Université de Montréal

Évaluation des facteurs influençant l'avortement des œufs de la punaise marbrée *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) par la guêpe parasitoïde *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae).

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Résumé

Les *parasitoïdes* se développent sur, ou, à l'intérieur d'un hôte qu'ils tuent au cours de leur développement. Ils sont souvent utilisés en lutte biologique car ils ont la capacité de réduire et de contrôler les populations d'organismes considérés comme nuisibles. Nous avons investigué la capacité de la guêpe parasitoïde *Telenomus podisi* à induire l'avortement des œufs de son hôte : la punaise exotique *Halyomorpha halys*. Cette nouvelle association d'espèce ne permet pas à la guêpe de se reproduire, mais elle induit un taux d'avortement des œufs de l'hôte, néanmoins variable d'une guêpe à l'autre. Nos trois expériences ont permis de déterminer que 1) le taux d'avortement suit la grosseur de l'œuf, qu'il soit parasité ou non. Que ce taux d'avortement est encore plus important lorsque parasité par des guêpes de plus petite taille. 2) Le taux d'avortement est expliqué à 18.2% par la génétique des guêpes ($h2 = 0{,}182$). 3) Il est difficile de générer une population de *T. podisi* capable d'induire un taux d'avortement élevé après 5 générations de reproduction sélective. Nous ouvrons, par ces résultats, une piste en matière de lutte biologique contre l'insecte ravageur qu'est *H. halys*. L'utilisation de petites guêpes permettrait de réduire la reproduction de la punaise. Mais aussi, qu'à la lumière du contenu de ce mémoire, un programme de reproduction sélective pourrait être optimisé pour produire des guêpes performantes en matière d'avortement des œufs de *H. halys*.

Mots clés: Avortement des œufs, hôtes, reproduction sélective, héritabilité, lutte biologique, mortalité non-reproductive.

Abstract

Parasitoids develop on, or, inside a host they kill during their development. They are often used in biological control because they have the ability to reduce and control populations of organisms considered harmful. We investigated the ability of the parasitoid wasp *Telenomus podisi* to induce the abortion of its host eggs: the exotic stink bug *Halyomorpha halys*. This new species association does not allow the wasp to reproduce, but it induces some abortion of the eggs of the host, nevertheless variable from one wasp to another. Our three experiments have determined that 1) the abortion rate follows the size of the egg, whether parasitized or not. That this abortion rate is even greater when parasitized by smaller wasps 2) The abortion rate is explained to 18.2% by wasp genetics (h2 = 0.182). 3) It is difficult to generate a population of *T. podisi* capable of inducing a high abortion rate after 5 generations of selective breeding. With these results, we are opening a track in biological control against the pest insect *H. halys*. The use of small wasps would reduce the stink bug reproduction. But also, at the light of the contents of this thesis, a selective breeding program could be optimized to produce efficient wasps in the abortion of *H. halys* eggs.

Keywords: Egg abortion, hosts, selective breeding, heritability, biological control, non-reproductive mortality

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Liste des sigles, abréviations et symboles

MAPAQ	Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec
OMAFRA	Ministère de l'Agriculture, de l'Alimentation et des Affaires Rurales (« Ministry of Agriculture, Food and Rural Affairs »)
RH	Humidité relative (« relative humidity »)
LM	Modèle linéaire (« linear model »)
GLM	Modèle linéaire généralisé (« generalized linear model »)
GLMM	Modèle linéaire généralisé mixte (« generalized linear mixed model »)
SD	Écart-type (« standard deviation »)
RHT	Tibia postérieur droit (« right hind tibia »)
$AIC_{(c)}$	Critère d'information d'Akaike corrigé (« corrected Akaike's information criterion »)
h^2	Héritabilité

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Chapitre 1 :

Introduction

1.1 Revue de littérature et contexte général

Les parasitoïdes sont, en général, des insectes parasites d'autres insectes. Les adultes ont un mode de vie libre et les stades larvaires se développent en se nourrissant de leur hôte, menant à la mort de ce dernier (Godfray, 1994). Leur capacité à contrôler les populations d'insectes nuisibles a été identifiée depuis longtemps comme une approche intéressante en lutte biologique. Plusieurs recherches sur les parasitoïdes sont motivées par la découverte de nouveaux agents de lutte biologique ou l'amélioration de leur utilisation (Godfray, 1994). En effet, les parasitoïdes ont un impact important sur les populations de différents groupes d'insectes et peuvent être ainsi utilisés comme agents de contrôle de populations d'organismes nuisibles (Greathead, 1986; Mills, 2003; Bompard et *al.*, 2013).

Bien que par définition le parasitoïdisme implique la mort de l'hôte par la larve qui le consomme, d'autres finalités existent (Maure et *al.*, 2011). De même, en plus d'utiliser leurs hôtes pour la reproduction, certains parasitoïdes peuvent les exploiter pour d'autres fins, lesquelles peuvent aussi entraîner la mort ou non de l'hôte. En effet, par exemple, un certain nombre de parasitoïdes adultes se nourrissent directement des fluides corporels de l'hôte (Giron et *al.*, 2004), ou infligent, par l'action mécanique de l'ovipositeur, des mutilations qui peuvent s'avérer fatales lors de l'évaluation de la qualité de l'hôte (Barrett

& Brunner, 1990; Martinez-Ferrer et *al.*, 2003). Une autre condition particulière a été identifiée, soit lorsque les parasitoïdes provoquent l'avortement des œufs hôtes (Abram et *al.*, 2016a). L'avortement de l'œuf induit par un parasitoïde se produit lorsqu'un parasitoïde d'œufs tue son hôte à la suite de l'insertion de son ovipositeur, mais que ni un parasitoïde, ni un hôte n'émerge (Abram et *al.*, 2016a). Ce phénomène constitue un cas de mortalité induite sans reproduction (Abram et *al.*, 2016a; Kaser et *al.*, 2018).

Les cas de mort de l'hôte induite par un parasitoïde ne contribuant pas à son succès reproducteur sont peu étudiés et restent peu compris (Abram et *al.*, 2016a). Ils devraient pourtant être pris en compte dans les programmes de lutte biologique puisqu'ils contribuent parfois de manière significative à la mortalité d'une forte proportion des hôtes attaqués, et donc à la réduction potentielle des populations des espèces nuisibles (Van Driesche et *al.*, 1987). Par exemple, plusieurs espèces de la famille des Trichogrammatidae sont couramment utilisées pour lutter contre diverses espèces de lépidoptères nuisibles pour l'agriculture et la foresterie (Smith, 1996). Le contrôle de leur population par les Trichogrammatidae se fait en partie par l'avortement des œufs de leurs hôtes (Abram et *al.*, 2016a). Lors de son étude, Abram et *al.* (2016a) ont identifié une grande variabilité de l'avortement des œufs de trois espèces de lépidoptères induit par différentes espèces et souches d'espèces de Trichogrammatidae. En effet, les pourcentages d'avortement allaient de 12,0 à 100,0% pour *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), 5,5 à 56,8% pour *Trichoplusia ni* (Hubner) (Lepidoptera: Noctuidae), et de 15,6 à 100,0% pour *Pieris rapae* (L.) (Lepidoptera: Pieridae) (Annexe Figure A1). Ces résultats montrent que non seulement une espèce n'a pas le même pouvoir avorteur sur un ou plusieurs hôtes, mais qu'en plus ce pouvoir est variable entre individus, et aussi pour un même individu.

Par contre, l'exploitation d'un hôte par un parasitoïde, menant à son avortement, ne contribue pas à la valeur adaptative (fitness) du parasitoïde puisqu'il ne peut se reproduire. Il perd à la fois une ressource (un œuf) ainsi que le temps et l'énergie investi à trouver et à exploiter un hôte non convenable. Ce scénario peut être considéré comme un cas de piège évolutif, qui définit un organisme contraint, par son passé évolutif, à commettre des erreurs, bien que des conditions adéquates (ou des choix adaptatifs) soient disponibles (Schlaepfer et *al.*, 2002). C'est par exemple le cas pour le papillon *Pieris oleracea* qui se reproduit en pondant ses œufs sur les feuilles de différentes plantes herbacée tel que la Cardamine carcajou, *Cardamine diphylla*. L'alliaire officinale, *Alliaria petiolatam*, est un plante exotique et envahissante en Amérique du Nord, qui ressemble aux autres herbacées que *P. oleracea* utilise pour sa reproduction. Keller et Chew (2008) ont identifié que *P. oleracea* pouvait pondre sur la plante envahissante, mais que ses larves ne pouvaient que très faiblement se développer. Les conséquences pour le papillon étaient d'autant plus grandes car il préférait même pondre sur plante invasive plutôt que sur son hôte naturel. Cette situation pourrait mener à l'extinction de *P. oleracea* si sa population descend sous une taille critique avant adaptation au nouvel environnement (Schlaepfer et *al.*, 2002).

Ce type de piège peut arriver dans différentes situations, notamment lors d'une nouvelle interaction entre une espèce indigène et exotique (Schlaepfer et *al.*, 2005; Keeler &

Chew, 2008; Abram et *al.*, 2014a). Les conséquences peuvent affecter les populations de parasitoïdes (Abram et *al.*, 2014a) qui pourraient se voir décimées et ainsi causer de possible déséquilibres dans l'écosystème puisque certains de leurs hôtes ne se trouveraient plus contrôlés (McPherson, 1982, Heimpel et *al.*, 2003).

Notre étude s'inscrit dans la continuation des travaux de doctorat de Paul Abram. Le système écologique se compose de la punaise marbrée *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) et de la guêpe parasitoïde d'œufs, *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae). La guêpe, indigène d'Amérique du Nord, est une espèce généraliste qui s'attaque aux œufs de plusieurs espèces de punaises de la famille des Pentatomidae. La punaise marbrée*,* une espèce envahissante originaire d'Asie, observée en Europe depuis 2004 (Wermelinger et *al.,* 2008, Haye et *al.*, 2014; 2015), a été découverte en 1998 aux États-Unis, où elle cause d'importants dommages à de nombreuses cultures. Cette espèce de Pentatomidae possède plusieurs ennemis naturels dont des espèces de punaises zoophages ou prédatrices, des diptères ou encore des aranéides (Jones, 2013; Abram et *al.*, 2014b; 2017). En Asie, de nombreuses guêpes parasitoïdes s'attaquent aux œufs de la punaise marbrée et effectuent un certain niveau de contrôle des populations (Kawada & Kitamura, 1992; Arakawa & Namura, 2002; Arakawa et *al.*, 2004; Lee et *al.*, 2013). En Amérique du Nord seules quelques espèces des genres *Trissolcus* et *Telenomus* exploitent la punaise marbrée comme hôte potentiel, mais elles sont incapables de s'y reproduire (Leskey et *al.*, 2012; Abram et *al.*, 2014a; Jones et *al.*, 2014). La situation est similaire en Europe où quelques espèces des genres *Trissolcus et de Telenomus* pondent leurs œufs dans ceux de la punaise marbrée mais sans succès de développement en conditions naturelles (Haye et *al.*, 2015; Cornelius et *al.*, 2016).

Toutefois, certaines guêpes, dont *T. podisi*, entraînent un fort taux d'avortement des œufs de *H. halys*, allant jusqu'à 30% de plus que le taux d'avortement naturel (~15%) (Abram et *al.*, 2014a; 2016a). Les principales étapes menant à la mort de l'hôte peuvent être décrites comme suivant : 1) Lors de la ponte, la femelle parasitoïde injecte dans l'hôte non seulement un œuf mais aussi 2) des éléments facilitateurs tel que du venin (Asgari & Rivers, 2011) ou des virus (Edson et *al.*, 1981; Rizki & Rizki, 1990) qui servent à inhiber le système immunitaire de l'hôte. De plus, 3) des cellules spécialisées telles que les tératocytes, issues de la membrane séreuse de l'œuf, procèdent à la séquestration d'énergie utilisée par l'embryon du parasitoïde et non l'hôte. Par contre, les raisons menant à l'avortement des œufs de l'hôte, sans succès reproducteur pour le parasitoïde, restent inconnues.

