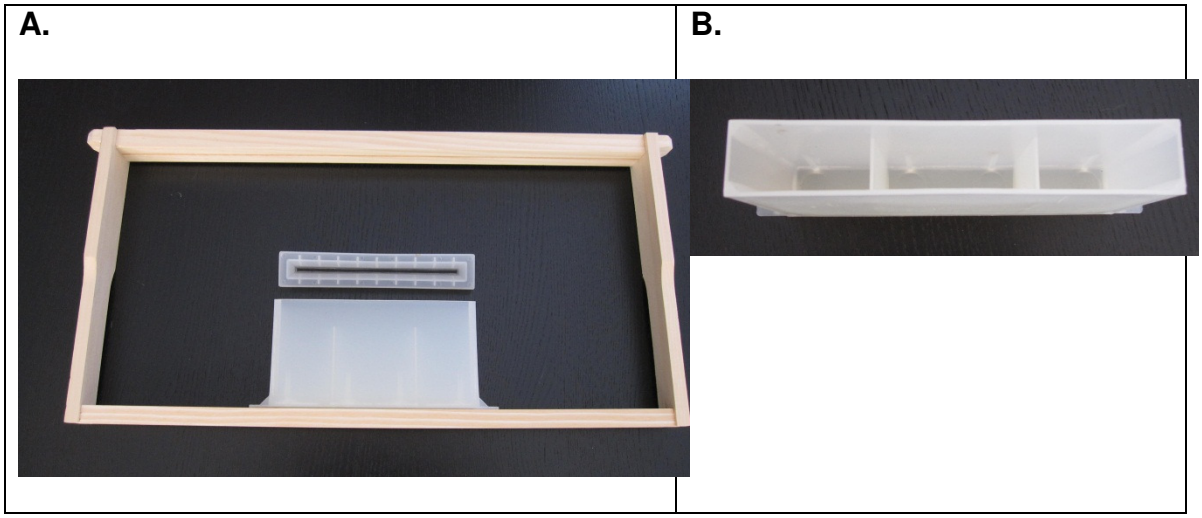


Figure 3 Hood™ trap (Rocky Mountain Bee Farm). A. Trap placed in an empty frame, container and lid. B. Inside the trap, the container has three compartments. Photo credits: Martine Bernier.

Figure 4 Teal™ trap placed at the entrance of the hive. Photo credits: Martine Bernier.

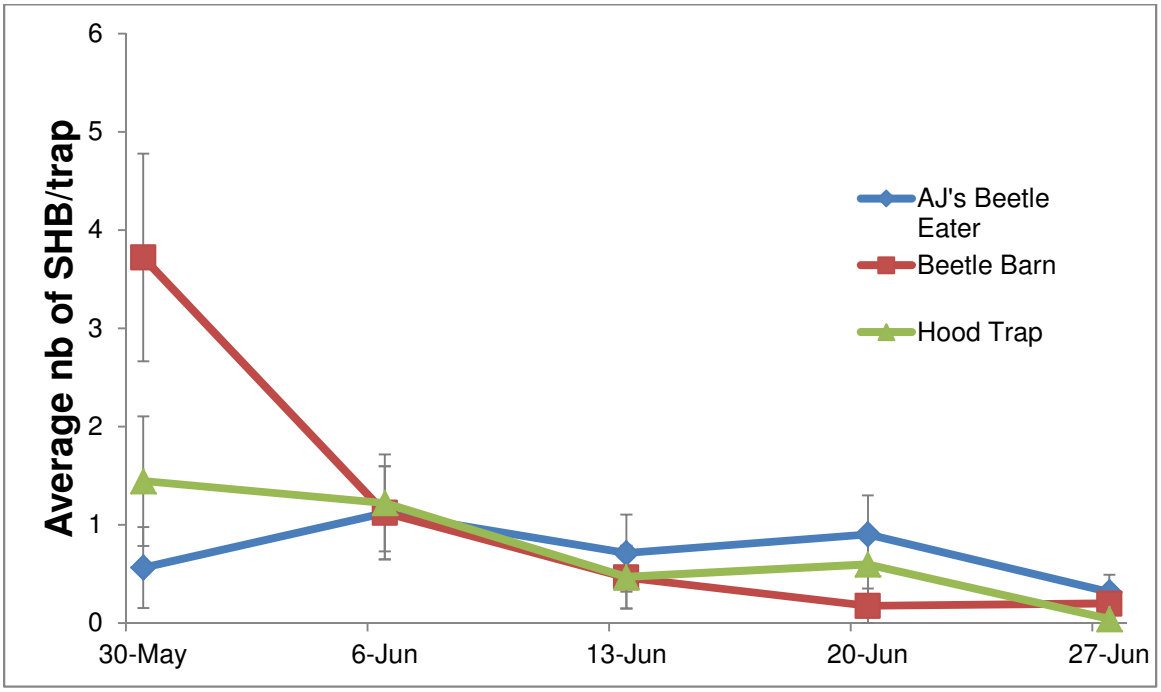


Figure 5 Average number of adult SHB caught in traps according to the type of trap, from May 30th to June 27th, 2011, in Montérégie-Ouest.

