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Laurence Arvanitakis

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# THÈSE

Pour obtenir le grade de

Docteur

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**Spécialité : Écologie et Biologie des Populations**

**Présentée par ARVANITAKIS Laurence**

# INTERACTION ENTRE LA TEIGNE DU CHOU *PLUTELLA XYLOSTELLA* (L.) ET SES PRINCIPAUX PARASITOIDES EN CONDITIONS TROPICALES : APPROCHE ETHOLOGIQUE, ECOLOGIQUE ET EVOLUTIVE.

**Soutenue le 19 décembre 2013 devant le jury composé de**

| | |
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## Résumé

L'espèce *Plutella xylostella* (L.) (Lepidoptera : Plutellidae) défoliatrice des choux constitue surtout un problème dans les régions tropicales et subtropicales. La lutte chimique a rapidement montré ses limites du fait de l'apparition de résistance dans les populations. Des moyens de lutte alternatifs ont été mis en place, impliquant principalement des insectes parasitoïdes, parmi lesquels *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera : Eulophidae) et *Cotesia vestalis* (Haliday) (Hymenoptera : Braconidae) qui sont les plus couramment utilisés en raison de leur spécificité envers *P. xylostella*. Afin de contribuer à une meilleure connaissance du contrôle de la teigne en région tropicale, nous avons étudié les relations hôte-parasitoïde entre *P. xylostella* et *O. sokolowskii* d'une part, et *P. xylostella* et *C. vestalis* d'autre part, en conditions de laboratoire et sur le terrain au Sénégal et au Bénin. Au Sénégal, quatre espèces de parasitoïdes sont présentes sur les chenilles : *O. sokolowskii*, *Apanteles litae*, *C. vestalis* et *Brachymeria citrae*. Au Bénin, seule l'espèce *C. vestalis* est présente. Au Sénégal comme au Bénin, les facteurs climatiques contribuent au développement de la teigne et les précipitations ne régulent pas les populations du ravageur. Dans ces deux pays, la teigne n'est pas contrôlée par ses ennemis naturels. La lutte biologique par conservation y est à prendre en considération et l'utilisation de plantes compagnes cultivées en association avec le chou peut être envisagée pour réduire les populations de la teigne. Les études en laboratoire ont montré qu'*Oomyzus sokolowskii* est un parasitoïde larvo-nymphal performant. Concernant *C. vestalis*, les femelles détectent et reconnaissent leur hôte grâce aux lipides cuticulaires émis par les chenilles. Des marqueurs moléculaires (isozymes et ISSR) ont confirmé une forte variabilité entre les populations de *P. xylostella* à l'échelle mondiale, les populations d'Australie et du Japon étant très différentes des autres et formant deux groupes distincts. La structuration des populations semble influencée par le type de climat : tropical et non tropical.

**Mots-clés** : *Plutella xylostella*, *Oomyzus sokolowskii*, *Cotesia vestalis*, interaction hôte-parasitoïde, agroécosystème tropical, Brassicacées, lutte biologique.

## Abstract

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the most destructive pest of Brassicaceae worldwide and poses particularly acute problems in tropical areas. Chemical control is impaired by multiple-insecticide resistance in this species. Alternative methods are based on biological control by parasitoids, such as *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) and *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae), which are commonly used due to their specificity towards DBM. To help to improve the biocontrol of the moth in the tropics, we studied host-parasitoid relationships between *P. xylostella* and these two parasitoids under both laboratory and field conditions in Senegal and Benin. In both countries, climatic conditions are favourable for the development of DBM and rainfall does not limit populations of this pest. In Senegal, four parasitoid species are present on DBM larvae: *O. sokolowskii*, *C. vestalis, Apanteles litae*, and *Brachymeria citrae*. In Benin, *C. vestalis* is largely dominant. In neither of these countries, the moth is sufficiently controlled by natural enemies. Conservation biological control might be combined with the use of companion plants in cabbage crops to reduce DBM populations. Laboratory studies have shown that *O. sokolowskii* is an efficient larval-pupal parasitoid. In *C. vestalis*, females detect and recognize their host using cuticular lipids produced by the caterpillar. Studies of molecular markers (isozymes and ISSR) have confirmed high variability among DBM populations around the world, those from Australia and Japan being distinct and very different from any other population. Population structure seems to be influenced by the type of climate (tropical vs. non-tropical).

**Keywords**: *Plutella xylostella*, *Oomyzus sokolowskii, Cotesia vestalis*, host-parasitoid interactions, tropical agroecosystems, Brassicaceae, biological control.

*Il est mon ami et mon maître,*

*Au-delà des apparences, il est profondément humain,*

*Au-delà de la passion, l'entomologie est sa vocation,*

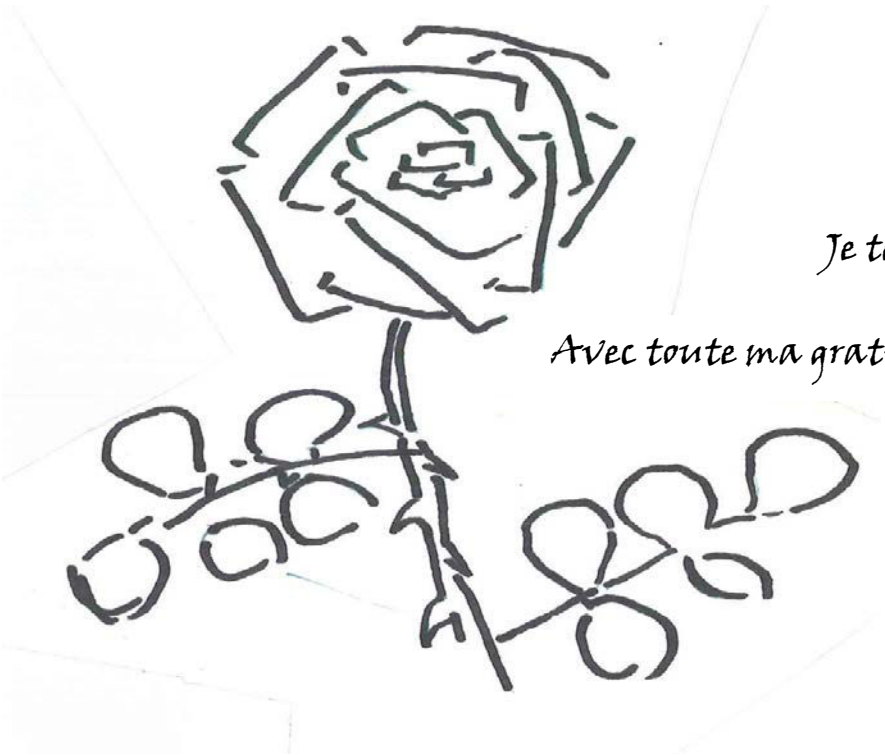
*Sincère, curieux, obstiné, battant et sensible,*

*Il cache ses talents d'artiste peintre et de photographe,*

*Il a souvent été seul contre tous,*

*Il m'a tout appris de mon métier,*

*Je ne lui serais jamais assez reconnaissante.*



*Dominique*

*Je te dédie ce manuscrit*

*Avec toute ma gratitude et mon amitié*

# *Ils ont aussi contribué à ce que je suis aujourd'hui…*

Cette icône appartenait à mon arrière grand-mère Parasquevi Arvanitakis. Je tiens à lui rendre hommage, ainsi qu'à tous mes aïeux. Depuis bien longtemps déjà, ils ne sont plus de ce monde, mais je sais, qu'ils seraient fiers de moi.

Cette icône représente la *Présentation du Christ au temple*. Si j'en fais une interprétation personnelle, ce manuscrit représentera le jour de ma soutenance, la présentation d'un travail de recherche de plusieurs années, à mes pairs.

Icône grecque du début du XX$^{e}$ siècle

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La cigale *Lyristes plebejus* (Scopoli)
Aquarelle 1808

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Feuille de *Ginkgo biloba* récoltée au jardin des plantes de Montpellier, 1984. Classe 2$^{nde}$, Lycée La Frondaie, Castelnau-le-lez

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*La connaissance n'est que provisoire et jamais absolue.*

*Socrate*

*Le commencement de toutes les sciences, c'est l'étonnement*

*de ce que les choses sont ce qu'elles sont.*

*Aristote*

*Il n'est pas d'un homme raisonnable de blâmer*

*par caprice l'étude des insectes,*

*ni de s'en dégoûter par la considération des peines qu'elle donne.*

*La nature ne renferme rien de bas.*

*Tout y est sublime, tout y est digne d'admiration.*

*Aristote*

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**A3** : Sow G., Arvanitakis L., Niassy S., Diarra K., Bordat D. 2013. Performance of the parasitoid *Oomyzus sokolowskii* (Hymenoptera: Eulophidae) on its host *Plutella xylostella* (Lepidoptera: Plutellidae) under laboratory conditions. *International Journal of Tropical Insect Science*, 33 (1): 38-45.

**A4** : Arvanitakis L., David J-F., Bordat D. Incomplete control of the diamondback moth, *Plutella xylostella*, by the parasitoid *Cotesia vestalis* in a cabbage field under tropical conditions. Soumis à *BioControl*.

**A5** : Roux O., Van Baaren J., Gers C., Arvanitakis L., Legal L. 2005. Antennal structure and oviposition behavior of the *Plutella xylostella* specialist parasitoid: *Cotesia plutellae*. *Microscopy Research and Technique*, 68: 36-44.

**A6** : Roux O., Gers C., Tene-Ghomsi J. N., Arvanitakis L., Bordat D., Legal L. 2007. Chemical characterization of contact semiochemicals for host-recognition and host-acceptance by the specialist parasitoid *Cotesia plutellae* (Kurdjumov). *Chemoecology*, 17: 13-18.

**A7** : Pichon A., Arvanitakis L., Roux O., Kirk A.A., Alauzet C., Bordat D., Legal L. 2006. Genetic differentiation among various populations of the diamondback moth, *Plutella xylostella* (Lepidoptera : Yponomeutidae). *Bulletin of Entomological Research*, 96: 137-144.

**A8** : Roux O., Gevrey M., Arvanitakis L., Gers C., Bordat D., Legal L. 2007. ISSR-PCR: Tool for discrimination and genetic structure analysis of *Plutella xylostella* populations native to different geographical areas. *Molecular phylogenetics and Evolution,* 43: 240-250.

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# INTRODUCTION GENERALE

La culture des Brassicacées est une des productions agricoles les plus importantes au monde. D'après les données FAO (FAOSTAT 2013), 37 millions d'hectares on été cultivés en 2011 avec une production annuelle globale de 152 millions de tonnes uniquement pour le chou, le chou-fleur et le colza. Elles sont essentiellement produites en Asie, en Europe et en Amérique du Nord (Tableau 1). Les deux tiers de cette production sont localisés en Asie, où ces légumes représentent parfois la principale source alimentaire des populations (Grzywacz et al. 2010). De nombreuses régions africaines et sud américaines en voie de développement dépendent principalement de cette culture.

Cependant, la production de Brassicacées est sérieusement affectée par un nombre important de ravageurs dont le principal est communément appelé « Teigne du chou », *Plutella xylostella* (Linné 1758) (Lepidoptera : Plutellidae). Les chenilles de ce micro-lépidoptère sont défoliatrices et peuvent causer jusqu'à 90% de perte de production (Talekar & Shelton 1993 ; Sarfraz et al. 2005). Cette espèce, originaire de la région Méditerranéenne (Hardy 1938), est devenue cosmopolite et se rencontre partout où sont présentes les Brassicacées cultivées et sauvages (Shelton 2004). Cette espèce envahissante est surtout un problème dans les régions tropicales et subtropicales où la culture de chou se conduit toute l'année. Les conditions climatiques très favorables à son développement, associées à un fort potentiel reproductif, permettent à *P. xylostella* d'avoir plus de 20 générations par an (Vickers et al. 2004).

La lutte engagée contre la teigne du chou fut d'abord axée sur l'utilisation de traitements chimiques à base d'insecticides de synthèses qui, très vite, ont montré leurs limites en se heurtant à l'étonnante capacité de résistance des populations de cette espèce à toutes les familles de produits existants (Cheng 1988), y compris les biopesticides à base de *Bacillus thuringiensis* (Berliner) (ou « *Bt* ») (Tabashnik et al. 1990 ; Sanchis et al. 1995). Cette course entre les agriculteurs et le ravageur conduit à l'augmentation incessante des doses de produits. L'estimation du coût annuel dans l'économie mondiale pour lutter contre la teigne est estimée à plus de 4 milliards de dollars (Zalucki et al. 2012). Pour faire face à ce problème, il a donc été nécessaire de trouver des méthodes alternatives permettant de contrôler le niveau des populations de *P. xylostella*, tout en respectant l'environnement et la santé des agriculteurs.

En région tempérée, la lutte intégrée (Integrated Pest Management ou IPM) a donné des résultats encourageant en combinant l'utilisation de plusieurs pratiques compatibles entre elles, telles que la rotation des cultures, les cultures intercalaires, la confusion ou le piégeage sexuel, la sélection variétale et la lutte biologique à l'aide d'antagonistes.

**Tableau 1**: Production de Brassicacées dans le monde en 2011 (FAOSTAT 2013)

**Production (en tonnes)**

| | Monde | Asie | Europe | Amérique du Nord |
|---|---|---|---|---|
| Chou | 68 840 531 | 51 859 189 | 12 267 797 | 1 154 102 |
| Chou-fleur et Brocoli | 20 876 817 | 17 000 754 | 2 355 945 | 359 253 |
| Colza | 62 454 482 | 22 544 274 | 22 306 360 | 14 863 410 |

**Surfaces cultivées (en hectares)**

| | Monde | Asie | Europe | Amérique du Nord |
|---|---|---|---|---|
| Chou | 2 373 818 | 1 741 982 | 428 962 | 34 077 |
| Chou-fleur et Brocoli | 1 209 106 | 895 725 | 137 042 | 17 232 |
| Colza | 33 645 342 | 14 641 931 | 8 809 291 | 7 893 920 |

Parmi ces stratégies, la lutte biologique est de plus en plus utilisée et elle constitue le moyen de lutte le plus efficace à grande échelle contre les populations de *P. xylostella* résistantes aux insecticides (Lim 1992). Plusieurs agents de lutte, comme les virus, les bactéries, les champignons, les prédateurs et les parasitoïdes, ont été recensés contre la teigne (Delvare 2004), mais les parasitoïdes sont de loin les plus utilisés, les plus étudiés et les plus efficaces.

Actuellement en Afrique, la teigne reste difficilement contrôlable, à l'exception de la région du Cap en Afrique du sud où l'on trouve une grande diversité de parasitoïdes et un taux de parasitisme proche de 90% (Smith & Villet 2004). Dans les autres régions, notamment en Afrique de l'Ouest, la faune parasitaire associée à *P. xylostella* est relativement pauvre. L'efficacité des parasitoïdes y est peu satisfaisante et les taux de parasitisme sont faibles (Lörh & Kfir 2004).

Parmi ces parasitoïdes, *Cotesia vestalis* (Haliday) (Hymenoptera : Braconidae) et *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera : Eulophidae) sont les plus couramment rencontrés en zones tropicales, notamment en raison de leur tolérance aux températures élevées, et aussi les plus utilisés en lutte biologique en raison de leur spécificité envers *P.*

*xylostella.* Pour autant, certaines de leurs introductions n'ont pas toujours été efficaces et les échecs ne trouvent pas toujours d'explications (Cock 1985 ; Chaves et al. 1993). Dans la pratique, le contrôle biologique est souvent aléatoire et les stratégies d'introductions sont basées sur des critères peu pertinents. Néanmoins depuis de nombreuses années, ces deux espèces sont présentes au Bénin et au Sénégal, avec des taux de parasitisme qui varient selon les saisons, entre 5% et plus de 80% (Bordat & Goudegnon 1997 ; Sall-Sy et al. 2004 ; Goudegnon et al. 2004). Cependant ces taux de parasitisme ne permettent pas pour autant un contrôle suffisant de la teigne.

C'est à partir de ce constat que nous avons construit la problématique de notre travail. Sans avoir la prétention de vouloir régler définitivement ce problème, nous voulons essayer de contribuer à améliorer le contrôle biologique de la teigne du chou en région tropicale.

En effet, il s'agit d'essayer de comprendre pourquoi ce ravageur est aussi difficile à contrôler dans ces conditions climatiques particulières et pourquoi ses parasitoïdes ne sont pas suffisamment efficaces. D'une manière générale, on a tendance à faire reposer entièrement le succès comme l'échec du contrôle biologique uniquement sur les caractéristiques biologiques du parasitoïde, sans se préoccuper de la part du ravageur dans l'interaction hôte-parasitoïde. Les hypothèses le plus souvent avancées mettent en cause des phénomènes de compétition interspécifique, de mal-adaptation du parasitoïde à son environnement ou encore l'usage d'insecticides par les agriculteurs. A ce jour, aucune réponse n'est satisfaisante et on ne s'était pas réellement penché sur ce problème, ce que nous avons essayé de faire ici.

Mieux comprendre le fonctionnement de l'interaction hôte-parasitoïde peut représenter à terme l'une des clés de la gestion raisonnée des introductions de parasitoïdes en lutte biologique classique ou par le maintien des espèces déjà établies (lutte biologique par conservation), et donc d'augmenter l'efficacité dans le contrôle du ravageur concerné.

Nous avons choisi d'étudier le modèle biologique *Plutella xylostella* - *Oomyzus sokolowskii* et *Cotesia vestalis.* Dans le contexte de cette relation hôte-parasitoïde, nous nous sommes intéressés d'une part à l'hôte et à ses deux parasitoïdes, et d'autre part à l'influence de l'environnement en condition tropicale sur l'interaction entre ces trois espèces.

Dans la mesure où ce travail traite essentiellement des relations hôte-parasitoïde, il nous a semblé important d'exposer d'abord les différentes caractéristiques qui régissent ce type de relation.

**Les insectes parasitoïdes**

Le « parasitoïdisme » (Wajnberg & Ris 2007) est un mode de vie à l'interface entre la prédation et le parasitisme. Comme les parasites, les insectes parasitoïdes dépendent aussi d'un hôte (le plus souvent un autre insecte) dont ils vont tirer les ressources nécessaires à leur développent (Godfray 1994). Cependant, seuls les stades pré-imaginaux nécessitent un tel mode de vie (Askew 1971). Les adultes mènent en général une vie libre pendant laquelle les femelles recherchent activement leurs hôtes pour y déposer leur progéniture. Les stades immatures tirent leur substance de ces hôtes et les tuent directement ou indirectement à l'issue de leur développement (Eggleton & Gaston 1990). Les parasitoïdes représenteraient entre 8 et 20 % des espèces d'insectes décrites à ce jour, la majorité appartenant soit à l'ordre des hyménoptères (environ 50 000 espèces décrites), soit à celui des diptères (environ 16 000 espèces connues) (Feener & Brown 1997). Des parasitoïdes ont été également signalés chez les coléoptères, les lépidoptères, les trichoptères, les neuroptères et les strepsiptères (Quicke 1997).

**Quelque caractéristiques écologiques des parasitoïdes**

Les parasitoïdes infestent principalement d'autres insectes. Cependant, certaines espèces sont capables de parasiter d'autres arthropodes mais aussi des plathelminthes, des annélides, des mollusques et même certains chordés (Feener & Brown 1997). Certains de ces hôtes sont parfois eux-mêmes des parasitoïdes et on parle alors d'hyperparasitisme. Le stade parasité est variable d'une espèce à l'autre, ce qui permet, lorsqu'il s'agit de parasitoïdes d'insectes, de définir globalement des parasitoïdes d'œufs, de larves, de nymphes, voire dans quelques cas d'adultes. Dans certains cas, le parasitisme peut avoir lieu au stade d'œuf, mais avec un développement du parasitoïde suffisamment lent ou différé pour s'achever beaucoup plus tardivement lorsque l'hôte a atteint un stade larvaire (parasitoïdes ovo-larvaires) ou le stade nymphal (parasitoïdes ovo-nymphals). Le nombre d'espèces susceptibles d'être infestées avec succès varie considérablement d'une espèce à l'autre. Par exemple, certains Tachinides sont hautement généralistes (ou polyphages) et peuvent parasiter plusieurs dizaines d'espèces hôtes dans des familles différentes (Stireman et al. 2006). De nombreuses espèces sont en revanche spécialisées sur une ou quelques espèces hôtes seulement. Selon les espèces, les stades immatures (œufs, larves) des parasitoïdes peuvent se développer soit à l'intérieur, soit à l'extérieur de leurs hôtes : on parle respectivement d'endoparasitoïdes ou d'ectoparasitoïdes. La possibilité de se développer ou non en présence d'un ou plusieurs congénères aux dépens d'un même hôte conduit à faire la distinction entre les parasitoïdes

solitaires ou grégaires. Chez certains parasitoïdes solitaires, les femelles ont une fonction discriminatoire qui leur permet de différencier les hôtes sains des hôtes déjà parasités par elle-même, par une autre femelle de leur espèce ou par celle d'une autre espèce**.** Il en résulte le développement d'une seule larve par hôte, sachant que la première pondue dévore ou élimine par des moyens chimiques les larves les plus jeunes (Wajnberg & Ris 2007). Chez les espèces grégaires, plusieurs adultes émergeront d'un seul hôte. Cela nécessite une évaluation par la femelle de la taille de l'hôte pour que les ressources qu'il représente puissent suffire au développement complet de tous les congénères (Wajnberg & Ris 2007). L'haplo-diploïdie est le système qui prédomine chez la plupart des hyménoptères parasitoïdes. Les femelles (diploïdes) sont issues d'ovocytes normalement fécondés tandis que les mâles fertiles proviennent d'ovocytes non fécondés (parthénogenèse arrhénotoque) et sont par conséquent haploïdes, avec un patrimoine génétique uniquement d'origine maternelle. Une conséquence de ce mode de reproduction est la possibilité, pour les femelles fécondées, de « choisir » la proportion des sexes dans leur descendance en fécondant ou non les œufs qu'elles pondent. La durée relative de l'interaction entre l'hôte et le parasitoïde est également importante et l'on distingue ainsi les espèces idiobiontes qui tuent et exploitent rapidement leurs hôtes, des espèces koïnobiontes qui permettent à leur hôte de continuer plus ou moins normalement leur développement avant de succomber sous l'effet du parasitisme (Askew & Shaw 1986). Chez l'adulte parasitoïde, la disponibilité des œufs varie également de façon très nette, certaines espèces disposant de la totalité de leurs œufs matures dès l'émergence de la femelle (espèces pro-ovogéniques) tandis que d'autres produisent des nouveaux œufs tout au long de leur vie (espèces syn-ovogéniques). En fait, la distinction est probablement beaucoup moins nette et il semble exister en pratique un continuum entre ces deux stratégies extrêmes (Jervis et al. 2001).

**Les étapes comportementales aboutissant au parasitisme**

Pour qu'un parasitoïde réussisse son infestation et son développement, il est communément admis que plusieurs étapes chronologiques doivent être franchies avec succès (Doutt 1959 ; Vinson 1976). Elles correspondent à deux grandes parties du cycle de vie de ces insectes. La première correspond à la perception, par une femelle, d'une série de stimuli qui vont lui permettre de réduire progressivement son aire de recherche pour aboutir à la découverte d'un hôte et à son acceptation en tant que site de ponte (on parle alors d'oviposition). Les étapes de cette première phase sont qualifiées de pré-ovipositionnelles et dépendent du comportement des femelles adultes (Vinson 1981). La seconde partie, qui

concerne les stades immatures se développant dans l'hôte, est qualifiée de post-ovipositionnelle et met en œuvre des mécanismes liés à la physiologie de l'association entre les deux partenaires (Vinson & Iwantsch 1980a, b).

Etant donné que nous nous sommes intéressés à l'une des étapes qui correspond à la reconnaissance de l'hôte par le parasitoïde (Cf. Chapitre III), nous décrirons uniquement la première phase (étapes pré-ovipositionnelles).

**Les étapes pré-ovipositionnelles**

C'est au cours de ces étapes qu'une femelle parasitoïde adulte partira à la recherche d'hôtes pour y déposer une descendance. Les mécanismes impliqués dans ces étapes pré-ovipositionnelles reposent sur les caractéristiques écologiques des niches occupées et les caractéristiques comportementales des deux partenaires. Ces mécanismes vont déterminer la capacité des femelles parasitoïdes à découvrir et à attaquer leurs hôtes et vont donc conditionner l'impact parasitaire sur la dynamique des populations hôtes (Wajnberg & Ris 2007).

- **Recherche de l'habitat de l'hôte**

Une femelle parasitoïde adulte récemment émergée doit, dans un premier temps, partir à la recherche d'habitats potentiellement colonisés par des hôtes. De nombreux travaux démontrent le rôle important joué par les caractéristiques visuelles, acoustiques et surtout olfactives de l'habitat des hôtes dans sa détection par les femelles parasitoïdes (Vinson 1976, 1981). Certaines plantes émettent, en cas d'attaque par des phytophages, des molécules caractéristiques, appelées synomones, qui peuvent être utilisées par des parasitoïdes pour trouver de façon précise un habitat infesté par leurs hôtes (Turlings et al. 1990).

- **Recherche de l'hôte**

Une fois un site potentiellement habitable trouvé, la femelle doit commencer à rechercher un hôte proprement dit à partir de stimuli parfois acoustiques ou visuels, mais généralement plutôt chimiques et olfactifs qui proviennent ici des hôtes eux-mêmes. Ces signaux sont qualifiés de kairomones (Wajnberg & Ris 2007). Il s'agit, par exemple, de phéromones sexuelles impliquées dans la recherche et la reconnaissance de partenaires pour la reproduction. Dans ce cas, la femelle parasitoïde agit comme un véritable espion en détournant à son avantage des signaux qui ne lui sont pas initialement destinés (Noldus 1989).

- **Acceptation de l'hôte**

Une fois l'hôte trouvé, la femelle doit encore s'assurer que celui-ci peut convenir au développement de sa descendance. Autant de questions auxquelles la femelle va devoir répondre grâce encore à des signaux physiques (taille, forme, couleur) ou chimiques provenant de l'hôte découvert (Wajnberg & Ris 2007). Ces signaux peuvent être situés à l'extérieur de l'hôte et sont perçus par le parasitoïde grâce à de nombreux récepteurs situés sur ses antennes qui sont richement innervées (Wajnberg & Ris 2007). Ils peuvent aussi se situer à l'intérieur de l'hôte et sont détectés grâce à des organes sensoriels situés sur l'ovipositeur (organe de ponte) lors de son insertion dans l'hôte (Wajnberg & Ris 2007). De puissantes interactions sélectives entre les deux partenaires ont probablement contribué à la riche diversité des stratégies adoptées par les femelles parasitoïdes.

Chez de nombreuses espèces, les informations recueillies par les femelles parasitoïdes permettent également de détecter des hôtes déjà parasités, soit par elles-mêmes précédemment, soit par une ou plusieurs autres femelles conspécifiques ou d'une autre espèce. La décision de pondre dans un hôte déjà parasité (on parle de superparasitisme) est généralement risquée pour un parasitoïde solitaire puisqu'elle entraîne une compétition à l'intérieur de l'hôte (van Alphen & Visser 1990 ; Plantegenest et al. 2004).

# Présentation des chapitres de la thèse

Le mémoire présenté s'articule en quatre grands chapitres. Tous les résultats obtenus sont présentés sous la forme d'articles scientifiques regroupés dans les trois derniers chapitres.

**Chapitre I : Présentation du modèle biologique étudié**

Il s'agit d'un chapitre introductif dans lequel est présenté un bilan des connaissances acquises sur les caractéristiques biologiques et écologiques des trois partenaires impliqués dans la relation hôte-parasitoïde étudiée, ainsi que les moyens mis en œuvre pour combattre le ravageur concerné.

**Chapitre II : Interaction entre *Plutella xylostella* et *Oomyzus sokolowskii***

Ce chapitre est consacré à l'interaction entre *P. xylostella* et le parasitoïde *O. sokolowskii.* Cette étude a été réalisée, dans un premier temps, en plein champ au Sénégal afin d'étudier l'effet des facteurs environnementaux sur les populations de la teigne et de son parasitoïde (article 1). Dans un second temps, les traits d'histoire de vie (article 2) ainsi que les performances d'*O. sokolowskii* (article 3) comme éventuel agent de lutte contre la teigne, ont été étudiés en conditions de laboratoire.

**Chapitre III** : **Interaction entre *Plutella xylostella* et *Cotesia vestalis***

Ce chapitre est consacré à l'interaction entre *P. xylostella* et le parasitoïde *C. vestalis*. Cette étude a été réalisée dans un premier temps en plein champ au Bénin afin d'essayer de comprendre pourquoi, malgré un fort taux de parasitisme, ce parasitoïde ne contrôle pas pour autant les populations de la teigne (article 4). Ensuite nous nous sommes intéressés au système de reconnaissance qui lie *C. vestalis* à *P. xylostella,* en conditions de laboratoire.

Cette étude porte d'abord sur le mode de détection et d'identification de l'hôte par le parasitoïde en recherchant chez *C. vestalis* quels sont les organes impliqués (article 5), puis nous avons tenté de déterminer la nature exacte du stimulus permettant cette identification et l'initiation de l'oviposition (article 6).

**Chapitre IV : Structuration génétique de *Plutella xylostella***

Dans ce dernier chapitre, il est question d'explorer la variabilité génétique au sein de *P. xylostella* à l'aide de deux marqueurs moléculaires (isoenzymes et ISSR), afin de différencier des populations d'hôtes d'origines géographiques différentes à l'échelle mondiale. Il s'agit également de voir si les populations sont structurées génétiquement et s'il existe une relation entre les distances géographiques et les distances génétiques. Les résultats de cette étude seront présentés dans les articles 7 et 8.

A l'issue des ces quatre chapitres, suivra ensuite une partie « discussion générale et perspectives », où nous ferons le point sur l'apport de nos travaux tant sur le plan théorique que sur le plan des applications.

# CHAPITRE I

# Présentation du modèle biologique étudié

# 1. Le ravageur : *Plutella xylostella* (L.)

## 1.1. Systématique

La teigne du chou (ou teigne des Brassicacées) *Plutella xylostella* (Linné 1758) est un lépidoptère appartenant à la superfamille des Yponomeutoidea et à la famille des Plutellidae. Par le passé, cette espèce a été appelée *Plutella maculipennis* (Curtis) avant d'acquérir son nom actuel (Moriuti 1986). C'est l'espèce la plus connue de son genre, à cause de son importance économique et aussi la seule à être cosmopolite (Kfir 1998).

Individu de collection monté sur paillette (×4)

## 1.2. Origine, répartition géographique et migration

L'espèce *P. xylostella* est originaire de la région Est du bassin méditerranéen (Hardy 1938) où l'on trouve aussi le plus grand nombre d'espèces de parasitoïdes et d'où sont originaires les principales Brassicacées cultivées (Tsunoda 1980). Cependant une origine en Asie mineure a été suggérée (Chu 1986), voire même en Afrique du Sud, où l'on a recensé de nombreuses espèces de Brassicacées sauvages et un important cortège de parasitoïdes et d'hyperparasitoïdes des populations de *P. xylostella,* dont certains sont endémiques et spécialistes de cette espèce (Kfir 1998).

Ce ravageur est cosmopolite et est l'une des espèces les plus répandues dans le monde. L'étendue de sa propagation est due essentiellement à son importante capacité de déplacement par migration passive et à l'extension des cultures de Brassicacées due à l'activité humaine. Zalucki & Furlong (2011) ont élaboré et validé un modèle bioclimatique pour *P. xylostella*, qui prévoit une distribution de base où elle persiste toute l'année, ainsi que les régions où elle peut être un ravageur saisonnier (Fig. 1).

C'est en effet un grand migrateur, capable de franchir plus de 3000 km à l'aide des vents (Chu 1986). Ceci explique qu'on la retrouve régulièrement au Canada ou au nord du Japon (Hokkaido), où elle ne peut survivre en hiver, mais où les vents du sud la ramènent chaque printemps (Honda et al. 1992). En Malaisie, la présence de ce ravageur est due à une introduction de variétés de choux originaires de Chine, d'Inde et d'Europe (Ooi 1986). En raison de ses grandes capacités d'adaptation à une large gamme de températures

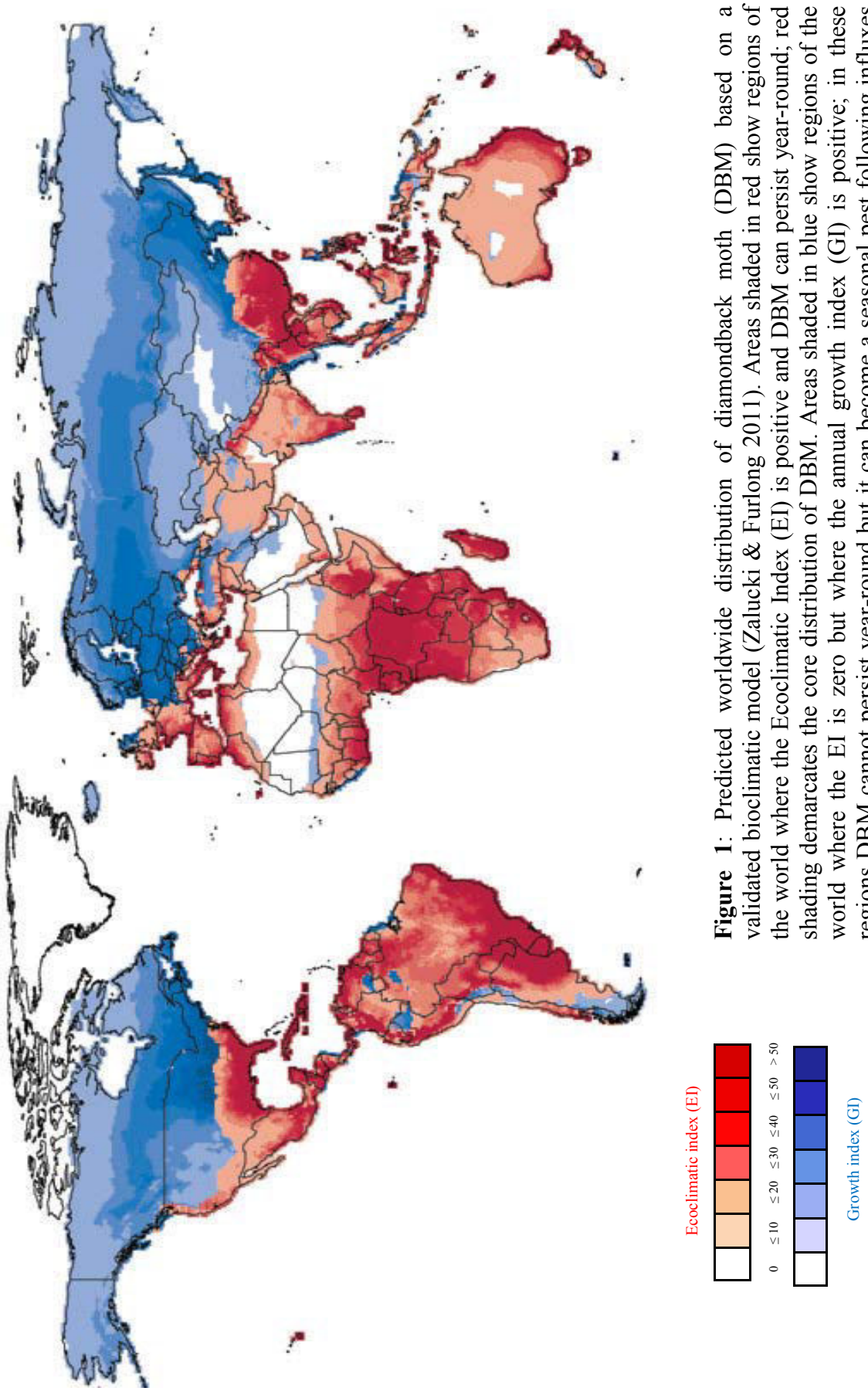


**Figure 1**: Predicted worldwide distribution of diamondback moth (DBM) based on a validated bioclimatic model (Zalucki & Furlong 2011). Areas shaded in red show regions of the world where the Ecoclimatic Index (EI) is positive and DBM can persist year-round; red shading demarcates the core distribution of DBM. Areas shaded in blue show regions of the world where the EI is zero but where the annual growth index (GI) is positive; in these regions DBM cannot persist year-round but it can become a seasonal pest following influxes of moths from elsewhere.

(développement de 5 à 35 °C et une survie à une exposition temporaire de - 9, - 14 et - 19 °C respectivement pour les adultes, les larves et les nymphes) (Honda 1992), *P. xylostella* est désormais présente partout où les Brassicacées sont susceptibles de pousser. Elle s'adapte même au climat subantarctique (Crafford & Chown 1987) et elle aurait réussi à atteindre l'île Marion (Chown & Avenant 1992). Dans certaines régions du monde, l'absence d'ennemis naturels susceptibles d'avoir un impact suffisant pour assurer le contrôle de ses populations lui permet de proliférer (Talekar & Shelton 1993). Seules les régions où la température ne dépasse pas 0 °C pendant plus de 60 jours ne sont pas infestées (Honda 1992).

### 1.3. Spectre de plantes-hôtes

*Plutella xylostella* est une espèce spécialiste oligophage : elle se développe exclusivement sur des plantes appartenant à la famille des Brassicacées. Elle est attirée par les composés soufrés appelés glucosinolates (Cf. Encadré I) caractéristiques de cette famille végétale. Elle vit essentiellement sur les choux et les autres Brassicacées cultivées (moutarde, colza, navet, etc.), mais on la trouve aussi sur les Brassicacées sauvages (bourse à pasteur, cardamine, ravenelle, etc.) qui peuvent servir de réservoir durant les périodes où les cultures ne sont pas disponibles (Muhamad et al. 1994).

Les Brassicacées sont distribuées dans de nombreuses régions du monde sous les climats tropicaux et tempérés selon les espèces. On les trouve principalement dans leur région d'origine en Méditerranée, ainsi que dans les régions sud-est et centrale de l'Asie (Koch & Kiefer 2006). Toutes ces plantes sont facilement cultivables quelque soient les différentes conditions climatiques. Selon les variétés, les températures de culture sont comprises entre 4 et 30° C. Certaines ont un cycle de vie annuel (le brocoli), d'autres sont bisannuelles (le chou) ou pérennes (*Arabidopsis lyrata*).

Anciennement nommées Crucifères, elles sont aujourd'hui divisées en 25 tribus et comprennent 3709 espèces appartenant à 338 genres (Warwick et al. 2003). Elles représentent une importante famille de plantes dicotylédones, essentiellement herbacées. C'est dans la tribu des *Brassiceae* que l'on trouve le plus grand nombre d'espèces à haute valeur économique. Ces espèces sont cultivées, entre autre, pour la production de légumes sous forme de feuilles et de fleurs (chou-fleur, chou pommé, chou de Bruxelles, brocoli, cresson, roquette, etc.) sous forme de racines (radis, navet, rutabaga), d'huiles à usage alimentaire et industriel (le colza), de condiments (moutarde, raifort, wasabi), de fourrage ou de plantes ornementales (Giroflée, Ibéris, etc.). Ce sont surtout les légumes du genre *Brassica* qui sont les plus consommés et essentiellement l'espèce *B. oleracea* (L.) (Tableau 2).

**Encadré I :** Les glucosinolates

Les glucosinolates sont des composés organiques dont la molécule de base comprend un élément dérivé du glucose et une chaîne soufrée et azotée. Ce sont des métabolites secondaires présents dans toutes les parties de la plante, que l'on trouve chez de nombreuses Brassicales et plus particulièrement chez les Brassicacées. De nombreux dérivés des glucosinolates sont impliqués dans la défense des plantes contre les herbivores. Ces glucosinolates sont toxiques pour la plupart des insectes. Cependant, certains d'entre eux, notamment *P. xylostella*, sont capables de désactiver ces molécules grâce à une glucosinolate sulfatase, rendant ainsi la plante comestible (Ratzka et al. 2002). Ils sont également responsable de goût âpre, amer ou piquant de certaines de ces plantes (moutarde, radis, choux…). Ces molécules sont synthétisées à partir d'acides aminés (méthionine, phénylalanine, tyrosine ou tryptophane). Les molécules actives (dérivées des glucosinolates) sont issues de l'action de la myrosinase qui, en présence d'eau, supprime le groupement glucose. La molécule restante est transformée en un thiocyanate, un isothiocyanate ou un nitrile qui sont les substances actives. Ces métabolites secondaires initialement prévus pour dissuader les ravageurs peuvent également être attractifs pour les mâles et les femelles de *P. xylostella*, être des stimulants de l'oviposition pour les femelles et des phagostimulants pour les chenilles (Spencer et al. 1999). Ils vont également participer aux défenses indirectes de ces plantes puisqu'ils sont également utilisés par les parasitoïdes spécialistes des phytophages inféodés aux Brassicacées pour localiser leurs hôtes.

**Tableau 2 :** Espèces de Brassicacées les plus consommées

| Genre | Espèce et variété | Nom commun |
|---|---|---|
| *Brassica* | *B. oleracea* var. *botrytis* | Chou-fleur |
| | *B. oleracea* var. *capitata* | Chou cabus ou pommé |
| | *B. oleracea* var. *gemmifera* | Chou de Bruxelles |
| | *B. oleracea* var. *gongyloides* | Chou rave |
| | *B. oleracea* var. *italica* | Brocoli |
| | *B. oleracea* var. *sabauda* | Chou de Milan |
| | *B. oleracea* var. *sabellica* | Chou frisé |
| | *B. rapa* var. *chinensis* | Chou chinois |
| | *B. rapa* var. *oleifera* | Navette |
| | *B. rapa* var. *rapa* | Navet |
| | *B. napus var. napus* | Colza |
| | *B. alba* | Moutarde blanche |
| | *B. juncea* | Moutarde chinoise |
| | *B. nigra* | Moutarde noire |
| *Raphanus* | *R. sativus* | Radis |
| | *R. niger* | Radis noir |
| *Armoracia* | *A. rusticana* | Raifort |
| *Nasturtium* | *N. officinalis* | Cresson de fontaine |
| *Eruca* | *E. sativa* | Roquette |
| *Wasabia* | *W. japonica* | Wasabi |

### 1.4. Morphologie, biologie et écologie

- **Les stades pré-imaginaux**

Les œufs sont jaunâtres, elliptiques et mesurent environ 500 µm. Ils sont pondus isolement ou en petits groupes, le plus souvent sur la face inférieure des feuilles (Fig. 2). La fécondité est élevée, la femelle pouvant pondre entre 150 et 300 œufs (Pichon 1999), avec cependant d'importantes variations dépendant de multiples facteurs (température, qualité de la nourriture de la femelle pendant les stades larvaires, densité des populations, etc.).

Les larves se développent en passant par quatre stades larvaires. Après éclosion, les larves néonates puis de stade L1 se dispersent. Elles sont endophylles et creusent des galeries ou « virgules » dans le parenchyme de la feuille (Fig. 2). Au stade L2, les larves sont de couleur jaune ivoire avec une capsule céphalique noire et mesurent de 2 à 3 mm de long (Fig. 2). Non mineuse, elles se nourrissent de l'épiderme des feuilles formant des « fenêtres » caractéristiques de l'espèce (Chua & Lim 1979). Dès ce stade, les chenilles se suspendent à un fil de soie au moindre danger. Au stade L3**,** elles sont de couleur jaune-brun, à pilosité plus visible. La capsule céphalique est brun clair à brun foncé. Au stade L4, les chenilles sont d'un vert vif et peuvent mesurer 8 mm de longueur. A ce stade, on observe un dimorphisme sexuel : une tâche blanche sur le cinquième segment abdominal révèle la présence de gonades visibles par transparence pour les chenilles qui donneront des mâles (Fig. 2) (Liu & Tabashnik 1997).

Les nymphes, d'une longueur de 5 à 7 mm, sont entourées d'un cocon fusiforme et étroit, constitué de mailles lâches (Fig. 2). Elles sont d'un vert pâle au début du stade nymphal, puis brunissent progressivement à l'approche de la mue imaginale.