La lutte biologique, dont le principe consiste à utiliser des ennemis naturels d'une espèce considérée nuisible pour en contrôler sa population, se décline sous trois principaux types d'applications. 1) La lutte biologique classique, qui peut être utilisée dans les cas où il est nécessaire de contrôler une population d'espèces exotiques en important un ennemi naturel présent dans la région d'origine de l'espèce envahissante. 2) La lutte biologique par conservation, qui consiste à utiliser des ennemis naturels déjà présents et de favoriser leur développement et leur reproduction par l'aménagement de leur environnement. 3) La lutte biologique par inondation, qui consiste à augmenter la population d'ennemis naturels indigènes ou exotiques à l'endroit où se trouve le ravageur à contrôler, par exemple en relâchant une grande quantité d'agents de lutte biologique produits commercialement (Helyer et *al.*, 2014; Heimpel & Mills, 2017).

Dans le cas de l'envahissement du territoire nord-américain par *H. halys*, la méthode en théorie la plus performante serait d'utiliser la lutte biologique classique puisqu'il s'agit d'une espèce exotique qui a peu d'ennemis naturels indigènes efficaces en Amérique du Nord (Abram et *al.*, 2014b; 2017). Il s'agirait par exemple d'importer des parasitoïdes comme, entre autres, des espèces de *Trissolcus* et de *Telenomus* ou encore des Pentatomidae prédateurs, et ce, depuis la Chine, le Japon ou la République de Corée (Lee et *al.*, 2013). Cette méthode présente un grand potentiel puisqu'elle repose sur l'utilisation d'associations hôtes/proies-parasites/prédateurs qui ont déjà fait leurs preuves en matière de contrôle dans les pays d'origine de la punaise marbrée. La lutte biologique classique présente néanmoins certains risques pour l'environnement local qui pourrait se retrouver bouleversé par l'introduction de nouvelles espèces d'ennemis naturels (Howarth, 1991; Mason et *al.*, 2017). Alternativement, *T. podisi* présente un grand intérêt puisque, comme décrit précédemment, la guêpe a la capacité d'induire l'avortement des œufs de *H. halys*. La lutte biologique par inondation de *T. podisi* capable d'induire l'avortement serait alors une stratégie intéressante là où il s'avère nécessaire de contrôler les populations de punaise marbrée. L'utilisation de cette guêpe n'a jusqu'à maintenant jamais été étudiée et non envisagée, elle pourrait néanmoins être une solution prometteuse.

Le recours à des parasitoïdes avorteurs et leur succès en lutte biologique dépendent essentiellement des niveaux d'avortement anticipés. En effet, bien qu'en moyenne *T. podisi* induit un taux d'avortement d'environ 30% chez les œufs de *H. halys* (Abram et *al.*, 2014a), il existe une très grande variation d'avortements induits, non seulement entre les femelles, mais aussi pour une même femelle, allant de 0 à 100% (Abram et *al.*, 2016a). Aucune étude n'a exploré l'origine de cette variation, il serait toutefois essentiel d'identifier les facteurs impliqués dans ce phénomène d'avortement des œufs, avec en ligne de mire la possibilité d'utiliser *T. podisi* de manière efficace dans un programme de lutte biologique contre *H. halys*. En particulier, il importe de déterminer s'il est possible par sélection génétique non seulement de bonifier le caractère avorteur d'une espèce de parasitoïde mais également de le stabiliser au sein d'une population. La capacité d'avortement de *T. podisi* pourrait ainsi être lié à un ensemble de traits menant à la mort de l'embryon de punaise dans son œuf.

La taille d'un organisme détermine souvent sa valeur adaptative, quant à 1) son succès reproducteur (Wiens, 1984; Madsen & Shine, 1993; Jones & Hutchings, 2001; Bangham et *al.*, 2002) 2) l'acquisition de nourriture (Brooks & Dodson, 1965; Brown et *al.*, 1978; Mittelbach, 1980; Sutherland & Stillman, 1988) et 3), ou sa longévité (King, 1987; Sogard, 1997). Chez *H. halys*, un œuf plus gros pourrait présenter un avantage pour le développement de sa larve puisqu'il offre plus de ressources en matière d'énergie (Parker & Begon, 1986; Sota & Mogi, 1992) et une plus grande résistance à la dessiccation (Sota & Mogi, 1992). Les parasitoïdes de grandes tailles se déplacent plus vite (Abram et *al.*, 2016b), vivent plus longtemps (King, 1987), sont plus féconds (Waage & Ning, 1984;

Rosenheim & Rosen, 1991; Abram et *al.*, 2016b) et, de manière plus générale, présentent une meilleure valeur adaptative que les individus de petite taille (Visser, 1994). La taille d'une guêpe influence de fait la taille de ses organes, dont l'ovipositeur (Brandl, 1987), des oocytes (Charnov & Skinner, 1985) ou de la quantité de venin injecté lors d'une ponte. Ainsi, la taille de *T. podisi* pourrait influencer sa capacité à faire avorter les œufs de *H. halys*.

Une guêpe qui induit un fort taux d'avortement pourrait en fait découler d'une combinaison de traits codés par sa génétique. La relation d'expression d'un ou plusieurs traits entre générations repose sur le concept d'héritabilité, qui exprime la part de la variation des facteurs génétiques impliqués dans l'expression d'un trait (Wray & Visscher, 2008). Autrement dit, l'héritabilité permet de quantifier la capacité d'un parent à transmettre ses traits à sa descendance, et donc d'engendrer des individus ayant un phénotype (un trait ou ensemble de traits) semblable aux parents. Par le fait même, la mesure de l'héritabilité permet d'évaluer la probabilité qu'un ou des traits se maintiennent au sein d'une lignée d'individus ayant les mêmes parents. Plusieurs méthodes permettent de mesurer l'héritabilité (exprimé h^2). La régression parents-enfants consiste à mesurer l'héritabilité par l'intermédiaire d'une régression linéaire. Elle nécessite de mesurer l'expression d'un trait chez des parents et leurs enfants, puis d'utiliser ces valeurs pour le modèle linéaire et de calculer la pente de la droite de tendance (r^2) qui sera alors la valeur d'héritabilité (h^2) (Doolittle, 1987; Conner & Hartl, 2004). Une autre méthode, *Breeder's equation* consiste à comparer l'expression d'un trait entre parents et enfants par l'intermédiaire de la moyenne du trait de l'ensemble d'une population (Conner & Hartl, 2004). L'équation s'exprime par $R = h^2S$ où R représente la réponse à la sélection, h^2 exprime l'héritabilité et S la différence de sélection. La valeur de S correspond à la différence de l'expression du trait mesuré entre une population et les individus possédant le trait en question. La valeur R correspond à la différence de l'expression du trait mesuré entre une population et la descendance des individus possédant le trait. Ainsi, si le trait mesuré est aussi fréquent chez les descendants qu'au sein de l'ensemble de la population, alors l'héritabilité sera de 0, signifiant que l'expression du trait est liée principalement aux facteurs environnementaux. À l'inverse, si le trait est autant présent dans la descendance que leurs parents, alors l'héritabilité sera de 1, signifiant que l'expression du trait est liée aux composantes génétiques des individus. Dans la mesure où un trait est héritable, il peut être sujet à la sélection dans l'optique de voir ce trait présent dans un ensemble d'individus d'une population. Dans le cas de l'effet d'avortement des œufs de *H. halys* par *T. podisi*, si le caractère avorteur est lié à des traits qui sont héritables, on devrait alors techniquement pouvoir sélectionner pour une population de guêpe dont tous les individus induiraient l'avortement des œufs de la punaise.

Il serait même possible d'envisager de sélectionner pour différentes capacités d'induction d'avortement grâce à la reproduction sélective (ou sélection artificielle) (Conner, 2003). Cette approche consiste à isoler les gènes responsables du trait d'intérêt en faisant se reproduire uniquement les individus le possédant (Gjedrem, 1997; Swallow & Garland, 2005; Purugganan & Fuller, 2009). Ainsi on sélectionne les individus ayant la génétique codant pour le trait recherché, et petit à petit on exclut de la lignée les individus qui l'expriment le moins. Le trait en question s'exprime par sa présence/absence ou par l'intensité de son expression. Ainsi, on pourrait sélectionner des femelles *T. podisi* possédant une forte capacité d'avortement des œufs de *H. halys*. Ce procédé a déjà été utilisé avec succès pour sélectionner ou amplifier des traits chez des espèces de différentes règnes (Mousseau & Roff, 2005). Chez les animaux, il est ainsi possible de sélectionner des traits comportementaux (Hyde & Sawyer, 1980; Ayres & Arnold, 1983; Kirpichnikov 1983), morphologiques (Cook, 1964; Lannan, 1972; McLaren, 1976) ou physiologiques (Gjedrem et *al.*, 2012; Newkirk, 1980). Pour qu'un tel programme soit efficace, il nécessite deux conditions essentielles : 1) qu'il soit héritable et 2) qu'une variation génétique soit suffisamment présente dans la population utilisée. En effet, il est essentiel de pouvoir tester un large spectre d'individus ayant une génétique différente pour sélectionner dès le départ des individus ayant le plus grand potentiel (Lommen et *al.*, 2017; Wajnberg, 2004). Par exemple, cette méthode a été utilisé afin d'augmenter l'affinité d'un parasitoïde envers un hôte non habituel. *Horogenes molestae* se reproduit naturellement dans la tordeuse orientale du pêcher, *Grapholitha molesta* (Busck). Allen (1954) a réussi à sélectionner une lignée ayant une préférence similaire pour la teigne de la pomme de terre *Gnorimoschema operculella* (Zeller), une espèce non hôte, après 39 générations de sélection. Dans la mesure où les facteurs menant à l'avortement des œufs de *H. halys* par *T. podisi* ont une composante génétique, un programme de reproduction sélective permettrait de non seulement de produire des guêpes avec un fort taux d'avortement, mais aussi que ce caractère soit présent dans toutes les guêpes de la population produite.

1.2 Objectif de l’étude

Tel que le suggère Abram et *al.* (2016a), les parasitoïdes avorteurs méritent plus d'investigation d’un point de vue théorique, pour la compréhension de la dynamique des interactions parasitoïdes-hôtes, et d’un point de vue appliqué, quant à leur potentiel en lutte biologique.

La présente étude, supportée par un financement du programme de Coopération Québec-Ontario pour la recherche en agroalimentaire (MAPAQ-OMAFRA), consiste à explorer la nature et les mécanismes de la mortalité des œufs de la punaise marbrée *H. halys* induite par la guêpe parasitoïde *T. podisi*. La réalisation de ce projet de recherche permettra de contribuer aux domaines de l’écologie fondamentale et appliquée, essentiellement par l’atteinte de deux objectifs:

a. Identification et exploration de l’influence du ratio de taille parasitoïde-œufs d’hôte sur l’avortement des œufs attaqués par le parasitoïde.
b. Évaluation du potentiel d’amplification de la capacité de *T. podisi* à avorter les œufs de *H. halys*. Cela afin d’examiner la possibilité de sélectionner des souches performantes de *T. podisi* avorteur comme agent de lutte biologique contre *H. halys*.

1.3 Hypothèses et prédictions

Les hypothèses quant au caractère avorteur du parasitoïde *T. podisi* se déclinent selon deux principes non-exclusifs:

1. La capacité de *T. podisi* à avorter les œufs de *H. halys* dépend de facteurs plastiques. C'est-à-dire que des facteurs biotiques (taille relative de l'hôte et du parasitoïde) modulent la capacité d'avortement de la guêpe.
2. La capacité de *T. podisi* à avorter les œufs de *H. halys* est héritable. C'est-à-dire que des traits ancestraux déterminent la capacité d'avortement de la guêpe.

Le projet comprend deux parties, chacune associée à un projet spécifique (Étude 1 – Chapitre 2 et Étude 2 – Chapitre 3).

Étude 1 :

Hypothèse: Le ratio de taille entre le parasitoïde et l'hôte influence le taux d'avortement.

Prédiction: L'incidence d'avortement des œufs de *H. halys* augmente avec le ratio de la taille entre *T. podisi* et son hôte. Le taux d'avortement sera plus important entre un parasitoïde de grande taille et un petit œuf.

Étude 2 :

Hypothèse: La capacité d'avortement dépend de caractéristiques transmissibles génétiquement.

Prédiction: Les individus à forte capacité d'avortement produiront une descendance ayant également une telle capacité. Grâce à la reproduction sélective, le taux d'avortement des œufs de *H. halys* par *T. podisi* augmentera au fil des générations.