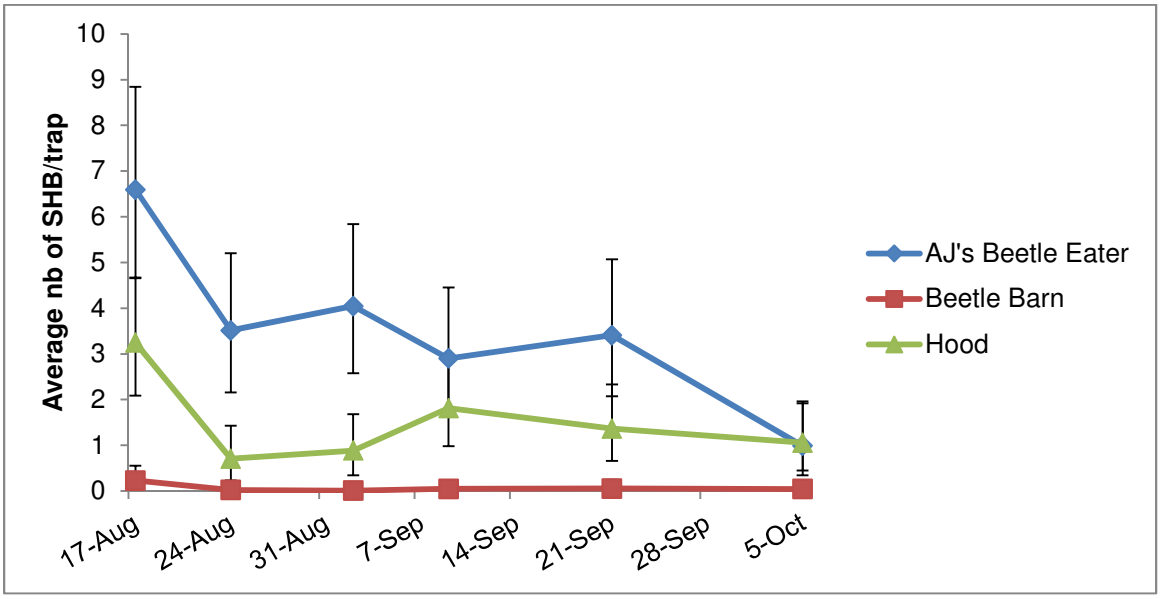


Figure 6. Average number of adult SHB captured per trap, from August 17th to October 5th, 2011, in Essex County.

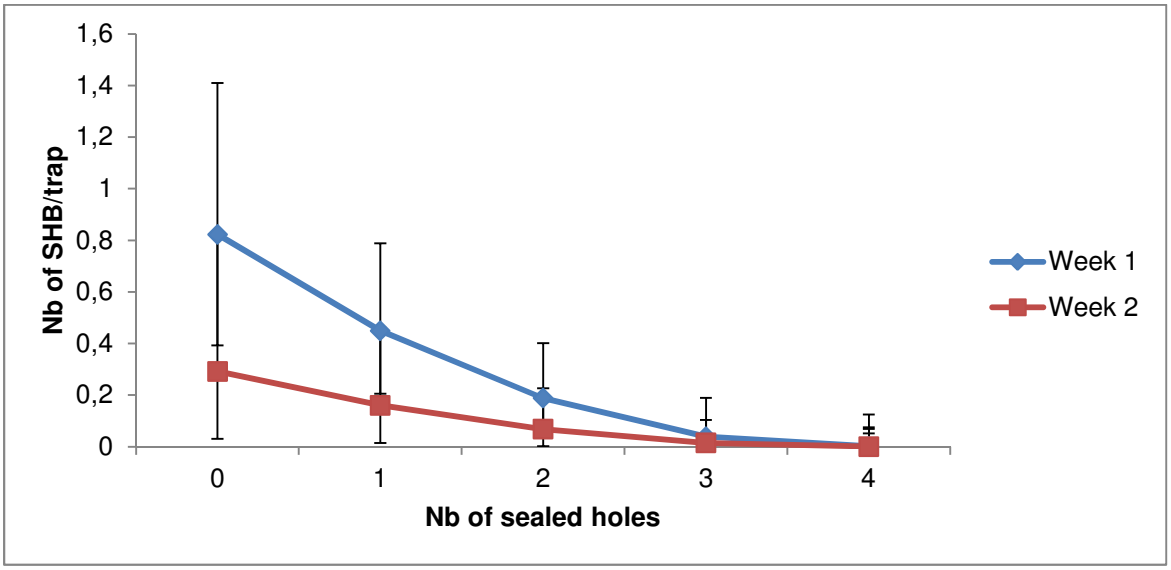


Figure 7. Average number of adult SHB captures by the Beetle Barn during week 1 and 2 in Essex County, Ontario.