- **L'adulte**

L'adulte est un papillon de taille inférieure à 10 mm, pour une envergure d'environ15 mm. Les ailes antérieures sont étroites, allongées et finement frangées. Au repos, il conserve les ailes plaquées contre le corps, en forme de toit. La frange claire le long des ailes antérieures forme une série de 2-3 losanges alignés dorsalement rappelant la forme d'un diamant, qui est à l'origine de son nom anglo-saxon de « Diamondback moth ». Ceci est plus visible chez le mâle où les couleurs sont contrastées (Fig. 3). C'est le seul dimorphisme sexuel visible. Pour différencier les sexes de façon certaine, une observation des valves génitales est nécessaire. Les femelles attirent les mâles à l'aide d'une phéromone sexuelle

(Chow et al. 1974 ; Maa 1986). L'accouplement (Fig. 3) se fait dos à dos dès l'émergence. La femelle ne s'accouple qu'une seule fois et le mâle de 1 à 3 fois (Talekar & Shelton 1993).

**Figure 2** : Les différents stades pré-imaginaux de *P. xylostella***.** En haut à gauche (× 2) et à droite (× 10), œufs disposés en petits groupes ou isolés autour des nervures sous la face inférieure de la feuille. Au centre, à gauche, des galeries creusées par des chenilles endophylles de stade L1, et à droite, des chenilles de stade L2 (× 7) caractérisées par une capsule céphalique noire et une chenille de stade L4. En bas à gauche, une chenille de stade L4 (× 6) avec une tâche blanche qui révèle l'emplacement des gonades (G), et à droite, une nymphe (× 6) dans son cocon.

**Figure 3** : Adultes de *P. xylostella*.
En haut, à gauche un mâle (× 5)
et à droite une femelle (× 5).
En bas, accouplement (× 3,5)

La température optimale de développement du ravageur se situe entre 17°C et 25°C (Atwal 1955), mais elle peut considérablement augmenter. Par exemple, des populations tropicales peuvent effectuer leur cycle larvaire à 35°C (Observation personnelle). A 25°C, le cycle complet peut durer 16 jours : trois jours pour l'incubation des œufs, neufs jours pour le développement larvaire et quatre jours pour la nymphose (Fig. 4). Cette rapidité de développement explique sa capacité à détruire rapidement les cultures attaquées sous les climats chauds, même si le nombre d'individus est faible au départ (cas de migrants amenés par le vent, par exemple). Le nombre de générations par an varie de trois dans les pays à climat tempéré, à plus de 20 dans les pays possédant un climat tropical. Aucune période de diapause n'a été mise en évidence chez *P. xylostella* (Harcourt & Cass 1966 ; Yamada & Umeya 1972).

### 1.5. Les moyens de lutte contre *Plutella xylostella*

C'est le plus important ravageur des cultures de Brassicacées dans le monde et en particulier dans les zones tropicales et subtropicales (Talekar & Shelton 1993 ; Furlong et al. 2013) (Fig. 5 et Fig. 6). L'estimation du coût annuel dans l'économie mondiale pour lutter contre la teigne est supérieure à 4 milliards de dollars. Ceci peut représenter de 30 à 50% des coûts de production, loin devant les coûts de fertilisation (Zalucki et al. 2012).

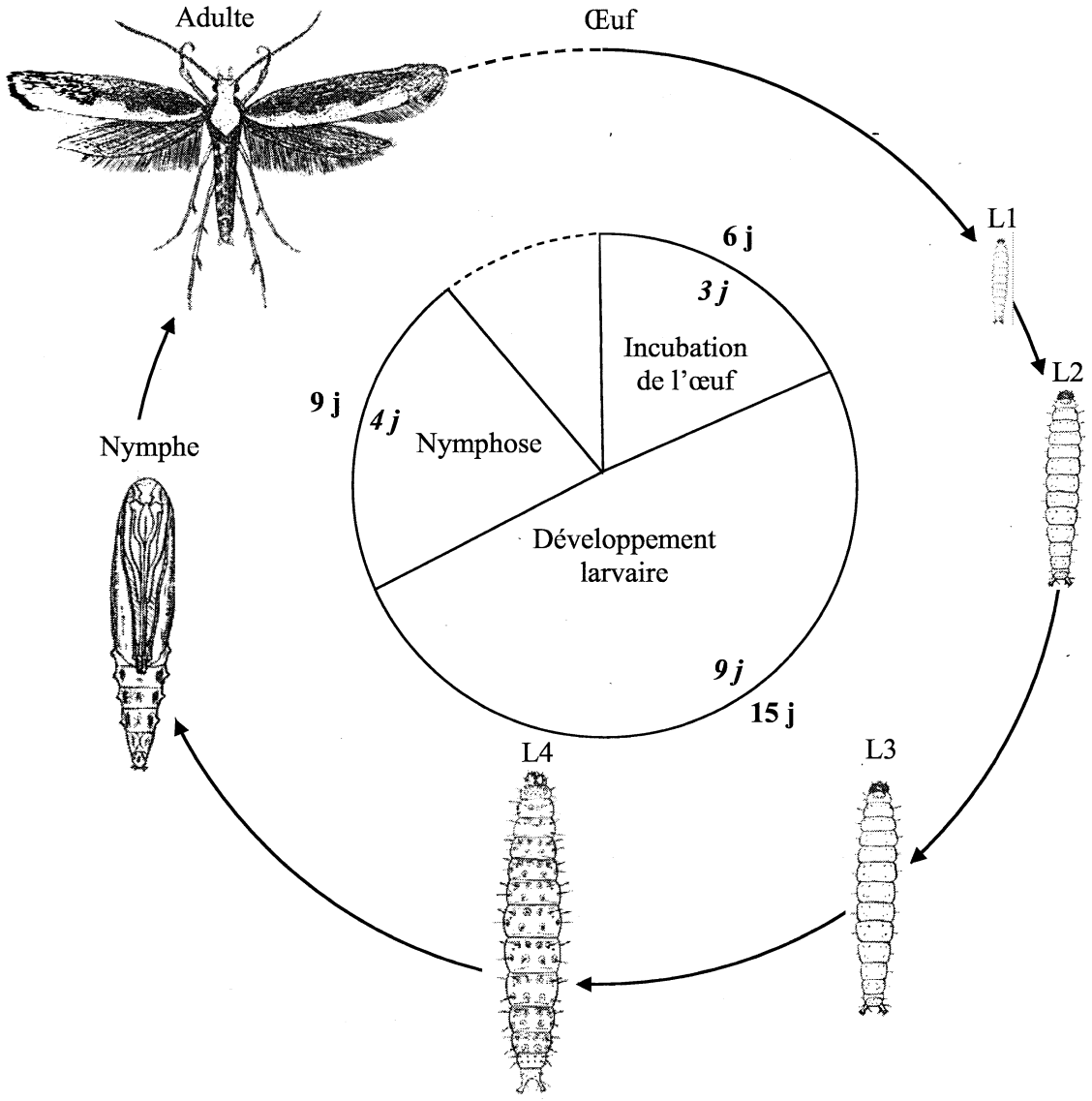


**Figure 4** : Cycle de développement de *P. xylostella* à 20° C (à l'extérieur du cercle) et à 25 °C (à l'intérieur du cercle) (Valeurs en jours, Salinas 1986) (Dessin : Carpenter 2005).

**Figure 5** : Culture de choux au Sénégal dont les feuilles sont entièrement perforées par les chenilles de *P. xylostella*

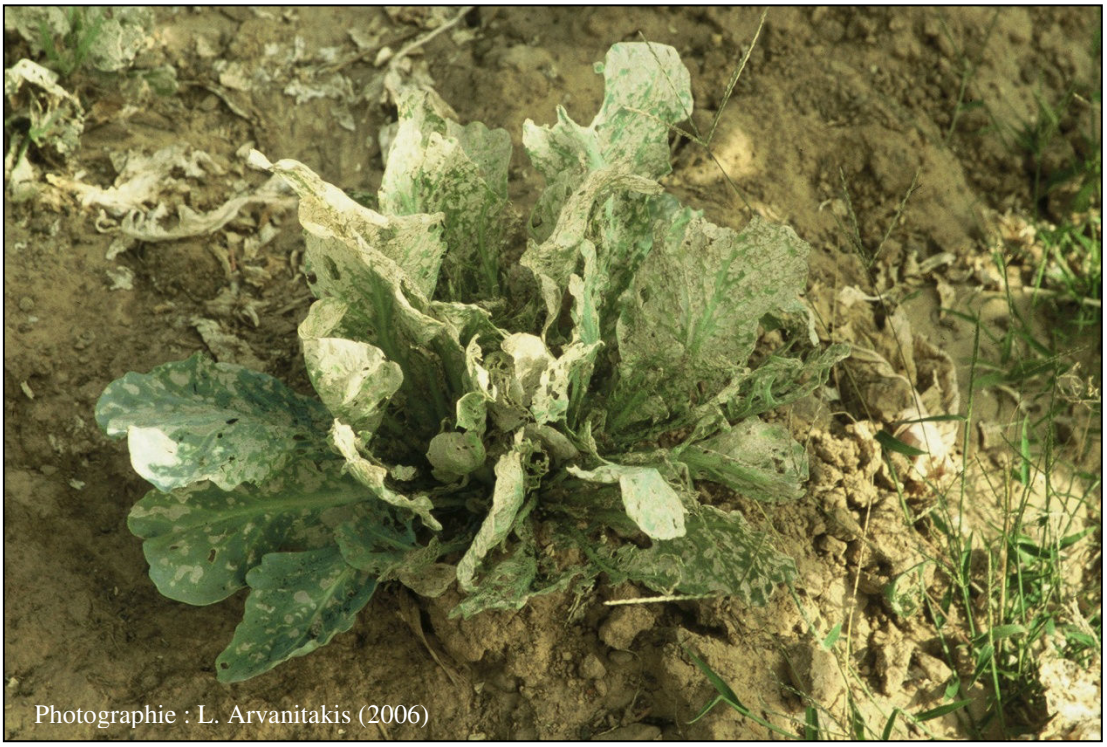


**Figure 6**: Chou entièrement détruit par les chenilles de *P. xylostella* au Bénin. Les feuilles sont réduites à de la dentelle

Dans le Sud-Est de l'Asie, des explosions démographiques de *P. xylostella* ont pu provoquer jusqu'à 90% de perte de récolte (Verkerk & Wright 1996). Dans les pays occidentaux, le seuil économique est atteint dès qu'il y a une seule chenille de stade L4 par chou car les trous créés par celle-ci suffisent à altérer l'aspect de la plante et à la rendre invendable (Shelton et al. 1983 ; Maltais et al. 1998). Dans certains pays où la culture du chou représente près de 90% des cultures maraîchères, la lutte contre ce ravageur est plus qu'un enjeu économique : c'est une condition de survie des traditions locales et cela concerne la protection de la santé des populations humaines. Encore aujourd'hui, l'utilisation de pesticides reste la méthode de lutte la plus répandue, bien que l'apparition de résistances à la plupart des matières actives rend la lutte peu efficace. Dans certains cas, des solutions alternatives sont conçues sous la forme de programmes de lutte intégrée ou Integrated Pest Management (IPM), basés sur la combinaison de plusieurs méthodes afin de gérer les résistances et de limiter la quantité de pesticides libérés dans l'environnement. Outre la lutte chimique, nous passerons en revue les principales méthodes de lutte dites alternatives basées sur différents critères : 1) la modification de la plante hôte (sélection variétale et transgénèse) ; 2) la modification des pratiques culturales (rotation, plantes pièges, plantes intercalaires, irrigation par aspersion) ; 3) le comportement du ravageur (phéromones sexuelles) ; 4) l'utilisation d'autres organismes vivants (lutte biologique).

### 1.5.1. La lutte chimique

L'usage d'insecticides reste la méthode la plus répandue. Dans la première moitié du XX$^{e}$ siècle, l'éventail des produits est resté limité : fumigants, composés minéraux (arsenic) et végétaux (nicotine, roténone, pyréthrines) (Huckett 1934). Peu efficaces et trop toxiques, ils sont supplantés à partir de 1945 par les insecticides de synthèse. Les organochlorés tels que le Dichloro-diphényl-trichlore éthane (DDT) ont prouvé leur efficacité et leur utilisation s'est alors largement répandue (Ankersmith 1953). Rapidement suivi du lindane, des organophosphorés, des carbamates (carbofuran et méthomyl), des pyrèthrinoïdes (cyperméthrine, deltaméthrine…), voire des cocktails de produits issus de plusieurs familles chimiques (Sun et al. 1986). Au cours des années 70, l'efficacité des formulations contenant les toxines de la bactérie *Bacillus thuringiensis* (*Bt*) (Berliner) a été démontrée et ces biopesticides qui ont une action sélective sont devenus très populaires.

Cependant la limite des insecticides est vite apparue en donnant naissance à des populations résistantes aux principales familles chimiques. Dès 1951, *P. xylostella* est résistant au DDT en Indonésie (Ankersmith 1953). En 1980, on recense des résistances envers

36 produits différents dans 14 pays (Miyata et al. 1986) et envers 51 produits en 1989 (Shelton et al. 1993b) dont les formulations de biopesticides à base de *Bt* (Syed 1992). La recherche de solutions alternatives a conduit à s'intéresser à des insecticides d'origine biologique, extraits de végétaux. Seuls les extraits de graines de « neem » issus de l'*Azadirachta indica* (Meliacea) (Goudegnon et al. 2000 ; Löhr & Kfir 2004) offrent la possibilité d'une utilisation à grand échelle, peu coûteuse et efficace. Néanmoins, ils présentent des effets phytotoxiques, modifiant la couleur des feuilles de choux (Schmutterer 1992 ; Leskovar & Boales 1996). Malgré tous les produits disponibles, des agriculteurs à Hawaii ou au Japon, ont été amenés à abandonner leurs cultures de Brassicacées, car la teigne était résistante à tous les produits autorisés aux doses sanitairement acceptables (Nakahara et al. 1986 ; Tanaka 1992).

### 1.5.2. La lutte biologique

- **Définition et généralités**

La lutte biologique est définie comme « l'utilisation d'organismes vivants (appelés auxiliaires) pour prévenir ou réduire les pertes ou dommages causés par des organismes nuisibles » (OILB-SROP 1973). Elle est également définie comme la suppression, le contrôle ou la régulation des populations de ravageurs à l'aide de prédateurs, de parasitoïdes, et de pathogènes (DeBach & Rossen 1991 ; Hawkins & Cornell 1999). Parmi les moyens de substitution aux insecticides chimiques, la lutte biologique est la plus employée et la seule qui soit efficace à grande échelle (Mills & Gutierrez 1999). Les opérations de lutte biologique réussies ont apporté des résultats significatifs pour l'agriculture et l'économie (Mills & Gutierrez 1999). Cependant des estimations concernant le contrôle d'arthropodes indiquent que seulement 34% des introductions ont abouti à l'installation de l'agent de lutte biologique et seulement 47% d'entre elles ont réduit les populations de nuisibles (Hall & Ehler 1979). Lynch et al. (2001) ont estimé à seulement 3 % le taux de succès des introductions. La lutte biologique ne se limite pas à la régulation de populations d'insectes nuisibles, mais peut également être utilisée contre les mauvaises herbes (Fraval & Silvy 1999) dont le contrôle connait de meilleurs taux d'installation (Roderick & Navajas 2003). L'échec de nombreuses introductions peut être en partie dû à la difficulté de prédiction des résultats potentiels des introductions. Les critères de recherche et de sélection des agents sont restés empiriques. Parmi ces critères, on peut citer celui qui prévoit qu'un agent de lutte biologique qui contrôle moins de 30% de la population de son hôte dans la zone de provenance n'aura qu'un succès limité, voire échouera dans la zone d'introduction (Hawkins & Cornell 1999). Les avantages

pratiques attribués à la lutte biologique par rapport à la lutte chimique sont : (1) les agents de lutte biologique n'ont pas d'effets phytotoxiques et ont peu ou pas d'effets nocifs pour la santé humaine et l'environnement ; (2) les applications d'agents sont plus simples et le travail est moins pénible ; (3) la lutte biologique est appréciée du public ce qui constitue un avantage pour la commercialisation des produits des cultures qui peuvent prétendre à des labels valorisables en terme de prix ; (4) elle peut représenter une alternative à la pérennité des traitements.

- **Différentes stratégies de lutte biologique**

**La lutte biologique classique** vise à introduire (on dit aussi acclimater) dans la culture à protéger un (ou plusieurs) auxiliaire(s) exotique(s) pour un établissement permanent et un contrôle durable des ravageurs. Cette stratégie fut la plus utilisées pendant plus d'un siècle avec des succès notables. Le ravageur est dans la plupart des cas lui-même exotique et pullule en l'absence de son cortège d'ennemis naturels, ou pour d'autres raisons écologiques (Colautti et al. 2004). Il s'agit alors d'importer un ennemi naturel sympatrique efficace (Eilenberg et al. 2001) et de recréer dans un nouveau contexte écologique l'équilibre démographique existant entre l'hôte et l'auxiliaire dans leur aire d'origine. D'un point de vue économique, cette stratégie par acclimatation est particulièrement intéressante puisque les coûts liés à son développement sont relativement limités par rapport à la durabilité du contrôle du ravageur et à la faible intervention humaine nécessaire.

**La lutte par lâchers inoculatifs** a pour objet d'établir une population d'auxiliaires suffisante pour contrôler le ravageur durant une période limitée dans le temps. L'utilisation de cette stratégie est particulièrement répandue pour les cultures sous serre puisque l'on estime que 5 % des 300 000 ha de serres dans le monde sont gérées grâce à la Protection Biologique Intégrée (PBI), notamment grâce à des lâchers inoculatifs de parasitoïdes ou d'autres auxiliaires (van Lenteren 2000). Le succès de cette stratégie sous serre s'explique en partie par la large gamme d'auxiliaires (plus d'une centaine d'espèces) disponibles et commercialisés dans le monde. Contrairement à la lutte biologique classique, la lutte biologique par lâchers inoculatifs nécessite un approvisionnement régulier et important d'auxiliaires. Cette étape supplémentaire de production de masse soulève plusieurs problèmes. En effet, les conditions de production et de stockage doivent préserver les qualités des individus, notamment leur fécondité, leur longévité, leur capacité de dispersion, etc. Par ailleurs, de nombreux auteurs soulignent l'importance d'évaluer l'impact des élevages de

masse au niveau génétique (van Lenteren 2003 ; Wajnberg 2004). En effet, les conditions imposées lors de la production de masse sont souvent très différentes de celles rencontrées dans l'agrosystème. Les pressions de sélection lors de la phase de production de masse peuvent à long terme altérer les performances des auxiliaires initialement choisis. De même, des phénomènes de dérive génétique ou de consanguinité importante pourraient également conduire à une réduction de la variabilité génétique avec une modification des caractéristiques initiales des souches utilisées.

**La lutte par lâchers inondatifs** vise à contrôler les populations de ravageurs par la seule activité de destruction réalisée par les auxiliaires lâchés en grand nombre dans l'agrosystème. Contrairement aux deux stratégies précédentes, l'action des auxiliaires sur la population du ravageur se veut donc beaucoup plus brutale et limitée dans le temps. L'effet des descendants des individus lâchés peut s'avérer également intéressant pour prolonger le contrôle du ravageur, mais il constitue ici rarement un objectif en soi.

**La lutte biologique par conservation** est de modifier l'agrosystème ou les pratiques culturales afin de protéger et de favoriser la présence d'ennemis naturels locaux, facilitant ainsi leur capacité à contrôler les populations d'insectes nuisibles. À l'heure actuelle, cette forme de lutte biologique est probablement la moins développée. Elle offre cependant des solutions pratiques efficaces (Landis et al. 2000). Trois tactiques non exclusives peuvent généralement être mises en œuvre dans cette stratégie. La première consiste à créer des abris ou des microclimats susceptibles de favoriser l'installation et la pérennisation des auxiliaires. La seconde stratégie consiste à mettre en place des sources de nourriture pour les auxiliaires adultes. Par exemple, l'influence de différentes espèces de fleurs sauvages sur le parasitoïde *Diadegma insulare* (Cresson) a été évaluée dans le cadre de la lutte biologique contre la teigne *Plutella xylostella* (Idris & Grafius 1995). Une dernière tactique cherche à maintenir des hôtes afin de maintenir la population d'auxiliaires sur la culture à protéger. D'un point de vue écologique, l'ensemble de ces pratiques a tendance à augmenter la biodiversité de l'agrosystème tant au niveau des espèces végétales qu'animales.

- **Les différents auxiliaires utilisés en lutte biologique**

La lutte biologique utilise des organismes vivants pour diminuer les niveaux de population d'autres organismes, généralement nuisibles. Les agents de la lutte biologique (appelés aussi auxiliaires) les plus souvent utilisés comprennent les microorganismes, les

nématodes, les prédateurs et les parasitoïdes (Lim 1992). On considère que la mortalité causée par les parasitoïdes est plus importante que celle attribuée aux prédateurs et aux microorganismes combinés. Des efforts importants sont menés en faveur de la lutte biologique contre *P. xylostella*, afin de ralentir l'apparition de résistances et de limiter la quantité de pesticides libérés dans l'environnement. Plusieurs agents de lutte biologique ont été étudiés, mais peu d'entre eux sont couramment utilisés contre *P. xylostella* (Fig. 7). Près de 135 espèces de parasitoïdes (Talekar & Shelton 1993 ; Delvare 2004), 25 prédateurs, au moins deux virus et deux bactéries, ainsi que sept champignons (Talekar 2004) ont été recensés comme ennemis potentiels des différents stades de développement de *P. xylostella.* Parmi eux, les parasitoïdes sont de loin les plus utilisés.

**Les virus** sont peu employés contre *P. xylostella* en raison d'un manque de virulence (Kolodny-Hirsch & Van Beek 1997 ; Greval et al. 1998). Toutefois, certains granulovirus semblent être très pathogènes, pouvant provoquer jusqu'à 90% de mortalité chez les larves de premier et second stade (Asayama 1986). Toutes les souches de *P. xylostella* n'ont pas la même sensibilité. Des épidémies apparaissent naturellement, mais elles restent limitées.

**Les bactéries :** la plus connue est *Bacillus thuringiensis* Berliner (Bacillales : Bacillaceae) ou *Bt.* Sa grande spécificité et les faibles risques qu'elle présente pour la santé humaine en ont fait un outil révolutionnaire dans la lutte contre les ravageurs de culture. Elle est utilisée sous forme de spores contenant des δ-endotoxines protéiques « Cry ». Plusieurs centaines de ces toxines sont connues, mais seules certaines classes sont actives contre les Lépidoptères (d'autres n'agissent que sur les Coléoptères ou les Diptères) (Chaufaux 1995 ; Monnerat 1995). Les variétés *Kurstaki* et *aïsawaï* (Eubactériales) sont utilisées pour le contrôle des populations de chenilles de *P. xylostella.* Elles tuent les insectes en se fixant sur la membrane des intestins et en créant des pores dans cette dernière (Gill et al. 1992). Quoique considéré au début de son utilisation comme une « arme absolue », *P. xylostella* est le premier insecte à avoir développé une résistance aux toxines de *Bt* en milieu naturel, avec des résistances croisées aux diverses classes de toxines (Tabashnik et al. 1997). Ces résistances sont la conséquence d'une utilisation intensive des traitements avec les formulations à base de *B. thuringiensis.* Les toxines de *Bt* présentent l'avantage d'être non toxiques vis-à-vis des parasitoïdes (Flexner et al. 1986 ; Lim 1992). En Malaisie, l'utilisation de ces toxines, combinée à celle des parasitoïdes est la pierre angulaire des programmes de lutte intégrée (Loke et al. 1992).

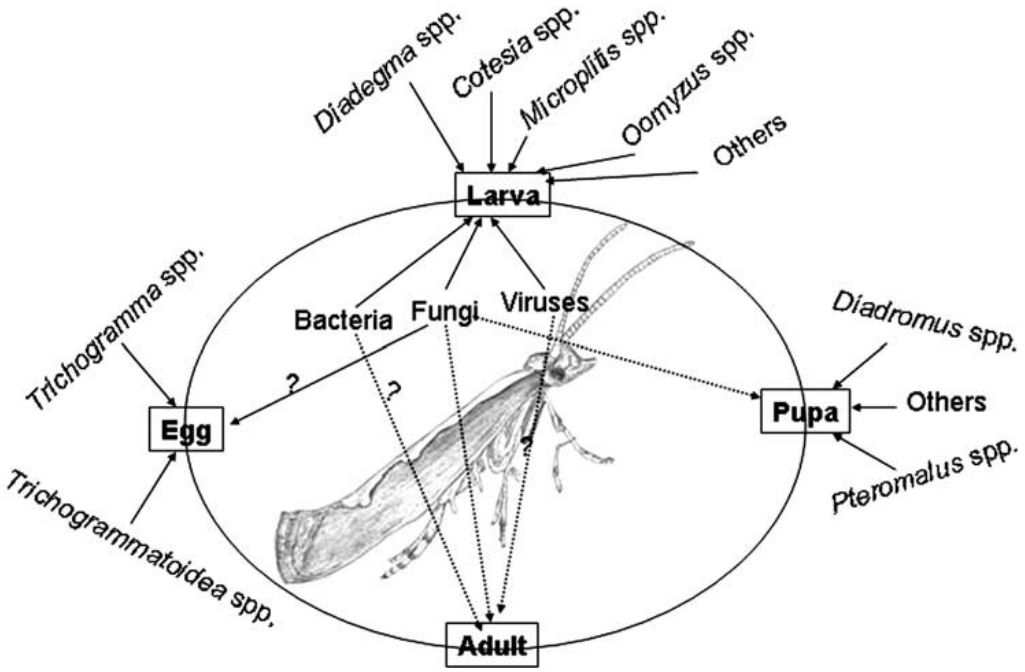


**Figure 7**: Principaux agents de lutte biologique utilisés contre les différents stades de *P. xylostella* (Sarfraz et al. 2005)

**Les champignons entomopathogènes** sont difficilement utilisables car ils ne prolifèrent qu'en milieu très humide et se développent plus facilement dans les pays tropicaux. Les deux champignons qui attaquent *P. xylostella* sont *Beauveria bassiana* (Balsano) (Hyphomycètes : Moniliaceae) et *Zoophtora radicans* (Brefeld) (Zygomycètes : Entomophthorales). Ils attaquent les larves et parfois les nymphes (Wilding 1986). Cependant, ces champignons sont peu spécialistes et peuvent aussi infecter les espèces de parasitoïdes et réduire de ce fait leur efficacité (Furlong & Pell 1996).

**Les microsporidies** appartenant au genre *Nosema* (Microspora : Nosematidae) peuvent infecter les larves de *P. xylostella* au niveau du système digestif et des tissus adipeux.

Cependant, les chenilles infectées peuvent nuire aux espèces de parasitoïdes vivant à leurs dépens en réduisant considérablement leur efficacité (Bordat et al. 1994 ; Gruarin 1998).

**Les nématodes entomopathogènes** sont peu utilisés car ils sont coûteux à produire et sensibles à la lumière et à la sécheresse (Baur et al. 1997 ; Lello et al. 1996). Au stade infectieux, ils portent des bactéries du genre *Xhenorhabdus* (Steinernematidae) ou *Photorhabdus* (Heterorhabitidae) qui infectent le ravageur en pénétrant dans l'hémocoele (Ratnasinghe & Hague 1995). Les isolats de *Steinernema carpocapsae* (Weiser) sont les plus efficaces contre les larves de *P. xylostella* (Baur et al. 1998).

**Les prédateurs** de *P. xylostella* peuvent être des Arachnides, des Insectes et des Acariens (Alam 1992 ; Lim 1992). Malheureusement, ils ne sont pas spécifiques à *P. xylostella* et leur taux de prédation est difficile à évaluer. D'après Goudegnon et al. (2004), les fourmis *Anomma nigricans* (Illiger) (Hym. : Formicidae) peuvent être des agents de contrôle de *P. xylostella* dans les zones périurbaines au Bénin.

**Les parasitoïdes** ont un mode de développement et des caractéristiques écologiques qui conduisent, au niveau individuel et dans la plupart des cas, à la mort de leur hôte. Au niveau populationnel, leurs caractéristiques contribuent également à la limitation des populations hôtes. A ce titre, les parasitoïdes se révèlent intéressants pour réduire l'impact des ravageurs des cultures. Ils sont responsables de nombreux succès en lutte biologique et occupent dans les écosystèmes naturels une place importante.

Les parasitoïdes restent de loin le groupe d'organismes le plus étudié, le plus utilisé et le plus efficace dans la lutte contre *P. xylostella* et souvent le moins coûteux (Waage & Greathead 1986). Parmi les espèces de parasitoïdes recensées sur *P. xylostella*, on retient principalement 6 parasites d'œufs, 38 de chenilles et 13 de nymphes (Lim 1986 ; Monnerat 1995 ; Delvare 2004). Ces espèces sont présentes un peu partout, mais individuellement chacune possède une aire de répartition plus restreinte que celle de la teigne. La plus grande diversité de parasitoïdes liés à *P. xylostella* se trouve dans la région méditerranéenne (Mustata 1992) mais également en Afrique du Sud (Kfir 1998). Dans son aire d'origine, l'espèce *P. xylostella* est relativement bien contrôlée par les populations locales de parasitoïdes (70 à 80%) (Löhr & Kfir 2004). Par contre, en zone tropicale, les successions de générations du ravageur sont loin d'être régulées par les parasitoïdes locaux souvent peu nombreux en termes d'espèces et peu efficaces (15 à 20% de parasitisme) (Löhr & Kfir 2004). Pour renforcer les

populations autochtones dans de nombreuses régions du monde, des introductions d'espèces exotiques ont été réalisées (Hardy 1938 ; Talekar & Shelton 1993). Bien que les résultats soient variables selon les régions, de nombreuses implantations ont réussi et la répartition actuelle de ces espèces n'a plus rien à voir avec leur aire d'origine. Certains échecs observés lors d'introductions sont liés à l'usage continu et abusif des traitements insecticides (Travis & Rick 2000). Ceci conduit à la destruction des parasitoïdes qui sont plus sensibles que leur hôte. L'utilisation d'insecticides reste donc incompatible avec la lutte biologique.

Les principaux parasitoïdes de *P. xylostella* appartiennent tous à l'ordre des Hyménoptères. On trouve des parasitoïdes d'œufs qui appartiennent tous à la famille des Trichogrammatidae. Ils ont fait l'objet d'études mais ne sont pas utilisés en lutte biologique contre *P. xylostella* (Klemm et al. 1992 ; Pak 1992). Ils contribuent faiblement au contrôle naturel et requièrent de fréquents lâchers de masse (Talekar & Shelton 1993). Par contre, les espèces qui s'attaquent aux stades larvaires sont les plus importantes et les plus efficaces et semblent donc offrir le meilleur potentiel de contrôle (Lim 1986). Elles appartiennent aux familles des Braconidae, des Ichneumonidae et des Eulophidae. Plusieurs genres sont particulièrement représentés : *Cotesia* et *Apanteles* (Braconidae), *Diadegma* (Ichneumonidae) et *Oomyzus* (Eulophidae). L'espèce *Diadromus collaris* (Ichneumonidae) est une espèce qui parasite les nymphes de *P. xylostella* (Tableau 3).

**Tableau 3 :** Principales espèces d'hyménoptères parasitoïdes de *P. xylostella*

| Stade parasité | Famille | Espèce | Caractéristiques |
|---|---|---|---|
| Oeuf | Trichogrammatidae | *Trichogramma* spp. | Généralistes, solitaires |
| Chenille | Braconidae | *Cotesia vestalis* (Haliday) | Spécialiste, solitaire |
| | | *Apanteles* spp. | Spécialistes, solitaires |
| | | *Microplitis plutellae* Musebeck | Spécialiste, solitaire |
| | Ichneumonidae | *Diadegma* spp. | Spécialistes, solitaires |
| | Eulophidae | *Oomyzus sokolowskii* (Kurdjumov) | Spécialiste, grégaire |
| Nymphe | Ichneumonidae | *Diadromus collaris* (Gravenhorst) | Spécialiste, solitaire |

### 1.5.3. Les autres méthodes de lutte

**La sélection variétale :** Il existe des variétés de choux moins sensibles aux attaques des chenilles de *P. xylostella*, par la production de toxines responsables de mécanisme d'antibiose (Eigenbrode et al. 1990 ; Eigenbrode & Shelton 1992) ou par des changements de la structure des cires épicuticulaires qui diminuent l'appétence des feuilles (Dickson et al.

1990 ; Stoner 1992 ; Shimabuku et al. 1997). Ces variétés sont peu employées car les modifications induites peuvent favoriser le développement d'autres ravageurs (tels que les altises *Phyllotreta* spp. (Coleoptera : Chrysomelidae) (Bodnaryk 1997) et donnent un aspect brillant aux feuilles (glossy leaves), peu apprécié des consommateurs (Lim 1992).

**Les plantes transgéniques :** La transgénèse « insecticide », avec intégration de gènes codant pour la production de diverses toxines d'origine bactérienne, végétale ou d'arthropodes venimeux, se développe de plus en plus (Schuler et al. 1998), surtout pour le maïs ou le coton. Les tests de toxicité montrent généralement une grande efficacité de ces plantes dans la suppression de leurs ravageurs (Stewart et al. 1996 ; Ramachandran et al. 1998). Cependant il existe déjà des cas où, des populations de *P. xylostella* résistantes au *B. thuringiensis*, consomment des choux transgéniques sans problème (Tang et al. 1999).

**Les phéromones sexuelles** de synthèse de *P. xylostella* peuvent être utilisées en pulvérisation pour désorienter les mâles qui recherchent les femelles (confusion sexuelle) ou en association avec des pièges englués (piège sexuel). Cependant, les résultats sont peu probants (Schroeder et al. 2000). Néanmoins, les pièges sexuels peuvent être utilisés pour estimer le nombre d'individus présents sur le site afin de réaliser des traitements lorsque le seuil économique est atteint ou dépassé (Reddy & Guerrero 2001 ; Lörh & Kfir 2004).

**L'irrigation par aspersion** réduit significativement les infestations de *P. xylostella,* de 40 à 60% en une année d'irrigation aux Etats-Unis (McHugh & Foster 1995). Lorsque les larves sont en présence d'une humidité relative très élevée (100%), le taux de mortalité est de 70%. Ces résultats sont confortés par la mise en évidence de la pluie en tant qu'un des principaux facteurs de mortalité chez *P. xylostella* (Wakisaka et al. 1992). Cette méthode de lutte est efficace mais coûteuse et favorise l'apparition de maladies cryptogamiques (Talekar & Shelton 1993).

L'utilisation de **plantes pièges** consiste à introduire, dans la parcelle cultivée, des plantes présentant une plus forte attractivité à la ponte pour les femelles. Dans le cas de *P. xylostella*, il s'agit d'introduire des plants de moutarde sur lesquels les femelles vont pondre préférentiellement (Charleston & Kfir 2000). Pour éliminer le ravageur, il suffit de détruire les plants de moutarde. Simple et efficace, cette méthode est utilisée en Inde (Srinivasan & Moorthy 1992 ; Talekar & Shelton 1993) et commence à se répandre en Afrique du Sud

(Charleston & Kfir 1999). Cependant certains agriculteurs sont réticents à utiliser cette technique qui implique de détruire volontairement une partie des plantes cultivées (observations personnelles.).

**La rotation culturale** consiste à ne pas laisser sur un cycle de culture se suivre deux fois la même plante ou des plantes de la même famille sur une parcelle. En milieu tropical, il est fréquent de trouver des cultures de choux toute l'année sur une même parcelle, ce qui permet à *P. xylostella* de se maintenir à des densités élevées. La rotation permettrait de priver temporairement le ravageur de sa plante hôte et donc de réduire ses populations (Talekar & Shelton 1993). Mais cette pratique a un coût financier pour les producteurs. Il leur est difficile d'arrêter de produire du chou qui constitue, dans certains cas, leur seule ressource.

**Les cultures intercalaires** (intercropping) **:** son principe consiste à cultiver des rangs alternés de choux et d'une autre espèce végétale comme l'ail ou la tomate (Talekar et al. 1986), dont l'odeur inhibe l'oviposition des femelles (Srinivasan 1984). Certaines plantes de grande taille peuvent agir comme une barrière physique limitant les déplacements du ravageur, les contacts visuels avec ses congénères ou encore avec sa plante hôte (Talekar & Shelton 1993). Morallo-Rejesus (1986) fait état de 88 plantes ayant un effet répulsif sur *P. xylostella*. Les plantes intercalées entre les plants de choux peuvent également servir de refuge pour les insectes parasitoïdes (Risch 1981 ; Sheehan 1986). Cette pratique s'avère peu intéressante car la plante intercalaire n'est pas d'un aussi bon rapport économique que le chou et ne convient pas aux agriculteurs spécialisés dans une seule culture. Il peut aussi y avoir des problèmes de compétition entre les deux plantes, ce qui nuit au rendement (Shellhorn & Sork 1997).

### 1.5.4. La lutte intégrée

Les résistances à tous les insecticides, le coût élevé de certaines pratiques, le manque d'efficacité de certaines autres et très souvent le manque de connaissance des populations locales sur les pratiques à suivre, rendent la lutte contre *P. xylostella* extrêmement difficile. Face à ces constats, l'importance d'une stratégie intégrée pour la gestion durable de *P. xylostell*a est au centre des préoccupations depuis plus de 20 ans (Grzywacz et al. 2010). La lutte intégrée aborde la lutte contre les ravageurs d'une façon économiquement et écologiquement saine, en utilisant un ensemble varié de techniques pour réduire et maintenir les populations de ravageurs à des niveaux acceptables. La plupart des programmes IPM

comportent des éléments de prévention et de prédiction qui s'efforcent de réduire, sinon d'éliminer, le besoin en mesures de lutte à grande échelle. Parmi les techniques utilisées dans un programme IPM se trouvent la lutte biologique qui prend une place fondamentale, les pesticides et les méthodes culturales de lutte. Le concept de lutte intégrée ou IPM (Integrated Pest Management) (Encadré II) est apparu dans les années 1980 et repose sur cinq grands points : 1) surveillance continue des parcelles par une présence constante sur le terrain et un suivi des populations de ravageurs (monitoring, piégeage sexuel…) ; 2) établissement des seuils économiques au-delà desquels un type bien déterminé d'intervention est préconisé ; 3) utilisation conjointe de méthodes non chimiques (principalement auxiliaires de cultures (parasitoïdes), moyens mécaniques, plantes pièges) ; 4) proscription des insecticides à large spectre au profit de produits plus sélectifs (*Bt*) dont les quantités employées sont réduites afin de faciliter l'implantation des auxiliaires utilisés ; 5) information et/ou participation des acteurs locaux sur les programmes de lutte raisonnée.

**Encadré II.** Integrated Pest Management (IPM)

Dans le **code international de conduite de la distribution et de l'utilisation des pesticides** adopté par la FAO en novembre 2002, la définition de l'IPM est la suivante :

**Integrated Pest Management (IPM)** means the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms.

L'application de tout ou partie de ces diverses recommandations ont donné de bons résultats. Par exemple, au Brésil, le seuil économique fixé à 6 trous sur la feuille centrale a permis de diminuer de 50% les quantités de *Bt* utilisées (Branco et al. 2004). A Cuba, le dépassement du seuil de 0,2 larves par plant préconise le lâcher de 50 000 *Trichogramma pintoi* Voegele par hectare. Près de 85% des fermiers cubains respectent ces conseils (Branco et al. 2004). L'application de tels programmes est bénéfique à de nombreux niveaux. En effet, en plus de la sauvegarde de la qualité des sols, de l'eau, de la santé humaine et des agro-écosystèmes, leur impact s'étend aux coûts de production et de manière concomitante aux bénéfices. En Malaisie et en Thaïlande, plus de 80% des vaporisations de pesticides ont été réduits et les profits ont été respectivement doublés et triplés (Lim 1992). Ces résultats sont

observés également à Hawaii et ont été obtenus en moins de sept années à Singapour (Ng et al. 2004).

# 2. Les parasitoïdes

## 2.1. *Oomyzus sokolowskii* (Kurdjumov)

### 2.1.1. Systématique

L'espèce a été décrite par Kurdjumov en 1912 et rattachée au genre *Tetrastichus*. En 1991, Graham l'a classée dans le genre *Oomyzus*.

| | | | |
|---|---|---|---|
| **Embranchement** | Arthropoda | **Famille** | Eulophidae |
| **Classe** | Insecta | **Sous-famille** | Tetrastichinae |
| **Ordre** | Hymenoptera | **Genre** | *Oomyzus* |
| **Super-famille** | Chalcidoidea | **Espèce** | *sokolowskii* |

### 2.1.2. Morphologie, biologie et écologie

L'œuf est de forme elliptique, transparent et mesure 0,3 × 0,06 mm. Les œufs sont souvent regroupés en amas de 3 à 15 unités dans la partie postérieure interne de l'hôte bien que la femelle n'ait pas de site de ponte préférentiel. Ces œufs se développent grâce aux éléments nutritifs de la chenille puis de la nymphe d'où ils émergent sous forme d'adultes. L'incubation des œufs dure en moyenne trois jours à 25°C (Saw et al. 2013).

La larve est de forme vermiforme, arrondie aux extrémités, quasi transparente et possède un tube digestif sur toute sa longueur. Leur nombre est variable dans la chrysalide parasitée. Au fur et à mesure de leur développement, les plus grosses larves colonisent la totalité de la nymphe, tandis que la compétition qui s'instaure entre elles provoque une diminution sensible de leur nombre au détriment des plus petites. En fin de développement, les larves ont vidé la chrysalide de *P. xylostella*. La durée du stade larvaire varie de quatre à sept jours à 25°C (Saw et al. 2013).

La nymphose se déroule en quatre étapes et dure en moyenne sept jours à 25°C. Une pré-nymphe de couleur blanche précède la nymphe caractérisée par l'apparition et la coloration des yeux et ocelles en rouges. Ensuite la nymphe se développe, grisaille puis prend une coloration définitive noire (Saw et al. 2013).

L'adulte est de très petite taille entre 1 à 2 mm. Son corps est de couleur noir brillant avec des reflets métalliques verts. La durée de vie moyenne d'un adulte est de sept jours (Hirashima et al. 1990). Un dimorphisme sexuel assez marqué permet de distinguer le mâle de la femelle à partir de la taille du corps (Fig. 8) et de la morphologie des antennes.
Le mâle a un abdomen cylindrique, de même diamètre que le thorax. Il possède des antennes de grande taille pourvues de quatre articles portant des soies nombreuses et longues. La femelle présente un abdomen plus renflé et anguleux en forme de losange. Elle est reconnaissable grâce à son ovipositeur (tarière) disposé dans une gouttière visible sur la face ventrale à l'extrémité de l'abdomen. Leurs antennes plus courtes possèdent des soies plus courtes et moins nombreuses.

**Figure 8** : Couple d'adultes d'*O. sokolowskii* (× 20) (femelle à gauche et mâle à droite)

L'accouplement, qui a lieu dès l'émergence, stimule fortement les capacités de parasitisme de la femelle. *Oomyzus sokolowskii* est un endoparasitoïde dont la femelle pond à l'aide de son ovipositeur dans les chenilles de *P. xylostella*. Tous les stades sont parasités, avec une nette préférence pour les chenilles L3 et L4 où les taux de parasitisme sont en moyenne respectivement de 54% et 76% (Saw et al. 2013). Bien qu'il soit généralement considéré comme un parasitoïde larvaire (Ooi 1988 ; Talekar & Hu 1996 ; Kfir 1997), certains auteurs le décrivent aussi comme un parasitoïde nymphal (Chelliah & Srinivasan 1986 ; Waterhouse & Norris 1987 ; Wakisaka et al. 1992 ; Noyes 1994).

Les larves du parasitoïde continuent en effet leur développement dans la nymphe de *P. xylostella* d'où émergeront les adultes. La durée du cycle biologique complet varie en fonction de la température (Tableau 4) ; elle est en moyenne de 15 jours à 25°C (Wang et al. 1999). Ce parasitoïde comme beaucoup d'hyménoptères, présente à la fois une reproduction sexuée et une reproduction asexuée par parthénogénèse arrhénotoque.