1.4 Contribution des auteurs

Les chapitres 2 et 3 sont rédigés en anglais sous forme d'articles scientifiques. Le développement des hypothèses de travail, la méthodologie, la réalisation et l'analyse des expériences associées à ces chapitres ont été réalisées par Frédéric Hadi, de même que leur écriture. Les chercheurs Jacques Brodeur et Paul Abram ont supervisé le projet, révisé les manuscrits et contribué aux idées importantes des deux articles. Les analyses statistiques ont été choisies et exécutées par Frédéric Hadi et Paul Abram. Plusieurs personnes ont également aidés à la collecte des échantillons ou ont agit comme soutient technique en laboratoire lors de ces expériences. Celles-ci sont mentionnées dans les remerciements des articles respectifs.

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1.6 Annexe

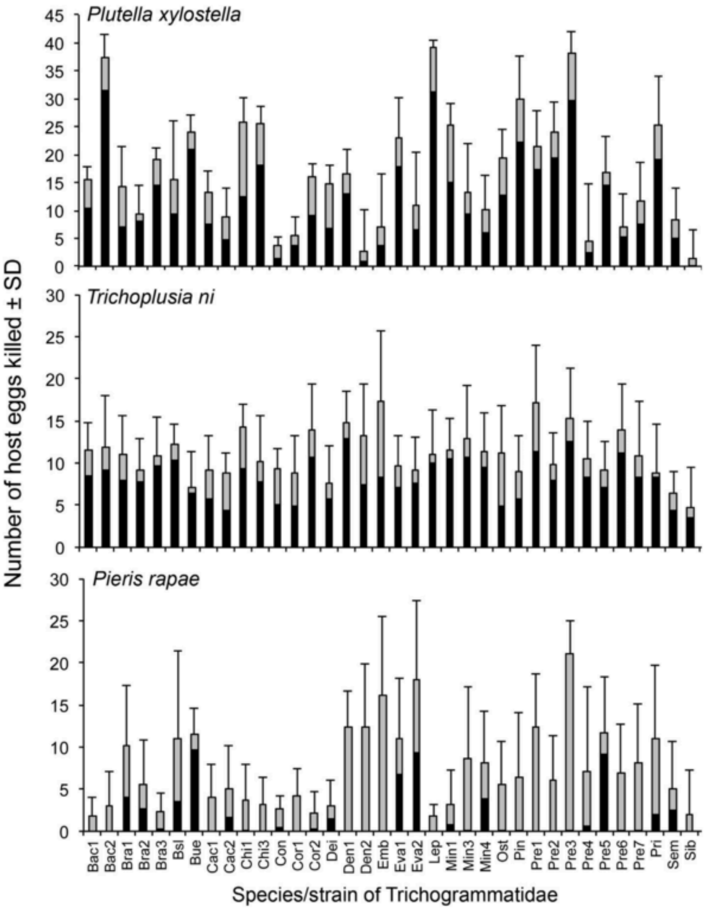

Figure A1. Extrait de Abram et *al.*, (2016a). Mean (±SD) mortality (black bars = parasitism, grey bars = abortion) of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), *Trichoplusia ni* (Hubner) (Lepidoptera: Noctuidae), and *Pieris rapae* (L.) (Lepidoptera: Pieridae) eggs induced by forty species/strains of Trichogrammatidae parasitoid wasps over a 24 h period.

Chapitre 2 :

Does size matter for natural and parasitoid-induced host egg abortion?

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Keywords: non-reproductive mortality, host-parasitoid interactions**,** *Halyomorpha halys*, *Telenomus podisi*, biological control

2.1 Abstract

Parasitoid-induced host egg abortion has recently been recognized as a common source of mortality for both the host and the parasitoid. This type of non-reproductive parasitism could affect host-parasitoid dynamics as well as biological control. The egg parasitoid *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) readily accepts the eggs of the exotic stink bug *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) as a host, but the parasitoid's offspring are unable to successfully develop in *H. halys* eggs. In addition, these parasitoids provoked variable levels of host egg mortality. In this study, we explore mechanisms behind egg abortion in both unparasitized and parasitized hosts by investigating the role of egg size, parasitoid adult size and the ratio between parasitoid females and host eggs. We hypothesized that abortion rates would be higher when large parasitoids attacked small host eggs and lower when small parasitoids attacked large host eggs. We found that large *H. halys* eggs, unparasitized or parasitized by *T. podisi*, were more likely to abort than small eggs. Contrary to our hypothesis, small parasitoids induced higher levels of egg abortion than large parasitoids, especially when ovipositing in large host eggs. Parasitoids inducing host egg abortion could represent a new type of natural enemy to be released in biological control programs. However, this time, the bigger parasitoids are not the most effective. Therefore, in biological control, "the rule of the game" would need to be reconsidered, using smaller parasitoids.

2.2 Résumé

L’avortement des œufs d’hôtes induit par les parasitoïdes a récemment été reconnu comme une source commune de mortalité à la fois pour l'hôte et pour le parasitoïde. Ce type de parasitisme non reproductif pourrait affecter la dynamique hôte-parasitoïde ainsi que la lutte biologique. Le parasitoïde d’œufs *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) accepte les œufs de la punaise exotique *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), mais la progéniture du parasitoïde n’est pas capable de se développer avec succès dans les œufs de *H. halys*. En plus de cela, ces parasitoïdes provoquent des taux variables de mortalité des œufs de l'hôte. Dans cette étude, nous explorons les mécanismes de l'avortement chez les hôtes non parasités et parasités en étudiant le rôle de la taille des œufs, la taille des adultes parasitoïdes et le ratio entre les femelles parasitoïdes et les œufs de l'hôte. Nous avons émis l'hypothèse que les taux d'avortement seraient plus élevés lorsque de gros parasitoïdes attaquaient des petits œufs hôtes et plus faible lorsque de petits parasitoïdes attaquaient de gros œufs hôtes. Nous avons constaté que les grands œufs de *H. halys*, non parasités ou parasités par *T. podisi*, étaient plus susceptibles d'avorter que les petits œufs. Contrairement à notre hypothèse, les petits parasitoïdes induisaient des taux plus élevés d'avortement des œufs que les gros parasitoïdes, en particulier lorsqu'ils pondaient dans de grands œufs hôtes. Les parasitoïdes qui induisent l'avortement des œuf hôte pourraient représenter un nouveau type d'ennemi naturel à relâcher dans les programmes de lutte biologique. Cependant, cette fois-ci, les plus gros parasitoïdes ne sont pas les plus efficaces. De ce fait, en lutte biologique, “la règle du jeu” devrait être reconsidérée, en utilisant des petits parasitoïdes.

2.3 Introduction

Insect parasitoids have a unique life history among parasitic organisms: adults are free-living and larvae develop on or within a single host, ultimately killing the host. Consumption of the host is not the only way of parasitoid-induced mortality (Van Driesche, 1983; Barrett & Brunner, 1990). Host death can also be caused by direct feeding of the adult parasitoid on the host (host feeding) (Jervis & Kidd, 1986). In addition, other modes of actions of parasitoids can negatively affect, and eventually kill their host without contributing to parasitoid reproduction. Following Abram et *al.* (2019), these non-reproductive effects can be subdivided into the following categories: *i)* Non-consumptive effects - the parasitoid presence induces a change in host behaviour, leading to fitness reduction or death (Sloggett & Weisser, 2011; Deas & Hunter, 2013); *ii)* Mutilation - the female parasitoid uses its ovipositor as a probe to assess host quality, resulting in mechanical injuries (Ingerslew & Finke, 2017; Cebolla et *al.*, 2018); *iii)* Pseudoparasitism – the female injects venoms, viruses and other chemical compounds while probing the host and/or laying an egg (Jones et *al.*, 1981; Tillinger et *al.*, 2004); *iv)* Immune defense costs - investment in immune defenses reduces host fitness (Kraaijeveld & Godfray, 1997); *v)* Aborted parasitism - a parasitoid egg is laid in or on the host, but neither the parasitoid or host develops (Abram et *al.*, 2016a).

Many parasitoid species are commonly used as biological control agents because they can regulate populations of insect pests (Greathead, 1986; Wajnberg et *al.*, 2008; Heimpel & Mills, 2017). In most instances, host death coincides with the successful development of the parasitoid's offspring. While non-reproductive mortality likely makes up a large component of biological control services provided by parasitoids (Van Driesche, 1983; Münster-Swendsen, 1994; Abram et *al.*, 2019), its occurrence and impact in host-parasitoid associations have often been underestimated, and factors that affect its magnitude rarely been studied. The idea of exploiting parasitoid non-reproductive effects for biological control purposes has recently been proposed (Abram et *al.*, 2016a).

Aborted parasitism has been reported by Abram et *al.* (2014; 2016a) for the parasitoid *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) attacking eggs of the brown marmorated stink bug *Halyomorpha halys* Stål (Hemiptera: Pentatomidae). Originally from Asia, *H. halys* has rapidly invaded North America and Europe, possibly because of a lack of efficient natural enemies (Rice et *al.*, 2014; Cornelius et *al.*, 2016). The brown marmorated stink bug potentially represent an evolutionary trap for the indigenous *T. podisi* because parasitoid females readily lay eggs in *H. halys* but successful development does not occur (Abram et *al.*, 2014). Oviposition by *T. podisi*, however, significantly increased levels of *H. halys* egg abortion (from 24.1% to 29.6%) relative to control individuals. Furthermore, the capacity to abort stink bug eggs varied greatly among parasitoid females (Abram et *al.*, 2016a). Egg abortion thus represents the primary mode of parasitoid-induced mortality in this new host-parasitoid association, and may have consequences in population dynamics and biological control.

Once a host has been discovered in the environment, the host killing efficiency of a parasitoid – for both successful parasitism and non-reproductive mortality – depends on the morphological and physiological suitability of the host (Vinson, 1976; 1984). The mechanisms behind parasitoid-induced egg abortion remain unexplored but they may result from (i) mechanical damage to the host egg during ovipositor insertion through the host egg shell, (ii) injection of chemical compounds (e.g. venom) by the parasitoid female that kill the host embryo or arrests its growth (Strand & Pech, 1995; Moreau & Guillot, 2005; Asgari & Rivers, 2011), or (iii) parasitoid embryo-derived damage to the host (e.g. teratocyte action, larval feeding (Dahlman, 1990; Volkoff & Colazza, 1992; Strand, 2014).

One source of variation in aborted parasitism could be the phenotypic variation in body size within a host-parasitoid association. Size is a key component of phenotypic variation in insects and is linked to a variety of fitness-related traits (Godfray, 1994; Kazmer & Luck, 1995; Ellers et *al.*, 1998). For parasitoids, the lethality of the various mechanisms potentially involved in egg abortion could be higher for larger individuals, which actually have stronger ovipositors (Brandl, 1987), larger accessory glands producing more chemical compounds and/or larger or more numerous oocytes (Charnov & Skinner, 1985) producing more teratocytes. From the hosts, egg size could influence survival rates and fitness components of both the host and the parasitoid (Sota & Mogi, 1992; Godfray, 1994) because of variations in the amount of resources and response to abiotic stress (Parker & Begon, 1986; Molles, 2002; Werf et *al.*, 2009).

In this study, we explored mechanisms behind egg abortion in both unparasitized and parasitized hosts by investigating the role of egg size, parasitoid adult size and the size ratio between parasitoid females and host eggs. For parasitized hosts, we hypothesized that the incidence of aborted parasitism depends on the size ratio between *T. podisi* and *H. halys*; i.e. aborted parasitism increases together with the size ratio and vice-versa).

Thus, we expected large parasitoids to induce a higher abortion rate when parasitizing small *H. halys* eggs, and small parasitoids to induce a lower abortion rate on large host eggs.

2.4 Materials and methods

2.4.1 Insect colonies

Insects were originally collected in the London and Hamilton (Ontario, Canada) areas in both 2011 and 2012. Stink bug and parasitoid colonies were maintained in 30 cm^3 ventilated polyester cages (BugDorm, Taiwan) at 24 ± 1°C, 50 ± 5% RH, 16L: 8D light cycle. Eggs of *P. maculiventris* and *H. halys* were taken from the cages every 24 h to 72 h and placed in Petri dishes (D: 9.0 cm, H: 1.4 cm) until hatching. *Halyomorpha halys* nymphs were then transferred to the colony and all stages were reared on potted soybean plants, fresh carrots, green beans and pumpkin seeds. For *P. maculiventris,* early-instar (I-III) nymphs were reared in plastic cylinders (D: 11.0 cm; H: 15.15 cm) and next transferred to the colony. All stages were fed with fresh green beans and yellow mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae and pupae.