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Aethina tumida (Coleoptera: Nitidulidae) pupal development in different thermo-hygrometric soil conditions

Martine Bernier, Valérie Fournier et Pierre Giovenazzo

Ce manuscrit sera soumis au ***Journal of Economic Entomology***.

Résumé

Le développement de la pupe d'*Aethina tumida* Murray (Coleoptera: Nitidulidae a été étudié dans un sol organique à des températures de 16, 18 et 20°C et à un contenu en eau gravimétrique de 0,150, 0,192 et 0,250 gg^{-1}. Ces valeurs représentent les conditions édaphiques retrouvées dans le sud-est du Canada. La survie de la pupe, son temps de développement, le sexe ratio et la survie des adultes émergents furent observés dans des chambres de croissance. Environ 50 larves du troisième stade furent placées dans des contenants en plastique contenant 0,12 kg de sol organique pasteurisé. Les résultats démontrent que la survie de la pupe augmente avec l'augmentation de température, mais diminue avec l'augmentation du contenu en eau du sol. Le temps de développement de la pupe augmente avec la diminution de température (69 à 78 jours à 16°C, 47 à 54 jours à 18°C et 36 à 39 jours à 20°C) et avec la diminution du contenu en eau du sol. Le contenu en eau du sol optimal pour le développement de la pupe est de 0,192 gg-1. Le sexe ratio des adultes émergents est influencé par le contenu en eau du sol. Dans un sol sec ou intermédiaire, le sexe ratio est de 1♀ :1♂ tandis qu'il est de 3♀ :1♂ dans un sol presque à saturation. La durée de vie des adultes est aussi influencée par le contenu en eau du sol. Celle-ci diminue avec l'augmentation du contenu en eau du sol. Cette étude contribue à une meilleure compréhension de la phénologie du petit coléoptère de la ruche en contexte climatique canadien.

Abstract

The pupal development of *Aethina tumida* was studied at various combinations of thermo-hygrometric soil conditions (16, 18 and 20°C and soil water contents of 0.150, 0.192 and 0.250 gg^{-1}) representative of soil conditions found in east-southern Canada. Survivorship and development duration of *A. tumida*'s pupa and sex ratio and lifespan of emerging adults were assessed. Assays were conducted in growth chambers on an average of 50 third instar larvae per termo-hygrometric combination. . Results show that survivorship of pupae decreased with lower temperature and higher soil moisture. 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 it was longer in a dryer soil. The optimal soil water content for the pupa development was 0.192 gg^{-1}. Sex ratio of the emerging adults was influenced by soil's water content. We measured one female to one male for dry and intermediate soil water content and three females to one male for wet soils. High soil moisture reduced lifespan of emerging adults. This study contributes to a better understanding of *A. tumida's* population dynamics in eastern Canada.

Introduction

Aethina tumida Murray (Coleoptera: Nitidulidae) or the small hive beetle (SHB) is a honey bees pest endogenous of South Africa (Lundie, 1940). Adults SHB, which are known to live several months (Lundie, 1940; Haque and Levot, 2005; Meikle and Patt, 2011; Murrle and Neumann, 2004) sneak into the colonies

where their eggs and larvae feed and develop. Important damages are made by the larvae, while their associated yeast, *Kodamaea ohmeri*, (Torto *et al.*, 2007) cause the honey to ferment (Lundie, 1940; Elzen *et al.*, 1999) and thus loses its nutritional value. High infestation rates will make the colony collapse (Elzen *et al.*, 1999).

This pest was accidently introduced in 1998 in Florida, United States (Thomas, 1998) and in 2002 in Australia (Somerville, 2003). First occurrences of the SHB in Canada were observed in 2002 (Manitoba) and 2006 (Alberta and Manitoba) without any signs of population survival after winter (Dixon and Lafrenière, 2002; Lafrenière, 2006; Nasr, 2006). In south eastern Canada (Southern Québec) an invasion of SHB was discovered during fall 2008 (Giovenazzo and Boucher, 2010). Presence of SHB in this region is caused by the invasion of flying beetles across the US border. More recently, it was also reported in Ontario (Kozak, 2010) and again in Manitoba (2012). The damages caused by SHB in Canadian honey bee colonies are not as important as in southern Unites States (Florida, Georgia and South Carolina). The colder Canadian climate could explain why SHB populations have not established yet.