**Tableau 4**: Durée du cycle biologique d'*O. sokolowskii* à différentes températures (Wang et al. 1999)

| **Température** (°C) | **Durée du développement** (jours) |
|---|---|
| 35,0 | 13,4 ± 0,15 |
| 32,5 | 11,0 ± 0,13 |
| 30,0 | 12,7 ± 0,15 |
| 25,0 | 15,6 ± 0,18 |
| 22,5 | 20,9 ± 0,09 |
| 20,0 | 26,5 ± 0,71 |

Dans le premier cas la descendance, issue d'œufs fécondés, se compose de femelles diploïdes. Dans le second cas, le développement embryonnaire des œufs non fécondés donne une descendance uniquement composée de mâles haploïdes. La femelle gravide détermine le sexe ratio de la descendance : elle pond en général plusieurs œufs fécondés, puis un petit nombre d'œufs non fécondés d'où un sexe-ratio en faveur des femelles (Uraichuen 1999). *Oomyzus sokolowskii* est une espèce grégaire (plusieurs congénères se développent aux dépens d'un même hôte) (Fig. 9). L'espèce est oïoxène, son spectre d'hôte se limitant à une seule espèce, mais cet hyménoptère peut aussi agir comme un hyperparasitoïde facultatif ou parasitoïde secondaire de *C. plutellae* (Waterhouse & Norris 1987 ; Liu et al. 2000).

### 2.1.3. Répartition géographique

Cette espèce est répertoriée sur les cinq continents, faisant suite à un certain nombre d'introductions, dont la première fut réalisée à Hawaii en 1953 avec une population originaire du Kenya. Sa répartition à l'échelle mondiale, prouve sa grande capacité d'adaptation face à des conditions climatiques variées, qualité nécessaire pour une lutte biologique efficace (Gruarin 1998) (Tableau 5).

**Figure 9** : Plusieurs adultes d'*O. sokolowskii* émergeant d'une nymphe de *P. xylostella*

**Tableau 5 :** Liste des pays où la présence d'*O. sokolowskii* est confirmée (Delvare 2004 ; Talekar 2004 ; Shelton et al. 2008)

| |
|---|
| Europe : France, Suisse, Italie, Hongrie, Roumanie, Russie |
| Asie : Pakistan, Inde, Sri Lanka, Japon, Chine, Corée du nord |
| Afrique : Egypte, Bénin, Sénégal, Kenya, Afrique du Sud, |
| Amérique du nord : Canada, USA |
| Amérique du sud : Brésil, Chili |
| Caraïbes : Martinique, Guadeloupe, Barbade, Jamaïque, République Dominicaine |
| Océanie : Australie, îles Fidji, Guam |

## 2.2. *Cotesia vestalis* (Haliday)

### 2.2.1. Systématique

*Cotesia vestalis* a subi quelques modifications taxonomiques : décrit au début du siècle par Kurdjumov (1912), redécrit en détail par Wilkinson (1939), il a d'abord été nommé *Apanteles plutellae*. Le genre *Apanteles* a éclaté et a vu une partie de ses espèces reclassées vers de nouveaux genres au sein de la sous-famille des Microgastrinae, entièrement révisée par Mason (1981). *A. plutellae* est devenu *Cotesia plutellae*. Fitton et Walker en 1992 ont proposé une synonymie avec *Cotesia vestalis* (Haliday), récemment confirmée par Shaw (2003).

| | | | |
|---|---|---|---|
| **Embranchement** | Arthropoda | **Famille** | Braconidae |
| **Classe** | Insecta | **Sous-famille** | Microgastrinae |
| **Ordre** | Hymenoptera | **Genre** | *Cotesia* |
| **Super-famille** | Ichneumonoidea | **Espèce** | *vestalis* |

### 2.2.2. Morphologie, biologie et écologie

Les stades pré-imaginaux sont décrits par Delucchi et al. (1954).

L'œuf est en forme de croissant, de couleur blanchâtre et mesure 0.3 mm de long. La femelle pond ses œufs sans préférence de localisation dans la chenille hôte.

Le premier stade larvaire est caractérisé par une larve à grosse tête, munie d'un prolongement caudiforme avec une vésicule caudale. La larve dispose de mandibules qui lui permettent de lutter contre les autres larves en cas de super-parasitisme au sein de la chenille hôte (Lloyd 1940).

Au second stade**,** la larve est vermiforme et possède une petite tête chitinisée avec des mandibules légèrement dentées. Elle vit en se nourrissant de l'hémolymphe de son hôte en évitant les organes vitaux pendant tout son développement.

La nymphose se réalise à l'extérieur de l'hôte**.** La larve émerge en perforant le tégument de la chenille-hôte mourante et tisse son cocon juste à côté de la dépouille. Le cocon long de 3 mm est de couleur blanc-jaune avec un aspect soyeux. A l'intérieur du cocon, la

nymphe se colore et se chitinise progressivement, avec séparation des appendices qui deviennent visibles, plaqués contre le corps.

L'adule émerge en découpant l'extrémité de son cocon. Il mesure entre 3 et 5 mm, son abdomen n'est pas pétiolé et son corps est de couleur marron-noir. Ses ailes sont transparentes et la paire antérieure porte une tache le long de la nervure costale. Cette espèce présente un dimorphisme sexuel. Le mâle, qui est haploïde, a une morphologie plus élancée et des antennes plus longues que le corps. La femelle qui est diploïde, est plus massive avec des antennes plus courtes ou égales à son corps. Son abdomen est terminé par un ovipositeur (ou tarière). Les adultes vivent généralement une à deux semaines (Verkerk & Wright 1996).
La durée moyenne du développement complet de l'œuf à l'émergence de l'adulte est de 13 jours à 20°C (Delucchi et al. 1954) (Fig. 10).

La vie des adultes s'organise autour des Brassicacées. La femelle détecte son hôte par l'intermédiaire de l'odeur caractéristique émise par le chou endommagé par les chenilles de *P. xylostella* (Bogahawatte & van Emden 1996 ; Potting et al. 1999). Il se constitue ainsi un complexe tri-trophique très spécialisé. Ceci est lié à la propre spécificité alimentaire de son hôte, qui ne se nourrit que de Brassicacées et il a dû s'adapter à la phytochimie particulière de cette famille végétale. Quant aux mâles, ils vont, outre les phéromones sexuelles, utiliser ce même signal pour trouver les femelles afin de s'accoupler. Le mâle manifeste un comportement de cour qui consiste en des vibrations des ailes. L'accouplement, très bref, peut se réaliser dès l'émergence des adultes. La femelle commence à pondre au cours des 24 heures qui suivent (Saw et al. 2013).

Comme de nombreux endoparasitoïdes, *C. vestalis* injecte lors de l'oviposition un polydnavirus qui permet d'éviter l'encapsulation de l'œuf et qui est donc chez cette espèce indispensable à sa réussite parasitaire. Comme la plupart des Hyménoptères parasitoïdes, *C. vestalis* a un mode de reproduction haplo-diploïde. La femelle a l'aptitude de pondre des œufs fécondés ou non. Les œufs non fécondés (haploïdes) donnent des mâles par parthénogénèse arrhénotoque et les œufs fécondés (diploïdes) donnent des femelles. En condition expérimentale, la femelle est capable de s'attaquer à tous les stades larvaires de son hôte. Cependant, elle préfère les L2 et L3 (Talekar & Yang 1991 ; Shi et al. 2002). Les L1 sont endophylles et donc difficilement accessibles compte tenu de la taille réduite de l'ovipositeur de la femelle. Les L4 au contraire sont plus grosses et plus agiles, et donc rarement parasitées

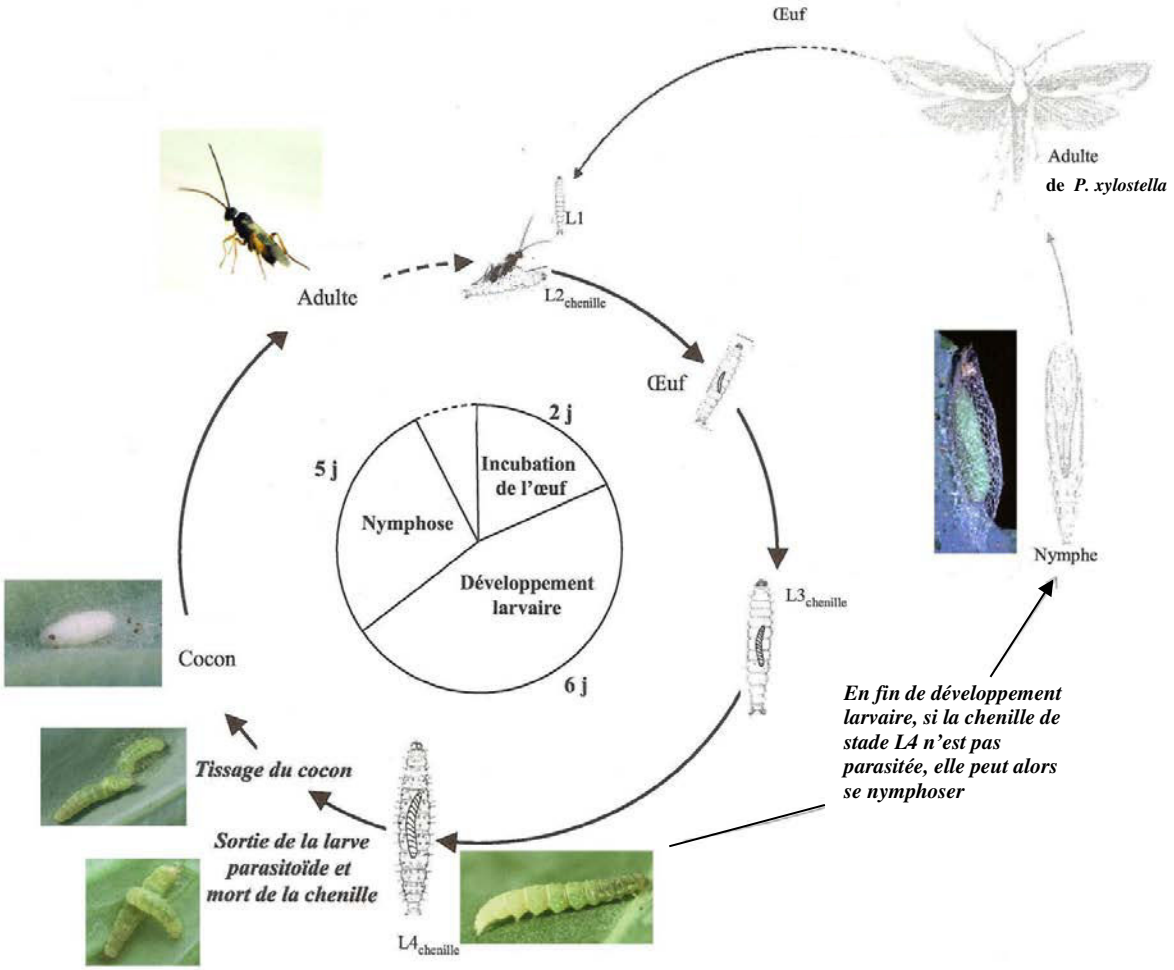


**Figure 10** : Cycle de développement de *C. vestalis à* 20°C (Delucchi et al. 1954).

(Lloyd 1940). Ce parasitoïde préfère donc pondre dans des hôtes plus petits que lui, ce qui en fait obligatoirement un koïnobionte. En effet, une fois parasitée, la chenille continue son développement jusqu'au dernier stade larvaire d'où sortira le parasitoïde ayant terminé son développement larvaire.

*Cotesia vestalis* est un endoparasitoïde solitaire qui s'attaque à un microlépidoptère qui ne fournit de ressources nutritives que pour une seule larve. La femelle ne possède pas de capacités de discrimination. Il n'y a pas de phéromone de marquage comme on l'observe chez d'autres Hyménoptères (Lloyd 1940). En effet, il n'est pas rare qu'une femelle ponde plusieurs fois dans le même hôte. Dans le cas de super-parasitisme, quand plusieurs œufs sont

pondus par différentes femelles dans un même hôte, il y a compétition et élimination des parasitoïdes surnuméraires (Mackauer 1990). Généralement, c'est la première larve à éclore qui détruit les autres œufs (Vinson & Hegazi 1998).

### 2.2.3. Répartition géographique

*Cotesia vestalis* est probablement originaire de la région Méditerranéenne comme son hôte. Sa répartition, bien que plus restreinte que celle de son hôte, reste relativement étendue. L'aire géographique que l'espèce occupe actuellement (Tableau 6) n'a plus grand-chose à voir avec sa répartition naturelle. De nombreux programmes de contrôle de *P. xylostella* ont conduit à introduire de nombreux parasitoïdes dans des zones qui n'en comptaient pas ou très peu. De ce fait, il est devenu l'un des parasitoïdes de *P. xylostella* le plus répandu au monde. Très tolérante à la chaleur (30-35°C), c'est une espèce qui est préférentiellement relâchée dans les zones tropicales. Introduite dans de nombreux pays depuis les années 70, elle a su s'acclimater rapidement, bien que certaines introductions se soient soldées par des échecs.

**Tableau 6 :** Liste des pays où la présence de *C. vestalis* est confirmée (Delvare 2004 ; Talekar 2004 ; Shelton et al. 2008)

| |
|---|
| Europe : Finlande, France, Autriche, Bulgarie, Serbie, Turquie, Russie, |
| Asie : Pakistan, Inde, Sri Lanka, Taiwan, Indonésie, Malaisie, Philippines, Vietnam, Chine, Japon, |
| Afrique : Egypte, Sénégal, Bénin, Kenya, Afrique du Sud, Réunion |
| Amérique du Nord : USA |
| Amérique du Sud : Brésil, Venezuela |
| Caraïbes : Martinique, Guadeloupe, Jamaïque, Barbade, Ste Lucie, République Dominicaine |
| Océanie : Australie, îles Fidji |

# CHAPITRE II

## Interaction entre *Plutella xylostella* et le parasitoïde *Oomyzus sokolowskii*

# 1. Introduction

Ce chapitre est consacré à l'interaction entre *P. xylostella* et ses auxiliaires naturels, particulièrement *O. sokolowskii.* Les travaux correspondant ont été publiés et sont présentés ici sous la forme de trois articles.

Nous avons choisi le Sénégal comme site d'étude pour étudier cette interaction, pour plusieurs raisons : 1) ce pays présente des caractéristiques climatiques tropicales ; 2) le chou y est cultivé toute l'année ; 3) *Plutella xylostella* en est le principal ravageur et ses dégâts sont importants ; 4) la lutte chimique y est pratiquée mais elle s'avère inefficace ; 5) à ce jour, aucune étude approfondie n'avait été réalisée sur la teigne.

Afin d'identifier les facteurs environnementaux (biotiques et abiotiques) pouvant favoriser ou inhiber le développement de la teigne, nous avons étudié les relations croisées entre les facteurs climatiques, *P. xylostella*, sa plante hôte et ses ennemis naturels. Cette étude a été réalisée en plein champ à Malika, dans la zone périurbaine de Dakar (article 1).

*Oomyzus sokolowskii* est l'un des parasitoïdes de *P. xylostella* et on le rencontre majoritairement au Sénégal (Delvare & Kirk 1999 ; Sall-Sy et al. 2004). Pour autant ce parasitoïde a été très peu étudié. Afin de connaitre son potentiel comme éventuel agent de lutte, nous avons étudié en conditions de laboratoire quelques traits de son histoire de vie (article 2), ainsi que les facteurs qui peuvent contribuer à connaitre et augmenter ses performances parasitaires vis-à-vis de son hôte (article 3).

Dans la mesure où une partie des travaux proposés dans ce chapitre a été réalisée en condition de plein champ au Sénégal, il nous a semblé important d'exposer d'abord les caractéristiques climatiques et agricoles de ce pays de l'Afrique de l'Ouest et de faire un point sur le statut de la teigne du chou. Ensuite nous avons fait une synthèse des résultats obtenus à partir des trois articles, puis une conclusion.

## 2. Etude de terrain : le Sénégal

### 2.1. Présentation générale

Situé dans la zone intertropicale (entre le tropique du cancer et l'équateur), le climat y est de type sahélien au nord (région du fleuve Sénégal), voire semi-désertique, de type subtropical (humide) au sud (Gambie et Casamance) et de type soudanien au centre. Il est également caractérisé par l'alternance de deux saisons :

- Une saison des pluies, appelée « hivernage », qui dure de juin à octobre, avec un pic de précipitations en août (250 mm). C'est la période des moussons. Les précipitations s'échelonnent entre 1500 mm au sud et 200 mm par an au nord. Les températures sont comprises entre 25°C et 35°C.

- Une saison sèche un peu plus fraîche, de novembre à juin avec des températures comprises entre 17°C et 25°C.

L'agriculture sénégalaise est largement dominée par des exploitations de très petite taille de type familial qui constituent la quasi-totalité des activités agricoles villageoises. Les cultivateurs produisent des choux, essentiellement dans les Niayes, toute l'année pour répondre à la demande nationale, mais aussi pour l'exportation dans la sous-région (Mauritanie, Mali, Burkina Faso, etc.). Cette région fournit 80% de la production maraîchère nationale. La région des Niayes (Fig. 11) couvre une bande dunaire littorale, d'une longueur de 180 km et d'une largeur d'environ 30 km, s'étendant de la banlieue de Dakar jusqu'à celle de Saint-Louis, au Nord. Appelées aussi "Poumons maraîchers du Sénégal", les Niayes bénéficient de conditions hydriques (nappes phréatiques nombreuses et superficielles) et pédoclimatiques (températures fraîches, faible amplitude thermique, dunes et dépressions) exceptionnelles, propices aux cultures maraîchères.

Le chou est un produit de grande consommation au Sénégal puisqu'il fait partie du plat national et il est consommé quotidiennement. C'est une culture importante parce que son cycle est relativement court (60-90 jours après repiquage). La possibilité de le cultiver toute

l'année, tant en saison sèche qu'en hivernage, permet de financer d'autres activités et d'autres cultures. En 2011, le Sénégal a produit 50 000 tonnes de choux sur une superficie de 2 444 hectares (FAOSTAT 2013). Ce pays est le second pays producteur de chou de l'Afrique de l'ouest, après le Niger.

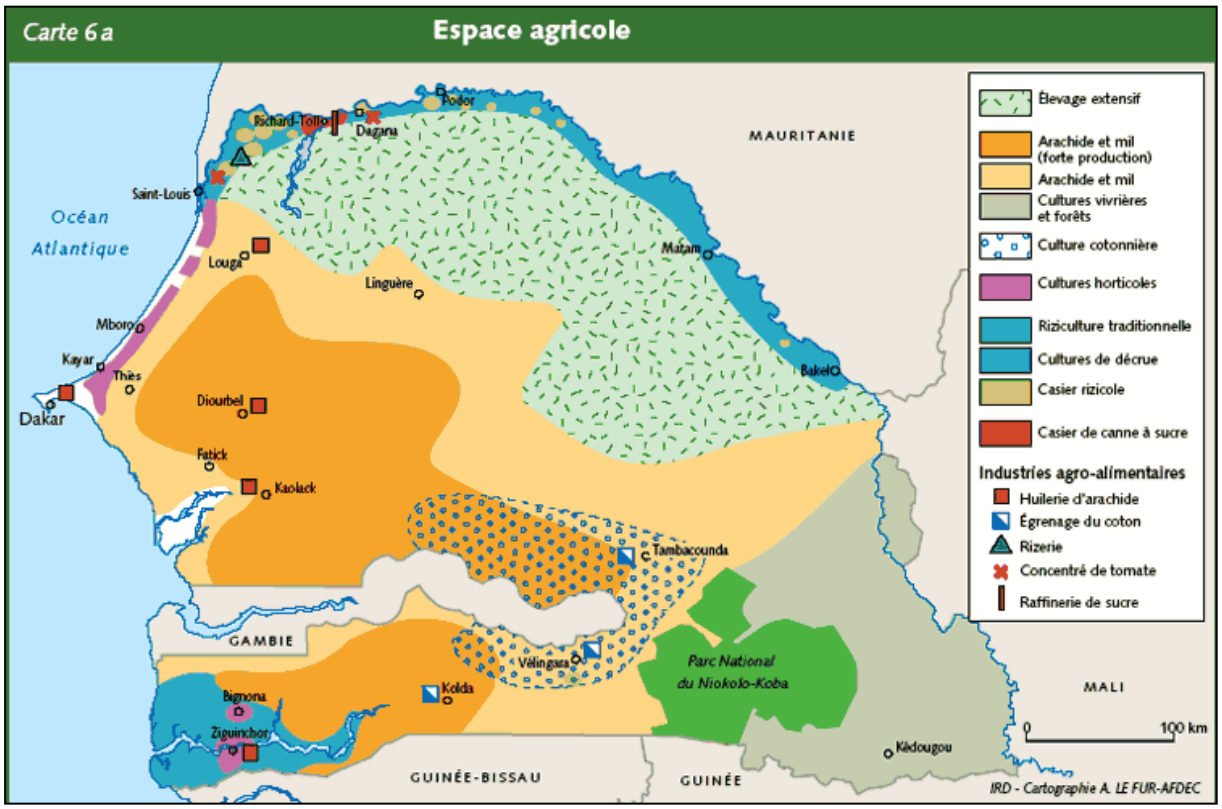


**Figure 11** : Les différents espaces agricoles du Sénégal. La région des Niayes (colorée en mauve) est une zone géographique du Nord-Ouest du Sénégal qui s'étend de Dakar jusqu'à Saint-Louis. Elle représente la zone des cultures horticoles la plus importante.

### 2.2. Statut de *Plutella xylostella* au Sénégal

Bien que les tendances de consommation de chou soient à la hausse, le potentiel de production est fortement freiné par l'impact négatif des ravageurs sur cette culture. D'après l'ISRA (Institut Sénégalais de Recherches Agricoles), *P. xylostella* est le principal ravageur. Il provoque d'important dégâts puisque les cultivateurs peuvent perdre jusqu'à 80% de leur production.

Face à ce problème, les agriculteurs utilisent beaucoup de pesticides, mais peu formés ils ne connaissent pas la réelle action des pesticides utilisés, ni leur mode d'utilisation, ce qui se traduit par une utilisation abusive et/ou mal adaptée des produits phytosanitaires. De telles pratiques ont pour conséquences d'affecter la santé des agriculteurs et des consommateurs, de contaminer l'environnement et les nappes phréatiques, d'induire des phénomènes de résistance chez les populations de la teigne tout en éliminant ses ennemis naturels.

Au Sénégal, les méthodes alternatives à la lutte chimique sont très peu développées. Cependant, les produits à base de *B. thuringiensis* (*Bt*) et d'insecticides naturels, dont les extraits de graines de « Neem » (*Azadirachta indica*), peuvent constituer une alternative efficace et plus respectueuse de l'environnement (Grzywacz et al. 2010). Bien que ces produits constituent des palliatifs aux pesticides chimiques, l'optimisation de leur application et la prise en compte du cortège parasitaire de *P. xylostella* sont souvent négligées. La lutte biologique est, de ce fait, très peu voire pas employée. L'insuffisance de données sur la biologie et l'écologie de la teigne du chou et de ses ennemis naturels dans les conditions tropicales que représente le Sénégal a ainsi justifié cette étude.

### 2.3. Site d'expérimentation

L'étude a été réalisée dans la zone périurbaine de Dakar, dans les Niayes, sur le site de Malika (latitude: 14°47'38 N et longitude: 17°20'20 W) (Fig. 12 et Fig. 13). Cette localité bénéficie d'un climat tropical de type côtier à deux saisons influencé par les alizés maritimes et la mousson. Les précipitations y sont peu abondantes et dépassent rarement 500 mm par an. Les températures moyennes varient entre 23°C et 30°C selon la saison. L'expérimentation a été réalisée pendant deux ans, sur la parcelle d'un agriculteur qui cultive du chou (*Brassica oleracea* L. var. *capitata*, cultivar « Marché de Copenhague ») toute l'année, sans utiliser d'insecticide.

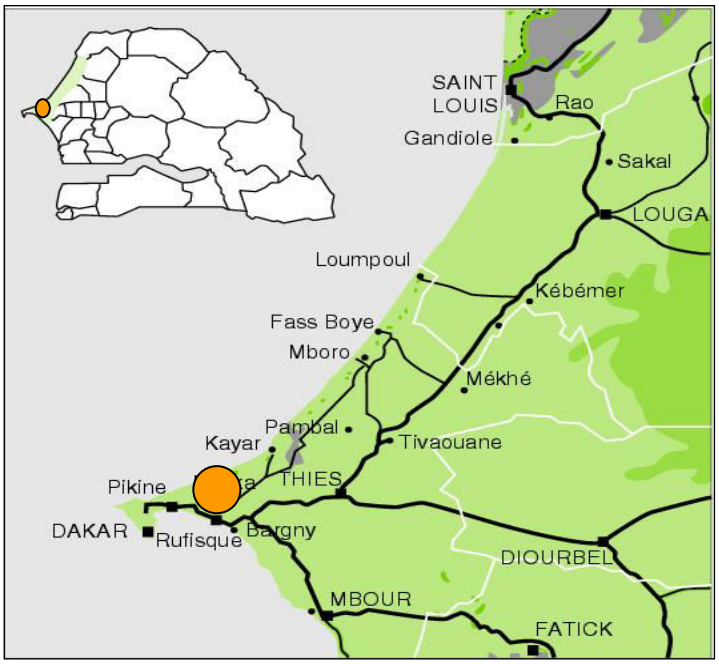


**Figure 12** : Localisation du site de Malika dans la zone périurbaine de Dakar

**Figure 13** : Parcelle de choux sur le site de Malika, dans la zone périurbaine de Dakar

## 3. Synthèse des résultats

Parmi les facteurs climatiques étudiés, la saisonnalité influence particulièrement la dynamique des populations de la teigne. En effet, l'abondance de *P. xylostella* est significativement plus importante pendant la saison sèche quand les précipitations sont peu importantes et les températures plus basses, comprises entre 18°C et 25°C. Les précipitations n'ont pas été un facteur de régulation du ravageur, contrairement aux températures élevées durant la saison chaude et humide (hivernage). La plante hôte influence également la dynamique de la teigne. L'abondance des chenilles est plus importante en début de culture quand les choux sont jeunes.

Quatre espèces d'hyménoptères ont été identifiées comme parasitoïdes de *P. xylostella*. Il s'agit d'*Oomyzus sokolowskii, Apanteles litae* Nixon (Braconidae), *Cotesia vestalis* et *Brachymeria citrae* Westwood (Chalcididae*). Apanteles litae* s'est révélée l'espèce la plus abondante en saison sèche, par contre *O. sokolowskii* a été l'espèce rencontrée le plus régulièrement pendant toute la durée de notre étude. Le taux de parasitisme total calculé a été de l'ordre de 10% et aucun contrôle de la teigne n'a été observé durant toute la période d'étude.

La durée totale du développement d'*O. sokolowskii*, entre l'oviposition et l'émergence de l'adulte, est relativement rapide (15 jours à 25°C), avec trois jours pour l'incubation des œufs, quatre jours pour le développement larvaire et huit jours pour la nymphose.

Cette espèce présente un dimorphisme sexuel basé sur la taille du corps et la longueur des tibias des adultes. La femelle est plus grosse et ses tibias sont plus longs que ceux du mâle. C'est une espèce synovogénique, puisque la femelle produit des œufs tout au long de sa vie. Son mode de reproduction est basé sur un système haplo-diploïde avec une parthénogénèse arrhénotoque.

La sex-ratio de la descendance est en faveur des femelles avec en moyenne huit femelles pour deux mâles. La femelle peut parasiter tous les stades larvaires (L2 à L4) et le stade pré-nymphal, avec une nette préférence pour le stade L4. Le taux de parasitisme est quatre fois plus élevé quand la femelle a été accouplée.

Une femelle isolée peut parasiter 14% de chenilles L4 et engendrer une descendance très faible. En présence de congénères, ce taux augmente significativement puisqu'elle peut parasiter jusqu'à 78 % des chenilles L4 mises à sa disposition et sa descendance peut être multipliée par cinq. La population du Sénégal a des nymphes significativement plus grosses

que celles de la population de Martinique qui nous a servi de référence. Les femelles *d'O. sokolowskii* du Sénégal parasitent d'avantage les chenilles L4 martiniquaises que sénégalaises (81% et 66%, respectivement). Le taux de parasitisme varie en fonction de l'âge de la femelle parasitoïde. Dès les premiers jours de sa vie, le taux peut atteindre 82% puis décroître jusqu'à 31% au bout de 28 jours.

## 4. Conclusion

Les facteurs climatiques en conditions tropicales ont une influence sur la dynamique des populations de la teigne du chou, en effet la saison sèche est plus favorable à son développement et les précipitations ne sont pas un facteur de régulation.

Malgré la présence de quatre espèces d'hyménoptères parasitoïdes, le taux de parasitisme est faible et le contrôle du ravageur reste insuffisant.

*O. sokolowskii* présente une durée de développement rapide (15 jours) et parasite préférentiellement les chenilles de stades L4. Ses performances parasitaires dépendent du nombre de congénères, de l'origine de l'hôte et de l'âge de la femelle et le taux de parasitisme obtenu en conditions de laboratoire peut atteindre 80%.

Pour conclure, *O. sokolowskii* est un parasitoïde de *P. xylostella* qui présente des performances parasitaires évidentes en conditions de laboratoire, ce qui n'est pas le cas en conditions de terrain. Malgré la présence des trois autres espèces, le taux de parasitisme reste faible et la teigne n'est pas contrôlée.

# ARTICLE 1

## The relationship between the diamondback moth, climatic factors, cabbage crops and natural enemies in a tropical area

**Gallo SOW, Karamoko DIARRA, Laurence ARVANITAKIS and Dominique BORDAT**

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# The relationship between the diamondback moth, climatic factors, cabbage crops and natural enemies in a tropical area

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**ABSTRACT**

The impact of abiotic and biotic factors (rainfall, temperature, host plant and natural enemies) on population dynamics of the *Plutella xylostella* L. diamondback moth was investigated. The experiments were conducted during the rainy and dry seasons for two years (June 2009-April 2011) on unsprayed cabbage plots in Malika (Senegal). Every 10 days, 10 cabbages were randomly selected. *Plutella xylostella* larvae, pupae and parasitoid cocoons were recorded on each plant. Before each sampling, the diameters and ages of plants were recorded. Temperature and rainfall were also recorded during this study. Larvae and pupae of *P. xylstella* were higher for the dry season than the rainy season. There was a negative correlation between temperature and *P. xylostella* populations, and a strong relationship between *P. xylostella* populations and the age of cabbages. Females oviposited on young cabbages where the presence of young larvae was important, whereas older immature stages were mainly found in older cabbage plants. Parasitoid populations were higher for the dry season than the rainy season. High temperatures did not increase the pest populations and parasitism rate. There was no effect found on pest, plants and natural enemies due to rainfall. There was a positive correlation between pest populations and parasitism. Four Hymenoptera species were found: *Oomyzus sokolowskii*, *Apanteles litae*, *Cotesia plutellae* and *Brachymeria citrae*, but they were not efficient to control the *P. xylostella* populations. These results are important for understanding the factors that promote or inhibit pest populations and their natural enemies, and therefore essential for effective crop protection.

Key words: biological control, parasitoid, plant phenology, *Plutella xylostella*, rainfall, temperature

## INTRODUCTION

Cabbage, *Brassica oleracea* L., is an important vegetable crop playing a key role in the economy of many countries, particularly in Asia and Africa (Grzywacz et al. 2010). The diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) is oligophagous and considered to be the most important pest for the Brassicaceae family (Talekar and Shelton 1993, Sarfraz et al. 2006). The proliferation of larvae pest populations is favoured by the short duration of the life cycle, with up to 20 generations per year under tropical conditions

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(Vickers et al. 2004) and a high reproductive potential of the females (Justus et al. 2000). The damage caused by this pest has been estimated globally to cost US$ 1 billion in direct losses and control costs (Grzywacz et al. 2010). The use of synthetic insecticides is the main control strategy (Kibata 1996). This pest has developed resistance against all major groups of pesticides, including *Bacillus thuringiensis* bacterial based bio-pesticides (Tabashnik et al. 1990, Zhou et al. 2011).

Several studies (Shelton et al. 1993, Hill and Foster 2000, Liu et al. 2000) have shown that the use of insecticides is not a sustainable pest management option for farmers, as it is fraught with problems such as the improper handling of pesticides, increased cost of pesticides, reduced control efficacy and contamination of the farming environment (Dobson et al. 2002). A possible alternative to pesticides in the development of an integrated management strategy against *P. xylostella* is biological conservation control using endemic parasitoids (Sarfraz et al. 2005). Parasitoids are particularly susceptible to chemical insecticides and understanding their role in the ecosystem is important for the implementation of an integrated pest management strategy (Shepard et al. 1999).

More than 90 insect parasitoids have been recorded, but less than 10 have bio-control potential for *P. xylostella* (Noyes 1994). Among these natural enemies, *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) is the most abundant larval parasitoid of *P. xylostella* in South Africa (Kfir 1997, Mosiane et al. 2003). In Ethiopia, *Oomyzus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae), *Diadegma* sp. (Hymenoptera: Ichneumonidae) and *Cotesia plutellae* are the most important ones, accounting for more than 90% of the parasitoid complex (Ayalew et al. 2004). However, total parasitism of *P. xylostella* rarely exceeds 15% in East Africa (Kfir 2003). According to Löhr and Kfir (2004), the diversity of the parasitoid fauna associated with *P. xylostella* in West Africa is relatively poor. Most common were *C. plutellae* and *O. sokolowskii* in Benin and Senegal (Goudegnon et al. 2004, Sall-Sy et al. 2004), while *Apanteles litae* Nixon (Hymenoptera: Braconidae) was predominant in Ivory Coast (Löhr and Kfir 2004).

The agro-ecological concept, which integrates agriculture into the natural ecosystem, has been found useful for population management of *P. xylostella* (Vandermeer 1995). Population management of pests integrates cultivated plants, endemic flora, natural enemies and climatic factors (Regnault-Roger 2005). Temperature and humidity are among the most important climatic factors affecting the biology of the diamondback moth (Guo and Qin 2010). According to Ansari et al. (2010), the development of *P. xylostella* depends on the host plants and temperature. The development rate in relation to temperature plays an essential role in pest management, especially in helping to predict the timing of the development of pests and natural enemies in field conditions (Roy et al. 2002).

The biology and ecology of the pest population and its relationship with the host plant and natural enemies must also be studied (Campos et al. 2003). Brassica IPM depends on a good understanding of factors affecting *P. xylostella* population dynamics. In this study, the impact of abiotic and biotic factors (rainfall, temperature, host plant and natural enemies) on the population dynamics of *P. xylostella* was investigated on cabbage plants in the field.

## MATERIAL AND METHODS

### *Study site*

The study was conducted in Malika, a district in the Niayes of Dakar, Senegal (N: 14°47’552, W: 17°19’818 and 189 m above sea level). The area is characterised by a long dry season from November to June with a temperature range of 15-20°C and a short rainy season from July to October, with temperatures ranging between 25 to 35°C (Pereira 1963). The yearly precipitations do not exceed 500 mm between August and September. The experiments were conducted during the rainy and dry seasons for two years from June 2009 to April 2011.

### *Cabbage crops*

The host plants (*Brassica oleracea* L. var. *capitata* 'Copenhagen Market' were grown in a small farmers’ field and no insecticide was used. Thirty-day old seedlings were transplanted to seven replicate plots. Plot size was six rows of 5 m length, each with a spacing of 40 cm between plants and 60 cm between rows. Spacing between plots was 1 m. In order to protect the plants from nematodes, Furadan at 500 g was applied in the soil prior to planting. Poultry manure at 50 kg was applied 10 days later with intensive irrigation. Additional fertilizers NPK (10:10:20) at 5 kg and poultry manure at 75 kg were applied 15 days after planting. The crops were watered daily using sprinkler irrigation.

### *Sampling methods*

The samplings started 10 days after transplanting and were performed every 10 days on unsprayed cabbage plots. Samples were collected randomly by selecting 10 cabbages in the central rows of each plot. Each plant selected was examined and numbers of *P. xylostella* larvae (second to fourth instar), pupae and parasitoid cocoons were recorded and left to develop in order to determine parasitism levels in the field (Nofemala and Kfir 2005). The eggs and larvae that were inside the leaves were not considered. The samples were taken to the laboratory where they were maintained at 25°C, 60% relative humidity and 12 h light/dark photoperiod. Emerging parasitoids were identified (by the taxonomy Laboratory from Cirad, Montpellier, France), and their incidence recorded.

The diameters and ages of cabbage plants collected during each sample were noted. The temperature of the air was recorded with the aid of an automatic tape recorder, "Tinytag", programmed via the software Tinytag Explorer 4.1 (Tinytag Explorer 2005). Parameters were recorded all 10 min and permitted to have a daily mean of temperature. Rainfall was also recorded daily using a rain gauge. The effects of these factors on the population dynamics of *P. xylostella* and parasitoid populations were assessed.

### *Statistical analysis*

The data were normalised by logarithmic transformation before being subjected to an analysis of variance (ANOVA). The abundance of *P. xylostella* larvae and pupae, parasitoid populations, temperature and rainfall among the seasons were analysed using one-way ANOVA (XLSTAT). Means were separated using the Student Newman Keuls test. Correlation analyses were performed to determine relationships between the abundance of *P. xylostella* and rainfall, infestation levels and temperature, plant age and infestation levels, rainfall and parasitoid populations, temperature and parasitoid populations, plant age and parasitoid populations, and abundance of *P. xylostella* and parasitoid populations using XLSTAT version 2012.1.01. In all statistical analyses, p = 0.05 was considered significant.

**Table 1.** Overall relationship between the abundance of *Plutella xylostella* larvae and pupae, parasitism, adults of parasitoid species, temperature and rainfall from June 2009 to April 2011

| Parameter | Season | |
|---|---|---|
| | dry | rainy |
| *P. xylostella* larvae/pupae | 474.0 ± 71.4 a* | 29.1 ± 9.2 b |
| Parasitism (%) | 5.5 ± 1.6 a | 0.4 ± 0.1 b |
| *Oomyzus sokolowskii* | 7.7 ± 1.5 a | 0.9 ± 0.3 b |
| *Apanteles litae* | 10.3 ± 2.3 a | 0.6 ± 0.2 b |
| *Cotesia plutellae* | 3.9 ± 2.1 a | 1.1 ± 0.7 a |
| *Brachymeria citrae* | 0.0 a | 0.1 ± 0.1 a |
| Mean temperature (°C) | 23.8 ± 1.5 b | 29.7 ± 2.6 a |
| Total rainfall (mm) | 0.0 b | 498.0 ± 0.0 a |

*Values marked with the same letters do not differ significantly at p = 0.05

## RESULTS

### *Relationships between P. xylostella populations and climatic factors*

*Effects of season*

The population density of the pest varied significantly between the dry and rainy seasons (F = 11.17, p = 0.002, Tab. 1). The abundance of *P. xylostella* was higher in the dry season than in the rainy season. Infestation levels by larvae and pupae were high at the middle of the dry season (February-April), fluctuating between 100 and 800 larvae and pupae per plant. The immature stages of *P. xylostella* were low in the rainy season (1 to 200 larvae and pupae per plant) and the beginning of the dry season (16 to 120 larvae and pupae per plant) (Fig. 1).

*Effects of rainfall and temperature*

No significant correlation was found between rainfall and infestation levels of *P. xylostella* (r = –0.198, p = 0.247). There was a significant difference between rainfall during the rainy and dry seasons (F = 13.75, p = 0.001, Tab. 1). Rainfall was higher during the rainy season than the dry season (Fig. 2). There was a significant correlation between temperature and *P. xylostella* populations (r = –0.405, p = 0.014). There was also a significant difference between temperatures during the rainy and dry seasons (F = 65.37, p = 0.0001, Tab. 1). Temperatures were higher (30 to 42°C) during the rainy season than the dry season (Fig. 2).