Telenomus podisi were reared on *P. maculiventris*, one of its most closely associated and suitable host species (McPherson, 1982; Cornelius et *al.*, 2016). Host eggs were glued on a filter paper with non-toxic glue and placed in the colony for parasitism during 48 h. Once removed from the cage, parasitized eggs were placed in Petri dishes (D: 9.0 cm, H: 1.4 cm) until parasitoid emergence. Newly emerged parasitoids were then transferred to the colony. Females used in the experiments were kept in Petri dishes with males for 24 h to allow mating and next individually put in 1.2 mL Eppendorf tubes with a drop of honey for 48 h. Experiments were conducted with naïve females having 9-15 mature eggs in their ovarioles (P. K. Abram, unpublished data). All experiments were conducted under the same conditions as described above for rearing.

2.4.2 *Halyomorpha halys* egg size variation

Prior to determining the effect of *H. halys* egg size on abortion rate by *T. podisi*, we conducted two preparatory experiments to characterize the intrinsic egg size variation and weight loss over time in *H. halys*. We first examined how egg weight varies within and between 77 egg clutches randomly selected from the colony, each clutch containing a minimum of 19 freshly laid eggs (<24 h). Eggs were carefully separated from the mass and weighed individually on a Mettler Toledo MT5 Micro Balance (± 1μg). Among all clutches, mean (±SD) egg weight was 1329.66 ±152.63 μg, ranging from 778 to 1927 μg (Figure 1). In the following experiments, individual egg weight was estimated by dividing total clutch weight by the number of eggs because in most cases, egg weights were similar within a clutch (Figure 1). Eggs were not removed from the clutch to avoid mortality. Egg size was categorized in three groups, based on the estimated individual egg weight: small (<1200 μg), medium (1200 to 1400 μg), and large (>1400 μg).

During preliminary tests to evaluate the repeatability of egg weight measurements, we observed that *H. halys* eggs tended to lose weight over time, which could adversely affect the accuracy of egg weight measurements. To further examine this trend, we conducted repeated daily measurements on 198 *H. halys* eggs to quantify weight loss when eggs were reared during 6 days at 24 ± 1°C, 50 ± 5% RH, and then placed at 4°C for 11 days (see experimental conditions below). The presence/absence of an "egg-burster", a small triangular structure that appears at the interior surface of viable eggs after 4 to 5 days (Javahery, 1994; Endo & Numata, 2017), was used to determine if an egg was viable (n = 152) or had aborted (n = 46). Egg weight decreased continuously over time for both viable and aborted eggs (Linear Model, $P < 0.001$, adjusted $R^2 = 0.62$), but occurred at different rates with viable eggs losing weight more rapidly than aborted eggs (LM, $P < 0.001$, adjusted $R^2 = 0.8025$) (Figure 2). Accordingly, to account for egg weight loss over time, a correction based on the above linear models was applied to *H. halys* egg weight to estimate eggs weight at the moment it was exposed to parasitism. We run a separate set of three linear models corresponding to three phases of weight loss (0–3, 3–5, and 5–17 days) for viable and aborted eggs. This "corrected" weight was used for statistical analyses.

2.4.3 *Telenomus podisi* body size variation

Adult *T. podisi* size is proportional to the volume of the egg in which it develops (Abram et *al.*, 2016b). To produce *T. podisi* females of different sizes for the experiments, 0-48 h old *P. maculiventris* eggs were collected from the colony and weighed individually as described above. Egg weight varied from 178 µg to 488 µg, with a mean (±SD) of 375.75 ±44.85 µg (n = 399). Host eggs were assigned to three weight categories: small (<350 µg), medium (350 to 400 µg), and large (>400 µg). Eggs were then glued on filter papers and placed in the *T. podisi* rearing cage for 24 h (Gaudreau et *al.*, 2017). Parasitized eggs were next placed individually into 1.5 mL microtubes and incubated under the rearing conditions described above until parasitoid emergence (~15 days).

2.4.4 Abortion rates

Nine treatments based on *T. podisi* adult size and *H. halys* egg size categories (3 x 3 factorial design) were considered to test abortion rates. *Halyomorpha halys* egg clutches were held in place on a 1 x 1 cm paper square with plasticine to prevent parasitoid females from hiding under the egg clutch. Clutches were then individually put in a Petri dish (D: 5.0 cm, H: 1.0 cm) with a 3-9 days old virgin female for 5 hours. It was previously determined that virgin and mated *T. podisi* females induce similar abortion rates (V. Burte, unpublished data). Each treatment was replicated 4 to 11 times, depending on egg availability in different weight classes. Parasitoid behavioral sequences were filmed using a Sony Handycam HDR-XR500 and we used host marking behavior to determine if a host had been parasitized or not (Abram et *al.*, 2014). Attacked but unmarked eggs (i.e. host rejection) and superparasitized eggs (i.e. more than two eggs laid per host, < 1% of parasitized eggs) were discarded from the analysis.

Following parasitoid exposure, host eggs were left in the Petri dishes and maintained under standard experimental conditions. The presence/absence of egg-bursters was used to determine if eggs were viable or had aborted. Eggs were next placed at 4°C until weight measurement.

To control for natural abortion in egg masses not exposed to *T. podisi*, 17 *H. halys* egg clutches were collected from the colony and assigned to each of the three egg size categories (3 to 9 clutches per category), in parallel with the experiments described above. Abortion rate was also calculated for eggs that had been exposed to parasitoids in the previous experiment but had not been attacked (no oviposition). Abortion rates were thus assessed for three treatments: (i) parasitized eggs (identified from videos), (ii) eggs exposed to parasitoids but not parasitized, and (iii) control eggs (not exposed to *T. podisi*).

Following the experiment, parasitoids were preserved in 1.5 mL microtubes containing 90% ethanol. They were next dissected to measure their right hind tibia (RHT) length; a linear proxy for body size (Roitberg et *al.*, 2001). The measurements were performed under 94.0 X magnification using digital measurement software (ZEN 2012, Blue Edition, version 1.1.1.0) in combination with a camera (ZEISS, Axiocam HRC) on a stereomicroscope (ZEISS, Stereo Discovery version 20).

2.4.5 Statistical analyses

Our general statistical approach, depending on the occurrence of repeated measures (random effects), was to fit generalized linear models (GLMs) or generalized mixed models (GLMMs) to the data, with proportion of aborted eggs as the response variable. Models were simplified to include only significant fixed factors ($p < 0.05$) with iterative likelihood ratio tests. The conditional coefficient of determination $R^2_{(c)}$ was calculated to assess the model fit (expressed $R^2_{LM(c)}$, $R^2_{GLM(c)}$ and $R^2_{GLMM(c)}$) (piecewiseSEM package

1.2.1) (Nakagawa & Schielzeth, 2013). Applied to GLMMs, this statistic describes the proportion of variance explained by both the fixed and random factors (Johnson, 2014). In addition, the corrected Akaike's information criterion (AIC_c) was used to identify the most appropriate model (Burnham & Anderson, 2002).

To test the effect of the treatments (parasitized, not parasitized, control) on egg abortion, we used a GLMM (*glmer* function (lme4 package 1.1-13) fit by restricted maximum likelihood REML) (Bates et *al.*, 2015) with a binomial error distribution. The effect of *T. podisi* parasitism on *H. halys* egg abortion was first examined using a GLMM with the exposure treatment as a fixed effect and individual egg clutch as a random effect. Differences between means were next analysed with Tukey multiple comparisons test (ghlt function in the "multcomp" package). Then, to consider the parasitoid size X treatment interaction, we ran a second model including treatments, *H. halys* egg weight, and their interaction as fixed effect variables in the model; individual egg clutch was included as a random effect variable.

To test the combined effects of parasitoid size and *H. halys* egg weight on egg abortion we used a GLMM with a binomial error distribution. We included whether a given egg was aborted or not as a binomial response variable. *Halyomorpha halys* egg weight, *T. podisi* RHT length and their interaction were included as fixed factors. Parasitoid individual was included as a random effect variable.

To confirm that larger *P. maculiventris* eggs produced larger *T. podisi* as intended, we evaluated the relationship between *P. maculiventris* egg weight and *T. podisi* RHT length using linear and polynomial (quadratic) regressions. We found significant positive relationship between *P. maculiventris* egg weight and *T. podisi* size (LM, adjusted $R^2 = 0.5144$, $P < 0.0001$) (Figure 3). The polynomial regression fit the data better than a linear regression ($R^2_{LM(c)} = 0.532$; AIC = 386.37).

In addition, before proceeding with our analysis, we tested for potentially confounding effects. First, we confirmed that there was no relationship between *H. halys* egg weight and the order attack by *T. podisi* (GLMM, $R^2_{GLMM(c)} = 0.506$, $\chi^2 = 0.0622$, df = 1, P = 0.803), i.e., *T. podisi* did not parasitize larger eggs at the beginning of the oviposition sequence. Additionally, the order in which *T. podisi* parasitized eggs in a clutch did not affect the proportion of eggs aborted (GLMM, $R^2_{GLMM(c)} = 0.251$, $\chi 2 = 0.046$, df = 1, P = 0.830); i.e., eggs parasitized earlier in the sequence were not more likely to abort than those parasitized later in the sequence. Finally, there was no relationship between the proportion of parasitized *H. halys* eggs per clutch and the size of *T. podisi* (GLM, $R^2_{GLM(c)} = 0.006$, F = 0.608, df = 50, P = 0.604); i.e., larger parasitoid females did not parasitize a greater number of eggs.

Statistical analyses were conducted using RStudio (version 1.0.143, R version 3.4.0 "You Stupid Darkness" under Mac OS X 10.11.6) (<www.r-project.org>).

2.5 Results

When considering only exposure treatment, *H. halys* eggs abortion levels varied significantly among conditions ($\chi2 = 11.31$, $p = 0.0035$), with parasitized eggs aborting more than unparasitized eggs (Tukey multiple comparisons; $p = 0.014$) and marginally more than control eggs ($p = 0.057$). However, including *H. halys* egg size in statistical models showed that it was the dominant factor explaining variation in host egg abortion: the probability of *H. halys* egg abortion increased strongly with *H. halys* egg size, but the effect varied by exposure treatment (i.e., the treatment x egg weight interaction was significant (Table I). The effect of egg weight on abortion rate was lower for parasitized than for unparasitized and control eggs because exposure to parasitoids increased abortion levels of lighter eggs (Figure 4).

Levels of parasitoid-induced egg abortion depended on the ratio between parasitoid size and host egg weight (Table I). Remarkably, smaller parasitoids induced overall higher abortion rates, but this trend was mostly apparent for larger host eggs. There was a very strong positive effect of host egg size on abortion when the eggs were parasitized by smaller parasitoids (Figure 5), but with increasing female size this effect became minimal - for eggs parasitized by the largest parasitoids, there was almost no effect of host egg size on abortion (Figure 5).

Table I. The influence of host egg weight and parasitoid body size on egg abortion in *Halyomorpha halys* under three experimental treatments: control eggs, eggs parasitized by *Telenomus podisi*, eggs exposed to *T. podisi* but not parasitized. Results from generalized mixed models (GLMM).

Model	R-squared of simplified model	AIC_c	Factor	χ2	P
Abortion by exposure treatment and *H.* halys egg weight	0.698	1107.3	Treatment	7.423	**0.0023**
			H. halys egg weight	72.090	**<0.0001**
			Treatment x *H. halys* egg weight	6.507	**0.025**
Abortion of parasitized eggs by *T. podisi* size and *H. halys* egg weight	0.552	740.3	T. *podisi* size	4.996	**0.019**
			H. hays egg weight	26.308	**<0.0001**
			T. podisi size x *H. halys* egg weight	6.950	**0.008**

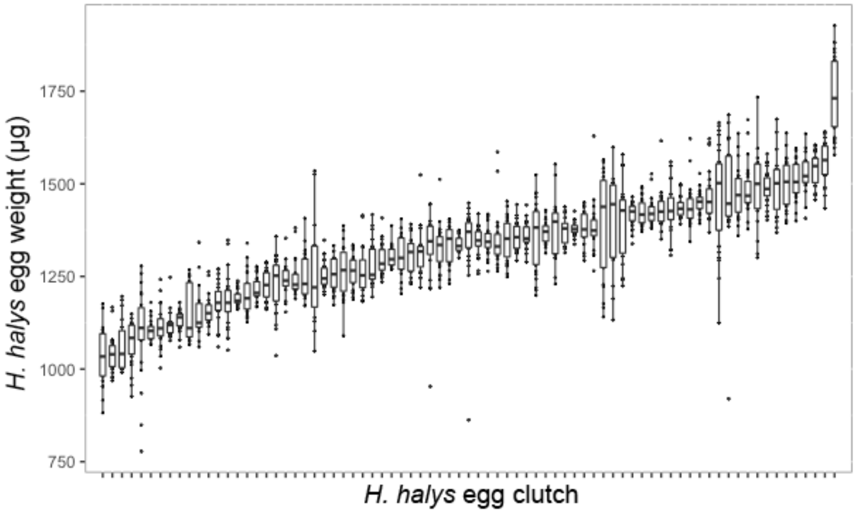

Figure 1. Variation in *Halyomorpha halys* eggs weight (*µg*) within and between 77 egg clutches (19-29 eggs per clutch, for a total of 1828 eggs). The horizontal line in each box shows medians, boxes contain the 25th–75th percentiles, whiskers display the upper and lower deciles, and dots identify outliers.