The SHB pupal stage is vulnerable because both climatic factors and predators have an impact on it. SHB death was only observed at the pupal stage while reared at 24-28 and 34°C (de Guzman and Frake, 2007). Thus, Lundie (1940) and Ellis *et al.* (2004) suggested that environmental factors might limit or improve the reproduction potential of SHB. Because SHB pupate in the soil, edaphic factors will greatly influence their development. Among them, de

Guzman *et al.* (2009) listed soil type, moisture and density, field slope, drainage, rainfall, and temperature. 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) have the greatest effect on SHB pupation. Soil humidity is required for the development and survivorship of the pupae (Lundie, 1940; Haque and Levot, 2005). Pupae of other beetles are also known to desiccate and die when exposed to dry weather, while moist soils are more favorable (*Cerotoma trifurcate* Forster, (Eddy and Nettles, 1930)). Finally, soil type does not seem to affect the development of SHB's pupae (Schmolke, 1974; Ellis *et al.*, 2004; de Guzman *et al.*, 2009).

Pupal developmental has been tested at temperatures between 21 and 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), which are representative of African, southern United States and Australian climates conditions. Moreover, Pettis (2010) observed that there were little to no development under 10°C. The SHB is also known to die at temperature below 0°C (Jacobson, 2005). Nonetheless, soils temperatures in Canada during beekeeping season can be 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 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 importance of soil moisture. Neumann *et al.* (2001), Murrle and Neumann (2004),

de Guzman and Frake (2007) and de Guzman *et al.* (2009) did not measured water content while Haque and Levot (2005) and Meikle and Patt (2011) did not compare several soil moistures. Ellis *et al.* (2004) compared two soil water levels (0% and 11% water by weight) and concluded that a dry soil was not suitable 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 South-eastern Canada. We also measured SHB sex ratio and lifespan of emerging adults.

Materials and Methods

Small hive beetle rearing

Adult beetles, males and females, were collected during May 2010 in infested honey bee colonies located in Montérégie-Ouest, 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), Laval University, Québec, Canada. The growth chambers were kept in darkness (0L:24D), at 30 ± 0.5°C and 50 and 60% relative humidity. Adult beetles were placed in 473 mL plastic containers (10.5 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 sufficient humidity in plastic containers. Adults and larvae were fed *ad libitum* with honey bee pollen.

Pupal development

Survival rate and duration of pupation in soil were measured in growth chambers (Conviron, model PGR15 and E15) at 16, 18 and 20°C and of 0.125, 0.192 and 0.250 g of water/g of dry soil [i.e. 0.05, 0.15 and 0.25 cm^3 of water/cm^3 of soil]. These values correspond to a dry, intermediate and wet (near saturation) soil. The organic potting soil (Pro-mix® by Premier Tech) was pasteurized (30 minutes at 60°C) and oven dried (40°C for 48 hours). Sterilized water (150 mL, 230 mL and 300 mL) was added to soil (0.12 kg) to obtain the different concentrations of 0.125, 0.192 and 0.250 g of water/g of dry soil. Theses concentrations correspond, in an organic soil to a dry, intermediate and wet (near saturation) soil. Probes were used to record soil temperature (12-Bit Temp Smart Sensor S-TMB-M006, Onset® HOBO® Data Loggers, Massachusetts, USA) and soil moisture (EC-5 Moisture sensor S SMC-M005, Decagon Devices, Pullman, Washington, USA). Mature larvae (wandering stage) were placed in plastic containers with specific soil thermo-hygrometric conditions and allowed to burrow naturally into the soil. Larvae of same age were obtained from five sexually mature females and males that mated during 24 hours. Eggs were laid over a period of 24h and young larvae were fed *ad libitum* with pollen and water for 15 days. On day 15, these larvae were equally distributed into the 9 different

experimental groups (3 different temperatures X 3 different soil water contents). Experimental design is explained below in the statistics section

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

Sex ratio and lifespan of emerging adults

Emerging adults were collected with an aspirator (Schmolke, 1974; Ellis *et al.*, 2004). They were then sexed by applying a gentle pressure on their abdomen with the finger tips to show either female's ovipositor or male's 8th tergite (de Guzman and Frake, 2007). All emerging adults were placed by couples in 50mL plastic tubes (Starstedt™) 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 by another adult of the same treatment and gender if available (Meikle and Patt, 2011). The date was recorded at the onset of egg laying and the couple was transferred into a 473mL plastic container used for the insect rearing. Young emerged larvae were removed daily and counted.