### *Relationship between P. xylostella populations and the age of the cabbage*

There was a negative correlation between the young larval stages of *P. xylostella* with the cabbage age (r = –0.340, p = 0.038). These young stages (L2 and L3) decreased when the age of the cabbage was 50 to 55 days in the dry season. On the other hand, the number of old stage (L4 and pupae) increased

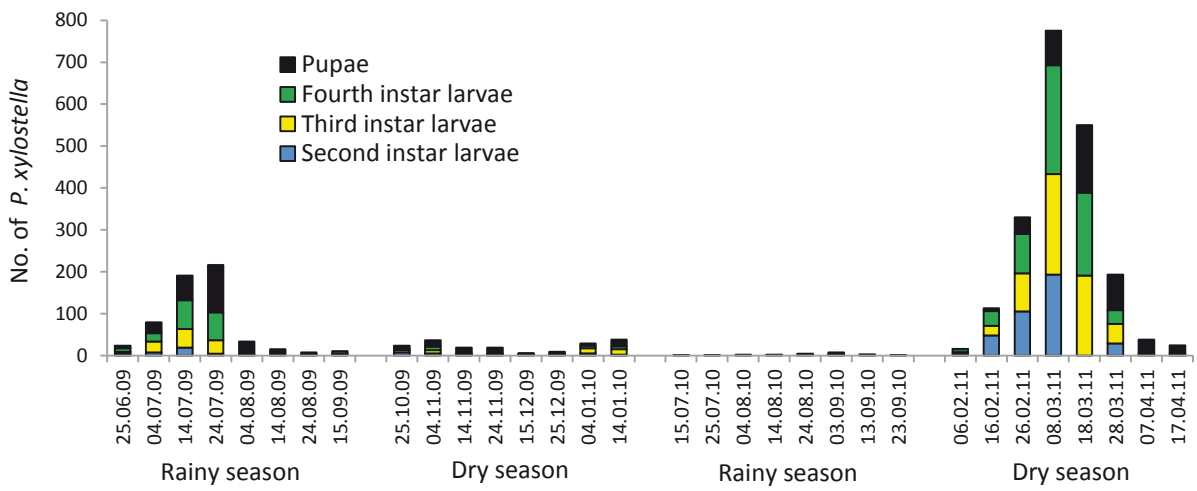


**Figure 1.** Abundance of *P. xylostella* larvae and pupae on unsprayed cabbage fields during the rainy and dry seasons from June 2009 to April 2011

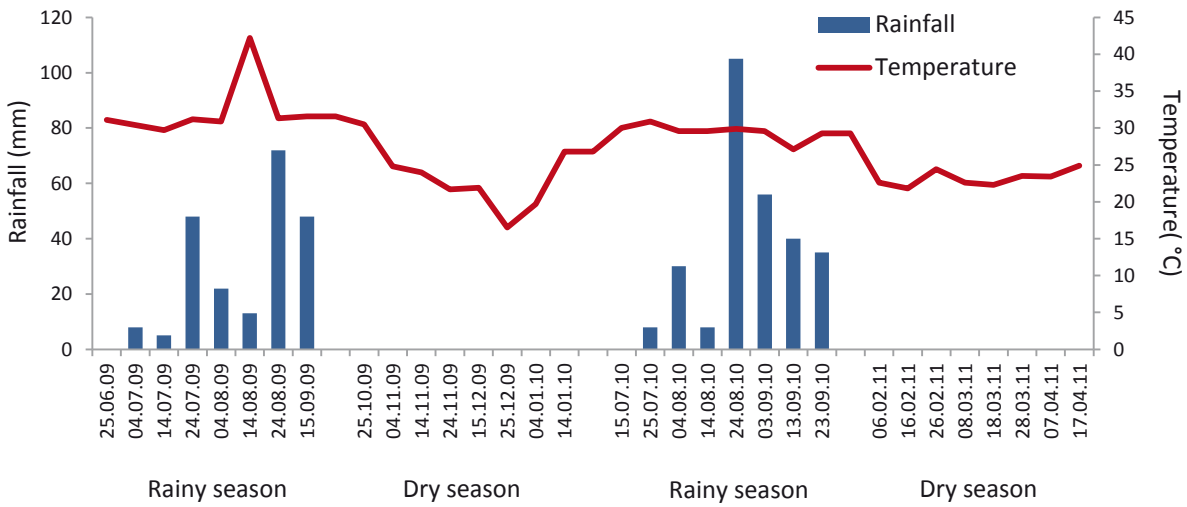


**Figure 2.** Total rainfall and mean temperature recorded at Malika (Senegal) during the rainy and dry seasons from June 2009 to April 2011

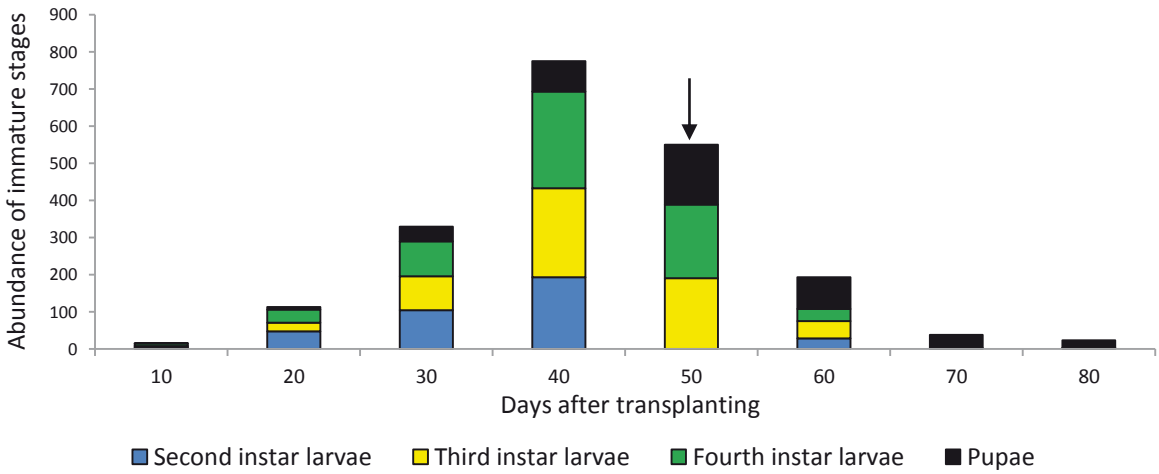


**Figure 3.** Relationship between the abundance of immature stages of *P. xylostella* and the age of the cabbage in the dry season. Arrow indicates the beginning of hearting (cabbage maturation)

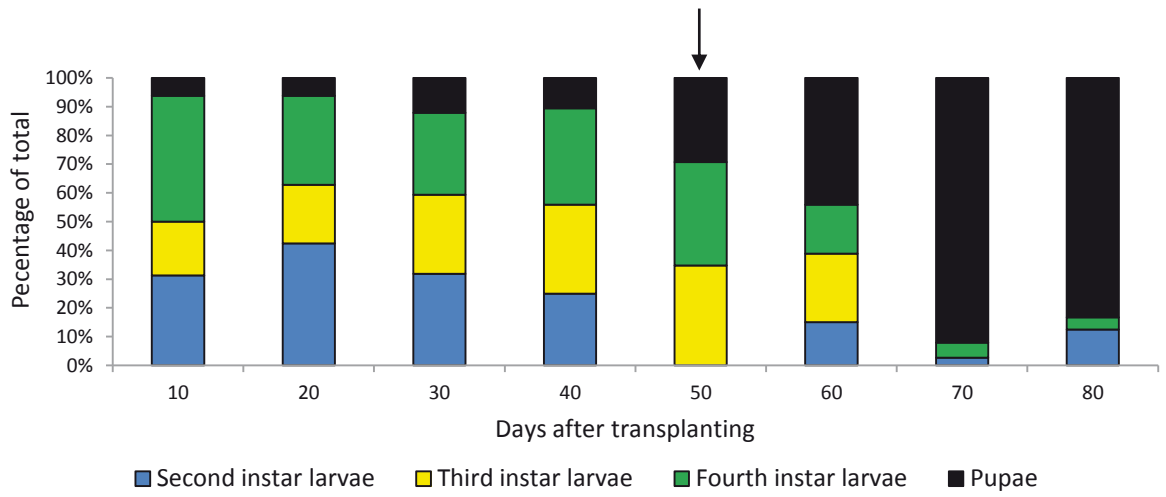


**Figure 4.** Relationship between the relative abundance of immature stages of *P. xylostella* and the age of the cabbage in the dry season. Arrow indicates the beginning of hearting (cabbage maturation)

with the age of the cabbage (r = 0.44, p = 0.007) (Figs 3 and 4). There was a significant correlation between the diameter of the cabbage plants and pest populations (r = 0.39, p = 0.018). The diameter of cabbage plants increased with plant age.

### *Relationships between natural enemies and climatic factors*

*Effects of season*

Parasitoid populations were significantly different depending on the seasons (F = 29.81, p = 0.0001). The average parasitism varied significantly between seasons. It was high in the dry season, and very low in the rainy season (Tab. 1).

Four indigenous parasitic Hymenoptera were found in the pest populations (Fig. 5). *Oomyzus sokolowskii* Kurdjumov (Eulophidae), a larval-pupal, was active throughout the year and dominated the parasitoid complex (Fig. 5). It was the only gregarious parasitoid recorded during this study. The population density of *O. sokolowskii* varied significantly between the dry and rainy seasons (F = 14.49, p = 0.001, Tab. 1). *Apanteles litae* Dixon (Braconidae), a larval parasitoid, was most predominant in the dry season (F = 7.55, p = 0.009, Tab. 1). *Cotesia plutellae* Kurdjumov (Braconidae), a larval parasitoid, was also recorded throughout the year, but its activity was sporadic (Fig. 5). There was no significant difference between the seasons (F = 1.71, p = 0.19, Tab. 1). Only two specimens of *Brachymeria citrae* Westwood (Chalcididae), a pupal parasitoid, were recorded and there were no significant differences between the seasons (F = 0.23, p = 0.06, Tab. 1). Hyperparasitoids were not recorded.

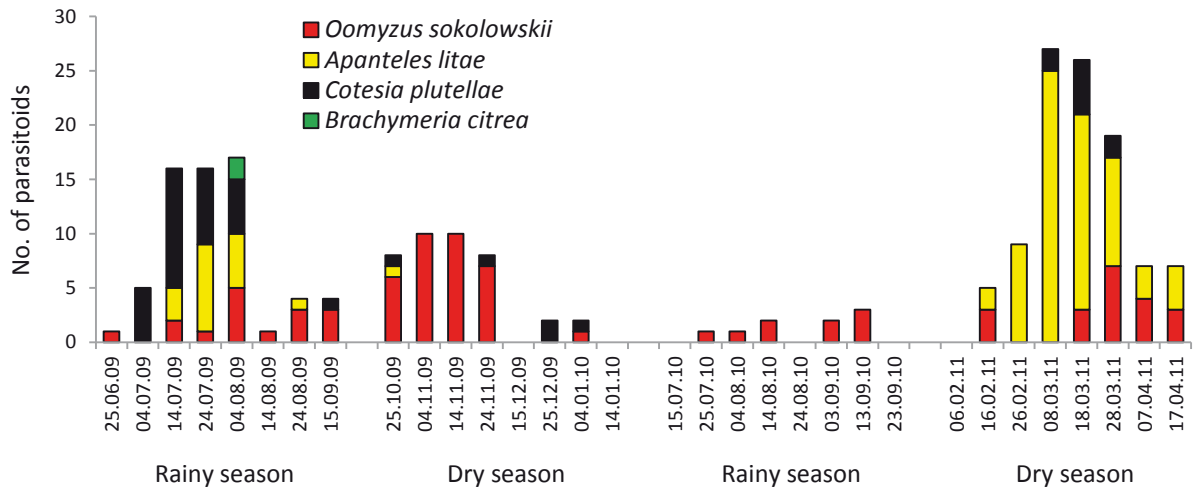


**Figure 5.** Abundance of parasitoids associated with *P. xylostella* on unsprayed cabbage fields during the rainy and dry seasons from June 2009 to April 2011

*Effects of rainfall and temperature*

No significant correlation was found between rainfall and parasitoid populations (r = –0.27, p = 0.1) (Fig. 2). There was a negative correlation between temperature and parasitoid populations (r = –0.34, p = 0.04). There was also a negative correlation between temperature and *A. litae* (r = –0.35, p = 0.032). There was a positive correlation between temperature and *B. citrae* (r = 0.36, p = 0.03). However, no significant correlation was found between temperature and *O. sokolowskii* (r = –0.29, p = 0.08). There was also no correlation between temperature and *C. plutellae* (r = –0.13, p = 0.4).

***Relationships between natural enemies and the age of the cabbage***

The correlation between the age of the cabbage and total parasitism was significant (r = –0.65, p = 0.0001, Fig. 6). There was a negative correlation between cabbage age and *O. sokolowskii* (r = –0.44, p = 0.007, Fig. 7). No significant correlation was found between the age of the cabbage and *A. litae* (r = –0.23, p = 0.16), *C. plutellae* (r = –0.32, p = 0.056) or *B. citrae* (r = –0.041, p = 0.8).

***Relationships between P. xylostella populations and natural enemies***

The correlation between pest populations and total parasitism was positive (r = 0.36, p = 0.003)(Figs 3 and 7). There was a positive correlation between *P. xylostella* populations and *O. sokolowskii* (r = 0.38, p = 0.02), *A. litae* (r = 0.98, p = 0.0001) and *C. plutellae* (r =0.77, p = 0.0001). No significant correlation was found between *P. xylostella* populations and *B. citrea* (r = 0.08, p = 0.6).

## DISCUSSION

The relationships between *P. xylostella* populations, climatic factors, cabbage phenology and the natural

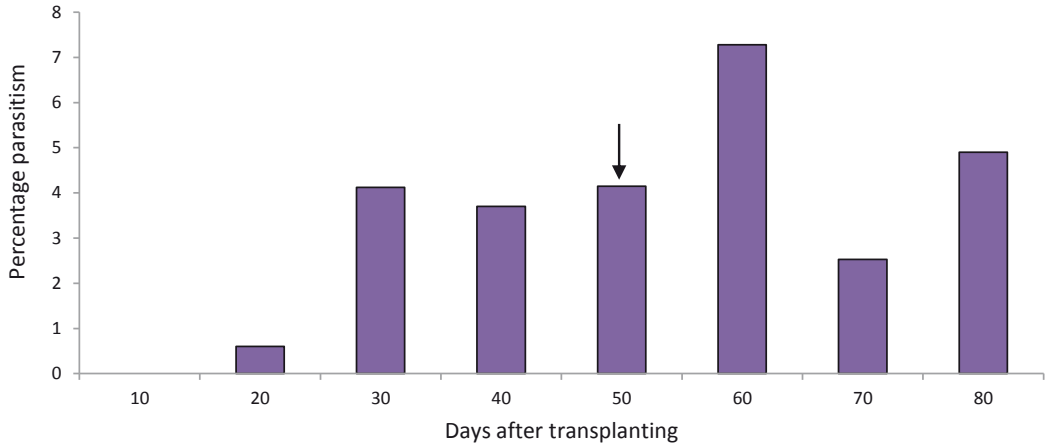


**Figure 6.** Relationship between *P. xylostella* parasitism and the age of the cabbage in the dry season. Arrow indicates the beginning of hearting (cabbage maturation)

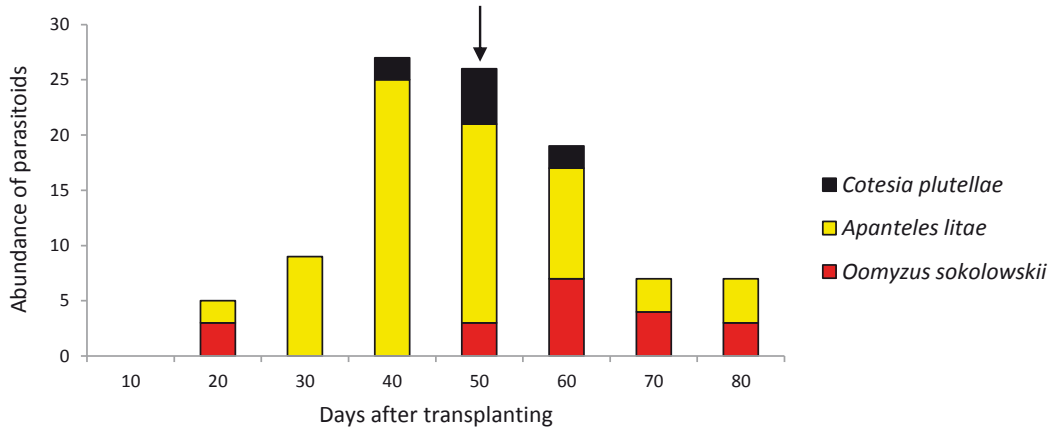


**Figure 7.** Relationship between the abundance of natural enemies and the age of the cabbage in the dry season. Arrow indicates the beginning of hearting (cabbage maturation)

enemy fauna were examined. The importance of climatic factors in the population dynamics of this pest has been emphasised by several authors (Cohen 1982, Vickers et al. 2004). The *P. xylostella* population was low in the rainy season. It is possible that rainfall may cause the mortality of immature stages of *P. xylostella*; but it is unlikely to be a major factor in the reduction of the pest populations during this period. The detrimental effect of rainfall and high temperature on pest populations has been reported by several authors (Wakisaka et al. 1992, Lui et al. 2000, Shirai 2000, Waladde et al. 2001). In the present study, we observed that the temperature influenced the dynamics of the *P. xylostella* population. The pest population increased when the temperature fell. In the rainy season, the mean temperature was 29.7°C, in the transient season it was 26.8°C and in the dry season it was 23.8°C. These data confirm Atwal (1955), where the optimal temperature for *P. xylostella* development was 17 to 25°C. However, several authors have reported that *P. xylostella* is a more important pest in tropical areas than in a temperate climate. This pest has a high number of generations per year in tropical areas; 20 in Taiwan and 28 in Malaysia (Miyata et al. 1986, Talekar and Shelton 1993). In Senegal (semi-urban Dakar area), *P. xylostella* larvae damage is most important in the dry season, probably due to many consecutive cabbage crops growing in this area for a long period of time. In the rainy season, vegetable farmers do not grow cabbages continuously.

A significant relationship was found between the immature stages of *P. xylostella* and the age of the cabbage. The number of young larval stages decreased on aged plants, whereas pupal stages increase considerably. According to Nofemela and Kfir (2005), the preponderance of younger individuals is an indication of a growing population, whereas the high incidence of older individuals is an indication of a declining population. This phenomenon is probably also due to the low attraction of old cabbages to ovipositing females because the glucosinolates produced by the cabbage decrease in concentration during tissue maturation (Hopkins et al. 1998, Spencer et al. 1999), and the effect of declining resource quality (Campos et al. 2006). According to Campos et al. (2003), plant ageing increased pre-imaginal mortality and reduced the larval development rate and fecundity.

Relationships were found between *P. xylostella* and parasitoid populations. Generally, the abundance of natural enemy populations increases with host populations (Elliott et al. 2002), and the parasitoid complexes develop more in relation to plant succession (Price 1973). Temperature can have considerable effects on host susceptibility and/or parasite virulence with parasitoids (Matthew and Blanford 2003). Our study showed that the temperatures in the dry season (20 to 25°C) were a favourable range for the development, survival, and reproduction of parasitoids particularly for *O. sokolowskii* (Wang et al. 1999).

However, the impact of parasitoids on *P. xylostella* populations was low. Parasitoid populations were not able to control this pest. Shepard et al. (1999) noted that in Southeast Asia, indigenous parasitoids of cabbage moth were not able to regulate populations of this pest. Many agro-ecosystems are unfavourable environments for natural enemies due to high levels of disturbance (Landis et al. 2000).

These results may be due to the particular location of the cabbage plots, but the importance of the selection pressures present in each agro-ecosystem and the effects of natural selection on the totality of viable species and the change in their behaviour during successive generations should be recognised. For example, *C. plutellae* populations control *P. xylostella* larval populations in South Africa (Smith and Villet 2002) and in some localities in Benin (Goudegnon et al. 2000), have decreased a few larval populations in Martinique Island (Smeralda 2000) and have had no incidence in Dakar Niayes.

Generally, the immediate environment of a cultivated plot is more influential (beneficial or not) on the population of pests than on natural enemy populations (Burel et al. 2000). Further studies in other environmental conditions in the field will be conducted to confirm the influence of the selection pressures on *P. xylostella* populations and their natural enemies in a cabbage crop agro-system. Despite four species of natural enemies, the low parasitism rates found on *P. xylostella* immature stages cannot control pest populations and may necessitate additional control measures.

## CONCLUSIONS

1. The present study showed that climatic factors influenced the dynamics of *P. xylostella* populations and their natural enemies. The density of pests increased when the temperature was low. Females of *P. xylostella* oviposit on relatively young cabbages where the presence of young larvae was important, whereas older

immature stages were mainly found in older cabbages.

2. Farmers can avoid chemical treatments 40 days after planting; the cabbages were not attractive to female pests. The limitation of these treatments promotes the survival of parasitoid fauna, despite its low observed incidence.
3. For treatments against larval populations, farmers should use bacterial insecticides, such as *Bacillus thuringiensis* (Bt) to control them. These products have no effect on natural enemies.

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# ARTICLE 2

## Life history traits of *Oomyzus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae), a parasitoid of the diamondback moth

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# Life history traits of *Oomyzus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae), a parasitoid of the diamondback moth

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In this study the life-history of *Oomyzus sokolowskii* (Kurdjimov), a parasitoid of the diamondback moth (DBM) *Plutella xylostella* (L. ) as characterized. The life cycle, adult size, fecundity, ovigeny, parthenogenesis, host age preference and host-searching behaviour by parasitoid females were studied under laboratory conditions. *Oomyzus sokolowskii*'s life cycle lasted 15. 6 days. Sexual dimorphism was recorded, with females being bigger than males. The species is synovigenic. The parasitism rate was significantly different between mated and unmated females, which implied that mating stimulated the behaviour of parasitism. Thelythokous parthenogenesis was not recorded. Females could parasitize all larval stages and pre-pupae, but the parasitism rate was higher in the fourth larval stages of DBM. The host-seeking behaviour was influenced by host spatial patchiness; *O. sokolowskii* females performed better when they were placed in a 7 $cm^3$ oviposition box. This study gives a better understanding of the life history traits of *O. sokolowskii*, which has been neglected in the biological control of DBM in tropical regions. The study suggests the use of *O. sokolowski* as a promising candidate for the management of DBM in cabbage in combination with other IPM strategies.

**Key words**: *Plutella xylostella*, sexual dimorphism, ovigeny, parthenogenesis, koinobiont, behaviour, biological control.

## INTRODUCTION

Parasitoids are insects that feed on the body of another insect or arthropod during the larval stage of their life cycle. The host organism will die as a result (Jervis *et al.* 2001). The study of parasitoid biology and behaviour was first motivated by their interest as auxiliaries in biological control programmes (van Alphen & Jervis 1996). The life-history traits are directly related to the organism's fitness, hence to its reproductive success and survival (Roitberg *et al.* 2001; Le Lann *et al.* 2011). For example, the hind tibia length is the best indicator of body size among parasitoids and is usually correlated with fitness (Riddick 2005; Da Rocha *et al.* 2007).

Parasitoids can be koinobiont, *i.e.* parasitoids whose larvae are associated with the development of their hosts and emerge at the end of their development, and idiobiont, which kill or paralyse their host at the time of parasitism and use the resources available at the time of oviposition (Quicke1997). Comparative studies on a wide number of species have shown that the mode of parasitoid development is correlated with parasitoid life history traits (Mayhew & Blackburn 1999; Jervis *et al.* 2003).

Most parasitic wasps reproduce sexually as well as parthenogenetically. Apart from a number of species or strains that reproduce by thelythokous parthenogenesis, most parasitic wasps reproduce by arrhenotokous parthenogenesis (Wenseleers & Billen 2000). In arrhenotokous species, mated females store sperm in their spermatheca and may have the ability to control the sex ratio of their offspring by modifying the proportion of fertilized eggs laid (Ratnieks & Keller 1998).

In parasitoids, egg production has been identified as an important life history trait (Rosenheim *et al.* 2000; Jervis *et al.* 2001). Flanders (1950) classified parasitic wasps into two groups: pro-ovigenic species whose eggs mature prior to laying, and synovigenic species that continue to mature eggs throughout their reproductive lives. Ovigeny is a concept that helps in the understanding of the evolution of life history strategies in insects, and it is measured by the ovigeny index which ranges

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from 0 to 1 and is defined as the ratio of the mature egg load at emergence (initial egg load) on maximum potential lifetime fecundity (Jervis *et al.* 2001).

Parasitoid foraging behaviour affects the number of successfully developing offspring (Mackauer & Völkl 1993; Godfray1994). For this reason, knowledge of parasitoid foraging behaviour can enhance the implementation of a successful biological control programme (Godfray 1994). To find their host, parasitoids may use chemical signals such as host sex pheromones (Leal *et al.* 1995) or aggregation pheromones (Yasuda & Tsurumachi 1995), or the volatile compounds produced by the infested plant (Choh *et al.* 2008; Kawazu *et al.* 2010).

*Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) is an important parasitoid and a potential biocontrol agent of the diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), a major pest of Brassicaceae (Fitton & Walker 1992). This gregarious parasitoid is adapted to high temperature conditions and has been introduced in tropical and subtropical zones to control DBM (Talekar & Hu 1996), where it has been recorded as an effective parasitoid (Ooi 1988; Liu *et al.* 1997). In Senegal, it is the most common parasitoid of DBM (Sall-Sy *et al.* 2004). Therefore, studies are needed to improve the knowledge of the biology and ecology of this beneficial for its integration in a DBM management programme in cabbage.

In this study the life history traits of *O. sokolowskii* were determined under laboratory conditions. These included the parasitoid development cycle, adult size, fecundity, ovigeny, parthenogenesis, host age preference and foraging behaviour of females. The establishment of these traits in *O. sokolowski* will be useful for the improvement of biocontrol strategies against DBM in tropical areas.

## MATERIAL AND METHODS

### *Insect rearing*

The study was conducted at the Entomology Laboratory of the Centre for International Cooperation in Agronomic Research in Development (CIRAD) in Montpellier, France.

The parasitoid population was obtained from parasitized pupae of DBM collected in 2011 from cabbage crops (*Brassica oleracea* L. var. *capitata*) in the Niayes area, situated in northwest Senegal (12°54′44″N 12°8′84″W, and at 189 m altitude).

Cultures of DBM were maintained by allowing the adult females to oviposit on brown mustard plants (*Brassica juncea* L. Czern. ) in oviposition boxes (50 cm × 50 cm × 50 cm). Egg clutches were collected every 24 hours. At hatching, larvae were placed on fresh leaves of cauliflower (*Brassica oleracea* L. var. *botrytis*). Mature larvae were transferred to new leaves in a large plastic box (28 cm × 27 cm × 8 cm) where they pupated. The pupae were collected daily. At emergence, adults were placed in oviposition boxes and fed with water and honey.

Cultures of *O. sokolowskii* were maintained by exposing fourth-instar DBM larvae to parasitoid females in a clear plastic container (5 cm high, 8 cm diameter). After 24 hours exposure, all larvae were removed and placed in an identical container. Adult parasitoids emerging from parasitized pupae were recovered and fed with honey.

All rearing and experiments were conducted at a constant 25 °C, 60 % relative humidity and 12L/12D photoperiod.

### *Development of* O. sokolowskii

Fifteen one-day-old females and 30 fourth-instar DBM larvae were placed in a clear plastic container (5 cm high, 8 cm diameter) daily. After 24 hours of exposure, all pupae were recovered and placed individually in clear plastic pill bottles (1 cm high, 3 cm diameter). To determine the immature stages of *O. sokolowskii*, 30 parasitized pupae were dissected and observed under a microscope. The development time in days of the parasitoid was measured from oviposition to adult eclosion.

### *Adult size and ovigeny*

The size of adult *O. sokolowskii* was assessed by measuring the length of the hind tibiae of 30 one-day-old males and 30 one-day-old females using a microscope equipped with an ocular micrometer.

The ovigeny index was calculated using the formula of Jervis *et al.* (2001). To determine the initial egg load, we used virgin females obtained by dissecting them as pupae out of parasitized DBM pupae and placing them in separate pill boxes (1cm × 3 cm). Upon emergence, 30 virgin females were dissected and the number of eggs in each was counted. Maximum potential lifetime fecundity was determined by presenting 30 one-day-old mated female parasitoids, each with two new fourth-instar DBM larvae every day until the

female died. After 24 hours of contact, formed pupae were placed individually in pill boxes. The number of eggs laid in host larvae and the duration of female oviposition were recorded. Each dead female was dissected and the number of eggs remaining was counted.

*Parthenogenesis*

Thirty parasitized DBM pupae were dissected to recover parasitoid pupae, and each was isolated in a pill box (1 cm high, 3 cm diameter). Thirty of the resulting unmated females were each presented with two fourth-instar DBM larvae. The same experiment was performed using 30 mated females. After 24 hours, each DBM larva that successfully pupated was placed individually in a pill box and monitored until the emergence of parasitoids or adult moths. Parasitoids were sexed, and the parasitism rate, the number of parasitoids produced and the sex ratio (% females) was calculated and compared with offspring of unmated and mated females.

*Host age preference*

Five stages of immature DBM (L2, L3, L4, prepupae, and pupae) were exposed to 24h-old unmated parasitoid females. Thirty individuals of one stage were exposed to 15 female parasitoids in a clear plastic container (5 cm high, 8 cm diameter). After 24 hours of exposure, the immature DBM were recovered and placed individually in pill boxes, where they were monitored until emergence. Five replicates were performed for each DBM stage. The parasitism rate was calculated from the number of parasitized larvae and pupae recovered.

*Foraging behaviour*

To study foraging behaviour, three different oviposition boxes; 3 $cm^3$ (A), 7 $cm^3$ (B) and 40 $cm^3$ (C) were used. In each box, one 24h-old female *O. sokolowskii* was exposed to two fourth-instar larvae of DBM for 24 hours. DBM pupae were placed individually in pill boxes, and were monitored until emergence. Ten replicates were performed for each oviposition box size. Parasitism rate, female productivity, number eggs laid per female, sex ratio (% female) and offspring development time were compared among the three oviposition boxes.

*Statistical analysis*

Data were normalized by logarithmic transformation before performing an analysis of variance (ANOVA) using Statview version 4. 55 (Statview 1996). The sizes of parasitoid adults, female productivity, parasitism rates, and progeny sex ratios from mated and unmated females were compared with *t*-tests. Parasitism rates of the different stages of DBM were analysed using one-way ANOVA. Female productivity, parasitism rate, sex ratio, and offspring development time from the three oviposition boxes were analysed using one-way ANOVA. Means were separated using the Student-Newman-Keuls test (XLSTAT software version 2012.1.01). The sex ratio (% female) was calculated using the formula by Silva-Torres *et al.* (2009). In all statistical analyses *P*-values < 0. 05 were considered significant.

## RESULTS

### Development of *O. sokolowskii*

The incubation period was 3.2 ± 20 days. The eggs were aggregated to each other forming one or more clusters of 5 to 20 units . Most eggs were located in the back of the host larvae but isolated eggs were sometimes observed. Vermiform larvae hatched three days after host parasitism and continued growing until filling the entire body of the host pupae. The duration of the larval stage ranged from four to seven days (mean 4.6 ± 0.24). The parasitoid prepupae were whitish with red eyes, and the pupae were dark. The nymphal stage lasted 7.8 days on average. Adults emerged from the pupae parasitized by drilling several holes through the host cuticle. Males and females of the parasitoid emerged simultaneously from DBM pupae. The overall development time of *O. sokolowskii* from egg to adult was 15.6 ± 0. 40 days (Fig. 1).

### Adult size and ovigeny

Females were significantly larger than males (Table 1), and the size difference was significant ($t$ = –8.71, d.f. = 58, $P$ < 0. 0001). After the emergence, *O. sokolowskii* females had an average initial egg load of 14 eggs. The average potential fecundity was four times higher. The ovigeny index of this species was 0.3 (Table 1).

### Parthenogenesis

The parasitism rate was significantly different between unmated and mated females ($t$ = 6.39, d.f. = 6, $P$ = 0. 0007). The mated females produced normal sexual offspring (males and females) while unmated females produced only males (Table 2).

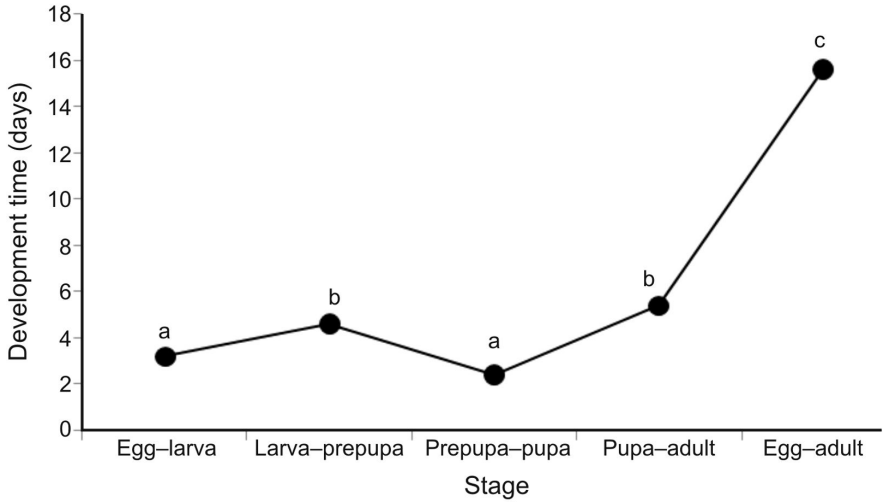


**Fig. 1**. Development time in days of *Oomyzus sokolowskii* from egg to adult at 25 °C. Different letters indicate significant differences (Student-Newman-Keuls, $P < 0.05$).

**Table 1**. Sexual dimorphism, fecundity and ovigeny index (mean ± S.E.) of *Oomyzus sokolowskii*.

| | Tibia length (mm) | Initial egg-load | Potential fecundity | Ovigeny index |
|---|---|---|---|---|
| Male | 0 3 ± 0 01 a | – | – | – |
| Female | 0 4 ± 0.01 b | 14. 1 ± 1 68 | 53. 6 ± 1. 60 | 0.3 |

Means in columns followed by the same letters are not significantly different (*t*-test, $P > 0.05$).

### Host age preference

The parasitism rate varied significantly with the host age ($F_{4.16} = 26.23$, $P < 0.0001$). It was significantly higher at the L4 larval stages ($P < 0.05$). However, there was no significant difference between L2 and L3 stages (Fisher, $P > 0.05$). The parasitism rate was significantly lower in prepupae and no parasitism was recorded on pupae (Table 3).

### Foraging behaviour

The parasitism rate was significantly different in the three oviposition boxes ($F_{2.18} = 15.87$, $P < 0.0001$). It was significantly higher in box B (Fisher, $P < 0.05$) than in the boxes A and C. Ten females laid in B, whereas in A and C, 3 and 1, respectively. The number of male and female offspring in box B was significantly different from those in boxes A and C ($F_{2.18} = 5.87$, $P = 0.008$, and $F_{2.18} = 10.00$, $P = 0.001$, respectively). Similarly, the total number of offspring was significantly higher in B ($F_{2.18} = 10.29$, $P = 0.001$). The sex ratio of offspring was not significantly different between the three oviposition boxes ($F_{2.18} = 1.42$, $P = 0.28$). The offspring development time was significantly higher in box C, not statistically different in A and B ($F_{2.18} = 9.01$, $P = 0.004$) (Table 4).

**Table 2**. Offspring productivity, parasitism rate and sex ratio (mean ± S.E.) between mated and unmated *Oomyzus sokolowskii* females.

| | Males | Females | Total progeny | Parasitism (%) | Sex ratio |
|---|---|---|---|---|---|
| Mated female | 1. 8 ± 0.41 a | 8. 4 ± 0.73 a | 10.2 ± 1.04 a | 45. 6 ± 3. 92 a | 83. 0 ± 2. 03 a |
| Unmated female | 10.3 ± 0.87 b | 0.0 ± 0.00 b | 10.3 ± 0.86 a | 12. 2 ± 0.14 b | 0.0 ± 0.00 b |

Means in columns followed by the same letters are not significantly different (*t*-test, $P > 0.05$).
Sex ratio corresponding to percentage females.

**Table 3**. Host age preference (immature DBM stages) of female *Oomyzus sokolowskii*.

| Host age (instars) | Parasitism (%) | Range (%) |
|---|---|---|
| Second | 39.9 ± 7.57 b | 23.3–63.3 |
| Third | 54.7 ± 8.71 b | 23.3–73.3 |
| Fourth | 75.9 ± 2.43 c | 70.0–83.3 |
| Prepupa | 15.3 ± 5.86 a | 0.0–36.7 |
| Pupa | 0.0 ± 0.00 a | 0.0–0.0 |

Means (± S.E.) in columns followed by the same letters are not significantly different according to the Student-Newman-Keuls test.

## DISCUSSION

*Oomyzus sokolowskii* is a larval-pupal endoparasitoid and its life cycle takes place entirely inside its host larvae, *P. xylostella*. Males and females emerge simultaneously, unlike in *Tetrastichus howardi* (Olliff) another Eulophidae parasitoid where females are the first to emerge (Birot *et al.* 1999). Under our experimental conditions, the cycle duration was 15.6 days, which is similar to the development time reported by Wang *et al.* (1999).

According to La Barbera (1989), sexual dimorphism in respect to size is commonly observed in the animal kingdom, including wasps. Our study confirms that females of *O. sokolowskii* are generally bigger than males. These results have been reported in many species of hymenoptera. For example, Bokonon-Ganta *et al.* (1995) and Aruna & Manjunath (2010) showed that females of *Anagyrus mangicola* (Noyes) (Hymenoptera: Encyrtidae) and *Nesolynx thumus* (Girault) (Hymenoptera: Eulophidae) are bigger than males. Mackauer & Sequeira (1993) and Thompson (1993) attributed the sexual dimorphism in the hymenopteran parasitoids to their development time and to the efficiency of their metabolism. The males emerge first and are smaller than females, though there are some exceptions (Harvey & Strand 2003; Da Rocha *et al.* 2007). The size of a parasitoid is considered one of the most important life-history traits contributing to the success of biological control of a pest (Aruna & Manjunath 2010). Moreover, it is related to other relevant factors such as fecundity, longevity and their fitness (Jervis & Copland 1996; Eijs & van Alphen 1999; Mayhew & Glaizot 2001).

Our results showed that mated females and virgin females produced the same number of offspring. They are similar to those reported by Metzger *et al.* (2008) on *Venturia canescens* Gravenhorst (Hymenoptera: Ichneumonidae) which produced the same number of offspring.

After emergence, *O. sokolowskii* females have few eggs to lay. However, they do not seem to be limited in the number of eggs for the first attack. Our results also show that female parasitoids continue maturing eggs throughout their reproductive life. According to Jervis *et al.* (2001), the number of eggs that a female lays is determined by the interaction of three factors: the number of suitable hosts encountered, the number of mature eggs during the female's life and the behavioural manipulation of the rate of egg laying. The work of Ellers & Jervis (2003) on parasitic wasps revealed an initial egg load between 15 and 435 eggs and potential lifetime fecundity between 40 and 835 eggs. In *Oomyzus sokolowskii*, the ovigeny index was less than unity; thus, it is a synovigenic species according to the classification of Jervis *et al.* (2001). However, the average number of eggs corresponding to the *O. sokolowskii* lifetime seems relatively low as compared to those described by Eller & Jervis (2003). Mayhew & Blackburn (1999) have shown that koinobiont species have a shorter adult lifetime, higher fertility levels and a greater oviposition rate than idiobiont species. According to Jervis *et al.* (2001), the ovigeny index seems to be related to the mode of development of the parasitoid. Koinobiont parasitoids tend to have an

**Table 4**. Oviposition box volume effect on the parasitism percentage and *Oomyzus sokolowskii* female production (mean ± S.E.).

| Laying box | % Parasitism | Females laid | Males | Females | Total adults | Cycles (days) | Sex ratio |
|---|---|---|---|---|---|---|---|
| 3(A) | 30.0 ± 15.31 b | 3 | 0.7 ± 0.34 a | 5.9 ± 3.01 a | 6.6 ± 3.43 a | 14.3 ± 0.35 a | 89.5 ± 0.84 a |
| 7(B) | 85.5 ± 7.60 c | 10 | 2.1 ± 0.63 b | 13.6 ± 1.70 b | 15.7 ± 2.04 b | 15.7 ± 0.30 a | 86.8 ± 2.36 a |
| 40(C) | 5.0 ± 3.93 a | 1 | 0.2 ± 0.12 a | 0.8 ± 0.54 a | 1.0 ± 0.61 a | 18.0 ± 0.04 b | 80.0 ± 0.02 a |

Means in columns followed by the same letters are not significantly different according to the Student-Newman-Keuls test.
Sex ratio corresponding to percentage females.

ovigeny index on average higher than idiobiont species. However, *O. sokolowskii* is a koinobiont gregarious larval-pupal parasitoid. According to Talekar & Hu (1996) and Wang *et al.* (1999), *O. sokolowskii* parasitizes all larval stages and even the prepupae of DBM, which is confirmed by our study. Koinobiont parasitoids have developed various adaptive mechanisms to operate a wide range of host stages, such as altering the host's behaviour (Slansky 1986), manipulating the host's development (Vinson & Iwantsch 1980) and controlling the host's immune responses (Strand & Pech 1995). This mode allows parasitoid development to increase diverted food resources. Koinobiont species can manipulate their physiology and feeding behaviour of their host to their advantage (Harvey *et al.* 1999). Strand (2000) found that koinobiont parasitoids have developed strategies to make the host resources more predictable.

The parasitism rate is higher in the fourth larval stages of DBM. Nakamura & Noda (2001) found that the larval stages were more appropriate for *O. sokolowskii* because they produce more interference than other stages. This could be explained by the increase in resources in the larger larvae. According to Harvey *et al.* (2004) large hosts are more profitable than small ones because they have more resources available for development of the parasitoid offspring. Older larval stages are preferable because they are likely to escape superparasitism close to pupation (Silva Torres *et al.* 2009).

Several authors have shown that volatile chemicals such as kairomones, pheromones or allomones from the host can influence the parasitoid's behaviour (Mattiacci *et al.* 2000; van Alphen *et al.* 2003). The stimuli diffuse through the air and the receivers are sensitive to very small amounts of products (Roux *et al.* 2007). In most parasitoids, patch quality is closely related to the proportion of good (unparasitized hosts) and bad (parasitized hosts). Parasitoid insects are able to evaluate the quality of the patch in which they are currently searching for hosts and the travel time between patches (Pierre *et al.* 2002). Insects may also rely on chemoreceptors found on the antennae, mouthparts, ovipositor and tarsi for host detection (Frings & Frings 1949; Weseloh 1972; Barlin & Vinson 1981). Therefore, host distribution on a given substrate may play an important role in the success of foraging and parasitism. Female *O. sokolowskii* seem more sensitive to the presence of the host in the 7 $cm^3$ box. In the 3 $cm^3$ box a saturation of chemoreceptors of the female due to a high concentration of chemical signals from the host may explain the decrease in the female ovipositions, resulting in a lower parasitism rate. Otherwise, a low concentration of chemical signals in the 40 $cm^3$ box could be the cause of the low parasitism rate and oviposition, resulting in lower female productivity. The behavioural process of host-seeking by females of *O. sokolowskii* is an important factor that determines the number of offspring of a female; therefore, it is directly linked to the success of reproduction and to fitness (Godfray 1994).

The results presented in this study provide valuable information on some life history traits of *O. sokolowskii*, which could be exploited efficiently for the management of DBM in Africa. For instance, in laboratory mass-rearing programmes, it is advisable to maintain *O. sokolowski* with late larval stages of DBM in adequate containers. In the case of mass-release programmes, the study encourages the release of mated females, which are shown to be more efficient in host foraging. Therefore, *O. sokolowskii* stands as a promising candidate for the control of DBM in Senegal and other tropical areas.

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# ARTICLE 3

## Performance of the parasitoid *Oomyzus sokolowskii* (Hymenoptera: Eulophidae) on its host *Plutella xylostella* (Lepidoptera: Plutellidae) under laboratory conditions

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# Performance of the parasitoid *Oomyzus sokolowskii* (Hymenoptera: Eulophidae) on its host *Plutella xylostella* (Lepidoptera: Plutellidae) under laboratory conditions

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**Abstract.** *Oomyzus sokolowskii* (Kurdjumov) is a gregarious larval–pupal parasitoid of the diamondback moth *Plutella xylostella* (L.). The objective of this study was to investigate the interactions between host and parasitoid by examining the effects of biotic factors such as gregariousness, host origin and stages, and female parasitoid age on the parasitism rate, developmental time, the number of offspring and the offspring sex ratio of *O. sokolowskii* under laboratory conditions. The percentage of parasitism and the number of parasitoids increased with the number of *O. sokolowskii* females. *Oomyzus sokolowskii* preferred fourth larval instars over other larval stages. The parasitism rate and the progeny production of *O. sokolowskii* decreased with parasitoid age; however, the developmental time and the sex ratio of the offspring were not significantly different. Our results confirm previous findings on larval preferences of *O. sokolowskii*. The study also confirmed the importance of geographical origin of the host on the performance of *O. sokolowskii*.

**Key words:** parasitoids, *Plutella xylostella*, cabbage, rearing, parasitism rate, host stage, biological control

## Introduction

Cabbage is an important agricultural crop that occupies a central role in the economy of many countries, especially in Asia and Africa (Grzywacz *et al.*, 2010). The diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is an oligophagous pest, considered as the most important source of crop losses in the Brassicaceae family (van Loon *et al.*, 2002; Shelton, 2004; Sarfraz *et al.*, 2006). Annual costs for pest management expenses are estimated at US$ 1 billion (Grzywacz *et al.*, 2010) exclusively for synthetic insecticide applications. Complete dependence on a chemical-based strategy for crop protection is, however, not sustainable. Also, the DBM has developed resistance to many synthetic insecticides and biopesticides, including *Bacillus thuringiensis* (Berliner) formulations (Liu *et al.*, 1997; Zhou *et al.*, 2011). As a result, attention has been directed towards alternative methods that could be used as components of integrated pest management systems.

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Biological control using parasitoids (Lim, 1992) is an alternative to chemical control. Among other natural enemies of DBM, the larval parasitoid *Oomyzus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae) is one of the most important endoparasitoids, used as a biological control agent for DBM management in various parts of the world (Wang *et al.*, 1999; Ferreira *et al.*, 2003).

Efficient biological control is only possible through knowledge of the biology and ecology of both the pest and its natural enemies (Andow *et al.*, 1997; Martinez-Castillo *et al.*, 2002). DBM populations from different localities present differences in biological behaviour (Arvanitakis *et al.*, 2002; Kirk *et al.*, 2004). Previous studies have demonstrated the existence of genetic variations between various DBM populations (Pichon *et al.*, 2004; Roux *et al.*, 2007). A parasitoid host can affect its performance, especially in gregarious species (Silva-Torres *et al.*, 2009*b*). To date, little is known about the performance of *O. sokolowskii*, taking into account those biotic factors. The knowledge of such differences could be used in biological control or in conservation biological control programmes against the DBM.

Hence, the aim of this study was to describe the interactions between *P. xylostella* larvae and *O. sokolowskii* females under laboratory conditions. We focused on the effect of gregarious behaviour, host origin and stages, and female parasitoid age on the parasitism rate, developmental time, the number of offspring and the sex ratio of *O. sokolowskii*.

## Materials and methods

### *Study site*

The experiments were conducted at the Laboratory of Entomology for International Cooperation in Agronomic Research for Development Centre (CIRAD) in Montpellier, France.