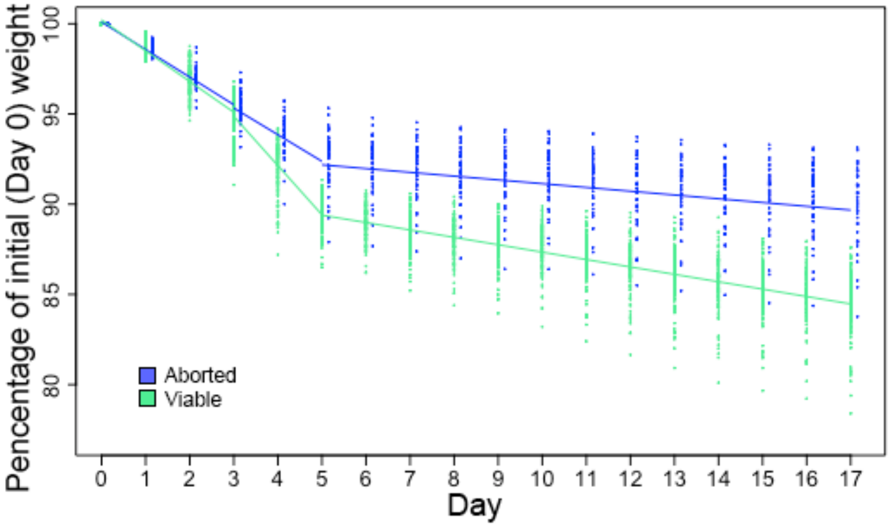

Figure 2. Linear regressions (6) describing three phases of weight loss (% of initial weight) over time of aborted (n = 46) and not aborted (n = 152) *Halyomorpha halys* eggs. Generalized linear models (GLM) for aborted eggs from day 0 to day 3 ($R^2 = 0.912$, $y = -1.5276x + 100.1$), day 3 to day 5 (egg-burster appearance) ($R^2 = 0.513$, $y = -1.4764x + 99.843$) and day 5 to day 17 ($R^2 = 0.166$, $y = -0.2061x + 93.431$). GLM for viable eggs from day 0 to day 3 ($R^2 = 0.910$, $y = -1.5542x + 100.16$), day 3 to day 5 ($R^2 = 0.886$, $y = -2.8979x + 104.03$) and day 5 to day 17 ($R^2 = 0.627$, $y = -0.4012x + 91.552$). The egg-burster in viable eggs appears around day 4, eggs were placed at 4°C on day 5 (see M&M for details).

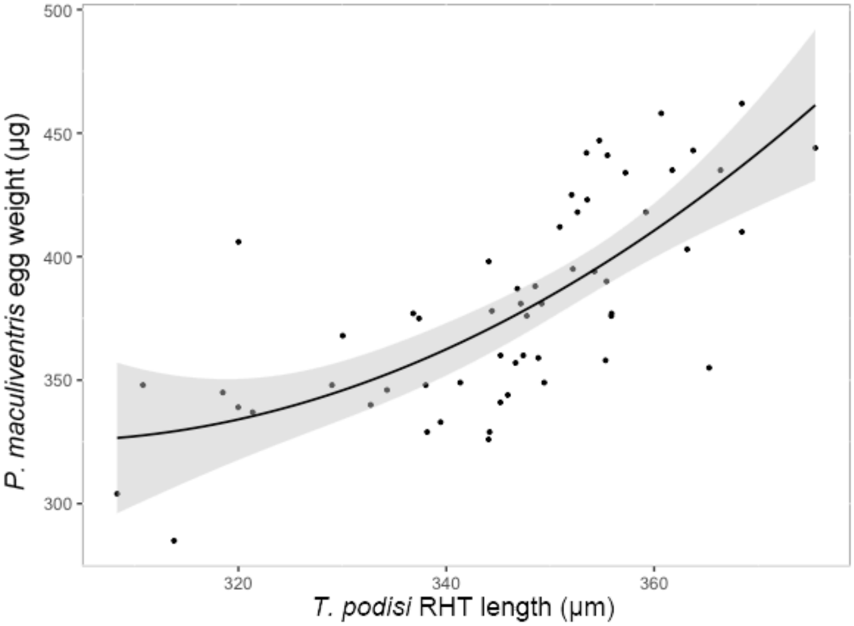

Figure 3. Relationship between *Telenomus podisi* size (right hind tibia length) and the weight of the *Podisus maculiventris* egg in which the wasp developed (+/- 95% CI, polynomial regression ($y = 0.0245x^2 - 14.779x + 2550.1$), $p < 0.001$, adjusted $R^2 = 0.5144$).

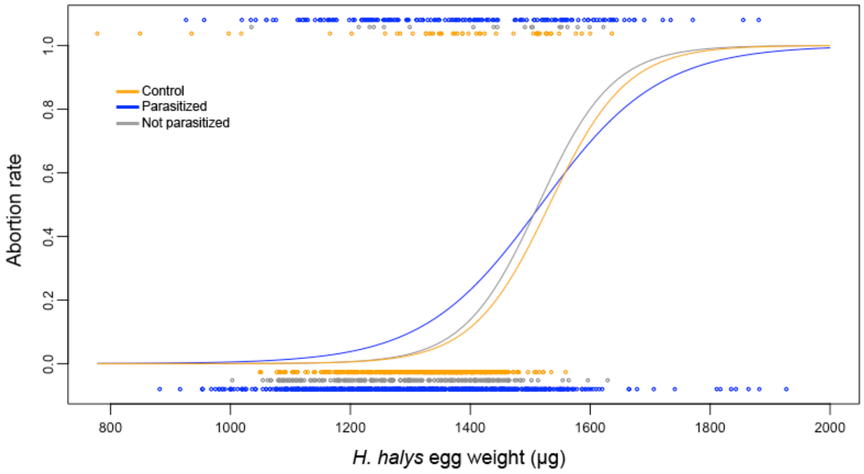

Figure 4. Probability of *Halyomorpha halys* egg abortion depending on weight and exposure to *Telenomus podisi*. N = 1560 eggs (438 unexposed controls (orange), 897 parasitized (blue), and 225 exposed to *T. podisi* but not parasitized (grey)). Statistical results are shown in Table I.

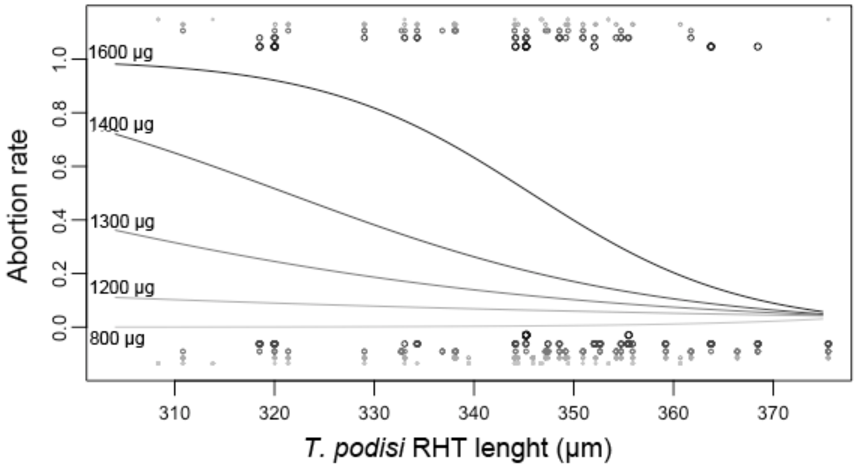

Figure 5. Probabilities of egg abortion in *Halyomorpha halys*, parasitized by *Telenomus podisi* based on host egg weight and parasitoid size (879 eggs parasitized by 47 *T. podisi*). Lines show predictions from a GLMM for different *H. halys* egg weight. Points refer to the eggs used for the analysis. The size of the point refers to the size of the egg. Statistical results are shown in Table I.

2.6 Discussion

Size matters for both natural and parasitoid-induced egg abortion in *H. halys*. Large stink bug eggs, unparasitized or parasitized by *T. podisi*, were more likely to abort than small eggs. However, contrary to our hypothesis, small wasps induce higher levels of egg abortion than large wasps, especially when ovipositing in large host eggs.

A general pattern for ovigenous animals is that large females produce bigger eggs than small females, and larger individuals hatching from bigger eggs have a better fitness [insects (Fox, 1994; Harvey et *al.*, 1994), copepods (Guisande & Harris, 1995), amphibians (Semlitsch & Gibbons, 1990), fishes (Hutchings, 1991; Marteinsdottir & Steinarsson, 1998), turtles (Gutzke & Packard, 1985) and birds (O'Connor, 1979, Moss et *al.*, 1981; Davis, 2008)]. However, for insects, several studies did not report a relationship between egg size and offspring performance, leading Fox and Mousseau (1996) to suggest that producing bigger eggs would only be advantageous when immatures are facing adverse conditions (e.g. desiccation, starvation, larval competition). The relationship between egg size and offspring's hatching success is by far less studied, with intrinsic egg mortality being either positively (Thomas, 1983; Wiklund & Persson, 1983; Eeva & Lehikoinen, 1995) or not related (Moss et *al.*, 1981; Bancroft, 1984; Karlsson & Wiklund, 1984; Pepin, 1991; Marteinsdottir & Steinarsson, 1998) to egg size. In *H. halys*, abortion of unparasitized eggs increased from 6.54% in small eggs (<1200 µg) to 23.18% in large eggs (>1400 µg).

Why large *H. halys* eggs were more likely to abort than small eggs? During their embryonic development insects pass through a sequence of maturation phases, from mitotic division of the zygote nucleus to hatching (Gillott, 2005). Although a diversity of embryonic developmental patterns has been identified in insects, much remains to be learned about embryos that abort before hatching. Egg mortality can arise from abiotic factors (e.g. lethal temperature, desiccation, UV radiation) (Pak et *al.*, 1990; Speight et *al.*, 1999), cytoplasmic incompatibility (e.g. infection by intracellular bacteria) or natural enemies (Price & Price, 1975; Yeargan, 1979). In our laboratory experiments, all abiotic factors were controlled; stink bug eggs being exposed to the same temperature, relative humidity and radiation. Differences in abortion rate between small and large eggs may be related to gas exchanges across the egg membranes. On one hand, large viable eggs had a lower weight loss percentage than small eggs (LM, $P = 0.006$, $R^2 = 0.131$), suggesting that larger eggs are more resistant to desiccation. The lower surface-to-volume ratio of larger eggs would reduce evapotranspiration rate (Sota & Mogi, 1992; Romesburg, 2011). On the other hand, such a reduction of gas exchange in large eggs could limit oxygenation of the embryos and induce more abortion. As suggested by Fox and Mousseau (1996), larger eggs could promote the development and survival of the embryo under harsh climatic conditions. The relationship between egg size and abortion rate in unparasitized insect eggs remain to be explored in more details.