Statistics

The experimental design was a split-plot with temperature as the main plot and water content of soil as subplot. The temperatures were randomized into a

3x3 Latin square with block (date) as row and growth chambers as column. There were three repetitions for the temperatures 18 and 20°C and two repetitions for 16°C. Each soil water content was repeated twice per growth chamber. Data were analysed using SAS Software (SAS Institute 2000, Version 9.2). Pupal development time and lifespan of emerging adults were analysed using proc Mixed with temperature and soil moisture as fixed effects. Estimation of parameters of regressions for pupal development time was made with proc Mixed. Data for pupal survival rate and sex ratio of emerging adults was transformed with arcsine square-root to reach normality and were analysed with proc GLIMMIX with a binomial distribution, with temperature and soil moisture as fixed effects. LSD's tests were used to compare treatment means. Estimation of parameters of regressions for survival rate was made with proc GLIMMIX. Logit model was used for the binomial distribution. Finally, for sex ratio results, a post hoc comparison was made for soil water content effect and Bonferroni adjustments were made for *t-value* probability to account for multiple comparisons. Below are the starting dates of the different experimental blocs, each experimental block consists of 9 experimental conditions (3 temperatures X 3 soil water contents):

- Block 1 (July 8th, 2011): total of 972 larvae were produced and 54 larvae were put into each treatment (3 temperatures x 3 soil water content).
- Block 2 (October 11th, 2011): total of 1224 larvae were produced and 68 were put into each treatment (T x soil water content).

- Block 3 (January 18th, 2012): total of 774 larvae were produced and 43 larvae were put into each treatment (T x soil water content).

Results

Pupal development

Analysis of variance showed significant soil temperature x moisture effect for survival rate of pupae ($F = 15.91$; $df = 4.28$; $P<0.0001$). The survival rate was higher at 20°C for water content of 0.125 and 0.192 water by weight (respectively 97.39% ± 1.73 and 97.82% ±1.49) and at 18°C for water content of 0.125 and 0.192 water by weight (respectively 90.25% ± 4.20 and 89.03% ± 4.62). The pupae that developed at 16°C had a significantly lower survival rate than the ones that developed at 18 and 20°C. However, at 16°C, the survival rate was higher for water content of 0.192 water by weight (22.86% ± 8.14) than for water content of 0.125 water by weight (14.71% ± 5.89) and 0.250 water by weight (12.46 % ± 5.80). In wet soils (0.250 water by weight), the survival rate was low (41.59% ± 11.21 at 18°C and 38.26% ± 13.34 at 20°C). High water content of the soil and temperature of 16°C are limiting factors on the development of *A. tumida*'s pupae (table 4 and fig. 5). The regressions for survival rate according to water content were significant ($F = 29.89$; $df = 1.28$; $P<0.0001$; AUROC = 0.91 for 0.125 water by weight; $r^2 = 0.88$ for 0.192 water by weight and $r^2 = 0.67$ for 0.250 water by weight) (fig. 5).

Pupal development time was affected by soil temperature and soil moisture ($F = 5.23$; $df = 4.28$; $P = 0.0028$). Mean development time was 72.9

days at 16℃, 50.3 days at 18℃ and 38.0 days at 20℃ (all moistures combined). At 16 and 18℃, the development time was longer for the pupae in a soil water content of 0.125 water by weight than at 0.192 water by weight and 0.250 water by weight. At 20℃, there was no significant difference between the three different soil water contents (38.32d ± 2.24 at 0.125 water by weight, 36.74d ± 2.24 at 0.192 water by weight and 38.97d ± 2.34 at 0.250 water by weight) Lower temperature increase the duration of pupal development and at temperature below 18℃, dry soil also increase the duration of development (table 5 and fig. 6). The regressions of development time of the pupae according to soil water content were significant ($F = 31.01$, $df = 1.28$, $P < 0.0001$; $r^2 = 0.97$ for 0.125 water by weight; $r^2 = 0.99$ for 0.192 water by weight and $r^2 = 0.98$ for 0.250 water by weight) (fig. 6). The development time of the females was not different from the males ($f = 0.1700$; $df = 1.28$; $P = 0.6807$). Not all un-emerged adults were recovered. Some of dead larvae were colonized by an unknown fungus.

Emerging adult SHB

A. tumida's sex determination was not affected by temperature ($P = 0.3683$) but soil moisture effect was almost significant ($P = 0.0659$). Post hoc contrast 0.250 vs 0.125+0.192 was conducted and was significant ($F = 5.97$; $df=1.28$; $P = 0.0211$) after a Bonferroni adjustement ($\alpha=0.025$). The proportion of females was 0.51 ± 0.03 at 0.125 water by weight cm^3/cm^3, 0.52 ± 0.03 at 0.192 water by weight and 0.73 ± 0.07 at 0.250 water by weight (table 6). In dry or

intermediate soils, the sex ratio is 1♀:1♂ while in wet soils, the sex ratio is 3♀/1♂.