### *Insect rearing*

DBM larvae were previously collected from cabbage plantations (*Brassica oleracea* var. *capitata* L.) in the Niayes area in Senegal (12°54′44″N and 12°8′84″NW; 189 m elevation). The colony was maintained on Chinese mustard (*Brassica juncea* (L.) Czern.), where gravid females were allowed to oviposit. Emerging larvae were placed on the fresh leaves of cauliflower (*B. oleracea* var. *botrytis* L.) for their development. The larvae were later transferred onto the fresh leaves placed on the bottom of a large transparent plastic box (28 × 26 × 15 cm), for pupation, and pupae were collected daily. Newly emerged adults were placed in cages (50 × 50 × 50 cm) and fed with water and honey.

The *O. sokolowskii* colony was obtained from parasitized DBM pupae collected from cabbage plantations in Pikine (near Dakar), Senegal. The rearing consisted of exposing fourth-instar DBM larvae to parasitoid females in transparent plastic boxes (8 × 5 cm) for oviposition for 24 h. The DBM larvae were removed after parasitism and placed in identical boxes for their development until pupation. Fresh leaves of cauliflower were provided as source of food. Adult parasitoids emerging from parasitized pupae were collected and maintained in a plastic box and fed with honey drops deposited on the mesh cover.

Climatic conditions of all insect rearing were maintained at 25 ± 1°C temperature, 60 ± 5% relative humidity and 12 h light-12 h dark photoperiod.

### *Gregariousness of O. sokolowskii*

Females of *O. sokolowskii* (24 h old) were used in this experiment. In brief, 1, 5, 10, 15 and 20 females were placed, respectively, with 2, 10, 20, 30 and 40 fourth-instar DBM larvae (from Senegal) for 24 h. The number of host larvae was increased to avoid the risk of superparasitism. The larvae were then transferred individually into clear plastic pill boxes (3.5 × 1 cm) with a leaf disc of cabbage as food supply, and monitored daily until the emergence of adult parasitoids. The parasitism rate was calculated from the number of parasitized larvae and pupae. The developmental time (from egg to adult), and the number of offspring and the sex ratio were also compared in each cohort. The experiment was replicated seven times.

### *Host origin effects*

Two populations of *P. xylostella* were used in this experiment: a population from Senegal (from the Niayes area) and another one from Martinique Island (Saint Pierre, 14°44′30″N and 61°10′33″W; 1200 m elevation). For each population, the size of the pupae was measured using a dissecting microscope equipped with an ocular micrometer. Thirty pupae were used for each DBM population.

Fifteen 24-h-old *O. sokolowskii* females were exposed to 30 fourth-instar DBM larvae from each of the regions. After 24 h of exposure, all larvae were collected and placed individually in clear plastic pill boxes and followed up until the emergence of adult parasitoids. The parasitism rate was calculated from the number of parasitized larvae and pupae of each population. The developmental time (from egg to adult), and the number and sex ratio (% females) of the offspring were calculated. Bioassays were replicated seven times.

### *DBM stage effects*

Thirty DBM larvae (second, third and fourth), pre-pupae and pupae were exposed to 15 *O. sokolowskii* females in a clear plastic pill box for 24 h. The DBM population in this experiment was native to Senegal. Honey drips were deposited on the cover mesh as food supply. Parasitized larvae were transferred separately into pill boxes, with a leaf disc of cabbage as food supply. The newly formed pupae were monitored until the emergence of parasitoid adults. The parasitism rate for each DBM stage was calculated from the number of parasitized pupae. The developmental time (from egg to adult), the number of offspring and the sex ratio (% female) of parasitoids were compared among the different host stages. The experiment was replicated 10 times for each DBM stage.

### *Effect of female parasitoid age*

Fifteen 1-, 5-, 15- and 28-day-old *O. sokolowskii* females were, respectively, exposed to 30 fourth-instar DBM larvae for 24 h. The larvae were collected and transferred individually into pill boxes. They were monitored until the emergence of parasitoids. The parasitism rate, developmental time, the number of offspring and the sex ratio (% females) were compared. For each cohort, the experiment was replicated five times.

### *Statistical analysis*

Data were normalized by logarithmic transformation before being subjected to an analysis of variance (ANOVA). Parasitism rate, the total number of adult offspring, developmental time and offspring sex ratios were compared for the different treatments using one-way ANOVA. Means were separated using the Student–Newman–Keuls test (XLSTAT software, version 2012.1.01). Parasitism rate, developmental time, parasitoid offspring number and sex ratio between host populations of different origins were compared with *t*-tests (STATVIEW, 1996). The sex ratio was calculated using the Silva-Torres *et al.* (2009*b*) formula as the proportion of females. In all statistical analyses, the level of significance was kept at 5%.

## Results

### *Gregariousness of O. sokolowskii*

The parasitism rate varied significantly according to the number of females ($F_{(4,24)} = 11.35$; $P < 0.0001$; Table 1). The parasitism rate was significantly lower at one female parasitoid and increased as the number of females increased. However, there were no significant differences between the 5, 10, 15 and 20 females ($P < 0.05$). There were significant differences between the number of female parasitoids on the number of adults ($F_{(4,24)} = 17.80$; $P < 0.0001$), and the number of females ($F_{(4,24)} = 16.50$; $P < 0.0001$). A single female produced fewer offspring than grouped females. The developmental time and the sex ratio of the progeny were not significantly affected by the number of female parasitoids ($F_{(4,24)} = 1.32$; $P = 0.29$ and $F_{(4,24)} = 3.94$; $P = 0.05$, respectively; Table 1).

### *Host origin effects*

The difference in size between DBM pupae from Senegal and DBM pupae from Martinique was significant ($t = 6.091$; degree of freedom (df) = 58; $P < 0.0001$). DBM populations from Senegal were morphologically bigger than those from Martinique (Table 2).

There was a significant difference in parasitism rate between DBM populations from Senegal and Martinique ($t = -2.62$; df = 12; $P = 0.022$; Table 3). However, there was no significant difference in the total production of adults and the production of females ($t = -1.31$; df = 12; $P = 0.215$ and $t = -0.52$; df = 12; $P = 0.61$, respectively). The number of males in the offspring was significantly different between the two populations ($t = -2.68$; df = 12; $P = 0.02$). There was no significant difference in developmental time between the two populations ($t = 0.60$; df = 12; $P = 0.56$). The sex ratio was significantly different between the two populations ($t = 2.52$; df = 12; $P = 0.026$; Table 3).

**Table 1.** Mean (±SE) parasitism rate, productivity, developmental time and sex ratio (% female) of *Oomyzus sokolowskii* on DBM larvae

| Number of females | Parasitism (%) | Females | Total number | Cycle (days) | Sex ratio (%) |
|---|---|---|---|---|---|
| 1 | 14.30 ± 4.27b | 1.30 ± 1.28b | 1.45 ± 1.42b | 15.00 ± 0.30a | 90.00 ± 0.04a |
| 5 | 52.86 ± 6.80a | 8.57 ± 0.72a | 9.86 ± 0.80a | 15.46 ± 0.26a | 86.91 ± 1.47a |
| 10 | 71.43 ± 5.64a | 9.72 ± 0.47a | 12.29 ± 0.68a | 15.19 ± 0.12a | 79.30 ± 1.51a |
| 15 | 74.29 ± 3.47a | 10.43 ± 0.65a | 12.57 ± 0.87a | 15.21 ± 0.10a | 83.30 ± 1.18a |
| 20 | 77.90 ± 3.43a | 10.71 ± 1.36a | 13.14 ± 1.67a | 15.10 ± 0.14a | 81.64 ± 1.35a |

Means in columns followed by the same letters are not significantly different ($P > 0.05$; Student–Newman–Keuls test).

**Table 2.** Mean size of DBM pupae (mean ± SE) from different geographic origins

| Host origin | Size (mm) | Range (mm) |
|---|---|---|
| Senegal | 6.14 ± 0.05a | 5.53–6.67 |
| Martinique | 5.65 ± 0.06b | 4.76–6.33 |

Means in columns followed by the same letter are not significantly different ($P > 0.05$; *t*-test).

### *DBM stage effects*

The parasitism rate varied significantly according to host stage ($F_{(4,36)} = 26.23$; $P < 0.0001$). It was higher in L4 larvae and lower in pre-pupae by 75.9 and 15.3%, respectively. However, there were no significant differences between the L2 and L3 larval stages (Table 4). Similarly, there were no significant differences in the total production of adults ($F_{(3,27)} = 0.50$; $P = 0.68$) and females ($F_{(3,27)} = 0.69$; $P = 0.57$) between the host stages. Developmental time and sex ratio were not different between the host stages ($F_{(3,27)} = 1.39$; $P = 0.28$ and $F_{(3,27)} = 0.56$; $P = 0.65$, respectively; Table 4).

### *Effect of female parasitoid age*

The parasitism rate was significantly affected by the age of *O. sokolowskii* females ($F_{(3,12)} = 21.32$; $P < 0.0001$; Table 5). It was higher in 1- and 5-day-old females. The number of females was significantly affected by the age of female parasitoids ($F_{(3,12)} = 4.70$; $P = 0.02$); it was higher in 5-day-old female parasitoids. The number of adults was also significantly affected ($F_{(3,12)} = 4.24$; $P = 0.03$). However, the developmental time and the sex ratio were not affected by the age of female parasitoids ($F_{(3,12)} = 1.01$; $P = 0.42$ and $F_{(3,12)} = 1.55$; $P = 0.25$, respectively).

## Discussion

Knowledge on the biology and ecology of biological control agents is of paramount importance in the management of pests such as DBM. Our results suggest that gregariousness promotes increased parasitism rates and increased number of offspring per female. In fact, gregariousness is related to superparasitism, which, in turn, favours a higher number of offspring (Gu *et al.*, 2003; Silva-Torres and Matthews, 2003; Keasar *et al.*, 2006). According to Silva-Torres *et al.* (2009*b*), gregariousness and superparasitism can adversely affect parasitoid fitness. These behaviours appear to be of advantage to the parasitoid (Yamada and Miyamoto, 1998). In our study, the parasitism rate and the number of offspring increased with the number of parasitoid females. These results corroborate those of Hirashima *et al.* (1990) who found that host availability was a favourable factor to high parasitism rates. The proportion of males in the offspring also increased with the number of female parasitoids. These results are similar to those reported by Chen *et al.* (2008).

The DBM population from Senegal seemed to be of higher fitness than the population from Martinique. According to Chown *et al.* (2009), large-sized insects have a higher chance of survival and reproduction. In our study, the parasitism rate was significantly higher in the population from Martinique; however, female productivity was similar in both populations. Our results do not seem to be in agreement with previous findings. Edwards (1954) has reported the effect of host size. The author discovered that most female parasitoids, especially in gregarious species, first estimate the size of the host before ovipositing. This trait is one of the most important indicators used by female parasitoids for reproduction (Aruna and Manjunath, 2010). Zaviezo and Mills (2000) made the same assertion; the offspring production in gregarious parasitoids is often correlated with host size. In our results, DBM pupae from Senegal are larger than the DBM from Martinique, which would imply that the female parasitoid would oviposit more in Senegalese larvae. Yet, DBM individuals from Senegal, which were bigger in size, were less parasitized (−15%). Although the number of females was similar, the number of males in the offspring was higher in DBM individuals from Martinique. As a result, the sex ratio was higher in Senegalese DBM, which implies that *O. sokolowskii* could be a more efficient biological control agent in Senegal than in Martinique. In addition to the difference in size between the DBM populations, the difference in altitude between the regions could explain the difference in the performance of

**Table 3.** Effect of host origin on the parasitism rate, progeny, developmental time and sex ratio (mean ± SE) of *Oomyzus sokolowskii*

| Host origin | Parasitism (%) | Females | Total progeny | Cycle (days) | Sex ratio (%) |
|---|---|---|---|---|---|
| Senegal | 65.94 ± 4.85b | 9.71 ± 0.61a | 11.57 ± 0.78a | 15.56 ± 0.21a | 84.23 ± 1.56a |
| Martinique | 81.43 ± 3.36a | 10.14 ± 0.55a | 12.86 ± 0.60a | 15.37 ± 0.22a | 78.76 ± 1.51b |

Means in columns followed by the same letters are not significantly different ($P > 0.05$; *t*-test).

**Table 4.** Effect of host stages on the parasitism rate, progeny, developmental time and sex ratio of the DBM parasitoid *Oomyzus sokolowskii*

| Instars | Parasitism (%) | Females | Total progeny | Cycle (days) | Sex ratio (%) |
|---|---|---|---|---|---|
| Second | 39.98 ± 7.60b | 10.40 ± 0.60a | 13.40 ± 1.03a | 17.58 ± 0.44a | 78.10 ± 1.74a |
| Third | 54.66 ± 8.74b | 10.20 ± 0.97a | 12.40 ± 1.03a | 16.14 ± 0.33a | 82.65 ± 5.04a |
| Fourth | 75.98 ± 2.45a | 14.40 ± 2.02a | 17.40 ± 3.30a | 15.08 ± 0.32a | 85.18 ± 3.51a |
| Pre-pupae | 15.32 ± 5.93c | 13.40 ± 4.53a | 17.70 ± 6.12a | 12.96 ± 3.24a | 81.80 ± 5.27a |
| Pupae | 0d | 0b | 0b | 0b | 0b |

Means in columns followed by the same letters are not significantly different ($P > 0.05$; Student–Newman–Keuls test).

*O. sokolowskii* (Shelly *et al.*, 2003). It has been shown that distributions of both hosts and their parasitoids are influenced by altitude (Sivinski *et al.*, 2000, 2004), which presumably is, in turn, due to temperature and moisture gradients.

The study revealed the importance of host stage as an important ecological parameter to be considered in the biology of parasitoids (Bai *et al.*, 1992; Godfray, 1994; Islam and Copland, 1997; Fidgen *et al.*, 2000). It has also been shown that the quality of the host affects the fitness of offspring in parasitoids (Godfray, 1994). Our results indicate that the parasitism rate was higher in the fourth larval stages of DBM, which seems to be the most suitable stage for the development of *O. sokolowskii*. Host stages of bigger size contain more resources for the parasitoid larval stages (Harvey *et al.*, 2004). These observations are similar to previous work reported by Talekar and Hu (1996).

There were no significant differences in the number of offspring, developmental time and progeny sex ratio of *O. sokolowskii* in relation to the age of host stages, corroborating findings of Wang *et al.* (1999), possibly due to the gregariousness and superparasitism behaviour of *O. sokolowskii*. Although there were differences in parasitism rate between the stages of DBM, potential competition for resources between larval parasitoids could affect the number of offspring. Gregariousness and superparasitism can adversely affect the number of offspring (Silva-Torres *et al.*, 2009*b*). Consequences of such behaviour could greatly affect the fitness of progeny. On the other hand, Nakamura and Noda (2002) reported that the number of *O. sokolowskii* tends to increase with host age and is significantly higher in the late fourth-stadium hosts. Yet, our results did not corroborate their findings.

The success of parasitism depends on the age of the female parasitoid (Medeiros *et al.*, 2000); generally, parasitism rate decreased with age as shown in our study that 5-day-old females lay more eggs in their host than older females. This decrease in productivity can be explained by a decrease in physiological activity (Giron and Casas, 2003). Silva-Torres *et al.* (2009*a*) showed that the parasitism rate was higher in 2- to 4-day-old females of *O. sokolowskii*. According to Rajapakse (1992), the ideal age for *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) to properly parasitize larvae of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) ranged from 48 to 96 h, whereas the ideal age of parasitism for *Ceratogramma etiennei* (Delvare) (Hymenoptera: Trichogrammatidae) varied from 1 to 2 days post-emergence (Amalin *et al.*, 2005). Our results are similar to the work of Silva-Torres *et al.* (2009*a*) and Chen *et al.* (2008), who showed that the progeny produced per female and the number of parasitoids emerging per host significantly decreased according to age. Similarly, Li *et al.* (1993) argued that offspring production from females of *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae) decreases with age. However, the developmental time and sex ratio of offspring were not different between the different age groups of the parasitoid. Our results are similar to the findings of Riddick (2003), who demonstrated that the age of *Anaphes iole* Girault (Hymenoptera: Mymaridae) does not affect the sex ratio of the parasitoid.

The parasitism rate tended to decrease drastically in the 5-days-old females, suggesting that beyond a certain period of maturity, *O. sokolowskii*

**Table 5.** Effect of female *Oomyzus sokolowskii* age on parasitism rate, the number of offspring, developmental time and sex ratio (mean ± SE)

| Female age (days) | Parasitism (%) | Females | Number | Cycle (days) | Sex ratio (%) |
|---|---|---|---|---|---|
| 1 | 82.10 ± 3.20a | 9.25 ± 0.25b | 11.25 ± 0.25ab | 15.34 ± 0.12a | 82.38 ± 3.26a |
| 5 | 71.38 ± 4.96a | 12.75 ± 1.60a | 15.50 ± 2.40a | 15.08 ± 0.42a | 83.43 ± 3.00a |
| 15 | 41.10 ± 4.23b | 8.00 ± 1.23b | 9.50 ± 1.50b | 15.75 ± 0.28a | 84.35 ± 1.05a |
| 28 | 31.35 ± 7.52b | 8.00 ± 0.41b | 9.00 ± 0.41b | 15.25 ± 0.25a | 88.85 ± 0.51a |

Means in columns followed by the same letters are not significantly different ($P > 0.05$; Student–Newman–Keuls test).

females are less efficient in controlling DBM populations. Therefore, biological control of DBM cannot rely entirely on the use of parasitoids when they are too old (Persad and Hoy, 2003; Amalin *et al.*, 2005), and other methods need to be envisaged.

## Conclusion

The present study confirms the importance of *O. sokolowskii* as a promising biological control agent that could be used in an augmentative approach for the management of DBM populations in cabbage production. However, performance could be affected by host geographical origin. Moreover, age of female parasitoids could be a limiting factor for parasitoid production; therefore, other parameters should be combined during DBM management. The results of this study show that it is necessary to know in real time the best period for parasitoid mass release to ensure successful parasitism of DBM populations.

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# CHAPITRE III

## Interaction entre *Plutella xylostella* et le parasitoïde *Cotesia vestalis*

# 1. Introduction

L'espèce *Cotesia vestalis* (Haliday) a subi des modifications taxonomiques et dans les articles elle peut avoir été dénommée *Cotesia plutellae* (Kurdjumov). Les travaux présentés dans ce chapitre sont présentés ici sous la forme de trois articles, dont un soumis et deux publiés.

Une étude a été réalisée dans un premier temps en plein champ au Bénin et présentée dans l'article 4. Nous avons voulu déterminer quels sont les ennemis naturels de *P. xylostella* qui peuvent jouer un rôle dans un environnement tropical, et comprendre quand et comment l'interaction hôte-parasitoïde pouvait influencer la dynamique des populations de *P. xylostella* en l'absence d'insecticide. En second lieu, nous avons voulu comparer l'impact direct des parasitoïdes avec celui indirect des conditions abiotiques tropicales, puisque les précipitations sont, d'après plusieurs auteurs, un facteur important de contrôle de la teigne. Pour atteindre ces objectifs, nous avons étudié pendant 39 mois consécutifs dans la zone périurbaine de Cotonou (Bénin), les variations d'abondance de *P. xylostella*, dans un champ de choux pommés non traités, ainsi que celles des autres ravageurs également inféodés aux Brassicacées et de leurs ennemis naturels.

Ensuite, nous nous sommes intéressés au système de reconnaissance qui lie *C. vestalis* à *P. xylostella,* en conditions de laboratoire. Cette étude porte d'abord sur le mode de détection et d'identification de l'hôte par le parasitoïde en recherchant chez *C. vestalis* quels sont les organes impliqués (article 5)**,** puis nous avons tenté de déterminer la nature exacte du stimulus permettant cette identification et l'initiation de l'oviposition (article 6).

En effet, pour qu'un parasitoïde réussisse son infestation et son développement, il est communément admis que plusieurs étapes chronologiques doivent être franchies avec succès (Doutt 1959 ; Vinson 1976). Nous nous sommes intéressés plus particulièrement à la phase qui correspond à la perception par une femelle, de la série de stimuli qui vont lui permettre de réduire progressivement son aire de recherche pour aboutir à la découverte d'un hôte et à son acceptation en tant que site de ponte (on parle alors d'oviposition). Les étapes de cette première phase, qualifiées de pré-ovipositionnelles, sont très importantes car elles dépendent du comportement des femelles adultes (Vinson 1981) et conditionnent l'impact du parasitisme sur la dynamique des populations hôtes.

Bien qu'une préférence ait été démontrée chez des femelles de *C. vestalis* d'une part pour l'odeur émise par le chou et par un régurgitât de chenille de *P. xylostella* (Shiojiri et al. 2000 a, b, 2001) et d'autre part par l'odeur des sécrétions laissée sur la feuille (Reddy et al. 2002), les mécanismes de reconnaissance au contact de l'hôte restent à ce jour encore mal connus.

Décrite comme spécialiste, cette espèce devrait pouvoir avec exactitude localiser et surtout reconnaitre son hôte. Pour comprendre le mécanisme par lequel les femelles effectuent cette tâche, nous avons procédé par étapes:

- (1) Observation du comportement de recherche et de reconnaissance à l'aide d'enregistrements vidéo afin de déterminer quels stimuli pouvaient être à l'origine du réflexe d'oviposition,
- (2) Essai d'identification des différents organes sensoriels des femelles de *C. vestalis* impliqués dans la perception de ces stimuli,
- (3) Etude de l'équipement sensillaire de *C. vestalis* en observant en microscopie électronique à balayage les antennes des mâles et des femelles.

Dans un deuxième temps, nous avons tenté de déterminer la nature chimique exacte du stimulus impliqué lors de la rencontre physique entre *C. vestalis* et *P. xylostella*.

Selon Nelson & Charlet (2003), les substances impliquées dans la reconnaissance de l'hôte par les parasitoïdes sont des lipides cuticulaires non volatils. Vinson (1976) annonçait déjà que des hydrocarbures cuticulaires étaient les substances principalement impliquées dans ces phénomènes. Pour vérifier cela, nous avons analysé par chromatographie à phase gazeuse couplée à une spectrométrie de masse (GC-SM) un extrait cuticulaire et réalisé une série de tests comportementaux visant à tester les réactions du parasitoïde vis-à-vis d'extraits cuticulaires de la chenille hôte appliqués sur des leurres (chenille non-hôte).

## 2. Etude de terrain : le Bénin

### 2.1. Présentation générale

Le Bénin (Fig. 14) se situe dans la zone intertropicale de l'Afrique de l'Ouest qualifiée de « Diagonale de sécheresse », caractérisée par la faiblesse relative des précipitations annuelles. De forme étirée entre le fleuve Niger au nord et la plaine côtière dans le sud, le relief de l'ensemble du pays est peu accidenté. Le nord du pays est principalement constitué de savane et de montagnes semi-arides. Le sud du pays est constitué d'une plaine côtière basse parsemée de marécages, lacs et lagunes. C'est un petit pays d'une longueur de 700 km et d'une largeur de 120 km au sud et de 300 km au nord. Le climat varie fortement du sud au nord. Le sud a un climat subéquatorial (type Guinéen) qui se caractérise par une forte humidité (1 200 mm de pluie par an), par deux saisons sèches et deux saisons pluvieuses (avril à juillet et septembre à octobre) et par une température comprise entre 25°C et 30° C. Au nord, le climat est tropical (type Soudanien), marqué par des températures plus élevées pouvant atteindre 46° C, des précipitations annuelles plus faibles (900 mm de pluie) et par l'alternance de deux saisons, dont une pluvieuse (mai à octobre). La partie nord-ouest, occupée par la chaîne de l'Atacora, bénéficie d'un climat particulier où les températures sont plus fraîches et les précipitations plus élevées que dans le reste du pays.

Depuis une quinzaine d'année, le chou pommé est rentré dans la cuisine traditionnelle du Bénin et constitue une composante importante des régimes alimentaires quotidiens. Ces feuilles coriaces sont bien adaptées aux cuissons longues de cette cuisine. Ce légume est devenu incontournable et est particulièrement consommé en temps que met traditionnel pour la période de Noël. Les pommes de ces choux sont de petite taille comparées à celles des variétés cultivées en Europe. C'est une culture à haute valeur ajoutée actuellement en extension qui génère des sources importantes de revenus, particulièrement dans les zones urbaines et périurbaines. Cependant les opportunités économiques offertes par ces légumes sont souvent affaiblies par des dommages, provoqués par des nuisibles affectant leur production et leur commercialisation.

### 2.2. Statut de *Plutella xylostella* au Bénin

Au Bénin, *P. xylostella* est le principal ravageur des cultures de chou. L'ampleur des dégâts qu'il provoque peut aller jusqu'à la destruction totale de la culture (observations

personnelles). Les agriculteurs appliquent de manière inappropriée des pesticides, utilisés souvent pour les cultures de coton, qui sont de toute évidence inefficaces contre la teigne du chou. Au Bénin comme dans la plupart des autres pays africain, des stratégies alternatives à l'utilisation de pesticides sont nécessaires mais peu ou pas développées à ce jour. A propos de la lutte biologique, les agriculteurs béninois sont peu sensibilisés à ce mode de lutte car la plupart ne connaissent pas les différences qu'il y a entre un auxiliaire et un ravageur.

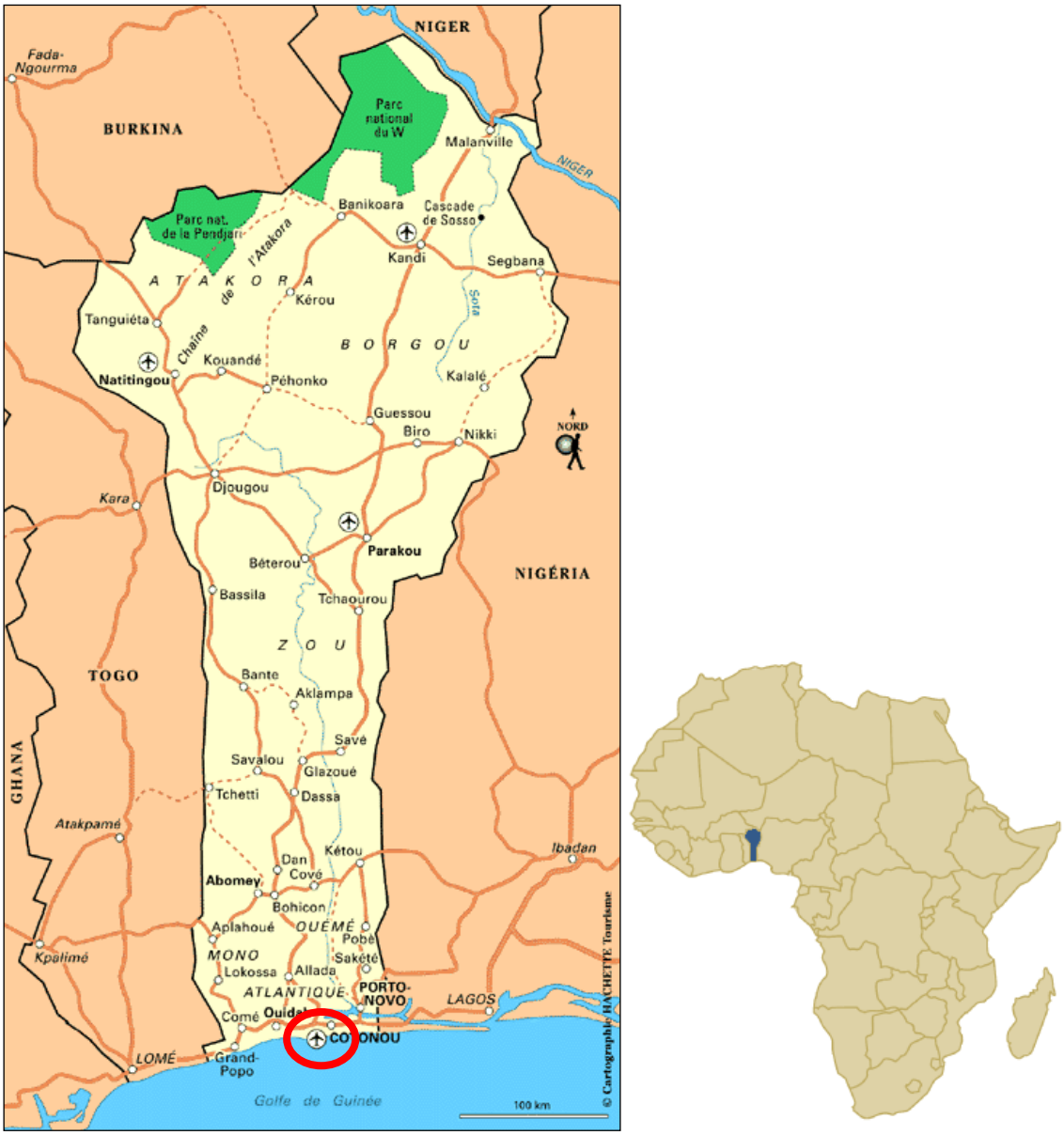


**Figure 14** : Carte du Bénin avec localisation (en rouge) du site d'étude dans la zone périurbaine de Cotonou

### 2.3. Site d'expérimentation

Notre étude s'est déroulée sur le site de Kouhounou (Fig. 15), dans la zone périurbaine de Cotonou. Nous avons effectué des prélèvements sur des parcelles de choux pommés non traités durant 39 mois consécutifs. Dans cette zone, les agriculteurs cultivent le chou toute l'année sans arrêt de culture.

**Figure 15** : Parcelle de choux sur le site de Kouhounou dans la zone périurbaine de Cotonou.

## 3. Synthèse des résultats

Nous avons pu établir la composition des communautés de ravageurs et d'ennemis naturels associées à la culture du chou au Bénin, dans la zone périurbaine de Cotonou. Le système hôte-parasitoïde comprend presque exclusivement *P. xylostella* et son parasitoïde larvaire *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae). Ces deux espèces ont des niveaux similaires d'abondance (en moyenne 7,5 ± 0,3 et 7,2 ± 0,3 individus par plante, respectivement). *Plutella xylostella* et *C. vestalis* ont montré des oscillations d'abondance couplées, avec un décalage d'environ deux semaines entre les pics de l'hôte et ceux du parasitoïde. Une forte abondance de parasitoïdes a entraîné une diminution significative de l'abondance de la teigne pendant plusieurs semaines. Toutefois, la population de parasitoïde diminuant à son tour n'a pas pu empêcher par la suite la remontée des effectifs de la teigne du chou. L'abondance de la teigne au cours des saisons n'est pas corrélée avec les variables météorologiques (précipitations et températures), même si de fortes précipitations durant la principale saison des pluies ont pu temporairement affecter le ravageur.

Des expériences d'ablation ont montré que les antennes jouaient un rôle prédominant dans l'induction de l'oviposition. L'analyse des séquences vidéo a révélé que l'oviposition ne pouvait avoir lieu sans un contact antennaire préalable. La femelle dispose ses antennes en crosse lors du comportement de recherche. Les observations en microscopie électronique à balayage ont révélé sept types de sensilles sur les antennes, parmi lesquels un type particulier est 4,5 fois plus présent chez les femelles que chez les mâles.

Le stimulus à l'origine de la réponse de la femelle parasitoïde (oviposition) est bien d'origine gustative. Les lipides cuticulaires des chenilles semblent impliqués puisque la femelle réagit à un extrait lipidique complet appliqué sur une chenille non-hôte. Le fractionnement de cet extrait inhibe l'effet des lipides cuticulaires car aucune des deux fractions engendrées (hydrocarbure et non-hydrocarbure) ne permet l'oviposition. L'analyse en chromatographie à phase gazeuse des deux fractions a révélé la présence de 44 composés. L'identification des composés cuticulaires en spectrométrie de masse n'a révélé que des produits très communs dans la fraction contenant les hydrocarbures, excepté un triterpenoïde apparenté à l'amyrine.

## 4. Conclusion

Aucun contrôle stable du ravageur n'a été observé durant la longue période de mesures effectuées. Dans les conditions tropicales du Bénin, les populations de *P. xylostella* croissent rapidement, avec une forte probabilité de recolonisation à partir des zones environnantes. La lutte biologique en utilisant un parasitoïde spécialiste bien établi, comme *C. vestalis,* montre ici ses limites et des recherches de mesures de contrôle supplémentaires paraissent nécessaires.

Seules les sensilles trichoïdes de type II révèlent un dimorphisme sexuel qui laisse sous-entendre qu'elles jouent un rôle tout particulier dans les processus menant à l'oviposition (localisation et identification de l'hôte). Leur absence chez d'autres espèces proches, comme *C. glomerata* et *C. rubecula*, suggère qu'elles sont spécifiques à l'espèce *C. vestalis*. Ces sensilles ont très certainement une fonction gustative (Vinson 1985 ; Schmidt & Smith 1989). Cependant, l'influence du contact chimique est difficile à séparer de l'influence du toucher et de la texture (Vinson 1976).

Nous avons pu identifier avec exactitude que le stimulus impliqué dans la reconnaissance de l'hôte et dans le déclenchement du réflexe d'ovipostion était bien d'origine gustative et qu'il était matérialisé par les lipides cuticulaires de la chenille hôte.

*Cotesia vestalis* est la seule espèce de parasitoïde rencontrée dans notre site d'étude dans le sud du Bénin. Malgré des abondances importantes et un fort taux de parasitisme, *C. vestalis* n'arrive pas à contrôler la teigne. Les précipitations ne sont pas un facteur de régulation des populations du ravageur. Nous avons pu mettre en évidence trois points importants du système de reconnaissance chez *C. vestalis* envers son hôte : (1) les femelles détectent et reconnaissent leur hôte à partir de leur signature chimique, composée par les lipides cuticulaires ; (2) le stimulus chimique constituant le signal de reconnaissance est composé de plusieurs molécules appartenant à deux classes de lipides et agissant en synergie ; (3) ce stimulus chimique est perçu par des sensilles gustatives implantées sur les antennes des femelles du parasitoïde.

# ARTICLE 4

## Incomplete control of the diamondback moth, *Plutella xylostella*, by the parasitoid *Cotesia vestalis* in a cabbage field under tropical conditions

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**Soumis à *BioControl***

# Incomplete control of the diamondback moth, *Plutella xylostella*, by the parasitoid *Cotesia vestalis* in a cabbage field under tropical conditions.

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## Abstract

Immature *Plutella xylostella* (Lepidoptera: Plutellidae) and parasitoids were sampled for 39 months in an unsprayed cabbage field near Cotonou, Benin, to determine how and when host-parasitoid interactions influence the population dynamics of the moth in a tropical environment. Eighty-three samples were taken at approximately two-week intervals. There were no seasonal patterns in moth abundance, which was not correlated with weather variables, although heavy rainfall during the principal rainy season may have temporarily affected the population. The host-parasitoid system consisted almost exclusively of *P. xylostella* and its larval parasitoid *Cotesia vestalis* (Hymenoptera: Braconidae), both species occurring at similar levels of abundance (on average 7.5 ± 0.3 and 7.2 ± 0.3 individuals per plant, respectively). The tendency for host-parasitoid dynamics to cycle was apparent in the field. *Plutella xylostella* and *C. vestalis* showed coupled oscillations in abundance, with a time lag of about two weeks between host and parasitoid peaks. High parasitoid abundance resulted in significant decreases in moth abundance over several weeks. However, the parasitoid population in turn decreased, could not prevent the moth from rebounding, and there was no stable control of the pest. We conclude that under tropical conditions in which *P. xylostella* populations grow rapidly, combined with a high probability of recolonization from surrounding areas, biological control by a well-established specialist parasitoid reaches its limits and additional control measures are necessary.

**Keywords**: *Plutella xylostella*, Plutellidae, *Cotesia vestalis,* Braconidae, Host-parasitoid dynamics, Tropical agro-ecosystems.

# INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the most destructive pest of Brassicaceae, especially *Brassica* vegetable crops, in many parts of the world (Furlong et al. 2013). Insecticide resistance is high in this species, which was the first crop pest to develop resistance to DDT and has now developed field resistance to a variety of products, including *Bacillus thuringiensis*-based products (Talekar and Shelton 1993; Furlong et al. 2013). This problematic situation has stimulated research activities on alternative methods of control, particularly on classical biological control programmes utilizing parasitoids.

Many species of parasitoid wasps (Hymenoptera) are known to attack *P. xylostella* (Delvare 2004; Sarfraz et al. 2005). Larval parasitoids in the genera *Cotesia* (Braconidae), *Diadegma* (Ichneumonidae) and *Microplitis* (Braconidae) have the greatest control potential, although a few prepupal and pupal parasitoids of the genus *Diadromus* (Ichneumonidae) also contribute to reduce populations of this pest (Sarfraz et al. 2005). Their impact on moths was clearly demonstrated in some studies. In South Africa, for example, Kfir (2004) showed that the suppression of parasitoids following the use of a selective insecticide in the field resulted in significantly higher levels of infestation by *P. xylostella*. In other studies, however, parasitism was insufficient to prevent severe outbreaks in unsprayed cabbage fields (Guilloux et al. 2003). Introductions of parasitoids against the diamondback moth in a number of countries also resulted in both successes and failures. Following releases of large numbers of the larval parasitoid *Cotesia vestalis* (Haliday) [= *C. plutellae* (Kurdjumov)] and the pupal parasitoid *Diadromus collaris* (Gravenhorst) on the island of St Helena, *P. xylostella* infestations remained so low that chemical control became unnecessary (Kfir 2011). However, unsuccessful introductions have also been reported and their causes are generally poorly understood (e.g. Mitchell et al. 1997; Upanisakorn et al. 2011). Failures have been mainly attributed to (1) the use of broad-spectrum insecticides that affect both the pest and its parasitoids; and (2) the inability of parasitoids to maintain abundant populations in the target population's environment, presumably due to the use of inappropriate species or ecotypes (Sarfraz et al. 2005; Kfir 2011; Furlong et al. 2013). However, host-parasitoid population dynamics is not straightforward, depends on many abiotic and biotic factors, and it is uncertain whether well-established populations of parasitoids necessarily translate into effective pest control (Vickers 2004). For example, host plant quality can indirectly influence

the development time and performance of specialist parasitoids (Sarfraz et al. 2009; Fathi et al. 2012), and obligate hyperparasitoids can limit the impact of primary parasitoids (Nofemela 2013). In Australia, Furlong et al. (2004) found largely unpredictable variations in the rates of parasitism among *P. xylostella* populations and concluded that more research was needed to understand the processes underlying the activity of natural enemies.

In the present study, we monitored changes in the abundance of the diamondback moth, other insect herbivores, and their natural enemies, in an unsprayed cabbage field in Cotonou, Benin, with the following objectives: (1) to determine which natural enemies of *P. xylostella* play a key role in this tropical area; (2) to clarify how and when host-parasitoid interactions can influence the population dynamics of *P. xylostella* in the absence of any insecticide; and (3) to compare the impact of parasitoids with the effects of hot and humid conditions in Benin, since rainfall has been reported to be an important factor of control of the moth in a number of studies (Guilloux et al. 2003; Kobori and Amano 2003; Tonnang et al. 2010). Some results of this field study were summarized elsewhere (Goudegnon et al. 2004) but the present paper gives the first detailed analysis of the data.

## MATERIAL AND METHODS

*Study site and sampling*

This study was conducted near Cotonou, Benin, in western tropical Africa, where the mean annual temperature is 27.2°C, with a low temperature range between the hottest month (28.9°C in March) and the coolest (25.6°C in August). Annual rainfall averages 1360 mm in the region, with two rainy seasons. The principal rainy season is from April to July, the peak rainfall occurring in June, and a less intense rainy period occurs in October. The driest months are December and January.

Cabbage (*Brassica oleracea* L.) is grown throughout the year in the periurban areas of Cotonou. Our study was conducted in an unsprayed cabbage field located at Kouhounou (6°24' N; 2°31' E), in which the KK Cross cultivar was grown in monoculture. Cultivation was done by local farmers who transplanted, watered and fertilized cabbages as usual, and were compensated for crop losses due to the presence of pests.

All lepidopteran larvae (from the second instar onwards) and pupae, as well as pupal cocoons of hymenopteran parasitoid wasps, were sampled over a 39-month period (January

1995-March 1998). Aphids (Hemiptera: Aphididae) and hoverfly larvae (Diptera: Syrphidae) were also sampled for 36 months over the same period (January 1995-December 1997). Eighty-three samples were taken, mostly at two-week intervals, with some temporal irregularity and two gaps in June and December 1996. The elapsed time between two samples was on average 14.0 ± 0.3 days but ranged from 7 to 20 days. On each sampling date, about 20 randomly selected cabbages were collected from even-aged plots 1.5 month after transplanting. In total, 1737 cabbages were examined, i.e. 21 ± 1 per sampling date. Leaves were thoroughly inspected and the numbers of insect larvae and pupae found in each plant were recorded. Aphid abundance was estimated semi-quantitatively on a scale of 1 to 5.

Sub-samples of lepidopteran larvae were taken to the laboratory, where they were maintained under ambient temperature, humidity and photoperiod conditions, and fed on fresh cabbage leaves until pupation. Pupae and parasitoid cocoons were then kept in plastic boxes (2.5 cm in diameter) until adult emergence to identify the primary parasitoids and hyperparasitoids associated with each species. Specimens were identified at the CIRAD laboratory, Montpellier, France.

*Statistical analyses*

Abundance data were expressed as mean numbers of individuals per plant (plus or minus the standard error of the mean). Relationships between moth abundance and weather variables were tested using the Pearson correlation coefficient ($r$). Shapiro-Wilk tests for normality were performed beforehand and variables were power-transformed when necessary. Pearson's $r$ was also used to test the correlations between host and parasitoid abundances after square-root transformation. These correlations were assessed for host and parasitoid abundances in the same samples (lag 0) and after shifting one variable several lags forwards or backwards (lagged correlation). Differences in moth abundance before and after parasitoid population peaks were tested using unpaired $t$-tests on square-root transformed data. All calculations were done using Stata statistical software (StataCorp 2005).

# RESULTS

*Community composition on cabbage leaves*

Larvae and pupae of four moth species were collected: *Plutella xylostella*, *Hellula undalis* (Fabricius) (Crambidae), *Spodoptera littoralis* Boisduval and *Chrysodeixis chalcites* (Esper) (both Noctuidae). Mean abundance during the study period was 8.0 ± 0.3 immature moths per plant, 93.4% of which belonged to *P. xylostella*. The other three species accounted for only 3.6%, 2.9% and 0.1% of the moths, respectively. Cocoons from three primary parasitoid wasps (Hymenoptera) were also collected: *Cotesia vestalis*, *Meteorus laphygmae* Viereck (Braconidae) and *Euplectrus laphygmae* Ferrière (Eulophidae). Mean abundance was 7.2 ± 0.3 cocoons per plant, 99.9% of which belonged to *C. vestalis*. Laboratory cultures showed that *C. vestalis* was strictly associated with *P. xylostella*, *M. laphygmae* and *E. laphygmae* with *S. littoralis*. No parasitois were found in *C. chalcites*. Therefore, the host-parasitoid system at this site was strongly dominated by the *P. xylostella-C. vestalis* association, both species occurring at similar levels of abundance.

The aphid *Lipaphis pseudobrassicae* Davis (Hemiptera: Aphididae) was often abundant on cabbage leaves (mean score 1.8 ± 0.1 on the scale of 1 to 5; range 0 to 5), as well as larvae of *Ischiodon aegyptius* (Wiedemann) (Diptera: Syrphidae) (on average 2.2 ± 0.1 larvae per plant), which is an important predator of aphids.

Only small numbers of hymenopteran hyperparasitoids were obtained from *C. vestalis* cocoons in the laboratory, the most common being *Aphanogmus reticulatus* (Fouts) (Ceraphronidae) and *Trichomalopsis orizae* (Risbec) (Pteromalidae). Five other hyperparasitoids were recorded sporadically: *Aphanogmus fijiensis* (Ferriere) (Ceraphronidae), *Elasmus* sp. (Elasmidae), *Hockeria* sp. (Chalcidae), *Notanisomorphella borborica* (Giard) and *Pediobius* aff. *afronigripes* Kerrich (both Eulophidae).