We expected large *T. podidi* females to provoke higher levels of egg abortion because their larger ovipositors would possibly induce more mechanical damages to the egg during oviposition or because females could inject larger quantity of venom or other regulating materials in the host egg. But *H. halys* eggs, especially large ones, were more likely to abort when attacked by small *T. podisi* females. Mechanisms underneath this pattern remain unclear. It might be that small wasps with a relatively short ovipositor have difficulties piercing the egg and that drilling significantly injured the shell and/or the chorion (Barrett & Brunner, 1990; Kaser et *al.*, 2018), possibly inducing desiccation (Lund, 1934; Calvin et *al.*, 1984; Pak et *al.*, 1990). Scanning and transmission electron microscopic studies of the surface of these parasitized egg could help identifying the amount of damage caused by the parasitoids ovipositors (Pak et *al.*, 1990).

Abram et *al.* (2016a) suggested that parasitoid-induced host egg abortion could be a desirable trait to exploit in both classical and inundative biological control. For one thing, the parasitoid inter-species and inter-individual variation for the capacity to abort host eggs could potentially be enhanced through artificial selection. Intriguingly, a paradigm arises from our study on the role of host-parasitoid size ratio in egg abortion. While larger parasitoids are considered to be more successful biocontrol agents than smaller individuals because they live longer, have greater fecundity, can disperse over longer distance and have better capacity to subdue their host (Waage & Ning, 1984; Godfray, 1994; Visser, 1994; Abram et *al.*, 2016b), our results show that smaller parasitoid females are more effective at inducing *H. halys* egg abortion than larger females. Aspects of non-reproductive parasitoid-induced mortality could thereby amend “the rule of the games” in biological control. Bigger natural enemies are not always the best.

In our study system, although *T. podisi* capable of reproducing successfully in the eggs of many indigenous stink bug species, *H. halys* eggs are three to four times bigger than North American *T. podisi* natural host eggs (e.g. *Podisus maculiventris Say (Hemiptera: Pentatomidae*)) (Arakawa et *al.*, 2004; Abram et *al.*, 2016b). The large size of *H. halys* eggs may be one of the contributing factors to failed parasitoid development, if the above mechanisms (e.g., venom injection) used by *T. podisi* to arrest host nymph development are “diluted” in a greater volume, or if parasitoid larvae (or their teratocytes) are unable to process the larger volume of resources (Abdel-latief & Hilker, 2008).

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Chapitre 3 :

Selective breeding in parasitoids: evaluation of the heritability of host egg abortion capacity in *Telenomus podisi*.

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3.1 Abstract

In biological control, parasitoids are commonly used to control populations of pest insects. Traits underlying their killing effectiveness can be selected and amplified over generations. Heritability measurement could help defining the proportion of a trait that is due to genetic variation. Selective breeding programs are also useful to amplify or homogenize a trait in a population. We ran two experiments to (i) evaluate to what proportion the abortion capacity of the parasitoid *Telenomus podisi* on the exotic stink bug *Halyomorpha halys* eggs is due to the additive genetic variation, and (ii) determine if the abortion capacity can be selected and amplified. We found a realized heritability of 0.182 using the 'Breeder's equation'. However, selection over four generations did not significantly increase abortion rates, although there was a trend. We suggest that it might be possible to amplify parasitoid-induced egg abortion by running the selection program over additional generations.

3.2 Résumé

En lutte biologique, les parasitoïdes sont couramment utilisés pour contrôler des populations d'insectes nuisibles. Leur capacité à tuer leur hôte peut être sélectionnée et amplifiée au fil des générations. La mesure de l'héritabilité peut aider à définir la proportion d'un trait due à la variation génétique. Les programmes de reproduction sélective sont également utiles pour amplifier ou homogénéiser un trait dans une population. Nous avons effectué deux expériences pour évaluer (i) dans quelle proportion la capacité du parasitoïde Tele*nomus podisi* à avorter les œufs de la punaise exotique *Halyomorpha halys* est due à la variation génétique additive, et (ii) si la capacité d'avortement peut être sélectionnée et amplifiée. Nous avons trouvé une héritabilité réalisée de 0,182 en utilisant l'équation Breeder's. Toutefois, le processus de sélection mené durant quatre générations n'a pas permis d'augmenter significativement la capacité d'avortement des œufs, bien qu'une tendance en ce sens se dessinait. Nous suggérons qu'il est possible d'amplifier l'avortement induit par le parasitoïde en poursuivant le processus de sélection sur un plus grand nombre de générations.

3.3 Introduction

Selective breeding (artificial selection) has been used for centuries to produce organisms with more desirable traits (Conner & Hartl, 2004). Selective breeding involves mating individuals with the ideal qualitative or quantitative trait values within a population, then selecting the offspring with improved values of the trait for future reproduction (Doolittle, 1987). Combined understanding of Mendelian inheritance (Fisher, 1919), quantitative genetics (Griffiths et *al.*, 2005; Saha et *al.*, 2013), and the genomics revolution (Gjedrem, 1997; Swallow & Garland, 2003; Conner & Hartl, 2004) have contributed to fine-tuning selective breeding techniques. It is now understood that successful selective breeding relies on two main conditions: (1) There is genetic variation in a population that is responsible for variation in the desirable traits, on which artificial selection can act; and (2) The traits are heritable; that is, they can be reliably transmitted from parents to offspring (Conner & Hartl, 2004; Sesardic, 2005). Heritability (h^2) is the most common way of measuring how well traits are transmitted with selective breeding, and indicates how much of the total genetic variation in a trait among individuals is due to additive genetic variation (Sesardic, 2005; Lommen et *al.*, 2017).

One of the underexploited applications of selective breeding is as a means of improving the traits of living organisms used to control pests (Boller, 1979; Hopper et *al.*, 1993). Arthropods, including insects, are commonly used as biological control agents, but selection programs have mostly focused on quantifying genetic variation as opposed to exploiting this variation by selectively breeding for specific traits (Wajnberg, 2004; Lommen et *al.*, 2017). However, some selective breeding experiments have been successful in improving a variety of traits (Hoy, 1976); these include extending parasitoid host range (Landaluce, 1950; Allen, 1954; Box, 1956); changing sex ratio (Wilkes, 1942; Simmonds, 1947), improving climatic tolerance (Wilkes, 1947; White et *al.*, 1970), and increasing pesticide resistance (Hoy, 1984; Roush & McKenzie, 1987). In selection programs, the focus has often been placed on traits that are associated with successful biological control agent reproduction (offspring production). However, some interactions between biological control agents and target pests do not add to the biological control agent's fitness, but still contribute to controlling target pests. This is exemplified in insect parasitoids, one of them most commonly used groups in biological control agents against insect pests (Godfray, 1994). While parasitoids, by definition, cause host death as a result of their offspring's development, many host attacks do not result in offspring development but still result in host death, and thus contribute to biological control ("non-reproductive mortality"). Recent studies (Abram et *al.*, 2016; Kaser et *al.*, 2018) have argued that this underappreciated component of biological control should receive more attention, especially in the context of inundative biological control where a second parasitoid generation is not necessarily mandatory for successful pest control. In some host-parasitoid associations where this type of mortality predominates, the ability of parasitoids to cause non-reproductive mortality could be a promising target for selective breeding.

An interesting case of non-reproductive mortality has been identified for a new host-parasitoid association comprising the brown marmorated stink bug *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) and the egg parasitoid wasp *Telenomus podisi* Ashmead

(Hymenoptera: Scelionidae). *Halyomorpha halys*, originally from Asia, was first positively identified in North America in 2003, Europe in 2008, and has since become a serious invasive pest of a wide range of agricultural crops (Rice et *al.*, 2014). In North America and Europe, native parasitoids (including *T. podisi* in North America) that normally reproduce in eggs of indigenous stink bug species, can rarely develop in *H. halys* eggs (Cornelius et *al.*, 2016), despite the fact that they accept them as hosts (i.e., lay their eggs inside them). Interestingly, in the case of *T. podisi*, up to 20% of *H. halys* offspring are unable to develop when parasitized (Abram et *al.*, 2014; Abram et *al.*, 2016). The level at which this "parasitoid-induced host egg abortion" is induced varies greatly among wasp individuals (Abram et *al.*, 2016). Some of this variation in abortion levels is due to aspects of wasp and host phenotype, namely size (Chapter 2 of this thesis), but it is unknown to what degree the ability to abort host eggs is underlain by heritable genetic variation. Understanding the factors involved in inducing *H. halys* egg abortion by *T. podisi* could lead to selective breeding of parasitoids causing a higher abortion rate, which could be useful in inundative biological control programs.

In this study, we tested the hypothesis that the level of *H. halys* egg abortion induction by *T. podisi* is influenced by traits that are heritable. Thus, we expected that offspring of individuals in the source population inducing the highest abortion levels of *H. halys* eggs would show greater levels of abortion than their parents. We also predicted that it would then be possible to further amplify the level of abortion induced by parasitoids through multiple generations of selection. Two experiments were conducted; one evaluating the realized heritability of the ability to induce host egg abortion in *T. podisi* (heritability measured by a response to selection; Lstibůrek et *al.*, 2018), and the other performing selective breeding through five generations of parasitoids.

3.4 Materials and methods

3.4.1 Insect colonies

Telenomus podisi parasitoids used in our experiments originated from stink bug eggs collected on the field in Canada and the USA (Table II) during the summer 2016 and maintained in separate laboratory colonies.

Telenomus podisi colonies were reared on *Podisus maculiventris* Say (Hemiptera: Pentatomidae) eggs at 24 ± 1°C, 50 ± 5% RH, 16L: 8D light cycle. Once glued to filter paper with white non-toxic glue (LePage White Glue), eggs were placed in *T. podisi* rearing cages for 48 h. For routine colony maintenance, the egg masses were then placed in Petri dishes (D: 9.0 cm, H: 1.4 cm) until parasitoid emergence, at which point emerged parasitoids were placed back into rearing cages with the rest of the colony.

Halyomorpha halys and *P. maculiventris* were collected between 2011 and 2012 in the vicinity of London and Hamilton (Ontario, Canada). Both stink bug species were reared on polyester ventilated cages (30 cm^3) (BugDorm, Taiwan). Eggs of both species were collected every 24 h to 72 h and incubated in Petri dishes (D: 9.0 cm, H: 1.4 cm). Once hatched, first-instar nymphs were moved to the 30 cm^3 polyester cage. All stages of *P. maculiventris* were fed with fresh green beans and yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae and pupae, while *H. halys* were fed with potted soybean plants, fresh carrots, green beans and pumpkin seeds. The quality of *H. halys* eggs produced by the colony was monitored over the course of the experiments by randomly taking masses from the colony, and they displayed a consistent natural abortion rate (Appendix Figure A2).

3.4.2 Realized heritability experiment

To measure the realized heritability of the capacity of *T. podisi* to induce *H. halys* egg abortion over one generation, we used the Breeder's equation formula ($h^2 = R / S$), where h^2 is relating to heritability, S is the selection differential (i.e. the mean difference of a trait between selected parents and the global population), R is the response to selection (i.e. the mean difference of a trait between the offspring of selected parents and the global population) (Lush, 1947). Each of these parameters was determined as follows:

For the selection differential (S), we generated a starting population using wasps originally collected in London (Ontario, Canada; Table II). We produced 49 females *T. podisi* by exposing *P. maculiventris* eggs to the *T. podisi* rearing colony (under the conditions described above). Once removed from the cages, each parasitized egg was placed individually into 1.5 mL microtubes, with drops of honey/water mixture, and incubated under the same rearing conditions until parasitoid emergence (~ 3 weeks).

To measure the trait of interest (ability to induce *H. halys* egg abortion) in the global population, we used <24 h *H. halys* masses of 28 to 24 eggs, randomly taken from the rearing cages. Each mass was separated into 1 pair of 12 eggs masses and held in place with plasticine on a 1 x 1 cm piece of paper, then placed in a Petri dish (D: 5.0 cm, H: 1.0 cm). One of the two masses of 12 *H. halys* eggs was exposed to the *T. podisi* females (which were naïve and virgin) for 5 hours, and the other half of the egg mass was used as a control, not being exposed to a parasitoid, (i.e., to evaluate natural level of abortion in the absence of parasitoids). After exposure, each wasp was removed from their Petri dish and placed in a 1.5 mL microtube. To obtain repeated measurements of wasp-specific abortion induction over several host egg masses (i.e., to dilute out effects that were due to individual egg mass characteristics) we repeated this procedure 3 times for each parasitoid (with 2 to 8 days intervals).