Lifespan of emerging adults was significantly affected by water content of the soil ($F = 8.34$; $df = 2.33$; $P = 0.0012$). Adults that developed at 0.125 and 0.192 water by weight lived twice longer than adults at 0.250 water by weight (table 7). However, lifespan of emerging adults was not affected by temperature ($F = 12.06$; $df = 2.1$; $P = 0.1995$) nor by sex ($F = 2.88$; $df = 1.28$; $P = 0.1006$).

Discussion

Pupal development

The survivorship of pupae is influence by both temperature and soil moisture. Survivorship is higher than 89% at 18 and 20°C and at low (0.125 g g^{-1}) and medium (0.192g g^{-1}) water content. Neumann *et al.* (2001) reported lower emergence (42.6 %) at similar temperature (17-24°C) and moist soil (unknown water content). However, they explained their high mortality with the narrow space provided to each larva (2.3 cm^3). In our experiment, each larva had between 6.0 and 9.5 cm^3 of soil available. At 20°C, we measured the highest survivorship (97.39% and 97.82%) in dry and intermediate water content of soil, which is similar to other experiments, even though the temperatures tested were higher than ours. Ellis *et al.* (2004) found an emergence of 91.5% in a mineral soil at 24.6 ± 1.3°C and 10% water by weight and de Guzman and Frake (2007) had a survivorship of 93% in moist potting soil at 24-28°C (unknown water content). Meikle and Patt (2011) had a proportion of emerged pupae of 92% at

21℃ (soil moisture of 5-8%, sandy soil). Marrone and Stinner (1984) also found high mortality in wet soils (10 kPa) with the pupa of the bean leaf beetle (*Cerotoma trifurcate* Forster) at all temperatures tested (20, 25 and 30℃). However, the total survivorship, from egg to adult, was higher in wet soils. The overall survivorship of *A. tumida's* pupae remains high even at 18℃, when the moisture is sufficient and the soil is not soaked. However, comparing soil moisture among experiments is still hazardous because the amount of available water of soil depends on soil texture (Villani and Wright, 1990) and organic matter (Buckman and Brady, 1960). Ellis *et al.* (2004) found an emergence of 0% in a completely dry soil (24.6±1.3℃), but this is not representative of field conditions, because soil always retains a certain amount of water (Buckman and Brady, 1960). They recommend placing colonies away from agricultural soils, which are moist and tilled and suitable for SHB pupation. .

Meikle and Patt (2011) and Pettis (2010) suggest that the minimal temperature for the development of the SHB is near 10℃. . In our preliminary studies, we measured no development at 12℃ and estimated a survival rate of 1% at 13℃. Our results suggest that minimal temperature needed for the development of the small hive beetle is higher than previously estimated by Pettis and Meikle and Patt (2011). This new information gives us insight that SHB development will be limited by cold soils temperatures that prevail in southern Canada in spring, winter and fall.

As seen among other insects (Samara *et al*., 2011), development time of SHB's pupae shorten as temperature increased. The range of emersion is also

tighter as temperature increase. At similar temperatures (17-24°C) and 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 emersion time. Murrle and Neumann (2004) found a pupation duration of 24.68±1.75 days at room temperature (18-25°C) and moistened mineral soil (unknown water content). At 21°C, Meikle and Patt (2011) found a pupation duration of 32.7 d in a moist (5-8% by weight) sandy soil. They also suggest a development time of 70 days at 15°C and 174 days at 12°C. At 16°C (lowest temperature tested) the pupal development time was between 69.06 and 78.14 days. Meikle and Patt (2011) were quite close with their prediction, but, at 15°C, we estimate that development time will be between 82 d and 93 d. Development time is also influenced by soil water content, as it is for other insects. For the pupa of the bean leaf beetle, *Cerotoma trifurcate* Forster (Marrone and Stinner, 1984), development time is shortest in wet soil (10kPa) and longest in dry soils (1000 kPa). These water content values are close to the values of our study. Finally, the knowledge of the development time of the small hive beetle at temperatures of soil measured in southern Canada allowed us to estimate the potential of generation of this pest. We estimate a potential of up to two generation per year, which is consistent with observations made by Eccles (Director of the Ontario Technical transfer team, personal communication) during summer 2012.

Emerging adult SHB

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 a 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 is different of 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 moisture for SHB. We suggest that males might be negatively affected by soil moisture and die more readily than females. They also might be more affected by fungus of soils.