*Seasonal aspects*

The monthly mean abundance of *P. xylostella* did not show consistent seasonal fluctuations (Fig. 1). Temporal variation followed a different pattern in each year and the highest monthly mean abundance values were observed under a variety of weather conditions, namely in August 1996 (cool with moderate rainfall), January 1998 (quite hot and very dry), March 1997 (hot and dry) and September 1996 (cool and dry). After two periods of heavy rainfall (> 500 mm) in June 1996 and 1997, abundance was very low in samples taken in late June-early

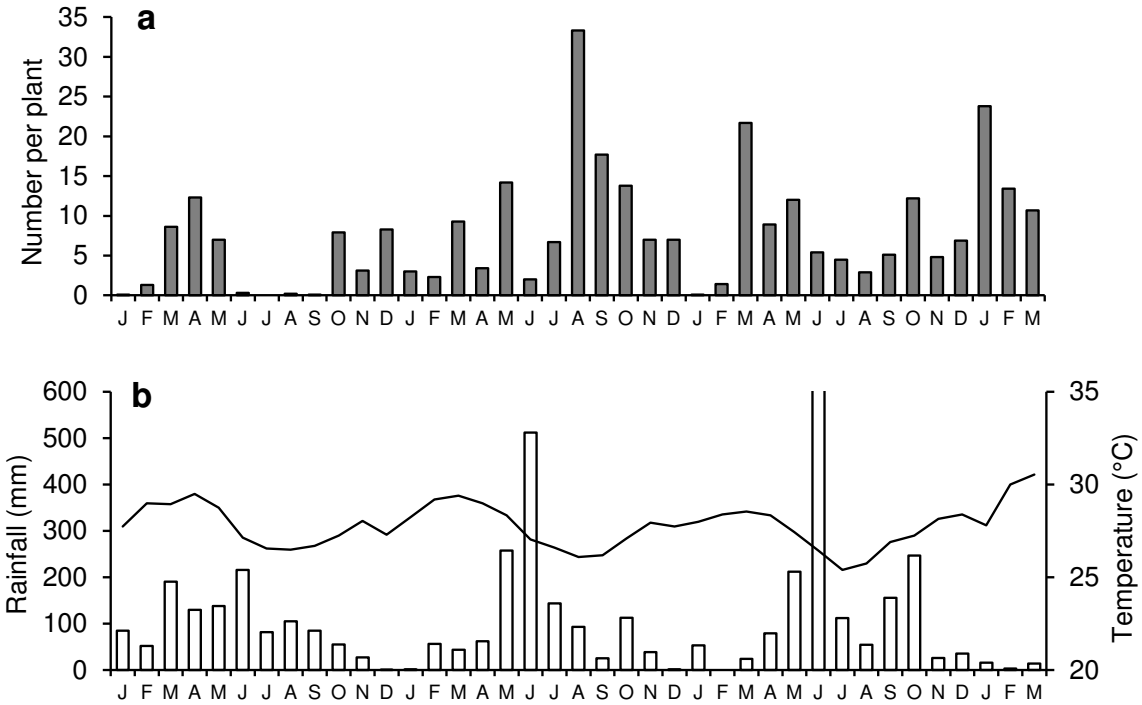


**Fig. 1** (**a**) Variation in the monthly mean abundance of *Plutella xylostella* (larvae 2 to 4 and pupae) on *Brassica* crops in Cotonou, compared to (**b**) monthly rainfall (bars) and mean temperatures (line) during the period of study (January 1995-March 1998)

July (0.3 ± 0.3 larvae and 1.3 ± 0.3 pupae per plant). However, there were no significant correlations between diamondback moth abundance and monthly precipitation ($r$ = -0.07), temperature ($r$ = 0.12) or relative humidity ($r$ = -0.13) during the study period.

*Host-parasitoid dynamics*

Changes in the mean abundance of *P. xylostella* and *C. vestalis* in the 83 cabbage samples are shown in Fig. 2. Populations of both species fluctuated markedly over short time intervals. Lagged correlation analysis showed that the abundance of *C. vestalis* was strongly correlated with that of *P. xylostella* in the preceding sample ($r$ = 0.74; $P$ < 0.001) (Fig. 2). This indicates that high and low population densities of the host tended to be followed two weeks later by high and low population densities of the parasitoid, respectively. There was a highly significant linear relationship between the abundance of the parasitoid and that of the host at lag 1 (Fig. 3).

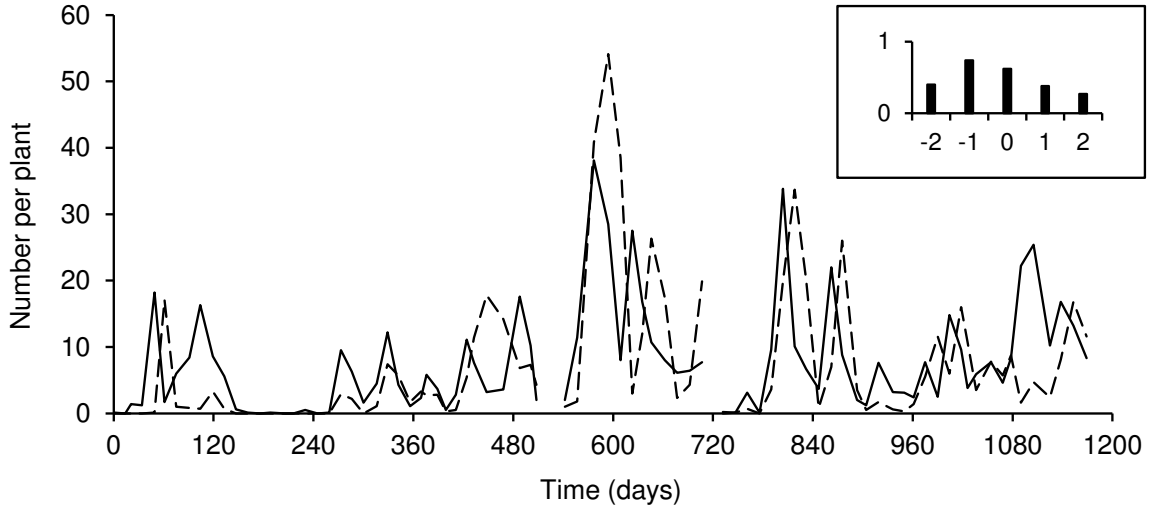


**Fig. 2** Variation in the mean abundance of *Plutella xylostella* (larvae 2 to 4 and pupae) (solid line) and *Cotesia vestalis* cocoons (dotted line) in 83 cabbage samples collected at *ca.* two-week intervals in Cotonou. The inset shows correlation coefficients between the two variables at lag 0 and at neighbouring lags

The impact of *C. vestalis* on *P. xylostella* can be assessed by comparing the mean abundance of immature moths before, during and after peaks in parasitoid abundance. For example, the results given in Table 1 were calculated for all peaks ≥ 10 *C. vestalis* cocoons per plant ($n$ = 20). They show that, on average, the abundance of *P. xylostella*, which was about 16 individuals per plant in samples immediately preceding parasitoid population peaks, decreased significantly in subsequent samples ($t \geq 2.54$; $P < 0.05$). The abundance of larvae decreased first, during parasitoid peaks and two weeks later, while the abundance of pupae was still significantly reduced four weeks after parasitoid peaks (Table 1).

*Relationships between P. xylostella and other insect herbivores*

There was no significant correlation between the mean abundance of immature *P. xylostella* and that of the other three moth species. There was however a significant negative correlation between the mean abundance of young larvae of *P. xylostella* (second instar) and that of the aphid *L. pseudobrassicae* ($r$ = -0.35; $P < 0.01$). Second-instar larvae were found in abundance on cabbage leaves only when aphids were absent or not very numerous.

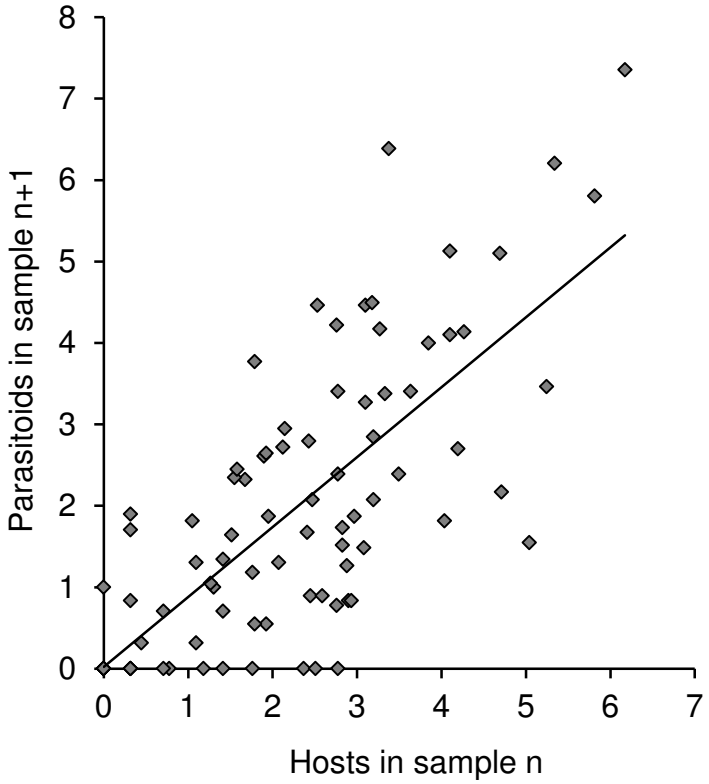


**Fig. 3** Linear relationship between the mean abundance of *Cotesia vestalis* in a sample and that of *Plutella xylostella* in the preceding sample (square-root transformed data). The equation of the regression line is y = 0.02 + 0.86 x ($P < 0.001$; $r^2 = 0.53$)

**Table 1** Variation in the mean abundance of *Plutella xylostella* (number of individuals per plant ± SE) before, during and after abundance peaks in *Cotesia vestalis* (≥ 10 cocoons per plant). Moth abundance was high in samples *n*, just before the occurrence of parasitoid peaks in samples *n*+1, and subsequently declined. Asterisks indicate significant decreases in moth abundance in samples *n*+1, *n*+2 and *n*+3 ($P < 0.05$)

| | samples *n* | samples *n*+1 (parasitoid peaks) | samples *n*+2 | samples *n*+3 |
|---|---|---|---|---|
| larvae and pupae | 15.9 ± 2.2 | 11.5 ± 2.3 | 9.4 ± 1.9 * | 9.4 ± 1.7 * |
| larvae | 9.6 ± 1.8 | 5.1 ± 1.7 * | 5.1 ± 1.8 * | 5.8 ± 1.8 |
| pupae | 6.3 ± 1.0 | 6.4 ± 0.9 | 4.3 ± 0.7 | 3.6 ± 0.6 * |

# DISCUSSION

Climate conditions in Cotonou are favourable for the year-round persistence of the diamondback moth. In the present study, samples that contained at least eight immature moths per cabbage plant were collected in any month of the year, even during the principal rainy season from April to July. Although several studies showed that large numbers of eggs and larvae could be washed off by rainfall or watering with sprinklers (Wakisaka et al. 1992; Kobori and Amano 2003), we found no correlation between diamondback moth abundance and monthly precipitation in Cotonou. The only obstacle to *P. xylostella* due to weather conditions in southern Benin may be heavy rains that occasionally pour down on the crops during the principal rainy season, which seems to affect moth populations for a few weeks, as in Ghana (Cobblah et al. 2012). Climate conditions are also favourable for the year-round persistence of the larval endoparasitoid *C. vestalis*, which is obviously an important natural enemy of *P. xylostella* in the system studied. This braconid wasp is common in western and southern Africa and has already been reported as the most abundant parasitoid of the diamondback moth in South Africa (Nofemela and Kfir 2008) and Ghana (Cobblah et al. 2012). Although *C. vestalis* was relatively less abundant in the first year of our study, possibly because of relatively dry conditions and former spraying of insecticides, the species subsequently reached higher population densities and its rate of parasitism in *P. xylostella* larvae was frequently well above 60% (Goudegnon et al. 2004).

At first sight, our results show aseasonal, apparently erratic fluctuations in the population of *P. xylostella*. However, the results also show that *P. xylostella* and *C. vestalis* populations are linked together by coupled oscillations in abundance, with a time lag of about two weeks between abundance peaks in the two species. This may reflect the inherent tendency of an insect host to show boom-and-bust cycles in the presence of a specialist parasitoid (Snyder and Ives 2009). Such oscillations are predicted by a number of models involving one prey species and one predator or parasitoid species (Ricklefs and Miller 2000; Begon et al. 2006). Similar oscillations were observed in laboratory microcosms involving an insect species and its parasitoid (Utida 1957; Begon et al. 1996), notably in laboratory cultures of *P. xylostella* and *C. vestalis* under controlled conditions (Karimzadeh et al. 2004). To our knowledge, this phenomenon has not been reported so far for a *P. xylostella* population attacked by a parasitoid under field conditions. In the cabbage field studied near Cotonou, the community of moths and parasitoids is strongly dominated by *P. xylostella* and

*C. vestalis* and close to a one-host-one-parasitoid system. Although *C. vestalis* can develop in other lepidopteran hosts in the laboratory (Cameron et al. 1997), it is generally regarded as highly specific to *P. xylostella*. In theory, this situation is conducive to oscillations. Indeed, abundance peaks in immature *P. xylostella* were often followed by abundance peaks in *C. vestalis* cocoons two weeks later, and high parasitoid population densities significantly reduced diamondback moth population densities over several weeks. When moths became scarce, the parasitoid population also declined strongly, which could make the crop susceptible to subsequent pest infestations.

In our field study, oscillations were much less regularly spaced out than some models predict, which can be explained in several ways: (1) not all samples were collected at regular time intervals; (2) due to the destructive sampling design, insects were not observed on the same individual cabbage plants on each date, which could influence the results when species distributions were patchy; (3) in the field, many biotic and abiotic factors can potentially interfere with host-parasitoid dynamics and cause erratic patterns of population density change; for example, our results indicate that the presence of aphids may affect the abundance of second-instar larvae of *P. xylostella* on cabbage leaves.

Coupled oscillations occurred repeatedly at our study site, in contrast with what occurs in laboratory studies, in which populations of hosts and parasitoids often crash rapidly (e.g. Karimzadeh et al. 2004 for *P. xylostella* and *C. vestalis*). The persistence of interactions in the field may be favoured by dispersal from and to surrounding areas. Adult diamondback moths move readily among fields (Shirai and Nakamura 1994; Schellhorn et al. 2008a). As cabbage fields are quite common around Cotonou, there are probably many places that may serve as sources of recolonization when population density is very low at a given site. *Cotesia vestalis* also moves readily among fields to find the host required for its reproduction. Feeding damage by *P. xylostella* leads to increased amounts of volatiles released by plants, which attracts the parasitoid (Potting et al. 1999). The host-parasitoid system may thus function not at the field scale, but as a metapopulation system at the landscape or regional scales, and further studies are needed to fully understand its dynamics in a spatial context (Schellhorn et al. 2008b).

As regards the efficiency of *C. vestalis* as a biological control agent, significant decreases in diamondback moth abundance after parasitoid population peaks suggest that *C. vestalis* is able to stop pest outbreaks. However, the parasitoid had only short-term effects at our study site and there was no stable control of the moth population at a sufficiently low density. The key problem is that the abundance of *C. vestalis* strongly decreases when there are few *P.*

*xylostella*, so that the parasitoid cannot prevent a resurgence of the pest. Under temperature conditions prevailing in Cotonou, the intrinsic rate of increase of *P. xylostella* is nearly at a maximum (Golidazeh et al. 2009) and, in the absence of parasitoids, small numbers of adult moths can produce considerable numbers of larvae in one or two generations. As *C. vestalis* numerically responds with a time lag roughly equal to two weeks, the pest can reach high densities before the parasitoid population becomes large enough to be effective. Therefore, *C. vestalis* is unable to control moth populations before damage exceeds acceptable levels. Although it is likely that cabbage infestation by *P. xylostella* would have been more severe in the absence of parasitism (Kfir 2011), the population density recorded in the presence of *C. vestalis* was on average 7.5 immature moths per plant in our study, which is hardly tolerable for *Brassica* vegetable growers. Clearly, additional control measures are necessary to reduce the damage caused by the diamondback moth in this system.

## CONCLUSION

The inherent tendency for host-parasitoid dynamics to cycle can become apparent in field populations of *P. xylostella* attacked by a specialist parasitoid. Although *C. vestalis* populations are well established in the Cotonou region and seem able to contain diamondback moth outbreaks by drastically reducing large populations, this parasitoid cannot prevent small populations of the pest from rebounding rapidly. This may be linked to the environmental conditions in southern Benin, i.e. a tropical climate in which moth populations can grow very rapidly when parasitoid density is low, combined with a high probability of recolonization from surrounding areas. Under such conditions, biological control by a specialist parasitoid reaches its limits and additional control measures – preferably compatible with *C. vestalis* in an integrated pest management programme should be taken against the diamondback moth.

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# ARTICLE 5

## Antennal structure and oviposition behavior of the *Plutella xylostella* specialist parasitoid : *Cotesia plutellae*

**Olivier ROUX, Joan Van BAAREN, Charles GERS, Laurence ARVANITAKIS and Luc LEGAL**

***Microscopy Research and Technique*, 2005, 68:36-44**

# Antennal Structure and Oviposition Behavior of the *Plutella xylostella* Specialist Parasitoid: *Cotesia plutellae*

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*KEY WORDS* SEM; sensilla; flow charts on factorial maps; diamondback moth; Braconidae

*ABSTRACT* Although several species of the genus *Cotesia* are used in biological control programs against insect caterpillars throughout the world, little is known of their oviposition behavior. We describe here the types and distribution of antennal sensilla in *Cotesia plutellae,* a larval parasitoid of *Plutella xylostella*, and we analyze its oviposition behavior. Seven types of sensilla were found on both males and females. Only sensilla trichodea type II, with a putative contact chemoreceptive function, was significantly more abundant in females than in males, and its morphology and position on antennomeres were linked to the antennation behavior used by females during host search. We conclude that gustatory stimulus following antennal contact is probably the key stimulus inducing oviposition behavior. The sensilla type assumed to be implied in oviposition behavior was present in *C. plutellae* but not in two closely related species (*C. glomerata* and *C. rubecula*), which is discussed. *Microsc. Res. Tech. 68:36–44, 2005.* © 2005 Wiley-Liss, Inc.

## INTRODUCTION

The genus *Cotesia* comprises 400 described species among an estimated total number of nearly 1,000 species worldwide (Shaw and Huddleston, 1991). Several *Cotesia* species are used for biological control of pest caterpillars throughout the world, while some are used as key model organisms in studies of the physiology and molecular biology of host-parasitoid interactions, behavioral ecology, and the ecology and genetics of metapopulations in fragmented habitats (Michel-Salzat and Whitfield, 2004).

*Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) is a primary solitary larval endoparasitoid, specialist of the Diamondback Moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) (Velasco, 1982; Verkerk and Wright, 1996). *C. plutellae* originates from Europe (Oatman, 1978) but has been reported worldwide. It is largely used as biological control agent for DBM management (Talekar and Shelton, 1993), and numerous attempts have been made to introduce it into different areas of the world, with mixed results (Talekar and Shelton, 1993; Velasco, 1982; Verkerk and Wright, 1996). In Asia, it is the only larval parasitoid of DBM, able to survive in tropical and subtropical plains (Talekar and Shelton, 1993; Verkerk and Wright, 1996).

Hymenopteran parasitoids commonly find their hosts, using chemical stimuli produced by the host or by the plant (Vet and Dicke, 1992; Vinson, 1976). When searching for hosts, wasps use different stimuli in a hierarchical process that lead them gradually to the host habitat, the host plant, and finally the host itself (see for review, Quicke, 1997; Vinson, 1976). During long range searching, *C. plutellae* females use monoterpenes and glucosilonate produced by wounds left by herbivores or mechanical damages (Potting et al., 1999; Reddy et al., 2002; Vuorinen et al., 2004a,b). After landing on a cabbage leaf, *C. plutellae* females appear to search randomly until they find a host (Guilloux, 2000). There is no fine description of the oviposition behavior in this species.

*Cotesia glomerata*, a gregarious and generalist parasitoid, is phylogenetically closer to *C. plutellae* (solitary and specialist) than to *Cotesia. rubecula*, a solitary parasitoid specialist of *Pieris rapae* larvae (Michel-Salzat and Whitfield, 2004). Significant differences have been shown between *C. glomerata* and *C. rubecula* in the number of some sensilla types, but not among types and topographical arrangement (Bleeker et al., 2004).

In the present study, we describe the antennal equipment of male and female *C. plutellae*, using scanning electron microscopy. The complete oviposition sequence of *C. plutellae* is also described and series of experiments were performed to determine which organs were really involved in host recognition. We hypothesized that a specific sensilla type was linked to oviposition behavior *sensus lato* and to the detection of the host *sensus stricto*. This possible adaptive link is discussed, as well as the possible contribution of comparative and phylogenetic analysis to the test of this hypothesis.

## MATERIALS AND METHODS

### Plants and Insects

*C. plutellae* and its host DBM originated from Cotonou in Benin and were collected on cabbage, *Brassica*

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*TABLE 1. Patterns of oviposition behavior of* Cotesia plutellae *and abbreviations used in text and figures*

| Pattern | Definition |
|---|---|
| walk | The female walks continuously with antennal movements (right/left, up/down, U-shape position with dorsal side or tip in contact with the leaf) |
| ant-c | Antennal contact with external, internal, or dorsal side or with the tip |
| line | The female takes place in line parallel to the caterpillar body |
| reab | The female moves toward the caterpillar and begins to bend the abdomen |
| ex-ca | Extension of abdomen, outspread wings, and antennal contact |
| dset-abd | The abdomen is deep-set in the host, wings are perpendicular to the body and antennal contact |
| ovip | Oviposition (the abdomen is quickly extended) |
| sting | Sting without oviposition (the abdomen is quickly extended, no egg was found) |
| b-ant | Antennae back to normal position (above the head) |
| b-abd | Abdomen back to normal position (in the same line as thorax) |
| b-wing1 | Wings back to outspread position |
| b-body | Body back to normal position |
| groo | Grooming (wasp rubs its antennae and abdomen with legs) |
| fly | The female flies away |
| ov-att | Oviposition attempt on the leaf |
| chase | The caterpillar escapes and the female chases it |
| wigg | The caterpillar wiggles to escape from the wasp after contact or sting |
| eject | The female is ejected by the caterpillar |

*oleracea* var. *capitata*. DBM (adults and larvae) was reared on Indian mustard, *Brassica juncea,* in Plexiglas cages of 50 × 50 × 50 cm, with water and honey provided separately *ad libitum* for adults. During oviposition of DBM, a new cabbage was placed in the cages every day to avoid larval stages overlapping. Larvae were transferred on a new cabbage every day. Cocoons were collected regularly and kept in a plastic box until emergence.

Parasitoids were reared in Plexiglas cages of 50 × 50 × 50 cm for mating and oviposition, with water and honey provided *ad libitum*. For oviposition, a cabbage with L2 larvae was introduced in the cage. Nymphs of parasitoid were collected and kept in a plastic box until emergence. DBM and parasitoids were maintained in a climatic room at 25 ± 1°C, 40–50% RH, and a 12 h light–dark photoperiod. All insects used were spring from second rearing generation.

### Scanning Electron Microscopy

Newly emerged parasitoids were killed by freezing and serially dehydrated in alcohol (70, 75, 80, 90, 95, and 100%), then placed in 100% acetone for 15 min, and in 100% chloroform at 60°C for 4 h. Preparations were kept in 100% acetone until the observation. Before observations, samples were critical point dried using $CO_2$ with a Balzers CPD-010 (Balzers, Liechtenstein) and gold/palladium coated using a JEOL JFC-1100 sputter unit (JEOL Ltd., Tokyo, Japan). Observations were performed at 2.5 kV with a JEOL SEM-6400.

We will use the sensilla denomination and classification of Bleeker et al. (2004), Zachuruck (1980, 1985), and Keil (1999).

### Behavioral Oviposition Sequences

Ten second instar caterpillars feeding on a piece of cabbage leaf (33 mm in diameter) were individually exposed to a parasitoid female in a glass Petri dish (33 mm in diameter and 8 mm depth). Each host was attacked once (a host was considered as being attacked when the female had stung and departed from it). All caterpillars were dissected in a saline solution a few hours after oviposition so as to confirm that an egg has been deposited by the female wasp. Ten females were tested (10 ovipositions per female for a total of 100 observations). Each oviposition sequence (from searching for host to departure of the female) was video-recorded with a camera (Canon Powershot A80), mounted on a binocular microscope (Wild Heerbrugg), and analyzed image by image (Pierre and Kasper, 1990; Van Baaren et al., 1993, 2002, 2003, 2004). On the basis of these observations, we obtained a description of the sequential structure of behavioral patterns (from the interpretation of factorial axes), and the patterns were placed in factorial space, their distance being inversely related to the frequency of their temporal succession. This analysis yielded a flow chart on factorial maps in which two patterns occurring frequently in succession will appear closely and linked with thick arrows. Conversely, two patterns occurring rarely in succession will be represented far apart and linked with thin arrows. The behavioral patterns, used in the analysis, are described in Table 1.

Sequences with oviposition (one egg was found) and with sting without oviposition (no egg was found) were analyzed separately and relative frequencies of patterns were compared with a $\chi^2$ test, using R statistical software (Ihaka and Gentleman, 1996). After a first analysis with all patterns, some rare patterns (i.e., recorded less than 10 times) were pooled to reduce the number of low values. Four classes of behavioral patterns were defined as follows: the first comprised rare patterns linked to preoviposition behaviors, the second, patterns linked to oviposition, the third, patterns linked to avoidance of defensive behaviors by the caterpillar, and the last, behaviors following oviposition, before the female returned to host location behavior.

### Determination of Organs Involved in Host Detection

Six tests were performed to evaluate the importance of chemical and contact detection of different organs, vision, hearing, and perception of movement in successful ovipositions: in each test, six second instar caterpillars were exposed to a parasitoid female in a glass Petri dish (50 mm in diameter) for 2 h. For the first test, females were untreated (control), for tests 2–6, antennae were cut off in test 2, tarsus on first pair of legs were cut off in test 3, palps were cut off in test 4, glass Petri dish were exposed in dark in test 5, and caterpillars were killed by freezing in test 6. Fifteen replicates were done for each test. To perform ablations, females were anesthetized on ice and manipulated under a binocular microscope (Wild Heerbrugg). All caterpillars were dissected in a saline solution a few hours after oviposition so as to determine the total number of eggs the female oviposited in each test. To compare tests, data were normalised by decimal loga-

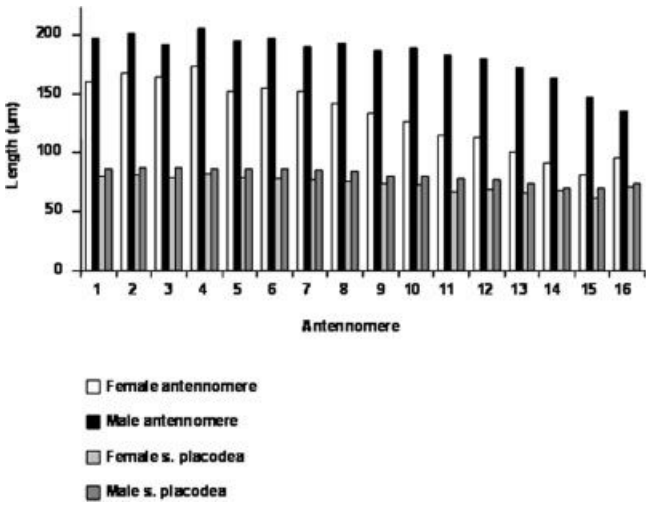


Fig. 1. Length (µm) of male and female antennomeres, and length (µm) of their sensilla placodea, from the proximal (1) to the distal (16) part of the antenna.

rithm (log $(x + 1)$) or square transformations ($\sqrt{x}$) tested with the Kolmogorov–Smirnov nonparametric test (Lilliefors method) (SYSTAT 8.0, 1998). An ANOVA was performed and the post hoc Bonferroni test were used (SYSTAT 8.0, 1998).

## RESULTS

### Microscopy

***Antennae.*** Antennae of males and females had a long uniform slender shape and had both 16 antennomeres whose length decreased from that of the proximal to the distal one (Fig 1). Antennae of males were thicker (diameter of antennomeres 52.6–82.0 µm) than those of females (49.2–65.5 µm), and their antennomeres were longer than those of females (Fig. 1). The space between antennomeres was larger in females than in males (Figs. 2a and 2b).

***Sensilla Types.*** Seven different types of sensilla were found on both male and female antennae, and the numbers on each antennomere are given in Table 2.

***Sensilla Trichodea Nonporous (NP).*** They were the most abundant on all antennomeres and were distributed in several rows between each sensilla placodea. They were inserted in a socket, had a grooved surface, and a droplet shape at the tip (Fig. 2c). Their length was about 35 µm and their diameter 2 µm at the base on the first antennomere, and they became thinner and shorter toward the distal end of antennae ($L \approx$ 24 µm, $Ø \approx 1$ µm).

***Sensilla Trichodea With Wall Pores (WP).*** These sensilla had a smooth cuticle, covered by small pores, and had a socket (Fig. 2d and Table 3). They were slightly bulbous at the base with a diameter of ~2 µm and had a conical tip. They were only distributed around the distal side of antennomeres and were absent in the last one (Figs. 3a, 3b, and Table 2).

***Sensilla Trichodea With Tip Pores (TP).*** Two different types were identified. They were both inserted in a socket, and had a grooved surface (Figs. 2c, 2e, 2f, 4e, and 4f). Type I had a classical conical shape with two pores on the tip (Fig. 4e). It had a length of ~22 µm on first antennomere vs. 18 µm on the last one in males, and 15 µm in females, with a diameter of 1 µm at the base. These type I sensilla trichodea were present on all antennomeres in two rings, one on the middle of the antennomere and one (with more sensilla) at the distal part of the antennomere. At the apex of the last antennomere stood three TP type I sensilla, longer than those on last distal antennomeres (Figs. 4a and 4b).

Type II sensilla trichodea was characterized by a cuticular projection over apical pores. This cuticular projection had an opercula shape and the opening was turned towards the proximal end of the antennomere (Fig. 2c). It had a length of 19 µm on proximal antennomeres vs. 16 µm on distal antennomeres in females, and a constant length of 16 µm in males. They were thicker than Type I sensilla (Fig. 2c) with a diameter of 2 µm at the base. Pores were numerous and distributed in an oval ring under the cuticular projection (Figs. 4e and 4f). These sensilla were 4.5 times more abundant in females than in males. They appeared on all antennomeres in females and only on antennomeres 6–16 in males (Table 2). In males, the cuticular projection seemed to be less developed than in females (Fig. 2f). These type II sensilla stood always in a ring on the middle part of antennomeres, but never on the ventral side. In females, they appeared in two rings on the last antennomere (Figs. 4a and 4b). They were distributed equally on dorsal and lateral sides. These sensilla appeared sometimes under a slightly different morphology (Fig. 4f): the cuticular projection seemed to be malformed or broken. This different morphological type appears everywhere on the antennomeres.

***Sensilla Placodea (or Multiporous Plate Sensilla).*** These sensilla were found on all antennomeres and were distributed into two regular rings on each antennomere, one proximal, and one distal (Fig. 3a). They were more numerous in males (Table 2). They had a carina shape and had a length of 61.6–81.5 µm in females and 69.5–87.0 µm in males, a width of ~3 µm and 2 µm high. In comparison to antennomeres, they had a relative constant length (Fig. 1). Therefore, the more the antennomeres shortened, the more sensilla placodea become imbricate (Fig. 3b). This phenomenon was more evident in females because of their smaller antennomeres. On the last female antennomeres, the repartition became random (Fig. 4a). They were covered by pores of ~20 nm in diameter. These pores were found in V-shaped rows (Fig. 4c).

***Sensilla Coeloconica Type I.*** They were described as a peg protruding from a pit in a donut-shaped ring (Bleeker et al., 2004). They had at maximal a width of ~1 µm and 4 µm in length, and the pit had an external diameter of ~6 µm. The peg was deeply striated (Fig. 4g). They were present in males and females on the ventral side of antennomeres 6–15 (only one on each) and were situated at the end of the proximal half part of them (Fig. 4d).

***Sensilla Coeloconica Type II.*** These sensilla had an oval donut shape of 7.5 µm long and 5 µm wide with

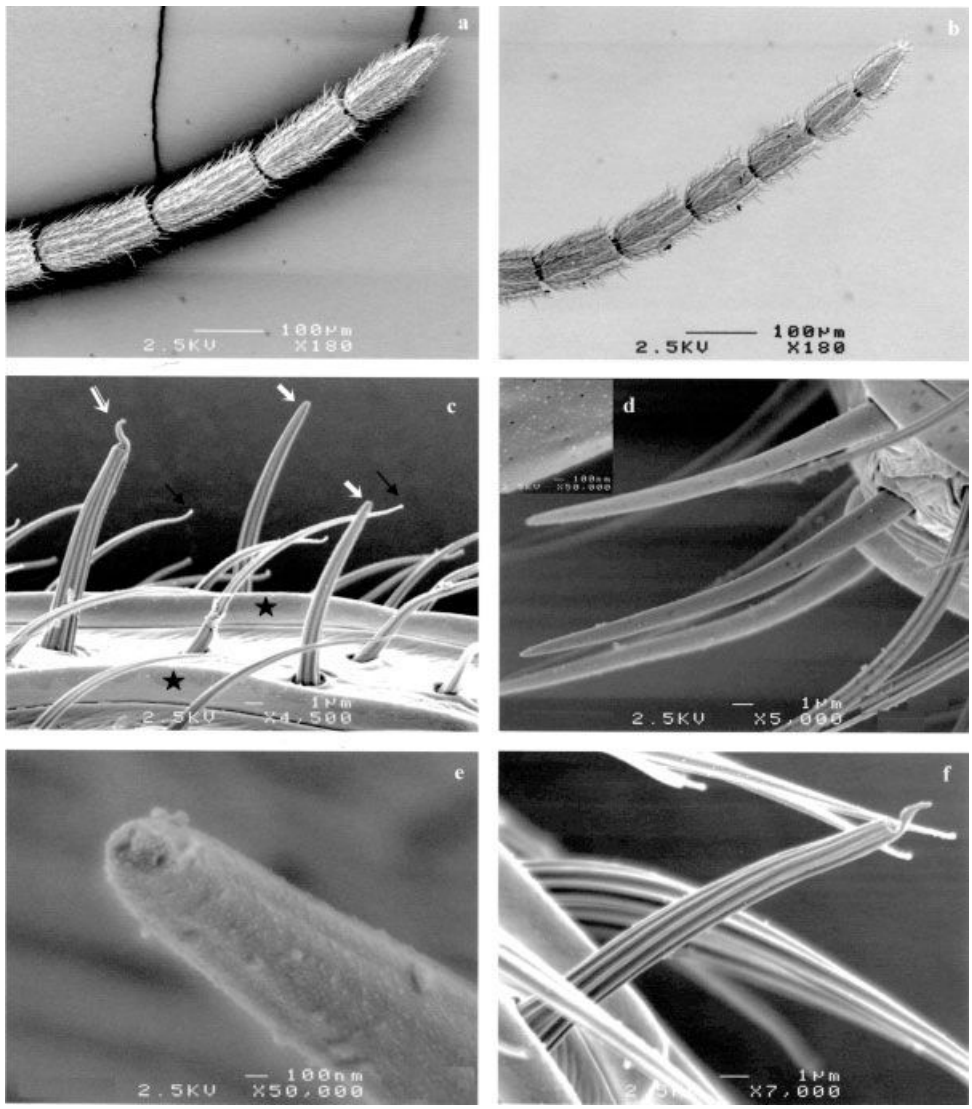


Fig. 2. Sensilla trichodea NP (thin black arrows), s. placodea (stars), s. trichodea TP type I (white arrows), sensilla trichodea TP type II (white double shafted arrow). **(a)** Apical part of male antenna. **(b)** Apical part of female antenna. **(c)** Detail of 16th female antennomere. **(d)** Detail of the apical part of the 2nd female antennomere showing s. trichodea WP. The box shows pores on the cuticle. **(e)** S. trichodea TP type I showing the double pores on the tip. **(f)** S. trichodea TP type II on the 9th male antennomere showing a thin cuticular projection.

*TABLE 2. Number of different types of sensilla on each antennomere in males (M) and females (F) of* C. plutellae, *from the proximal (1) to the distal (16) part of antenna*

| Antennomere | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| s. trichodea NP | F | Numerous on each antennomere | | | | | | | | | | | | | | | | |
| | M | | | | | | | | | | | | | | | | | |
| s. placodea | F | 19 | 22 | 22 | 23 | 23 | 25 | 25 | 26 | 26 | 27 | 25 | 24 | 22 | 20 | 20 | 13 | 362 |
| | M | 37 | 39 | 40 | 41 | 42 | 40 | 38 | 38 | 40 | 37 | 35 | 35 | 30 | 28 | 26 | 24 | 570 |
| s. trichodea WP | F | 20 | 14 | 14 | 11 | 12 | 11 | 13 | 16 | 17 | 22 | 18 | 21 | 22 | 17 | 21 | — | 249 |
| | M | 15 | 15 | 11 | 9 | 8 | 11 | 10 | 13 | 17 | 13 | 14 | 15 | 16 | 14 | 10 | — | 191 |
| s. trichodea TP type I | F | 7 | 6 | 7 | 7 | 8 | 8 | 8 | 10 | 10 | 9 | 8 | 8 | 11 | 10 | 10 | 24 | 151 |
| | M | 5 | 6 | 6 | 6 | 5 | 7 | 6 | 7 | 8 | 8 | 9 | 7 | 8 | 9 | 9 | 17 | 123 |
| s. trichodea TP type II | F | 1 | 1 | 2 | 2 | 2 | 2 | 3 | 4 | 6 | 6 | 6 | 6 | 6 | 6 | 8 | 13 | 74 |
| | M | — | — | — | — | — | 1 | — | 1 | 1 | 1 | 1 | 2 | 2 | 3 | 2 | 2 | 16 |
| s. coeloconica I | F | — | — | — | — | — | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | — | 10 |
| | M | — | — | — | — | — | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | — | 10 |
| s. coeloconica II | F | — | — | 1 | — | 1 | — | 1 | — | 1 | — | 1 | — | 1 | — | — | — | 6 |
| | M | — | — | 1 | — | 1 | — | 1 | — | 1 | — | 1 | — | 1 | — | — | — | 6 |

*TABLE 3. Differences between sensilla of* C. glomerata, C. rubecula *(Bleeker et al., 2004), and* C. plutellae *(this article)*

| | *C. glomerata*[a] | *C. rubecula*[b] | *C. plutellae*[b] |
|---|---|---|---|
| Body size | 6–7 mm | 6–7 mm | 4–5 mm |
| Length of antennae in female and male | 2.8–3.7 mm | 3.8–3.9 mm | 2.1–2.9 mm |
| s. trichodea NP | No differences | | |
| s. trichodea WP | Length: 30 μm; pore diameter: 20 nm | Length: 30 μm; pore diameter: 20 nm | Length: 23 μm; Pore diameter: 30 nm |
| s. trichodea TP type I | Length: 30 μm; diameter 2 μm | Length: 30 μm; diameter 2 μm | Length: 15–22 μm; Diameter: 1 μm |
| s. trichodea TP type II | Absent | Absent | Present |
| s. placodea | Length: 70–132 μm Arrangement of pores: in rows | Length: 81–132 μm Arrangement of pores: in rows | Length: 61–87 μm Arrangement of pores: in V-shape |
| s. coeloconica type I | Peg: 2–3 μm width; pit diameter: 8 μm on antennomeres 2–15 | Peg: 2–3 μm width; pit diameter: 8 μm on antennomeres 2–15 | Peg: 1 μm width; pit diameter: 6 μm on antennomeres 6–15 |
| s. coeloconica type II | Diameter: 9 μm | Diameter: 9 μm | Maximal diameter: 7.5 μm |

[a]Generalist and gregarious.
[b]Specialist and solitary.

a small bulb of 0.8 μm of diameter in the middle (Fig. 4h). Only one was present on distal ventral side of antennomeres 3, 5, 7, 9, 11, and 13 (Fig. 4d) in males and females.

### Behavioral Sequences

The typical oviposition sequence was divided into three parts that appear clearly on the factorial map: (1) host detection and self positioning before oviposition, (2) oviposition, and (3) return to normal position. The first axis separated the patterns linked to host detection and positioning (negative side) from the patterns linked to the behaviors following oviposition (positive side). The second axis separated the searching pattern (walk) (negative side) from oviposition (positive side).

Once the female landed on a leaf, it started to walk rapidly in a random pattern with antennal contact with leaf, feces, and silk. Most of the time, the antennae were U-shaped with the dorsal side of apical antennomeres (or sometimes only the tips) in contact with the leaf. This typical searching behavior (walk) (Table 1, Fig. 5), ended when the female had an antennal contact (ant-c) with the host. This antennation always occurred with the dorsal or lateral side or the tip of antennae, but never with the ventral side. Immediately after this contact, the female moved towards the caterpillar and began to bend its abdomen under the thorax (reab). The abdomen was then pushed farther on and the wings were slightly outspread above the abdomen (ex-ca). Oviposition was divided into two patterns. During the first one, the abdomen was firmly deep-set in the host, the wings were perpendicularly erected under the thorax, and the antennae were always in contact with the host (dset-abd). The second pattern was the oviposition *sensus stricto*. The female quickly and violently extended the abdomen (ovip), and during this movement antennal contact was lost. After oviposition, the body returned to a normal position in three steps, starting with antennae moved back above the head (b-ant), abdomen back in line with the thorax (b-abd), and finally wings starting to regain their resting position (b-wing 1). Then, the female regained normal position (b-body), began grooming (groo), and walked again (walk).

This sequence of patterns shows a clear Guttman effect (Guttman, 1941) on the factorial map (Fig. 5). This effect results in a hyperbola-shape of majority patterns corresponding to the ideal sequence of oviposition. This effect is representative of a low variability in pattern succession between different repetitions. However, this sequence was sometimes modified by the defensive behavior of the host. DBM larvae can react violently to the presence of the parasitoid (wigg) or can let themselves fall along a silk line. Although these defensive behaviors could temporarily interrupt the sequence, the female parasitoid rapidly reacted by the insertion of the ovipositor (dset-abd) before the caterpillar escaped, or chased it (chase).

Dissections of host larvae showed that 84% of ovipositions were successful. No behavioral differences were observed between ovipositions and stings without oviposition, except for the following patterns: at the end of the sequences, the grooming frequency (groo) was significantly superior in case of oviposition and the walk frequency (walk) was superior in case of sting without oviposition ($\chi^2$ (5) = 11.53; $P = 0.042$).

### Organs Involved in Host Detection

The ANOVA performed on rate of parasitism and on the number of eggs by the female revealed that treatments were significantly different ($F_{5,84} = 14.116$ ($P < 0.001$); $R^2 = 45.7\%$ and $F_{5,84} = 12.974$ ($P < 0.001$); $R^2 = 43.6\%$, respectively) and the Bonferroni post hoc adjustment showed that treatments 2 and 3 were different from the control (Table 4).