To calculate the abortion rate of each *H. halys* egg mass, we used the absence/presence of egg-bursters (black triangles) on the surface of the stink bug egg as an index of abortion (Endo & Numata, 2017). Egg-bursters appear only when the stink bug embryo develops successfully (5 days under our experimental conditions), but do not when the egg is aborted (Javahery, 1994). The corrected abortion rate induced by each wasp was calculated using Abbott's (1925) formula (subtracting the natural abortion rate measured in the control egg mass from the abortion rate of the exposed eggs to *T. podisi*) for each wasp exposure to *H. halys* eggs. Then we calculated the mean value of the 3 exposures to define each wasp's abortion rate.

To calculate the selection differential (S), we measured the corrected abortion rate for the global population (6.75 ± 8.1%; mean ± SD). Then we subtracted the corrected abortion rate of the selected parents among the global population (Tabashnik, 1992) (the 17 females with an abortion rate over 10%; 14.51 ± 4.37%). We obtained a selection differential (S) of 7.76

For the response to selection (R), in order to generate the offspring of the selected parents, one to two males haphazardly taken from the rearing cages were added to the microtubes containing the selected females for mating. Males were removed from the microtube after 48 h, and a filter paper strip with 10 *P. maculiventris* eggs glued onto it was added for 24h. Then, each parasitized egg was placed individually into 1.5 mL microtubes and incubated until offspring emergence. A total of 16 female *T. podisi* emerged from these eggs, each of which were exposed to *H. halys* eggs and the corrected abortion rate was calculated as described above.

To calculate the response to selection (R), first we measured the mean corrected abortion rate of the offspring of the selected parents (8.16 ±8.93%). Then we subtracted the global population corrected abortion rate (6.75 ±8.1%) for a response to selection (R) of 1.41 (Tabashnik, 1992).

3.4.3 Selective breeding experiment

We measured the abortion rate of these 11 females (F_0) by exposing them once to *H. halys* egg masses of 28 eggs (<24 hours since laying) randomly taken from the rearing cages under otherwise the same conditions as described for the heritability experiment.

As described above, the quality of *H. halys* eggs produced by the colony was monitored over the course of the experiments (Appendix Figure A2).

To produce the F_1 generation, we selected the five females showing the highest abortion rate, and added one to two males haphazardly taken from the rearing cages to the microtubes containing females for mating for 48 h. Next, a filter paper strip with 10 *P. maculiventris* eggs was placed in the microtubes with females (all under the same conditions as described for the heritability experiment) and incubated until the emergence of F_1 females.

In each subsequent generation (up to generation five, F_4) we randomly selected between four and 10 *T. podisi* (depending on availability) from the new generation of emerged females, and evaluated the abortion rate they caused on *H. halys* eggs under the same conditions as described above. We then selected four to five of the best females and mated them with one to two males produced by a different selected female from the previous generation, under the conditions described above.

To obtain more robust measurements of the abortion capacity of the final (F_4) generation, we selected 34 female *T. podisi* and evaluated their corrected abortion rate under the same conditions as described for the heritability experiment (exposing each female to egg masses of 12 eggs three times in sequence). In order to compare any potential increase in induced abortion rate caused by the selection process, the corrected abortion induced by a non-selected population of 29 *T. podisi* females was measured. Non-selected females, produced by the source colony from which the F_0 population originated (but had been undergoing non-directed reproduction over the course of the experiment), emerged at same time as the selected population, and were reared under the same conditions as for the initial F_0 population (from *P. maculiventris* eggs directly put in the rearing cages). Their corrected abortion rate induced on *H. halys* eggs was evaluated in the same way as for the selected population.

3.4.4 Statistical analyses

For the heritability experiment, in order to evaluate whether there was a difference in the level of induced *H. halys* egg abortion between the global population and the offspring of the selected parents, we performed a statistical comparison between these two groups. First, we visually confirmed normality of the data distribution with quantile-quantile and density plots. We confirmed that there was no significant difference of variance between treatments with two-tailed F-tests ($P = 0.5506$). Because the sample size was different between both treatments ($n = 53$ for the global population; $n = 16$ for the offspring of the selected parents), we performed a Welch Two Sample t-test.

For the selective breeding experiment, in order to evaluate if there was a difference in abortion between the F_4 selected population and the non-selected population, we performed a Welch Two Sample t-test, and tested the assumptions as described above (two-tailed F-tests, $P = 0.3047$, population of F_4 $n = 34$, $n = 29$ for the control).

All statistical analyses were performed using R software version 3.5.0 (R Core Team, 2018).

3.5 Results

Telenomus podisi did not exhibit a high capacity to transmit its capacity to abort *H. halys* eggs to its offspring, with a calculated realized heritability (h^2) of 0.182. In addition, no difference was observed in the induction of *H. halys* egg abortion between the initial source population (6.75 ±8.07%) and the offspring of selected parents (8.16 ±8.93%) (Welch t-test: t = 0.62, df = 63, P = 0.54) (Figure 6).

After four generations of selective breeding, there was no observed difference in abortion rate between the selected population (15.69 ±12.19%) and parasitoids from the source colony which were not subjected to selective breeding for abortion performance (12.07 ±10.21%) (Welch t-test: t = 1.26, df = 61, P = 0.21) (Figure 7).

Table II. Information about *Telenomus podisi* used for heritability and selection breeding experiments, with the estimated number of generations in the laboratory of each colony before starting the experiments, the species of eggs in which they were collected, the geographic origin of collection, and GPS coordinates.

Experiment	Laboratory generations before the experiment	Hatched from Pentatomidae eggs of	Location of origin	GPS coordinates
Heritability	18	*Podisus maculiventris* Say (13 egg masses)	London, ON Canada	N 43.028610 W 81.213339
Selective Breeding	15	*Euschistus servus* Say (3 egg masses)	Spartanburg, SC USA	N 35.428855 W 82.565024

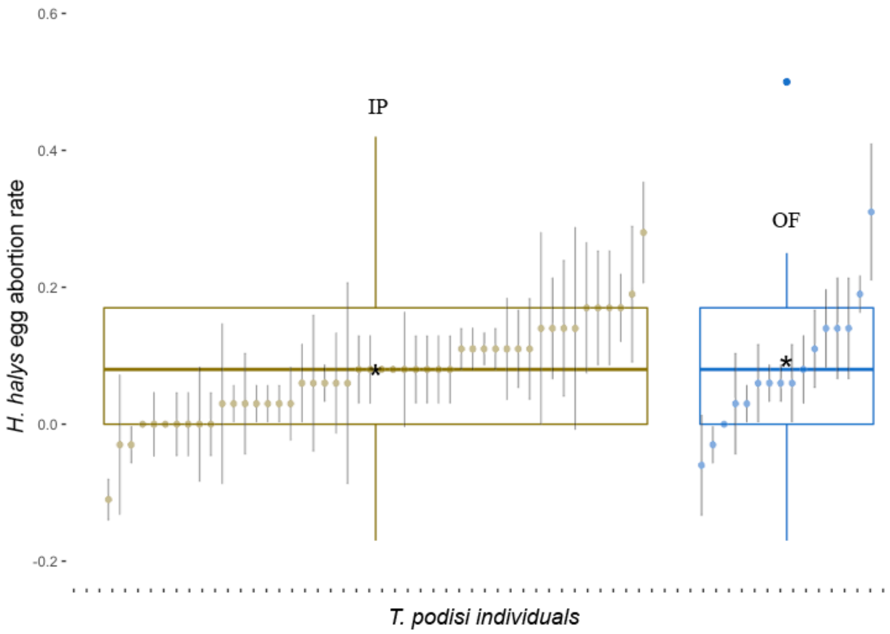

Figure 6. Abortion rate of *Halyomorpha halys* eggs induced by *Telenomus podisi* from the initial source population of parasitoids (IP, brown plot) and the best individuals of the population offspring (OF, blue plot). Horizontal line in each box show median, boxes contain the 25th–75th percentiles, whiskers show the upper and lower deciles, starts indicate means, and the darker blue point is an outlier. Dots represent each was abortion rate of each group; error bars show SD.

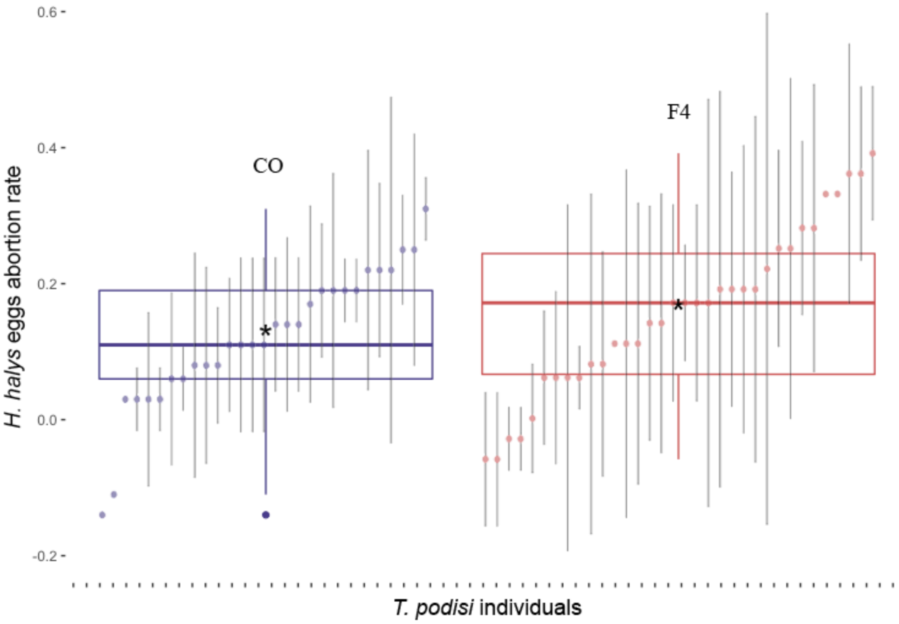

Figure 7. Abortion rate of *Halyomorpha halys* eggs induced by *Telenomus podisi* from the laboratory source population with no intentional selection (CO, purple plot) and a population after four generations of selection for increased abortion capacity (F4, red plot). Horizontal line in each box show median, boxes contain the 25th–75th percentiles, whiskers show the upper and lower deciles, starts indicate means, and the darker blue point is an outlier. Dots represent each was abortion rate of each group; error bars show SD.

3.6 Discussion

To our knowledge, our study is the first attempt to understand the heritability of traits linked with non-reproductive mortality in a parasitoid, and to attempt selective breeding to increase levels of non-reproductive host mortality (parasitoid-induced host egg abortion). Overall, however, we found weak support for our hypothesis that the capacity to induce abortion of *H. halys* eggs is heritable in *T. podisi*. While a relatively low realized heritability was calculated after one generation of selection, this did not translate to a statistically significant increase in abortion rate induced by the offspring of the best individuals. Likewise, there was no evidence that five generations of artificial selection improved abortion capacity.

Our heritability experiment found heritability ($h^2=0.182$) over one generation, but did not result in a statistically significant improvement in the selected parasitoid F_1 population relative to the global F_0 population. While at first glance this value is quite low, it is within the same range as other studies on insects testing the heritability of physiological (Collins, et *al.*, 1984; Holloway, 1986; Tanaka & Noppun, 1989; Tabashnik, 1992; Hawthorne, 1998) and behavioural (Caldwell & Hegmann, 1969; Duhrkopf & Young, 1979; Collins et *al.*, 1984) traits in insects. For example, Hawthorne (1997) evaluated the survival of leaf miner fly *Liriomyza trifolii* (Burgess) on a resistant cultivar of *Chrysanthemum morifolium*. He reported a relatively low heritability (h^2 of 0.22) after five generations, but significantly different than for previous generations. This suggests that even if heritability is relatively low when measured over a single generation, it may still be amplifiable though selection over a greater number of generations. However, in our experiment, the fact that the targeted trait of abortion capacity did not appear to be heritable at a level that resulted in significant improvement in the target trait, at least under our experimental conditions, may imply that host characteristics or environmental effects may predominate over heritable parasitoid traits in determining host egg abortion levels.

Our selection program for improved abortion capacity of *T. podisi* on *H. halys* eggs over five generations did not result in a statistically significant improvement in the selected parasitoid population relative to the non-selected population. However, there was a slight tendency towards increased abortion rates, possibly implying that, with more generations, a significant difference could become evident. Allen (1954) experienced a similar situation when selecting the parasitoid *Horogenes molestae* (Uchida), which naturally reproduces on the Oriental fruit moth *Grapholitha molesta* (Busck), to accept the potato tuber moth *Gnorimoschema operculella* (Zeller) as new host. Parasitism rate of the novel host was only 1.3% after three generations; however by manipulating host diet and use of chemical attractants over the course of subsequent generations, parasitism rate of the new host was increased to 7.1% after seven generations and after 28 more generations, they found evidences that the parasitoid would accept the novel host without attractants. In another parasitoid/moth system, Weseloh (1986) parasitism was enhanced on instar III host larva after ten generations, and instar IV after seventeen generations.