In our study, the lifespan of adult beetles was significantly affected by soil moisture. However, our highest average lifespan of emerging adults was 12.3 ± 1.2 days in dry soils, which is quite lower than what was reported in other studies. In intermediate soils, lifespan of emerging adults was 11.9 ± 1.2 days while it was 6.0 ± 1.2 days in wet soils. Lundie (1940) reported a lifespan of up to 188 days while adult SHB reared by Murrle and Neumann (2004) lived more than nine weeks. Our rearing methods for the emerging adults (in plastic tubes) might have reduced lifespan of adults or kill them faster. Sometimes, cotton balls were soaked instead of being moistened and dead beetles were found in the liquid underneath them. Sometimes, larvae that were produced by beetles were not removed quickly enough and were clogging the perforations in the lid with their dejection. The fermentation produced in the tube made the adults died by asphyxiation. In conclusion, this study offers new knowledge on pupal

development of the SHB in south-eastern Canadian climate. Under these conditions, SHB pupal development is limited when soil temperatures drop below 16C.

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Table 1. Mean percent survival ± SE for pupae of *Aethina tumida* at 16, 18 and 20℃ and soil water content of 0.125, 0.192 and 0.250 gg-1

Temperature (℃)	Soil water content (gg-1)	Survival rate (%)			
16	0.125	14.71	± 5.89	B	b
	0.192	22.86	± 8.14	A	c
	0.250	12.46	± 5.80	B	b
18	0.125	90.25	± 4.20	A	a
	0.192	89.03	± 4.62	A	b
	0.250	41.59	± 11.21	B	a
20	0.125	97.39	± 1.73	A	a
	0.192	97.82	± 1.49	A	a
	0.250	38.26	± 13.34	B	ab

Note: Means followed by the same letter are not significantly different at p = 0.05 (LSD test). Capital letters are comparison among moistures within one temperature. Lower case letters are comparisons among temperature within one moisture level.

Table 2. Mean development time of *Aethina tumida*'s pupae ± SE at 16, 18 and 20℃ and soil water content of 0.125, 0.192 and 0.250 water by weight

Temperature (℃)	Soil water content (gg^{-1})	Development time (d)			
16	0.125	78.14	± 2.09	A	a
	0.192	69.06	± 2.09	B	a
	0.250	71.55	± 2.31	B	a
18	0.125	54.40	± 2.09	A	b
	0.192	48.94	± 2.09	B	b
	0.250	47.58	± 2.19	B	b
20	0.125	38.33	± 2.24	A	c
	0.192	36.75	± 2.24	A	c
	0.250	38.97	± 2.34	A	c

Note: Means followed by the same letter are not significantly different at p = 0.05 (LSD test). Capital letters are comparison among moistures within one temperature. Lower case letters are comparisons among temperature within one moisture level.

Table 3. Proportion of *Aethina tumida*'s females in soil water content of 0.125, 0.192 and 0.250 water by weight

Soil water content (gg^{-1})	Proportion of females		
0.125	0.51	± 0.03	A
0.192	0.52	± 0.03	A
0.250	0.73	± 0.07	B

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

Table 4. Lifespan of emerging *Aethina tumida* adults in water content of 0.125, 0.192 and 0.250 water by weight

Soil water content (gg^{-1})	Lifespan (days)			
0.125	12.3	±	1.2	A
0.192	11.9	±	1.2	A
0.250	6.0	±	1.2	B