## DISCUSSION

We described the antennal sensilla of the larval parasitoid *C. plutellae*, and its behavioral oviposition on its host DBM. Seven different types of sensilla were found to be common in both sexes. Six of these sensilla types had already been described in other hymenopteran families (Ochieng et al., 2000; Van Baaren et al., 1996, 1999). The putative function of sensilla can be deduced from the number of pores (Bleeker et al., 2004; Keil, 1999). Sensilla trichodea NP are considered to be mechanoreceptors, TP type to be contact chemosensitive and involved in gustatory function, and WP type, such as sensilla coeloconica type I, to be olfactory sensilla. Sensilla coeloconica type II could be thermo- or hygroreceptors (Altner and Prillinger, 1980), while sensilla placodea have probably an olfactory function. The remaining type described in our study, the sensilla trichodea TP type II, had a particular tip that we can describe as a series of wide pores protected by a cuti-

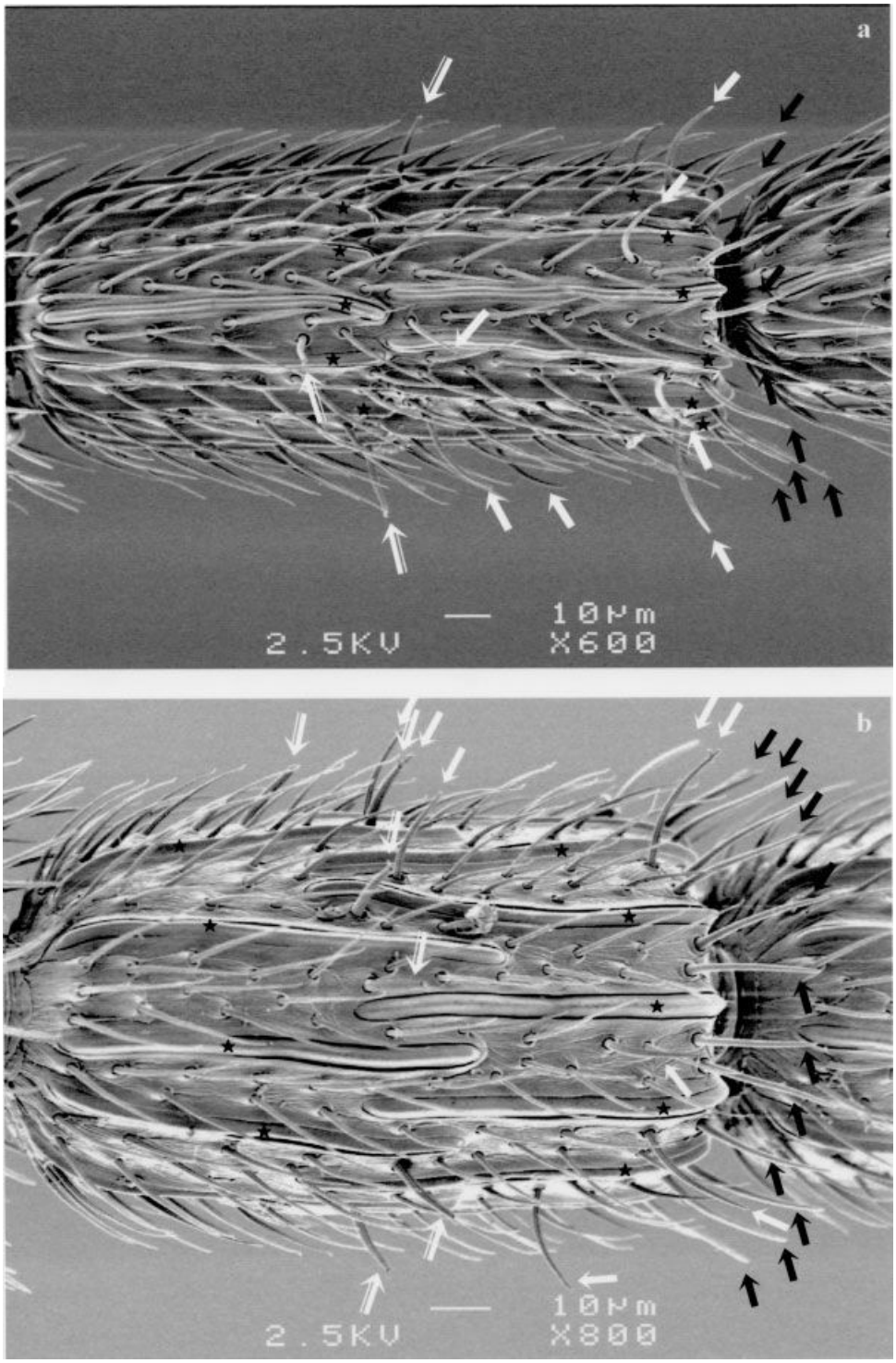


Fig. 3. Dorsal side of the 14th male **(a)** and female **(b)** antennomeres showing classical disposition of s. trichodea TP II (white double shafted arrows), s. trichodea TP (white arrows), s. trichodea WP (thick black arrows), and imbrications of s. placodea (stars) in females.

cular excrescence. Similar sensilla tips have been described in Braconidae and Encyrtidae and were considered to be contact chemosensitive (Ochieng et al., 2000; Van Baaren et al., 1996).

Ablation experiments showed that antennae were essential in host detection. The effect of ablation of tarsus on first pair of legs was also significantly different from controls, but observations revealed that these dif-

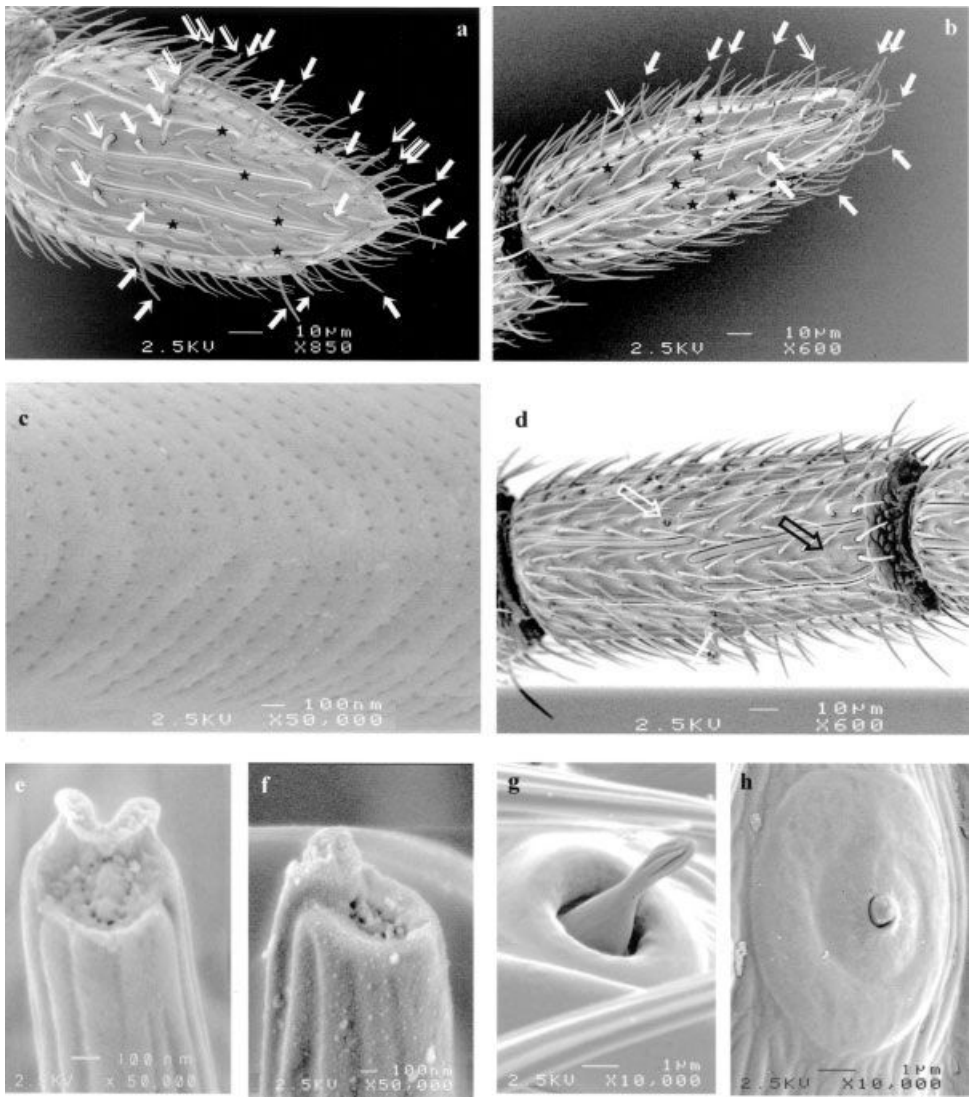


Fig. 4. Sensilla placodea (stars), s. trichodea TP type I (white arrows), sensilla trichodea TP type II (white double shafted arrows), s. coeloconica type I (white open arrow), s. coeloconica type II (black open arrow). **(a)** Dorsal side (on top) of the 16th female antennomere. **(b)** Dorsal side (on top) of the 16th male antennomere. **(c)** Detail of s. placodea showing the V-shape stripe pores organization. **(d)** Ventral side of the 9th female antennomere. **(e)** Detail of the tip of a s. trichodea TP type II showing the pore series in oval shape disposition. **(f)** S. trichodea TP type II showing a broken cuticular projection. **(g)** Detail of s. coeloconica type I on 7th male antennomere. **(h)** Detail of s. coeloconica type II on 7th female antennomere.

ferences are probably due to a physical handicap when females tried to oviposit on their host.

### Antennal Sensilla and Behavior

Our results showed that antennal structure, sensilla number, and topography were different between males and females and were probably linked to the use of antennae during the searching behavior of *C. plutellae*. First, when the female walks searching for its host, its antennae form a U-shape. This position was never observed in male antennae (pers. obs.) that had most often a straight erected or slightly curved position. The antennomeres in the distal half part of the male antennae are more narrowly spaced than in females, probably preventing the male's antennae from adopting the U-shape position.

We also showed that an antennal contact was obligatory to initiate the oviposition sequence. This antennal contact was the last step of host research and the first step of positioning for oviposition, and it was always done using the external side, the tip, or the dorsal side of the antennae. It should be noted that the sensilla trichodea TP type II are distributed on the dorsal and lateral sides of antennomeres. These sensilla, that are probably contact chemosensory sensilla, are perpendicularly erected from the cuticle and emerge from the layer of other sensilla, and are more numerous in females than in males. This suggests that the sensilla trichodea TP type II are closely related to the gustatory recognition of the host. Moreover, this type of sensilla have normally their opening oriented backwards, but in the U-shape antennal position, these opening are oriented toward the front and are the more exposed to

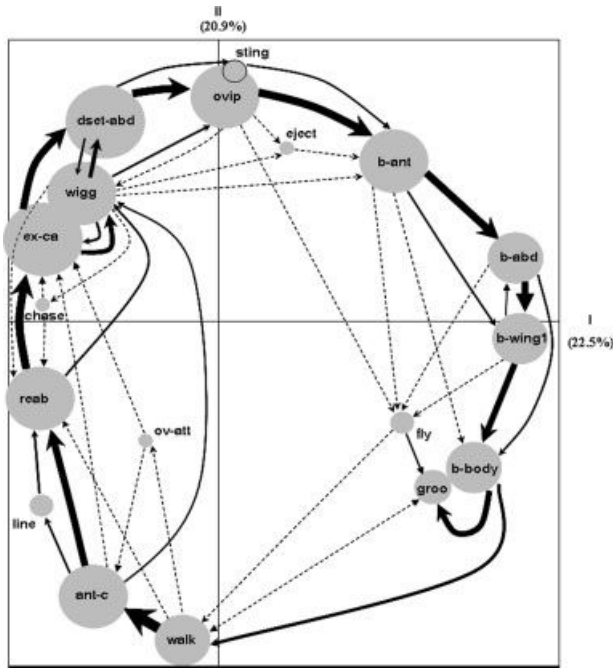


Fig. 5. Flow chart on factorial map obtained with 100 oviposition sequences of *Cotesia plutellae*. Behavioral patterns correspond to the abbreviations given in Table 1. Axis I and II are the two first axes of the Factorial Correspondence Analysis. The circles represent, from the smallest to the largest, respectively less than 10, 11–30, 31–60, 61–80, 81–90 and more than 90 behavioral patterns. The arrows represent the number of successions between two patterns. Small dashed lines represent less than 10 successions; solid lines are directly proportional to the number of successions (10–90).

chemical contacts. The dorsal location of contact chemosensory sensilla is uncommon. Usually, in parasitoids (Encyrtidae, Mymaridae, Trichogrammatidae), sensory equipment linked to oviposition are present on the tip or on the ventral side of apical antennomeres (Cônsoli et al., 1999; Isidoro et al., 2001; Van Baaren et al., 1995, 1996) and an increase in the variety of types is observed from the proximal antennomeres to the distal ones (Sen and Mitchell, 2001; Van Baaren et al., 1996, 1999). In the present case, we observed five sensilla types present from the first antennomere on, including sensilla trichodea type II involved in oviposition behavior. Moreover, the ablation of antennae suppressed totally the oviposition behavior.

The egg passage through the ovipositor may be observed in several parasitoid species, enabling the observer to distinguish oviposition from insertion of the ovipositor without oviposition (Van Baaren et al., 1995). Although dissection of DBM larvae showed that some females inserted their ovipositor without depositing an egg, in behavioral sequences, no differences were found between sequences with or without oviposition. Only the pattern "grooming" was frequently linked to true oviposition, but this connection was not exclusive.

### Comparison With *C. glomerata* and *C. rubecula*

In *C. glomerata* and *C. rubecula*, six types of sensilla were found: sensilla trichodea NP, WP, TP type I, sensilla placodea, and sensilla coeloconica type I and II (Bleeker et al., 2004). They were similar to those observed in *C. plutellae*, in which they were slightly smaller. The distribution of sensilla on antennomeres was very similar in the three species except for sensilla coeloconica type I. In *C. glomerata* and *C. rubecula*, a single sensilla coeloconica type I was found on each antennomere from the 2nd to the 15th one. In *C. plutellae*, they were less numerous and were found distributed from the 6th to the 15th antennomere (Table 4).

In *C. plutellae*, an additional sensilla was described, the sensilla trichodea TP type II, which represent the major difference with *C. glomerata* and *C. rubecula*. This sensilla type seems to be important in oviposition behavior (detection and identification of host).

In *C. glomerata*, as in *C. plutellae*, female antennae are shorter than in males, but not in *C. rubecula* where the length of antennae is equal in both sexes. This sexual dimorphism seems to be common in other hymenopteran (Ochieng et al., 2000; Van Baaren et al., 1999). The length of antennae is directly linked to the length of the antennomeres in the three species and with the length of sensilla placodea in both *C. glomerata* and in *C. plutellae.* Nevertheless, the decrease in antennomere length toward apical side of female antennae was not proportionally reflected in the length of s. placodea. Sensilla were slightly shorter, but it was the imbrications of proximal and distal s. placodea that allowed the narrowing of antennomeres. The length difference of s. placodea between sexes was greater in *C. glomerata* and *C. plutellae* than in *C. rubecula*.

A particular type of sensilla has been observed in *C. plutellae*, and neither in *C. rubecula* nor in *C. glomerata*. It is tempting to hypothesize a correspondence between sensillar equipment and types of parasitism (e.g., generalist vs. specialist). In the present case, *C. plutellae* and *C. rubecula* are specialists, while *C. glomerata* is a generalist, hence it does not seem that these sensilla are a general adaptation to specialization in parasitism. However, it could be an adaptation to a special character of the specific host DBM, like kairomones or some defensive behavior. To test these points further on, other close relative species should be studied concerning their type of parasitism, host characteristics, and sensillar equipment. Also, a phylogenetic perspective could help determining whether the presence of some particular sensilla types is a historical novelty (apomorphy), hence, a possible adaptation relatively to other species equipment. Phylogenetic hypotheses are available for the genus *Cotesia* (Michel-Salzat and Whitfield, 2004; Papp, 1986, 1987). The presently available data concern only three species, which does not allow any phylogenetic conclusion concerning evolutionary scenarios of biological characters (Brooks and MacLennan, 1991; Harvey and Pagel, 1991; Martins, 1996). A promising research program would consist in analyzing the relevant biological traits in species of the *C. rubecula* and *C. glomerata* group (Michel-Salzat and Whitfield, 2004) so as to both check for further possible correspondence between morphology and specialization in this parasitoid group, as already shown in Aphidiinae, Encyrtidae, Aphelinidae, Trichogrammatidae, Pteromalidae, Eucoilidae, Charipidae, and Megaspilidae by Le Ralec et al. (1996), and polarize the evolutionary scenarios for traits of interest into possible adaptations.

*TABLE 4. Effects of the six treatments performed on female parasitoids and described in Material and Methods section, on the proportion of parasitized hosts and the mean number of eggs laid by each tested individuals*

| | Treatments (sense or organ tested) | | | | | |
|---|---|---|---|---|---|---|
| | Control | Antennae | Tarsus | Palps | Vision | Movement and hearing |
| $N^a$ | 89 | 90 | 90 | 90 | 88 | 90 |
| % of parasitized caterpillars | 62.92 | $0.00^{**}$ | $20.00^{*}$ | 56.67 | 52.27 | 34.44 |
| Mean number of eggs laid by female ± SEM | 5.2 ± 3.6 | $0.0 \pm 0^{**}$ | $1.5 \pm 2.1^{**}$ | 8.9 ± 12.1 | 4.2±3.2 | 3.6 ± 1.7 |

[a]The number of caterpillars.
Values significantly different from control (ANOVA, followed by post hoc Bonferroni tests for 2 × 2 comparisons): $^{*}P \leq 0.01$; $^{**}P \leq 0.001$.

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# ARTICLE 6

## Chemical characterization of contact semiochemicals for host-recognition and host-acceptance by the specialist parasitoid *Cotesia plutellae* (Kurdjumov)

**Olivier ROUX, Charles GERS, Josephe Nathan TENE-GHOMSI, Laurence ARVANITAKIS, Dominique BORDAT and Luc LEGAL**

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CHEMOECOLOGY

# Chemical characterization of contact semiochemicals for host-recognition and host-acceptance by the specialist parasitoid *Cotesia plutellae* (Kurdjumov)

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**Summary.** *Cotesia plutellae* is a specialist parasitoid of *Plutella xylostella*. This specificity is potentially under the control of several factors before and after oviposition. Thereby, the stimuli that lead female parasitoids to host locations and to oviposition, might be at the basis of the specificity. We explore here the response of *C. plutellae* females exposed to host cuticular lipids. A total cuticular lipid extract of host caterpillars was fractionated into a hydrocarbon fraction and a non-hydrocarbon fraction. Neither fraction alone had any effect on oviposition behaviour in *C. plutellae* but the hydrocarbon fraction alone did seem to have a positive effect on the rate of antennal contact by the females. To induce oviposition behaviour, both fractions were necessary and reflect cooperation between at least one compound in each fraction. Identification of cuticular lipids shows that hydrocarbons were dominant (77%). Non-hydrocarbon compounds were mainly represented by 15-nonacosanone (18% of the total lipid extract). This ketone is rare in insect cuticle lipids and is thought to originate from the cabbage epicuticle where it is dominant with $n$-$C_{29}$ and 14- and 15-nonacosanol also found among the cuticular lipids of the host caterpillar.

**Key words.** *Cotesia plutellae* - *Plutella xylostella* - cuticular lipids - host-recognition - host-acceptance - parasitoid.

## Introduction

Hymenopteran parasitoids commonly find their hosts using various chemical stimuli produced by the host or by the plant (Vinson 1976; Vet & Dicke 1992). These different stimuli lead the wasps to the host habitat, then, in a hierarchical process, to the host plant and finally to the host itself (Vinson 1976; Quicke 1997).

*Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) is a primary solitary larval endoparasitoid, and a specialist of *Plutella xylostella* (L.), the diamondback moth (DBM) (Lepidoptera: Plutellidae), the greatest pest of crucifers in many parts of the world (Velasco 1982; Verkerk & Wright 1996). It is largely used as a biological control agent for DBM management and numerous attempts have been made to introduce it into different areas of the world, with mixed results (Velasco 1982; Talekar & Shelton 1993; Verkerk & Wright 1996). In Asia, it is the only larval parasitoid of DBM able to survive in tropical and subtropical plains (Talekar & Shelton 1993; Verkerk & Wright 1996).

The females of *C. plutellae* are primarily attracted by green leaf volatiles produced by wounds left by herbivores or mechanical injury (Potting *et al.* 1999; Liu & Jiang 2003). They also tend to show a specific response toward the host/plant versus non-host/plant complex odour (Shiojiri *et al.* 2000a; 2001). After landing on a damaged cabbage leaf, *C. plutellae* females have the ability to recognize a plant infested by its host through antennal contacts with kairomones. Host regurgitant, frass and plant contain the infochemicals and are important in host recognition (Shiojiri *et al.* 2000b; Reddy *et al.* 2002). In contrast, encounters with non-host kairomones put an end to the searching behaviour of females (Shiojiri *et al.* 2001). Despite all these mechanisms of host location, females are likely to encounter non-host insects on cabbage. To ensure that eggs are laid in a suitable host, cues perceptible at very short range are necessary.

Cuticular lipids are often involved in relationships between insects (Howard & Blomquist 1982; Blomquist *et al*. 1998). In social Hymenoptera, the cuticular lipids used in insect/insect relationships are mainly hydrocarbons and are involved in a variety of functions such as nest-mate recognition, reproduction, or parasitism (Dettner & Liepert 1994; Turillazzi *et al*. 2000; Ruther *et al*. 2002; Dani *et al*. 2005). In parasitoid wasps, non-volatile host cuticular lipids are used in very short range and in specialist parasitoids serve as chemical recognition signals to identify host species (Vinson 1976; Rutledge 1996; Howard *et al*. 1998; Padmavathi & Paul 1998; Kumazaki *et al*. 2000) or to discriminate suitable individuals for oviposition (Vinson & Guillot 1972; Buckner & Jones 2005).

We have recently shown that the females of *C. plutellae* detect their hosts through a short antennal contact, and we

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hypothesize that gustatory stimuli are elicitors of oviposition behaviour (Roux *et al.* 2005). In the present paper, we investigate the role of cuticular lipids as gustatory stimuli for host acceptance. We characterized the cuticular lipid composition of DBM caterpillars and assessed their biological activity on the oviposition behaviour of *C. plutellae* females through behavioural tests. We used bioassays to isolate active fractions and to detect additive or cooperative processes among them in eliciting behavioural responses in *C. plutellae.*

## Methods and Materials

*Plants and Insects. Cotesia plutellae* and its host DBM originated from Cotonou (Benin) and were initially collected in the field on *Brassica oleracea* var. *capitata*. DBM (adults and larvae) were reared in the laboratory on Indian mustard, *Brassica juncea,* in Plexiglas cages of 50 × 50 × 50 cm, with water and honey provided separately *ad libitum* for adults. During oviposition of the DBM, a new potted plant was placed in the cages every day to avoid overlapping of larval stages. Larvae were transferred to a new potted plant every day to provide them sufficient fresh leaves for optimal development. Cocoons were collected regularly and kept in a plastic box until emergence.

Parasitoids were also reared in 50 ×50 ×50 cm Plexiglas cages for mating and oviposition, with water and honey provided separately *ad libitum*. For oviposition, a cabbage with second stage larvae (L2) was introduced into the cage. Larvae in their final stage leave the host to form cocoons; these were collected and kept in a plastic box until emergence of the imago. DBM and parasitoids were maintained in a climatic room at 25±1 °C, 40-50 % RH and a 12L:12D photoperiod.

*Cuticular Lipid Extraction and Filtration.* Cuticular lipids of 200 *P. xylostella* larvae (stage L2) were extracted in 600 µl of hexane. Of this 200µl were put aside and used as total lipid extract. The 400 µl remaining were fractionated on a silica gel column (1 cm, 70-230 mesh, 60Å). Elution was done successively with 2 ml of hexane, 1 ml of chloroform and 2 ml of methanol. The hexane fraction contained only hydrocarbons. The chloroform and methanol fractions contained non-hydrocarbon lipids and were pooled. The two resulting fractions (hydrocarbon and non-hydrocarbon) were dried under nitrogen and re-dissolved in 200 µl of hexane.

*Bioassay*. All bioassays were performed with dead caterpillars (killed by freezing) or dummies. Dummies were made of second instar DBM larvae killed by freezing, washed twice in hexane for 1 min and dried under a stream of nitrogen. In order to exclude any ambiguities that could be linked to the possibility of incomplete washing of the host caterpillars, a second kind of dummy was prepared similarly but using *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae), a non-host caterpillar from a mass-reared laboratory strain maintained by the *Institut National de Recherche Agronomique* (INRA) "Le Magneraud" (Aquitaine, France).

Nine different treatments were performed: (1) Untreated DBM caterpillar (Host); (2) Untreated *O. nubilalis* caterpillar (Non-Host); (3) DBM dummies + 3 µl pure hexane added per dummy (Washed Host); (4) *O. nubilalis* dummies + 3 µl pure hexane (Washed Non-Host); (5) DBM dummies + 3 µl total lipid extract (Host + total extract); (6) *O. nubilalis* dummies + 3 µl total lipid extract (Non-Host + total extract); (7) *O. nubilalis* dummies + 3 µl hydrocarbon fraction (Non-Host + Hc); (8) *O. nubilalis* dummies + 3 µl non-hydrocarbon fraction (Non-Host + n-Hc) and (9) *O. nubilalis* dummies + 3 µl recombined fractions (Non-Host + Hc + n-Hc). The amount of 3 µl was the equivalent to the extract obtained from one caterpillar giving the dummies physiological concentrations of lipids.

For each treatment, 3 identical dummies or untreated caterpillars were exposed to a mated naïve parasitoid female in a glass Petri dish (33 mm in diameter and 8 mm depth) for 3 min. Caterpillars or dummies were placed on corners of a triangular piece of cabbage leaf with 1.5 cm edges. Two kinds of response were observed and counted: short antennal contact followed by an oviposition movement, as described in Roux *et al.* (2005), and short antennal contact without oviposition movement. Any female which showed no searching behavior during the first minute (i.e. inactive or running around the box) was discarded and replaced by another one.

*Statistical Analysis.* Data were analysed in two manners: (1) Counting the number of each response and (2) the proportions of each response. The number and proportions of each response to the treatments were analyzed using one-way ANOVA. Before analysis the number of events was normalised by square root transformation ($\sqrt{x}$) and proportions by the arcsine of the square root (*arcsine* $\sqrt{p}$). Data normalisation was tested with the Kolmogorov-Smirnov non-parametric test (Lilliefors method) (SYSTAT 8.0, 1998). ANOVA were performed and the *post-hoc* Bonferroni test used with the two data sets (SYSTAT 8.0, 1998).

*Chemical Analysis.* The total extract and the two fractions was analysed on an HP 5890 series II gas chromatograph (GC) with a split/splitless injector at 280 °C, an apolar HT5 capillary column (25 m × 0.22 mm ID, 0.1 µm film thickness, 5 % diphenyl and 95% dimethylpolysiloxane), and a flame ionization detector (FID) at 320 °C. The GC was coupled to a computer and data were processed with HP millennium software. One µl was injected into the column and elution was carried out with helium at 1 ml/min. The oven temperature was programmed from 50 °C (for 1 min) to 140 °C at 20 °C/min, from 140 °C to 280 °C at 4 °C/min and 280 °C for 5 min.

The determinations and checks were performed on a Finnigan Trace GC-MS 2000 Series chromatograph directly coupled to a mass spectrometer quadrupole detector. The whole system was controlled by the Xcalibur data system, version 1.2. MS spectra were recorded in EI mode (70 eV), over a mass range of 50-550 mass units with 2 scans per second. One µl was injected in splitless mode with an injector temperature of 280 °C and a detector temperature of 300 °C. An apolar Rtx®-5MS capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness, 5 % diphenyl and 95 % dimethylpolysiloxane) was used. Carrier gas conditions and oven temperature were the same as for GC analyses. Identifications were processed with a spectral database and with a series of standard linear alkanes. The standard linear alkanes were also used for alignment of GC profiles.

## Results

*Bioassay.* The behaviour of 27 females of *C. plutellae* was observed for each treatment. Results of the oviposition proportions (Fig. 1) are presented in two separate figure panels for better readability of two-by-two comparisons, but only one ANOVA was performed. The first part (Fig. 1a) presents positive (total hexane extract) and negative (washed) controls on host and non-host dummies and the second part (Fig. 1b) presents tests of the different host extract fractions applied to non-host dummies. ANOVA showed that oviposition proportions differed between treatments ($F_{8,\ 234}$ = 18.011; $P$ = 0.001). As expected, non-host caterpillars were significantly less attractive than hosts. Washed host caterpillars were also less attractive than untreated hosts (Fig. 1a.). When the total extract was applied to host dummies, no significant difference was observed with untreated host caterpillars. Nevertheless, activity tended to be not fully recovered. The application of total extract on non-host dummies provided the same activity as an application on host dummies. In fact, washed host caterpillars were not significantly different from host dummies with total extract. To test fractionated extract, we used only non-host dummies to be sure that no active compounds remained on them. The two fractions of total extract tested separately on non-host

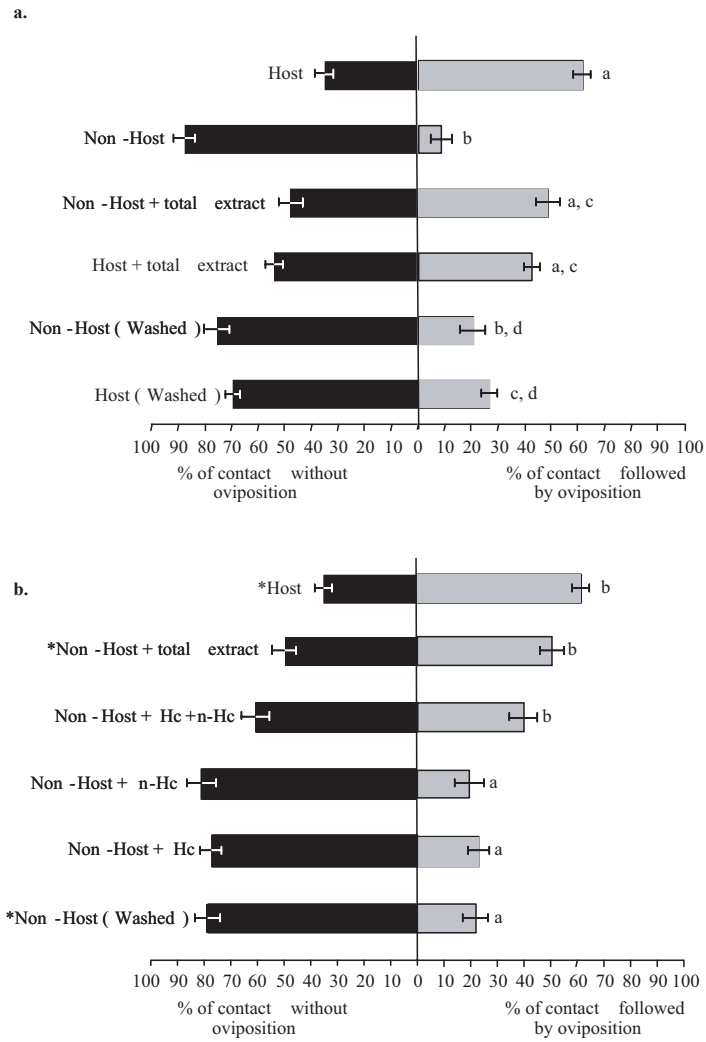


**Fig. 1** Relative proportions of oviposition (± SE) produced by *Cotesia plutellae* females after antennal contacts with different treatments applied to caterpillars. Treatments with different letters were significantly different (P<0.01). The results are separated into two parts for a better readability of comparisons.
**a.** Comparison of effects of total lipid extract when applied to host and non-host dummies.
**b.** Comparison of effects of hydrocarbon fraction (Hc) and non-hydrocrabon fraction (n-Hc) when applied to non-host dummies. Treatments marked by an asterisk were the same as in (a.) and were repeated to facilitate comparison

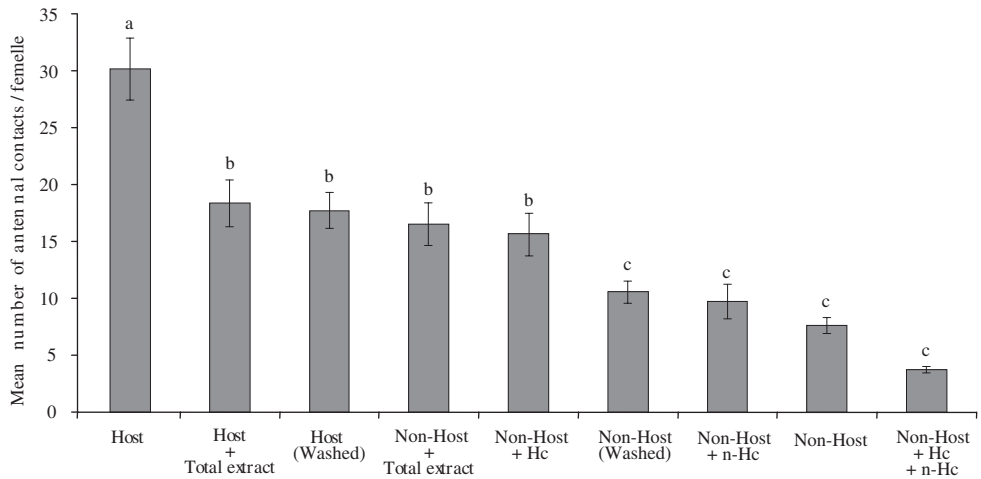


**Fig. 2** Mean number of antennal contacts (± SE) produced by *Cotesia plutellae* females with the different treatments applied to caterpillars. Treatments with the same letter were not significantly different (P< 0.01). Hc: hydrocarbon fraction; n-Hc: non-hydrocrabon fraction

dummies were inactive. The hydrocarbon and non-hydrocarbon fractions had the same activity as solvent alone applied to washed non-host caterpillars (Fig. 1b). However, when the two fractions were recombined, the oviposition proportion was the same as with the total lipid extract.

Mean number of antennal contacts (Fig. 2), also indicated that the treatments differed in acceptability to the females. The ANOVA revealed that the mean number of contacts produced differed among treatments ($F_{8,\ 234}$ = 29.521; $P$ = 0.001). Greater the numbers of antennal contacts associated with greater oviposition responses, suggesting the female's interest in the caterpillars. The results were slightly different from those obtained with the observation of oviposition proportions. As previously, it was for

**Table 1.** Cuticular lipid compounds of *Plutella xylostella* caterpillars detected by GC analysis and identified by GC-MS analysis

| Compound | Retention time | Relative abundance | Diagnostic ions m/z |
|---|---|---|---|
| HC fraction | | | |
| *n*-tricosane ($nC_{23}$) | 19.63 | 0.6 | – |
| 9-methyltricosane ($9MeC_{23}$) | 20.52 | 0.2 | 140/141, 224/225 |
| Unknown | 21.45 | 0.4 | – |
| *n*-tetracosane ($nC_{24}$) | 22.15 | tr | – |
| n-pentacosane ($nC_{25}$) | 24.72 | 1.3 | – |
| 9-methylpentacosane ($9MeC_{25}$) | 25.61 | 2.5 | 140/141, 252/253 |
| 7-methylpentacosane ($7MeC_{25}$) | 25.74 | 2.2 | 112/113, 280/281 |
| 5-methylpentacosane ($5MeC_{25}$) | 25.94 | 1.4 | 84/85, 308/309 |
| 3-methylpentacosane ($3MeC_{25}$) | 26.58 | 1.6 | 56/57, 336/337 |
| *n*-hexacosane ($nC_{26}$) | 27.33 | 0.4 | – |
| 9-methylhexacosane ($9MeC_{26}$) | 28.04 | 0.9 | 140/141, 266/267 |
| Unknown | 28.21 | 0.3 | – |
| *n*-heptacosane ($nC_{27}$) | 29.75 | 0.8 | – |
| 11-methylheptacosane ($11MeC_{27}$) | 30.64 | 13.1 | 168/169, 252/253 |
| 9-methylheptacosane ($9MeC_{27}$) | 30.71 | 3.6 | 140/141, 280/281 |
| 7-methylheptacosane ($7MeC_{27}$) | 30.82 | 3.7 | 112/113, 308/309 |
| 9,14-dimethylheptacosane ($9,14diMeC_{27}$) | 31.22 | 3.2 | 140, 211, 224, 296 |
| 7,16-dimethylheptacosane ($7,16diMeC_{27}$) | 31.42 | 6.8 | 112, 182, 252, 323 |
| 10,16-dimethylheptacosane ($10,16diMeC_{27}$) | 31.62 | 1.9 | 182, 253, 267, 281 |
| *n*-octacosane ($nC_{28}$) | 32.25 | 1.1 | – |
| 11-methyloctacosane ($11MeC_{28}$) | 32.92 | 0.7 | 168/169, 266/267 |
| *n*-nonacosane ($nC_{29}$) | 34.80 | 21.4 | – |
| 13-methylnonacosane ($13MeC_{29}$) | 35.29 | 1.8 | 196/197, 252/253 |
| 7,16-dimethylnonacosane ($7,16diMeC_{29}$) | 36.12 | 3.1 | 113, 211, 253, 351 |
| Unknown | 36.92 | 0.6 | – |
| Unknown triterpenoid[b] | 39.24 | 2.3 | – |
| Unknowns[a] | – | $0.9^{3}$ | – |
| Non-HC fraction | | | |
| 15-nonacosanone | 40.36 | 18.2 | 224/225 |
| 14+15-nonacosanol | 40.50 | 0.9 | 213, 226/227, 241 |
| Unknown | 43.13 | 1.4 | |
| Unknowns[a] | – | $2.7^{9}$ | – |

[a]Unknowns which were present in traces in GC analyses and were not detected by GC-MS analyses were pooled, the superscript digit indicating the number of pooled compounds.
[b]non-hydrocarbon compound but not retained in a silica gel column on elution with hexane.

untreated host caterpillars that the interest of females was highest and significantly different from other treatments with a mean of 30 antennal contacts per females ($P = 0.001$). Host dummies, non-host dummies with total extract, washed host and non-host with hydrocarbon fraction showed a high number of antennal contacts. Female *C. plutellae* produced fewer antennal contacts for non-host caterpillars, washed non-host dummies and non-host dummies with the non-hydrocarbon fraction. With approximately 4 antennal contacts recorded per females, non-host dummies with recombined fractions were the less attractive.

*Chemical Analyses.* Forty compounds were detected by the GC analyses, but only 28 were sufficiently concentrated for analysis by GC-MS (Table 1). The chain length of these compounds ranged from 23 to 29 carbon atoms. The cuticular lipid pool was mainly composed of linear, monomethyl-branched, or dimethyl-branched alkanes with an odd-number of carbons in the chain. Four compounds were dominant and represented more than 59 % of the total cuticular lipid extract: $nC_{29}$ (21.4 %), 15-nonacosanone (18.2 %), $11MeC_{27}$ (13.1 %) and $7,16diMeC_{27}$ (6.8 %). The hydrocarbon fraction represented more than 77 % of the amount of total cuticular lipids. The non-hydrocarbon fraction, which contributed to 22.5 % of the cuticular lipids, was composed of 11 compounds, but despite pooling the cuticular lipids extracted from 200 caterpillars, only 2 compounds were present in sufficient quantities to be identified by GC-MS. Two compounds were unusual in insect cuticular lipids, a ketone (15-nonacosanone) and an unspecified triterpenoid belonging to the amyrin family.

## Discussion

Most of the compounds identified by GC-MS analyses are common in cuticular lipids of insects. Only two compounds (15-nonacosanone and a triterpenoid belonging to the amyrin family) present in high quantities are unusual in insects. 15-Nonacosanone is one of the most abundant lipids in the epicuticular waxes of cabbage together with $n$-$C_{29}$ and 14- and 15-nonacosanol (Terroine 1936; Eigenbrode *et al.* 1991). The triterpenoid was eluted with the hydrocarbon fraction on the silica gel column. To our knowledge, only α- and β-amyrin (triterpenol) have been described as constituents of the epicuticular waxes in cabbage (Eigenbrode *et al.* 1991). These two compounds were not identified in

our extract but two unknown non-hydrocarbon peaks (detected only in small quantities in GC analyses) were present in the right shoulder of our unknown triterpenoid peak and could be the two isomers of amyrin. These compounds are maybe not synthesized by caterpillars but transferred by simple contact or ingestion (Espelie & Bernays 1989; Liang & Silverman 2000).

Our data confirm that contact kairomones, extractable from the host in hexane, stimulate oviposition by *C. plutellae*. The absence of particularities and specificities in cuticular lipid semiochemicals of DBM caterpillars led to a need of several compounds to elicit a specific response of *C. plutellae* females. Moreover, the compounds involved may act in specific proportions which could explain the lower activity of the recomposed total cuticular lipid extract. Bioassays indicate that a combination of hydrocarbons and more polar components elicited oviposition rate responses which were statistically indistinguishable from oviposition responses from total extract. The small non-significant difference could well have been caused by the imperfect distribution of lipids on the dummies (Ruther *et al.* 1998). The native host produced significantly more antennal contact than all other treatments. This could be explained in several ways. Despite the fact that dummies were dried under nitrogen stream, a residual odour of hexane can be last after the washing and may make dummies less attractive. Also, the washing of dummies with hexane changes the tonicity of cuticle from these fragile caterpillars by inducing dehydration. Therefore, a minor tactile cue may be implied even if there is only one short antennal contact and not several long palpations before oviposition. It still very difficult to separate the influence of contact chemicals cues from that of tactile cues (Vinson 1976). Concerning visual cues as shape or colour, these have been excluded of host-recognition mechanism by Roux *et al.* (2005).

The recombined fraction produced 5 times fewer antennal contacts than native total extract on non-host dummies. The successive filtrations, the relative proportions of the compounds, their concentrations and/or the physiological state of the females could all be involved. Despite the necessary need of compounds coming from the two fractions for oviposition, the hydrocarbon fraction alone was seen to have a positive effect on searching behaviour in *C. plutellae* females.

Several authors have underlined the need to identify the semiochemicals involved in host location and recognition and their importance in the design of programs of biological control (Bottrell *et al.* 1998; Lewis *et al.* 1998; Cortesero *et al.* 2000; Reddy *et al.* 2002), but the potential of such an approach remains unexplored (Turlings & Wäckers 2004). It would be very interesting to find the exact compounds involved in the recognition of DBM larvae by its parasitoid *C. plutellae*.

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# CHAPITRE IV

# Structuration génétique de *Plutella xylostella*

# 1. Introduction

Dans les deux précédents chapitres, nous nous sommes plus particulièrement penchés sur la biologie et l'écologie de deux parasitoïdes de la teigne. Nous avons également étudié l'influence des facteurs biotiques et abiotiques de l'environnement tropical, au Sénégal et au Bénin, sur la dynamique des populations du ravageur et de ses ennemis naturels. Nous allons dans ce dernier chapitre nous intéresser tout particulièrement au ravageur.

*Plutella xylostella* est une espèce cosmopolite dont l'aire de répartition est mondiale. L'étendue de sa propagation est due essentiellement à son importante capacité de déplacement par migration passive, à l'extension des cultures de Brassicacées due à l'activité humaine et à son importante capacité d'adaptation à des conditions de vie les plus extrêmes et les plus variées. Cela a pour conséquence de former un véritable « patchwork » de populations aux contours souvent indéterminés, ce qui rend son contrôle encore plus délicat. Pour autant, ce ravageur est toujours considéré comme une seule et même espèce. Quelques études ont montré une certaine variabilité génétique au sein de populations de *P. xylostella* originaires d'une même région (Corée, Sud-Est de l'Australie et de la Chine) (Kim et al. 2000 ; Endersby et al. 2005 ; Wei et al. 2013). Cependant, aucune étude sur la variabilité et la structuration des populations de *P. xylostella* à l'échelle mondiale n'avait été menée à ce jour.

On peut supposer que les populations présentes dans des régions géographiques éloignées peuvent être différenciées génétiquement. Des études sur la résistance aux pesticides chez *P. xylostella* ont mis en évidence des niveaux de sensibilité différents entre des populations séparées par moins de dix kilomètres dans des régions des îles Hawaii et de Taïwan (Cheng 1981 ; Liu et al. 1982 ; Tabashnik et al. 1987). Ces variations sont probablement induites par des variations de la pression de sélection dues aux différents composés insecticides utilisés. A Hawaii, les flux géniques entre les populations ne sont pas suffisants pour réduire les différences de sensibilité aux insecticides. Toutefois, les adultes des populations situées en zone tempérée sont capables de migrer sur de longues distances, par exemple entre le sud de la Finlande et l'Angleterre (McKenzie 1958) ou entre le sud des Etats-Unis et le Canada (Harcourt 1986). Dans certaines régions, les flux de gènes entre les populations pourraient donc réduire les effets d'une dérive génétique au sein de ces populations.