There were some important limitations to our study. First, we conducted heritability measurements and artificial selection on populations of parasitoids originating from a small number of collected individuals reared in the laboratory for several generations, likely resulting in inbred lines with low levels of genetic variation, possibly limiting the range of heritable genetic variation in traits that contribute to increasing host egg abortion by parasitoids. Second, due to difficulties with parasitoid rearing, we did not replicate our selection lines and did not have a rigorous control consisting of replicated control lines that were subjected to the same regime as selected lines but with random selection of individuals for the next generation (McGonigle et *al.*, 2017). As a result, we cannot exclude the possibility that genetic drift in either or both of our treatments may have contributed to the results.

Although our study did not demonstrate a high potential for selective breeding for abortion induction capacity of *T. podisi* on *H. halys* eggs, we believe that our results are an important first step for improving parasitoid traits related to non-reproductive mortality for use in biological control. The lack of a strong trend towards higher abortion rates with selection and its limited heritability extend upon our previous work (Chapter 2), which demonstrated that factors weakly linked to heritably genetic variation in the parasitoid – the size of *T. podisi* and weight if the *H. halys* eggs – have a comparatively large influence on egg abortion. Based on these results, we suggest that selective breeding programs that involve the interactions between the phenotypes of two organisms (e.g., host-parasitoid interactions) should be designed to take into account the phenotype of both the natural enemy and the host. Because we identified that the phenoptype of the parasitoid (size) shows different abortion rates depending on the phenotype (weight) of the host, host egg weight should then be controlled.

3.7 Acknowledgments

We thank Josée Doyon, Université de Montréal, for technical assistance and Dr. Tara Gariepy, London Research and Development Centre, Agriculture and Agri-Food Canada, for molecular identification and collection of the *T. podisi* strain from London. The Mountain Horticultural Crops Research and Extension Center of Mills River, North Carolina for the collection of the *T. podisi* strain from South Carolina. This study was supported by Québec-Ontario cooperation for Agri-Food Research Program to J. Brodeur.

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3.9 Appendix

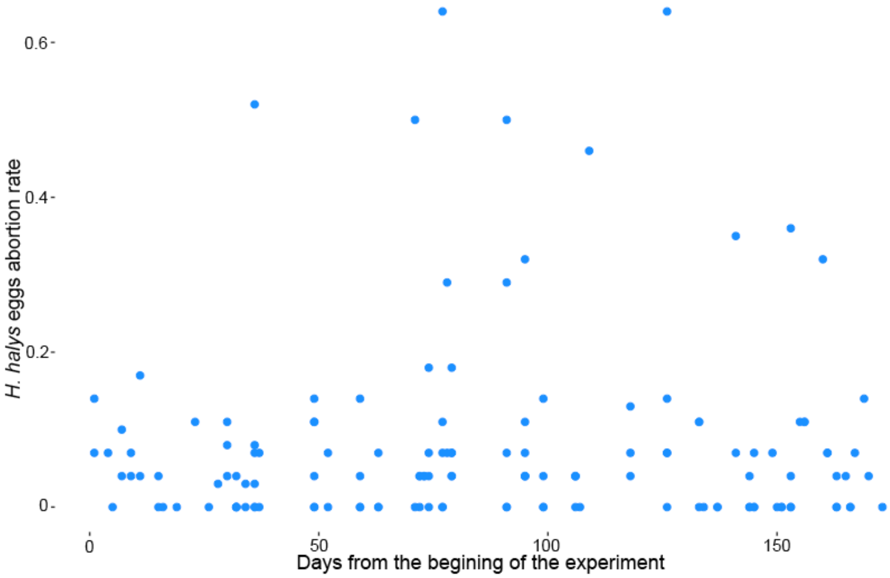

Figure A2. Natural abortion rate of *Halyomorpha halys* eggs between the beginning to the end of the selective breeding experiment. with no significant change in abortion levels over time (polynomial regression +/- 95% CI, p = 0.098, adjusted $R^2 = 0.007$).

Chapitre 4 :

Conclusion générale

4.1 Contributions

L'objectif principal de ce projet de maîtrise était d'identifier la nature et les mécanismes liés à l'avortement des œufs de la punaise marbrée *Halyomorpha halys* lorsque parasité par la guêpe parasitoïde *Telenomus podisi*. Nous avons pour cela mené une expérience permettant de déterminer si une composante phénotypique, ici la taille, chez l'hôte (l'œuf) et/ou le parasitoïde avait une part à jouer dans l'explication de la performance d'avortement. Au chapitre 2, nous démontrons que 1) les plus gros œufs de punaise sont plus sujet à avorter que ceux de plus petite taille, et ce, qu'ils soient parasités ou non par *T. podisi*. Par la même occasion, nous avons pu confirmer les observations de Abram et *al.* (2014; 2016a), à savoir que *T. podisi* a bien une incidence sur l'avortement. Contrairement à nos hypothèses, 2) ce sont les guêpes de petite taille qui induisent le plus d'avortement. C'est en augmentant le ratio de taille entre taille d'œuf / taille de guêpe que l'induction de l'avortement est le plus important.

Nous avons ensuite mené deux expériences pour évaluer les liens entre génétique de *T. podisi* et la capacité d'avortement. Au chapitre 3 nous montrons que la capacité d'avortement découle de traits qui sont à 18% codés par leur génétique. Puis, nous avons mené un programme de reproduction sélective sur 5 générations dans le but de produire une population de guêpes générant un taux d'avortement accru. Ce programme n'a néanmoins pas permis de produire une population induisant un taux d'avortement statistiquement amélioré par rapport à une population qui n'a pas été sélectionnée basé sur les performances des guêpes.

Un objectif secondaire était de comprendre la raison qui explique la variation d'induction de l'avortement. Au chapitre 2 nous avons trouvé une des composantes qui peut expliquer la variation d'avortement entre les guêpes comme l'a identifié Abram et *al.* (2016a). En effet, la taille de *T. podisi* est lié à sa capacité d'avortement, et une guêpe plus petite induira un avortement plus important. Une population de guêpe de même taille devrait alors avoir une variation de taux d'avortement moins importante qu'une population de guêpe de différentes tailles. En plus du lien avec sa taille, si une guêpe verra le taux d'avortement qu'elle induit varier en fonction de la taille des œufs qu'elle parasite. Si la biologie n'est pas aussi simple que ça à démystifier, dans les grandes lignes ont pourrait suggérer que la variation entre les guêpes dépend de leur taille, et que la variation d'avortement d'une même guêpe pourrait dépendre de la taille des œufs qu'elle parasite.

Au-delà des questions pour lequel le projet est né, de nombreuses petites confirmations et découvertes ont été faites. La plus importante était celle montrant qu'un 1) œuf de plus grosse taille a plus tendance à avorter qu'un œuf de petite taille (Chapitre 2). C'est une situation assez unique puisque la littérature fait état inverse. Nous montrons aussi que 2) les œufs de punaise perdent du poids au cours de leur développement, et que cette perte est constante, mais différente entre un œuf viable et un œuf avorté (Chapitre 2). Ainsi, il est possible de déterminer l'état d'un œuf de *H. halys* en évaluant la progression de sa perte de poids (Figure 2). Nous montrons d'autres éléments mineurs, mais qui peuvent servir à d'autres fins comme 3) le poids des œufs dans une masse d'œufs de *H. halys* varie grandement, et il varie aussi entre les masses (Figure 1). 4) Que la taille de *T. podisi* est proportionnelle au poids de l'œuf dans lequel la guêpe se développe (Figure 3). Abram et *al.* (2016b) avaient fait la même démonstration, mais avec le volume de l'œuf. 5) L'ordre d'attaque de *T. podisi* ne dépend pas du poids (taille) des œufs de H. halys (Chapitre 2). 6) L'ordre dans lequel les œufs sont attaqués par *T. podisi* n'affecte pas la probabilité d'avorter (Chapitre 2). 7) La taille d'une guêpe n'influence pas le nombre d'œufs qu'elle parasite (Chapitre 2).

Si le chapitre 2 est pour nous un grand succès avec de nombreuses découvertes, le chapitre 3 ouvre de belles perspectives. Nous en parlons dans la section suivante.

4.2 Limitations

La reproduction sélective est un processus long et exigeant qui, parfois, pour des raisons de mortalité des organismes évalués, ne peut être mené à son terme. Parfois, ce sont les résultats qui ne sont pas significatifs alors qu'une tendance est observée (Weseloh, 1986). Néanmoins, en produisant plus de générations et en adaptant son protocole, Allen (1954) a obtenu des résultats significatifs en matière de sélection après 3 ans et 39 générations. Nous suggérons qu'un programme reproduction sélective, particulièrement dans des systèmes complexes, nécessite beaucoup de temps et de générations; particulièrement lorsque l'héritabilité est faible. Avec plus de temps et de ressources, nous aurions aimé pouvoir étendre le programme de sélection à plus de générations, car une tendance était observé.

Nous aurions voulu avoir un « pool » génétique plus important. Nous avions fait l'acquisition de 6 souches de *T. podisi* issues de différentes régions d'Amérique du Nord que nous avions soumis à une évaluation génétique au « barcoding » moléculaire. Nous avions distingué 3 souches présentant des différences importantes que nous avons utilisées pour notre expérience de sélection. Malheureusement des problèmes de mortalité n'ont permis que de n'avoir s'une seule souche de *T. podisi* jusqu'à la fin du programme de sélection.

Pouvoir comparer notre population F_4 de guêpes sélectionnées à un « vrai » témoin aurait permis une plus grande précision dans nos comparaisons, ou en tout cas éviter de possibles biais causés par dérive génétique. Nous avions aussi des lignées de *T. podisi* qui ont suivi le même protocole que pour la sélection, sauf que les guêpes étaient choisies aléatoirement au lieu de l'être basé sur leurs performances d'avortement. Malheureusement, là encore, des problèmes de mortalités ne nous ont pas permis de voir ces lignées survivre jusqu'à la fin du programme.

Comme montré dans le chapitre 2, le phénotype des œufs et des guêpes influent sur l'avortement. Pour notre expérience de sélection, nous n'avons pas isolé ces incidences. Afin de s'assurer des guêpes en fonction de leur génétique et non de leur phénotype, il aurait été pertinent de standardiser les tailles, de la guêpe et des œufs. En utilisant les phénotypes les moins propices à avorter, il y a plus de chance de sélectionner pour le phénotype. De ce fait, utiliser des petits œufs avec des grosses guêpes permettrait au programme de reproduction sélective d'être plus efficace.

4.3 Perspectives

Comme nous le décrivons dans la section précédente, un programme de sélection reproductive pourrait être reconduit en prenant en compte nos remarques et découvertes. Ainsi il est en perspective de pouvoir, par la suite, produire des guêpes performantes de matière d'avortement.

Son utilisation en lutte biologique permettrait de contrôler la population, ou de limiter l'expansion de *H. halys* qui n'a toujours pas, en 2018, d'ennemi naturel identifié comme performant.

Notre découverte concernant la taille vis-à-vis de l'avortement permettrait dès maintenant d'agir face à *H. halys*. En effet, la production de guêpes de petite taille est quelque chose que l'on peut contrôler (utiliser des œufs d'hôte naturel de petite taille). L'utilisation de *T. podisi* de petite taille dans un programme de lutte biologique par inondation permettrait d'induire une certaine mortalité dans les œufs de *H. halys* de grande taille. Certaines questions pourraient être à élucider avant d'effectuer ce type de programme, telles que :

- Est-ce la taille de *H. halys* dépend de la taille de l'œuf dans lequel elle se développe ?
- Est-ce que la taille de la punaise influe sur sa charge en œufs ?
- Est-ce que la taille de la punaise influe sur la taille de ses œufs ?

Répondre à ces questions avant de lancer un programme visant les gros œufs de *H. halys* permettrait de s'assurer que l'élimination des petits œufs ne va pas diriger les populations de *H. halys à* une production de petits œufs, pour lequel *T. podisi* n'induirait plus d'avortement.

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