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

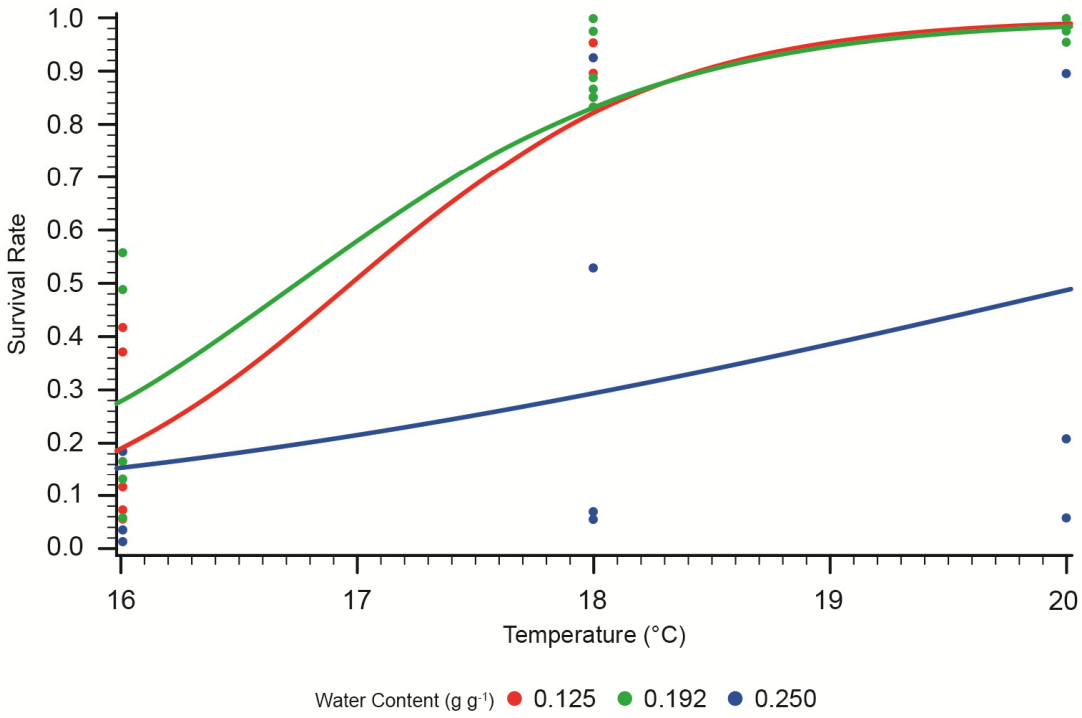

Figure 1. Survival rate of *Aethina tumida*'s pupae at temperature of 16, 18 and 20℃ and water content of 0.125, 0.192 and 0.250 water by weight (Linear equations in logit model for regression).

Equations:

Survival rate in soil of 0.125 gg^{-1}: [EXP (1.5320 + 1.4950 (t-18))] / [1 + EXP (1.5320 + 1.4950 (t-18))]

Survival rate in soil at 0.192 gg^{-1}: [EXP (1.6041 + 1.2786 (t-18))] / [1 + EXP (1.6041 + 1.2786 (t-18))]

Survival rate in soil at 0.250 gg^{-1} : [EXP (-0.8795 + 0,4152 (t-18))] / [1 + EXP(-0.8795 + 0,4152 (t-18))]

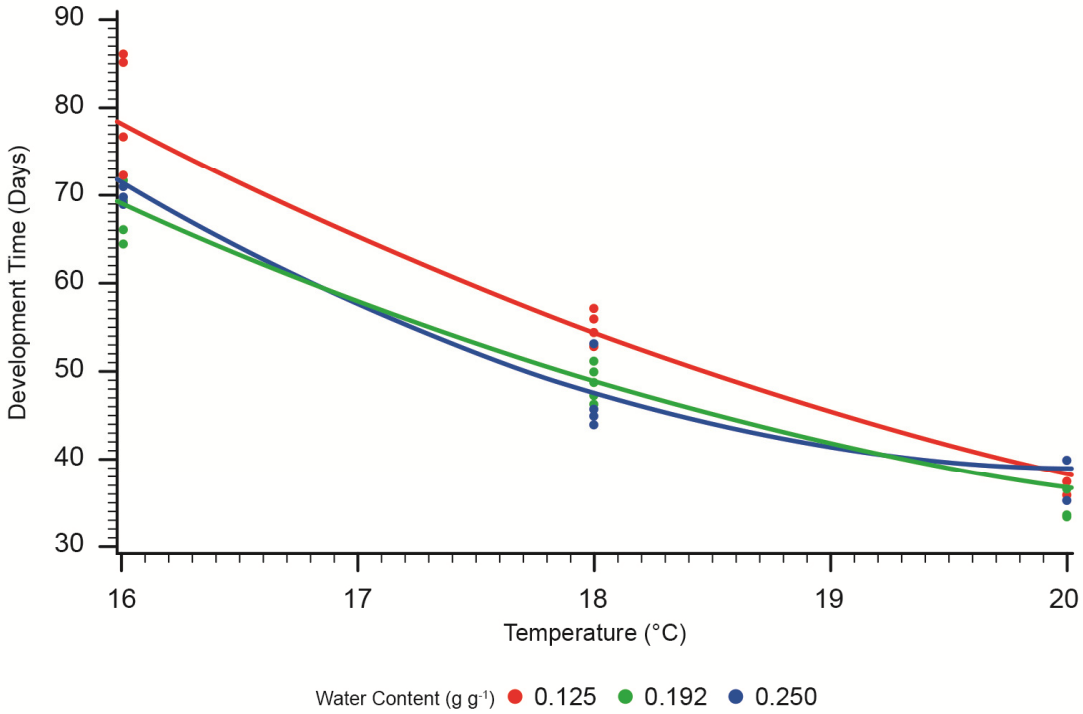

Figure 2. Development time of *Aethina tumida*'s pupae at temperature of 16, 18 and 20℃ and water content of 0.125, 0.192 and 0.250 water by weight (Quadratic equations for regression).

Equations:

Development time in soil of 0.125 gg^{-1}: $54.4041 - 9.9536\ (t\text{-}18) + 0.9576\ (t\text{-}18)^2$

Development time in soil at 0.192 gg^{-1}: $48.9409 - 8.0786\ (t\text{-}18) + 0.9911\ (t\text{-}18)^2$

Development time in soil at 0.250 gg^{-1} : $47.5765 - 8.1446\ (t\text{-}18) + 1.9216\ (t\text{-}18)^2$

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