Notre étude a pour objectif de déterminer si des populations d'origines très différentes au niveau mondial sont génétiquement différenciées, d'évaluer lesquelles semblent les plus isolées génétiquement et de déterminer s'il existe une relation entre les distances génétiques et les distances géographiques entre les populations.

Nous avons pour cela comparé des populations de *P. xylostella* d'origines géographiques différentes à l'échelle intercontinentale (Ile de la Réunion, Afrique du Sud, Bénin, Egypte, Brésil, Etats-Unis, Canada, Martinique, France, Roumanie, Autriche, Ouzbékistan, Japon, Philippines, Hong Kong, Laos, et quatre localités australiennes). Pour réaliser cette étude, nous avons utilisé deux marqueurs moléculaires : les isozymes et les ISSR (Inter Simple Sequence Repeat). Ces résultats font l'objet des articles 7 et 8.

## 2. Synthèse des résultats

Marqueur enzymatique : sur 21 enzymes étudiées, seulement sept (IDH, MDHP, G6PDH, MPI, PGM, HK, AAT) ont révélé des loci polymorphes avec des bandes clairement lisibles permettant une interprétation. Les tests d'Hardy-Weinberg ont montré que les populations de *P. xylostella* sont en déséquilibre pour de nombreux loci. Les déviations de l'équilibre sont dues à un déficit d'hétérozygotes pour quatre loci (IDH, MDHP, G6PGH et MPI) et à un excès pour un locus (AAT).

Marqueur ISSR : sept amorces ont été testées mais trois d'entre elles n'ont produit que des « smears » en raison d'une trop faible spécificité. A partir des quatre amorces restantes, 188 bandes ont été sélectionnées et utilisées dans les différentes analyses. La variabilité observée entre les populations est maximale (100% de polymorphisme) et les populations sont hautement différenciées sur le plan génétique ($Gst_{=}$ 0,238). Toutefois, la plus grande part de la variabilité est exprimée entre les individus au sein des populations mais les 19 populations étudiées restent cependant parfaitement identifiables.

Avec les deux marqueurs, on ne constate pas de corrélation entre les distances géographiques et les distances génétiques. Par contre, on observe clairement une structuration génétique des populations à l'échelle mondiale. Cependant avec les isozymes, cette structuration génétique semble plus cohérente. En effet, on distingue un premier groupe réunissant toutes les populations australiennes, un second groupe représenté uniquement par la population du Japon et un troisième groupe réunissant le reste des autres populations

étudiées. Si on fait un lien avec le type de climat, les populations sont réparties en deux groupes : les populations originaires des régions tropicales (Brésil, Philippines, Bénin et Réunion) et les populations originaires des régions tempérées (Australie, Ouzbékistan, France, USA, Afrique du Sud).

## 3. Conclusion

Les résultats obtenus avec les deux marqueurs moléculaires ont décrit une très forte variabilité au sein de chacune des populations. Malgré tout, la variabilité entre les populations reste très importante puisqu'elles sont parfaitement bien séparées. Il existe une structuration génétique des populations de *P. xylostella* à l'échelle mondiale. On peut penser que cette structuration est influencée par le type de climat : tropical ou tempéré.

Ces résultats étaient attendus en raison de la grande échelle géographique couverte par notre étude. Cependant, même des populations relativement proches géographiquement (moins de 600 km) présentent une forte différenciation là où Endersby et al. (2006) concluaient à des populations identiques en utilisant des microsatellites comme marqueurs.

Cette très haute variabilité intra et inter-populations trouve son origine dans la nature du marqueur utilisé (ISSR) ainsi que dans la biologie du ravageur. En effet, les ISSR étant des fragments d'ADN situés sur des portions non-codantes (marqueurs neutres), ils tendent à accumuler les mutations qui apparaissent au cours des générations. Ces mutations peuvent apparaitre en très grand nombre en raison, d'une part, de l'usage d'insecticides, agents mutagènes par excellence, d'autre part, d'un grand nombre de générations (plus de 20) susceptibles de se succéder au cours d'une même année sous des conditions climatiques de type tropical.

# ARTICLE 7

## Genetic differentiation among various populations of the diamondback moth, *Plutella xylostella* (Lepidoptera : Yponomeutidae)

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# Genetic differentiation among various populations of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae)

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**Abstract**

Genetic variation among 14 populations of *Plutella xylostella* (Linnaeus) from USA (Geneva, New York), Brazil (Brasilia), Japan (Okayama), The Philippines (Caragan de Oyo), Uzbekistan (Tashkent), France (Montpellier), Benin (Cotonou), South Africa (Johannesburg), Réunion Island (Montvert), and five localities in Australia (Adelaide, Brisbane, Mareeba, Melbourne, Sydney) were assessed by analysis of allozyme frequencies at seven polymorphic loci. Most of the populations were not in Hardy–Weinberg equilibrium and had a deficit in heterozygotes. The global differentiation among populations was estimated by the fixation index (Fst) at 0.103 for the 14 populations and at 0.047 when populations from Australia and Japan, which differed most and had a strong genetic structure, were excluded from the analysis. By contrast, the populations from Benin (West Africa) and Brazil (South America) were very similar to each other. Genetic differentiation among the populations was not correlated with geographical distance.

**Keywords:** *Plutella xylostella*, allozyme, population differentiation

## Introduction

*Plutella xylostella* (Linnaeus) (Lepidoptera: Yponomeutidae), the diamondback moth, is a major pest of *Brassica* crops and has a worldwide distribution (Talekar & Shelton, 1993). Its geographical origin is considered to be in the eastern Mediterranean region or South Africa because of the number of wild and endemic brassicas in these regions and the presence of a high number of known parasitoid species (Tsunoda, 1980; Kfir, 1998).

Populations of diamondback moth in tropical areas cause severe damage (as in Southeast Asia), but in temperate areas (e.g. Canada, USA, UK) damage to crops is usually less dramatic (Lim, 1986). Measures to control this pest were estimated globally at US$ 1 billion in 1992 (Talekar & Shelton, 1993). The main strategy of management has traditionally been insecticides (Talekar & Shelton, 1993). As a major consequence, resistance to synthetic insecticides has appeared in a relatively short period of time in the field (Sun *et al*., 1986). In addition *P. xylostella* has become the first species to develop field resistance to some of the toxins of *Bacillus thuringiensis* Berliner (Eubacteriales) (Tabashnik *et al*., 1987; Talekar & Shelton, 1993).

Genetic differences and gene flow between populations of *P. xylostella* have been investigated in the context of

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Table 1. Location and date of collection of *Plutella xylostella*.

| Country | Locality | Latitude | Longitude | Abbreviation | Date of collection |
|---|---|---|---|---|---|
| South Africa | Johannesburg | 26°08′ S | 27°54′ E | SA | 07/1999 |
| Benin | Cotonou | 6°29′ N | 2°37′ E | BEN | 09/1998 |
| Réunion Island | Montvert | 20°52′ S | 55°28′ E | REU | 08/1998 |
| Brazil | Brasilia | 15°52′ S | 47°55′ W | BRA | 08/1998 |
| United States | Geneva, NY | 42°51′ N | 76°59′ W | USA | 08/1999 |
| France | Montpellier | 43°38′ N | 3°53′ E | FRA | 08/1998 |
| Uzbekistan | Tashkent | 41°19′ N | 69°15′ E | UZB | 06/1999 |
| Japan | Okayama | 34°39′ N | 133°55′ E | JAP | 07/1999 |
| The Philippines | Caragan de Oyo | 7°04′ N | 125°36′ E | PHI | 01/1999 |
| Australia | Adelaide | 34°46′ S | 138°32′ E | AUAd | 06/1999 |
| Australia | Brisbane | 27°30′ S | 153°10′ E | AUBr | 05/1999 |
| Australia | Mareeba | 17°00′ S | 145°26′ E | AUMa | 08/1999 |
| Australia | Melbourne | 37°52′ S | 145°08′ E | AUMe | 03/1999 |
| Australia | Sydney | 33°55′ S | 151°17′ E | AUSy | 06/1999 |

insecticide resistance. Differences have been shown in the level of susceptibility to insecticide between populations less than 10 km apart, both in Taiwan and in Hawaii (Cheng, 1981; Liu *et al*., 1982; Tabashnik *et al*., 1987). These differences were probably induced by local selection pressure due to local variations in the insecticide treatments and the compounds used. Gene flow might not have been sufficient to overcome the differences in insecticide susceptibility between the populations in Hawaii (Tabashnik *et al*., 1987). Therefore it could be assumed that in some regions, isolated populations of *P. xylostella* may differentiate from each other, because of a strong selection pressure and because of reduced migration and therefore reduced gene flow.

However, adults can migrate over very long distances; mass migrations have been reported in temperate climates, between the south of Finland and England (Mackenzie, 1958), The Netherlands and England (Chapman *et al*., 2002) and between the southern USA and Canada (Harcourt, 1986). In these areas, the gene flow may be sufficient to overcome differences between the existing populations; however, differences in levels of insecticide resistance between populations may also result from different selection pressures due to local variation in insecticide use.

The genetic variation among world populations of *P. xylostella* using neutral alleles was not estimated. It was assumed that environmental factors, such as variations of temperature and rainfall could affect the selection pressure. Therefore it could be considered that genetic differences between populations of *P. xylostella* could also occur on genes which were not linked to insecticide resistance. In this study, these genetic differences between widely separated populations of *P. xylostella* were investigated with the objective of determining whether they were related to the geographical distance between populations. An earlier study of the estimate of gene flow from neutral or quasi-neutral allelic variation among populations from Hawaii, Wisconsin and Florida in the USA showed low levels of differentiation and suggested a substantial gene flow occurring between them (Caprio & Tabashnik, 1992). The enzyme electrophoresis technique, used in the study has been used to analyse geographical variations among populations of lepidopterous species (Daly & Gregg, 1985; Buès *et al*., 1994; Wainhouse & Juke, 1997).

The aims of the present study were to measure differences among *P. xylostella* populations worldwide and investigate possible reasons for these differences. Allelic variation was measured in populations from 14 different areas: USA, Brazil, South Africa, Benin, Réunion Island, France, Uzbekistan, Japan, the Philippines and five areas in Australia (Sydney, Brisbane, Melbourne, Mareeba, and Adelaide).

## Materials and methods

### *Sampling*

Strains of *P. xylostella* were obtained from 14 localities worldwide (table 1). Each sample comprised a minimum of 100 larvae and pupae. One of us (A. Kirk) and foreign collaborators collected pupae and larvae (second and third instars) by hand from plants from one field at each locality. They were immediately placed in plastic dishes with leaves of their host plant (cabbage) and dispatched by air to the CIRAD laboratory (Centre de Coopération Internationale en Recherche Agronomique pour le Développement, France) within 48 h. Samples received were reared at 25°C, ±2°C, 75% relative humidity, and a photoperiod of 12L/12D on a sequence of cultivated *Brassica* species. Different *Brassica* species were used in order to maintain moths in good condition at each stage of their development. We observed that second instar larvae developed better when placed on fresh leaves of cabbage (*B. oleracea* L. var. Chateaurenard), whereas third instar larvae did best on cauliflower leaves (*B. oleracea* L. var *botrytis*) until pupation. All pupae were placed in a plastic box until emergence of adults (males and females) which were placed in a Plexiglas cage with Chinese mustard *B. juncea* (L.) plant. Chinese mustard was used for oviposition and first instar larval development. When the number of eggs was sufficient to establish a laboratory colony, the adults were deep frozen in liquid nitrogen and conserved at −80°C. If the number of individuals was insufficient for a complete analysis, offspring of the moths were reared under the same conditions for another generation. Adults were deep frozen in liquid nitrogen and conserved at −80°C.

### *Enzyme electrophoresis*

Horizontal starch gels (13%) were prepared following the protocol of Pasteur *et al*. (1987) and stored at 4°C for up to

Table 2. Enzymes screened in populations of *Plutella xylostella* and running conditions for electrophoresis.

| Buffer | Enzyme stained | EC | Abbreviation |
|---|---|---|---|
| Tris-citrate pH 8 | Isocitrate dehydrogenase | 1.1.1.42 | IDH |
| | $NADPH^+$ malate dehydrogenase | 1.1.1.40 | MDHP |
| | Glucose-6-phosphate dehydrogenase | 1.1.1.49 | G6PDH |
| Histidine pH 6 | Mannose-6-phosphate isomerase | 5.3.1.8 | MPI |
| | Phosphoglucomutase | 5.4.2.2 | PGM |
| Tris-maleate EDTA pH 7.4 | Hexokinase | 2.7.1.1 | HK |
| | Adenylate kinase | 2.7.4.3 | AK |
| | Acid phosphatase | 3.1.3.2 | ACP |
| | Glucose-6-phosphate isomerase | 5.3.1.9 | GPI |
| | Glutamate dehydrogenase | 1.4.1.2 | GTDH |
| | Hydroxybutyrate dehydrogenase | 1.1.1.30 | HBDH |
| | Phosphogluconate dehydrogenase | 1.1.1.44 | PGDH |
| | Glycerol-3-phosphate dehydrogenase | 1.1.1.8 | G3PDH |
| | (S)-2-hydroxy-acid-oxidase | 1.1.3.15 | HAOX |
| Tris-ctrate pH 8.7 | Aspartate amino transferase | 2.6.1.1 | AAT |
| | Superoxide dismutase | 1.15.1.1 | SOD |
| | Glucose dehydrogenase | 1.1.1.118 | GCDH |
| | Xanthine dehydrogenase | 1.1.1.204 | XDH |
| | Alcohol dehydrogenase | 1.1.1.1 | ADH |
| | Gluthatione reductase | 1.6.4.2 | GR |
| | Pyruvate kinase | 2.7.1.40 | PK |

one day. Gel buffers used were Tris-citrate pH 8, histidine pH 6, Tris-citrate pH 8.7, Tris-maleate-EDTA pH 7.4.

Each entire frozen adult was homogenized in 20 µl of a NADP 2.3% solution using a pestle and mortar. This solution was then centrifuged for 10 min at 13000 rpm and 4°C. Fifteen µl of the resulting supernatant was maintained at −20°C or used immediately.

Each sample (15 µl of supernatant) was loaded on a wick of Whatman no. 3 filter paper and placed into a well of the gel. Each gel consisted of 15 wells equivalent to 15 adults from five populations, therefore three adults per population. Migration (150 V, 70 mA) lasted 6 h at 4°C. Migration buffers used were: Tris-citrate pH 8, Tris-maleate EDTA pH 7.4, borate/NaOH pH 8.25, Tris-citrate pH 8.7 (Pasteur *et al.*, 1987; Hillis *et al.*, 1996).

Soon after migration, each gel was horizontally sliced to four layers, 2 mm thick. Stains were prepared as required following the procedure of Pasteur *et al.* (1987) and Hillis *et al.* (1996). Different enzymes were tested (table 2) and each enzyme was stained in one slice. The following seven enzymes exhibited discernable bands and polymorphic loci: isocitrate dehydrogenase, $NADPH^+$ malate dehydrogenase, glucose-6-phosphate dehydrogenase, mannose-6-phosphate isomerase, phosphoglucomutase, hexokinase and aspartate aminotransferase. The banding patterns characteristic of the seven enzymes were observed in moths. Soon after their appearance, bands were noted and a scan of the gel was done to conserve a permanent recording. For each population and each enzyme, a minimum of 30 adults was used.

### *Data analysis*

Bands were interpreted in terms of loci and alleles and the allelic frequencies were determined. GENEPOP version 1.2 (Raymond & Rousset, 1995) was used to test for: (i) departures from the Hardy–Weinberg equilibrium (using Fisher's exact test); and (ii) the proportion of heterozygotes estimated under a Hardy–Weinberg equilibrium (He) and the proportion of heterozygotes observed (Ho). Differences among allelic frequencies for all pairs of populations were tested using Fisher's method, i.e. when genotypic frequencies were not independent, due to a linkage disequilibrium between loci, these were not maintained for the analysis of population structures. Deviations from equilibrium were estimated using Wright's F statistics (Weir & Cockerham, 1984). Values of Fis (deviation from random mating within a population), Fit (deviation from random mating in all populations) and Fst or 'fixation index' (deviation from random mating among populations) were given. The mean number of migrants (Nm) from each generation was estimated using the inverse relationship between Nm and Fst (Wright, 1951): $Nm = [(1/Fst) - 1]/4$, where N is the size of the population and m the mean migration ratio.

An unrooted neighbour-joining tree was constructed with the values of Fst among each population pair, using DARwin version 3.6.40 (Perrier & Jacquemoud-Collet, 2000).

## Results

Of 21 enzymes screened in *P. xylostella*, four loci did not show a band: superoxide dismutase (EC 1.15.1.1), alcohol dehydrogenase (EC 1.1.1.1), glutamate dehydrogenase (EC 1.4.1.2) and phosphogluconate dehydrogenase (EC 1.1.1.44). Some loci had diffuse bands: xanthine dehydrogenase (EC 1.1.1.204), acid phosphatase (EC 3.1.3.2), adenylate kinase (EC 2.7.4.3), hydroxybutyrate dehydrogenase (EC 1.1.1.30), (S)-2-hydroxy-acid oxidase (EC 1.1.3.15), glucose dehydrogenase (EC 1.1.1.118), glutathione reductase (EC 1.6.4.2) and glucose-6-phosphate isomerase (EC 5.3.1.9). Sometimes, bands had a good resolution but loci appeared to be monomorphic in each population: glycerol-3-phosphate dehydrogenase (EC 1.2.1.12) and pyruvate kinase (EC 2.7.1.40). Enzymes with polymorphic loci that could be clearly interpreted were: isocitrate dehydrogenase (IDH, EC 1.1.1.42), $NADPH^+$ malate dehydrogenase (MDHP, EC 1.1.1.40), glucose-6-phosphate dehydrogenase (G6PDH, EC

Table 3. Allelic frequencies of the seven polymorphic loci in the 14 *Plutella xylostella* populations.

| Locus | | SA | BEN | BRA | FRA | JAP | USA | UZB | PHI | REU | AUAd | AUBr | AUMA | AUMe | AUSy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | 32 | 31 | 30 | 30 | 33 | 35 | 32 | 34 | 32 | 33 | 34 | 35 | 35 | 35 |
| | Allele | | | | | | | | | | | | | | |
| IDHf | 1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | 2 | 0.984 | 1.000 | 0.983 | 1.000 | 0.924 | 1.000 | 0.984 | 0.812 | 1.000 | 0.985 | 0.941 | 0.943 | 0.871 | 0.900 |
| | 3 | 0.016 | 0.000 | 0.017 | 0.000 | 0.076 | 0.000 | 0.016 | 0.188 | 0.000 | 0.015 | 0.059 | 0.057 | 0.129 | 0.100 |
| IDHs | 1 | 0.016 | 0.000 | 0.017 | 0.000 | 0.045 | 0.043 | 0.016 | 0.191 | 0.000 | 0.000 | 0.059 | 0.057 | 0.000 | 0.086 |
| | 2 | 0.984 | 1.000 | 0.983 | 1.000 | 0.955 | 0.928 | 0.937 | 0.794 | 1.000 | 0.970 | 0.941 | 0.929 | 1.000 | 0.914 |
| | 3 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.029 | 0.047 | 0.015 | 0.000 | 0.030 | 0.000 | 0.014 | 0.000 | 0.000 |
| MDHPf | 1 | 0.433 | 0.383 | 0.517 | 0.383 | 0.576 | 0.469 | 0.464 | 0.375 | 0.219 | 0.803 | 0.206 | 0.000 | 0.500 | 0.500 |
| | 2 | 0.567 | 0.617 | 0.483 | 0.617 | 0.424 | 0.531 | 0.536 | 0.625 | 0.781 | 0.197 | 0.794 | 1.000 | 0.500 | 0.500 |
| MDHPs | 1 | 0.203 | 0.339 | 0.167 | 0.100 | 0.182 | 0.057 | 0.000 | 0.221 | 0.047 | 0.470 | 0.044 | 0.057 | 0.029 | 0.071 |
| | 2 | 0.484 | 0.548 | 0.550 | 0.467 | 0.455 | 0.586 | 0.516 | 0.632 | 0.406 | 0.485 | 0.897 | 0.943 | 0.971 | 0.929 |
| | 3 | 0.313 | 0.113 | 0.283 | 0.433 | 0.363 | 0.357 | 0.484 | 0.147 | 0.547 | 0.045 | 0.059 | 0.000 | 0.000 | 0.000 |
| G6PDH | 1 | 0.078 | 0.210 | 0.217 | 0.100 | 0.000 | 0.029 | 0.125 | 0.103 | 0.094 | 0.030 | 0.191 | 0.043 | 0.029 | 0.143 |
| | 2 | 0.359 | 0.371 | 0.367 | 0.350 | 0.227 | 0.143 | 0.234 | 0.279 | 0.297 | 0.318 | 0.279 | 0.329 | 0.200 | 0.343 |
| | 3 | 0.422 | 0.419 | 0.416 | 0.550 | 0.697 | 0.529 | 0.547 | 0.618 | 0.563 | 0.652 | 0.471 | 0.500 | 0.557 | 0.514 |
| | 4 | 0.141 | 0.000 | 0.000 | 0.000 | 0.076 | 0.300 | 0.094 | 0.000 | 0.047 | 0.000 | 0.059 | 0.129 | 0.214 | 0.000 |
| MPI | 1 | 0.141 | 0.113 | 0.000 | 0.033 | 0.000 | 0.100 | 0.000 | 0.059 | 0.031 | 0.000 | 0.015 | 0.257 | 0.000 | 0.043 |
| | 2 | 0.141 | 0.113 | 0.200 | 0.300 | 0.455 | 0.129 | 0.188 | 0.221 | 0.141 | 0.318 | 0.088 | 0.357 | 0.014 | 0.114 |
| | 3 | 0.359 | 0.323 | 0.333 | 0.300 | 0.545 | 0.214 | 0.531 | 0.265 | 0.344 | 0.500 | 0.882 | 0.329 | 0.714 | 0.443 |
| | 4 | 0.203 | 0.290 | 0.317 | 0.200 | 0.000 | 0.457 | 0.188 | 0.412 | 0.250 | 0.152 | 0.015 | 0.043 | 0.229 | 0.300 |
| | 5 | 0.078 | 0.129 | 0.100 | 0.117 | 0.000 | 0.086 | 0.031 | 0.044 | 0.109 | 0.030 | 0.000 | 0.000 | 0.043 | 0.100 |
| | 6 | 0.078 | 0.032 | 0.050 | 0.050 | 0.000 | 0.014 | 0.063 | 0.000 | 0.125 | 0.000 | 0.000 | 0.014 | 0.000 | 0.000 |
| PGM | 1 | 0.016 | 0.016 | 0.017 | 0.000 | 0.000 | 0.014 | 0.016 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | 2 | 0.062 | 0.032 | 0.117 | 0.017 | 0.000 | 0.114 | 0.047 | 0.000 | 0.016 | 0.031 | 0.000 | 0.000 | 0.014 | 0.043 |
| | 3 | 0.656 | 0.597 | 0.583 | 0.450 | 1.000 | 0.715 | 0.656 | 0.647 | 0.734 | 0.939 | 0.985 | 0.986 | 0.986 | 0.671 |
| | 4 | 0.250 | 0.307 | 0.200 | 0.533 | 0.000 | 0.143 | 0.187 | 0.235 | 0.219 | 0.030 | 0.015 | 0.014 | 0.000 | 0.200 |
| | 5 | 0.016 | 0.048 | 0.083 | 0.000 | 0.000 | 0.014 | 0.094 | 0.118 | 0.031 | 0.000 | 0.000 | 0.000 | 0.000 | 0.086 |
| HK | 1 | 0.000 | 0.097 | 0.000 | 0.050 | 0.000 | 0.071 | 0.031 | 0.015 | 0.031 | 0.000 | 0.103 | 0.000 | 0.014 | 0.014 |
| | 2 | 0.828 | 0.790 | 0.900 | 0.900 | 0.424 | 0.843 | 0.844 | 0.926 | 0.844 | 0.697 | 0.735 | 0.800 | 0.700 | 0.757 |
| | 3 | 0.172 | 0.113 | 0.100 | 0.050 | 0.576 | 0.086 | 0.125 | 0.059 | 0.125 | 0.303 | 0.162 | 0.200 | 0.286 | 0.229 |
| AAT | 1 | 0.234 | 0.452 | 0.417 | 0.100 | 0.000 | 0.057 | 0.016 | 0.471 | 0.422 | 0.091 | 0.000 | 0.186 | 0.086 | 0.214 |
| | 2 | 0.766 | 0.532 | 0.583 | 0.850 | 0.803 | 0.943 | 0.984 | 0.529 | 0.578 | 0.909 | 0.897 | 0.814 | 0.914 | 0.686 |
| | 3 | 0.000 | 0.016 | 0.000 | 0.050 | 0.197 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.103 | 0.000 | 0.000 | 0.100 |

See tables 1 and 2 for abbreviations.
N, number of adults analysed for each population.

1.1.1.49), mannose-6-phosphate isomerase (MPI, EC 5.3.1.8), phosphoglucomutase (PGM, EC 5.4.2.2), hexokinase (HK, EC 2.7.1.1) and aspartate aminotransferase (AAT, EC 2.6.1.1).

Some enzymes had several loci: IDH with loci IDHf and IDHs; MDHP with loci MDHPf and MDHPs. Allelic frequencies obtained for each locus in each population are given in table 3. Tests of the Hardy–Weinberg equilibrium showed that populations of *P. xylostella* were unbalanced at numerous loci. Frequencies observed differed from those estimated in 70 of the 126 comparisons ($P < 0.05$). They were caused by heterozygote deficits in the loci IDHs, MDHPs, G6PDH and MPI, and by an excess of heterozygotes in locus AAT (table 4). Fis values were significantly different from 0 for the loci MDHPs ($\chi^2 = 194.4$, df = 28, $P = 0.000$), G6PDH ($\chi^2 = 268.2$, df = 28, $P = 0.000$), MPI ($\chi^2 = \infty$, df = 28, $P = 0.000$), and AAT ($\chi^2 = 96.1$, df = 26, $P = 0.000$).

The loci IDHf and IDHs were monomorphic in populations of *P. xylostella* from Benin, France, Reunion Island and Melbourne. A low number of heterozygotes was observed for the locus PGM: it was monomorphic in the population from Japan and some of its alleles had low frequencies in populations from Adelaide, Brisbane, Mareeba and Melbourne in Australia. Allelic frequencies of the locus MDHPf showed variation between relatively close populations such as those from Adelaide and Mareeba. The mean heterozygosity observed (Ho) ranged from 0.183 (Brisbane) to 0.424 (Philippines). These values were lower than those estimated from the allelic frequencies, following the Hardy–Weinberg equilibrium (He), except for the populations from Melbourne, Sydney and Adelaide (table 5).

Allelic frequencies were significantly different for all pairs of populations using the Fisher test, except for the pair Benin/Brazil ($\chi^2 = 28.029$, $P = 0.06162$).

Some loci showed unbalanced linkages: IDHs and IDHf, MEf and G6PDH, MEf and MPI. Loci IDHf and MEf were not used in later analyses.

For all populations, Fst = $0.103 \pm 0.025$, and $P$ (Fst = 0) < 0.001. Values of Fst obtained for pairs of populations ranged from 0 (Benin–Brazil) to 0.230 (France–Melbourne) (table 6). In the Australian population group, Fst ranged from 0.039 (Brisbane–Melbourne) to 0.126 (Adelaide–Brisbane). The values of Fst between the populations from Australia and the other populations were over 0.100, except for the population from Sydney (table 6). Fst calculated for the population of Japan was also relatively high, it ranged from 0.072 (Japan–Adelaide) to 0.195 (Japan–Philippines). The estimated Fst for other pairs

Table 4. F statistics for all *Plutella xylostella* populations.

| Locus | Fis | Fst | Fit |
|---|---|---|---|
| IDHf | −0.001 | 0.056 | 0.047 |
| IDHs | 0.103 | 0.049 | 0.147 |
| MDHPf | −0.024 | 0.140 | 0.120 |
| MDHPs | 0.191* | 0.173 | 0.331 |
| G6PDH | 0.484* | 0.020 | 0.494 |
| MPI | 0.227* | 0.092 | 0.298 |
| PGM | 0.008 | 0.136 | 0.144 |
| HK | 0.026 | 0.087 | 0.111 |
| AAT | −0.417* | 0.155 | −0.197 |
| All | 0.151* | 0.103* | 0.238 |

See table 2 for abbreviations.

Table 5. Mean observed and expected heterozygosity at seven loci in *Plutella xylostella* populations.

| Population | Ho | He | $\chi^2$ | df | *P* |
|---|---|---|---|---|---|
| South Africa | 0.315 | 0.425 | ∞ | 14 | 0.000 |
| Benin | 0.406 | 0.436 | 73 | 14 | 0.000 |
| Réunion Island | 0.323 | 0.384 | 95.1 | 14 | 0.000 |
| Brazil | 0.374 | 0.429 | 77.2 | 14 | 0.000 |
| USA | 0.311 | 0.373 | 70 | 16 | 0.000 |
| France | 0.289 | 0.378 | 70.8 | 14 | 0.000 |
| Uzbekistan | 0.271 | 0.363 | 63.6 | 14 | 0.000 |
| Japan | 0.307 | 0.350 | 57.2 | 16 | 0.000 |
| The Philippines | 0.424 | 0.451 | 93.1 | 18 | 0.000 |
| Australia | | | | | |
| Adelaide | 0.316 | 0.310 | 45.7 | 16 | 0.000 |
| Brisbane | 0.183 | 0.253 | 50 | 16 | 0.000 |
| Mareeba | 0.222 | 0.261 | 34 | 14 | 0.002 |
| Melbourne | 0.314 | 0.274 | 62.6 | 14 | 0.000 |
| Sydney | 0.409 | 0.406 | 89.4 | 18 | 0.000 |

Ho, observed heterozygosity; He, estimated heterozygosity under Hardy–Weinberg equilibrium.

of populations ranged from 0.007 (Brazil–South Africa) to 0.104 (Uzbekistan–Philippines). The genetic differences between the populations were shown in an unrooted tree calculated with the values of Fst, using the neighbour-joining method (fig. 1). No relationship was observed between the geographic distances and the values of Fst. Populations from Benin and Brazil exhibited no differences (Fst = 0.0002), or were not considered as under reproductive isolation; whereas populations from Australia and Japan had a higher value of Fst, suggesting non-random mating and a low degree of gene flow with other populations. When populations from Australia and Japan were excluded from the analysis, Fst was 0.047 ± 0.020, with *P* (Fst = 0) < 0.001.

Populations differed the most about the loci MDHPs (Fst = 0.173), PGM (Fst = 0.136) and AAT (Fst = 0.155). Of the loci analysed, AAT was the only one that exhibited an excess of the heterozygotes observed, compared to the estimate. To assess its impact on the values of Fst, analyses were conducted without the allelic frequencies of AAT. The values of the heterozygosity observed were inferior to the values expected from the allelic frequencies, except in the population from Adelaide in Australia (table 7). The estimate of Fst for all the populations was 0.095 ± 0.027, with P (Fst = 0) < 0.033. Concerning population pairs, Fst ranged from 0 (Benin–Brazil) to 0.241 (France–Melbourne). The values of Fst were within the same estimates as previous analyses, but lower in the majority of the pairs of populations. When the populations from Australia and Japan were excluded from the analysis, the mean Fst = 0.02. However, the populations from Australia and Japan remained very different from the other populations.

The estimated comparative numbers of migrants per generation each year (Nm) has been calculated for some populations. When a population is in a suitable environment (25°C), a new generation is produced every four weeks. Nm was from 2 to 6 between populations in Australia and was estimated at 15 between populations from Benin and South Africa. The mean number of migrants was around seven individuals per generation for the populations from France and Uzbekistan. The mean numbers of migrants estimated between widely separated populations were not significantly different. The South African population exhibited a relatively high migration potential.

## Discussion

The enzymes corresponded to 11 loci of which nine were polymorphic. *Plutella xylostella* can therefore be considered a highly polymorphic species. No alleles were found that discriminated one population from another. The analyses did not reveal a locus linked to the sex, or the presence of null alleles.

Table 6. Fst estimated for all pairs of *Plutella xylostella* populations.

| Population | SA | BEN | BRA | FRA | JAP | USA | UZB | PHI | REU | AUAd | AUBr | AUMa | AUMe |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BEN | 0.017 | | | | | | | | | | | | |
| BRA | 0.007 | 0.000 | | | | | | | | | | | |
| FRA | 0.024 | 0.064 | 0.043 | | | | | | | | | | |
| JAP | 0.124 | 0.186 | 0.175 | 0.185 | | | | | | | | | |
| USA | 0.032 | 0.093 | 0.067 | 0.067 | 0.166 | | | | | | | | |
| UZB | 0.026 | 0.102 | 0.063 | 0.036 | 0.122 | 0.035 | | | | | | | |
| PHI | 0.042 | 0.019 | 0.013 | 0.079 | 0.195 | 0.081 | 0.104 | | | | | | |
| REU | 0.016 | 0.040 | 0.013 | 0.049 | 0.150 | 0.068 | 0.052 | 0.046 | | | | | |
| AUAd | 0.067 | 0.101 | 0.107 | 0.139 | 0.072 | 0.117 | 0.098 | 0.116 | 0.127 | | | | |
| AUBr | 0.136 | 0.180 | 0.173 | 0.217 | 0.166 | 0.176 | 0.119 | 0.196 | 0.197 | 0.126 | | | |
| AUMa | 0.086 | 0.121 | 0.117 | 0.169 | 0.151 | 0.115 | 0.128 | 0.113 | 0.146 | 0.097 | 0.103 | | |
| AUMe | 0.131 | 0.176 | 0.172 | 0.230 | 0.175 | 0.135 | 0.132 | 0.180 | 0.193 | 0.124 | 0.039 | 0.081 | |
| AUSy | 0.047 | 0.044 | 0.043 | 0.097 | 0.159 | 0.082 | 0.085 | 0.044 | 0.092 | 0.096 | 0.088 | 0.056 | 0.069 |

See table 1 for abbreviations.

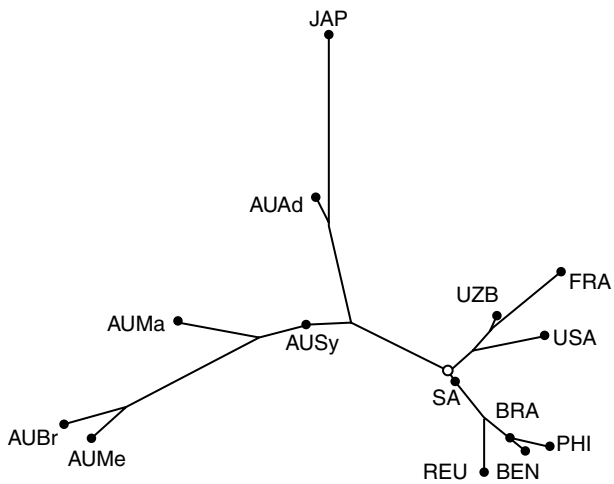


Fig. 1. Unrooted tree for fixation index (Fst) among pairs of populations of *Plutella xylostella*, calculated using the unweighted neighbour-joining method. See table 1 for abbreviations.

Table 7. Mean observed and expected heterozygosities at six loci (AAT excluded) in *Plutella xylostella* populations.

| Population | Ho | He | $\chi^2$ | df | *P* |
|---|---|---|---|---|---|
| South Africa | 0.365 | 0.489 | ∞ | 10 | 0.000 |
| Benin | 0.445 | 0.586 | 57.8 | 10 | 0.000 |
| Réunion Island | 0.400 | 0.523 | 53.2 | 10 | 0.000 |
| Brazil | 0.311 | 0.472 | 56 | 10 | 0.000 |
| USA | 0.457 | 0.523 | 59.5 | 10 | 0.000 |
| France | 0.320 | 0.531 | 63 | 10 | 0.000 |
| Uzbekistan | 0.370 | 0.438 | 37 | 10 | 0.000 |
| Japan | 0.394 | 0.449 | 43.9 | 10 | 0.000 |
| The Philippines | 0.350 | 0.501 | 56.6 | 12 | 0.000 |
| Australia | | | | | |
| Adelaide | 0.379 | 0.374 | 35 | 10 | 0.000 |
| Brisbane | 0.191 | 0.275 | 41.6 | 10 | 0.000 |
| Mareeba | 0.248 | 0.318 | 32.8 | 10 | 0.000 |
| Melbourne | 0.276 | 0.300 | 18.8 | 10 | 0.043 |
| Sydney | 0.324 | 0.418 | 40.1 | 12 | 0.000 |

Ho, observed heterozygosity; He, estimated heterozygosity under Hardy–Weinberg equilibrium.

Deviations from the Hardy–Weinberg equilibrium were caused by heterozygote deficits, except for one locus (aspartate aminotransferase) where an excess of heterozygotes was found. However, the results seemed to reveal a Walhund effect (resulting from a mix of several panmictic sub-populations for which initial allelic frequencies are fairly different) and may be explained by the various following causes which are not mutually exclusive. Departures from the Hardy–Weinberg equilibrium could be attributed to the sampling method, which included several populations of related species with different allelic frequencies. Another explanation was that the low heterozygosity could be derived from a small founding population. An example would be that a low number of migrants could have established a new population in an area. This hypothesis was an explanation for the reduction of the heterozygosity observed in the corn earworm *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Mallet *et al*., 1993). This phenomenon might have happened in the population from the USA since Geneva, New York is in northeastern USA, on the migration pathway of adults of *P. xylostella* from the southern states to Canada (Dosdall *et al*., 2002). Another example would be that climatic conditions could considerably reduce the size of a population in an area. In the northern region of Japan during winter, densities of *P. xylostella* are around five individuals to ten cabbage plants (Honda *et al*., 1992). In Benin, where cabbage production is maintained throughout the year, the population size decreases from June to September, because of the impact of heavy rains (Bordat & Goudegnon, 1997). A third explanation is that heterozygote deficits could be a consequence of reproductive isolation. Kim *et al*. (1999) considered this hypothesis, as they observed a high Fis in populations from South Korea. In the present study, there were not sufficient data to evaluate population dynamics throughout the year, or to ascertain the level of migration. Therefore, none of the three hypotheses could be confirmed.

The global Fst was relatively high (Fst = 0.103), compared to the values obtained in previous studies on *P. xylostella*. Caprio & Tabashnik (1992) obtained Fst values of 0.028–0.034 and Kim *et al*. (1999) estimated Fst = 0.0215. The differentiation index for populations excluding those from Australia and Japan (Fst = 0.047) was similar to these values. Values of Fst for populations of other lepidopterous species vary from 0.109 for pine beauty moth *Panolis flammea* (Denis & Schiffermüller) (Lepidoptera: Noctuidae) (Wainhouse & Juke, 1997), Fst = 0.080 for bog fritillary *Proclossiana eunomia* (Esper) (Lepidoptera: Nymphalidae) (Nève *et al*., 2000), Fst = 0.007 in the populations of the migrating black antworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) (Buès *et al*., 1994) and among African and European populations of the cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Nibouche *et al*., 1998).

In the present study, estimates of Fst between the populations were not correlated with the geographic distances between them. Populations from Australia and Japan were the most different, whereas populations from Brazil and Benin exhibited very similar allelic frequencies. This result has to be confirmed using DNA data to elucidate the phylogeny of these populations. Results of the enzyme electrophoresis were not informative enough to assume that these populations had the same geographical origin, or if the allelic frequencies were related to a high gene flow.

Gene flow between populations was limited, as confirmed by the number of migrants, particularly between populations in Australia. Nevertheless, migrations in tropical and subtropical areas (e.g. South Africa/Benin) appeared to be more important than in temperate areas (e.g. Uzbekistan/France).

Populations from Australia and Japan were different from other populations and between themselves. The mean number of migrants estimated among the Australian populations was reduced despite the short distances separating these populations. The differences could be the consequence of a small size of the population and a reduced gene flow. When populations are small, effects of genetic drift are more important. As a consequence, a higher number of migrants are necessary so gene flow can counterbalance genetic drift (Allendorf & Phelps, 1981). These populations could be in a condition of high reproductive isolation.

In addition, variations of the genetic structure of populations observed through their allelic frequencies could

be linked to insecticide resistance. The role of esterases in resistance to organophosphorous compounds has been demonstrated in *P. xylostella* (Liu *et al*., 1982; Miyata *et al*., 1986). In a study on the cotton whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), the allelic frequencies of esterases were different in an insecticide treated sample and in an unsprayed control sample (Wool *et al*., 1993). The enzymes studied here were not directly involved in the insecticide resistance mechanism. However, Herrero *et al*. (2001) has found a correlation between the presence of an allele in the locus MPI and the resistance to the toxin Cry1A of *Bacillus thuringiensis* (Bt) in *P. xylostella*. The change in frequency of the isozyme is an argument in favour of a linkage at the locus MPI to the Cry1A resistance locus. The appearance of this isozyme was caused by genetic selection and not by a physiological induction. As in the present study, no data on resistance in the populations sampled (to Bt toxins or other insecticides) were available, the link between the resistance and the allelic frequencies observed has not been confirmed. But, considering the results, it is possible that the isozyme linked to the locus of Bt resistance corresponded to the allele 1 or 2 of the locus MPI. The presence of this isozyme could be used to detect and to identify a resistance to Bt toxins in a strain and to evaluate its dispersal ability in the area. Because of the MPI linkage to *Bt* toxins, this locus might not be neutral, as required for a study of geographic variations. Results obtained in the present study show that populations are not most differentiated at this locus.

In conclusion, estimates of Fst suggested that the degree of differentiation could vary within a population of *P. xylostella* (migrations, reduction of size due to environmental conditions), and that gene flow could be important in tropical and sub-tropical populations. Diamondback moth population sizes can vary as a function of their local environment and migration in temperate areas where they probably do not overwinter. Gene flow could maintain the low level of allelic variation between most populations. Enzyme genotypes were not informative enough to precisely evaluate either the gene flow between populations a short distance apart or the gene flow over long distances, probably by way of mass migrations. Allelic frequencies may vary in time, according to population size, which itself varies due to genetic drift and migration. These factors could then change the frequency of enzyme genotypes.

Recent work on the genetic basis of insecticide resistance has shown that the frequencies of some isozymes could be related to a resistant genotype (Herrero *et al*., 2001). Further studies are needed to confirm this hypothesis and to consider enzyme genotypes as markers to detect resistance in a newly established population. The enzyme electrophoresis technique was not accurate enough to gain insights into the phylogeny of *P. xylostella*. Molecular markers are more powerful tools to assess relationships at the population level.

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# ARTICLE 8

## ISSR-PCR: Tool for discrimination and genetic structure analysis of *Plutella xylostella* populations native to different geographical areas

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# ISSR-PCR: Tool for discrimination and genetic structure analysis of *Plutella xylostella* populations native to different geographical areas ☆

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**Abstract**

The diamondback moth (DBM), *Plutella xylostella* (L.) is considered as the most destructive pest of Brassicaceae crops world-wide. Its migratory capacities and development of insecticide resistance in many populations leads to more difficulties for population management. To control movement of populations and apparitions of resistance carried by resistant migrant individuals, populations must be identified using genetic markers. Here, seven different ISSR markers have been tested as a tool for population discrimination and genetic variations among 19 DBM populations from Canada, USA, Brazil, Martinique Island, France, Romania, Austria, Uzbekistan, Egypt, Benin, South Africa, Réunion Island, Hong Kong, Laos, Japan and four localities in Australia were assessed. Two classification methods were tested and compared: a common method of genetic distance analyses and a novel method based on an advanced statistical method of the Artificial Neural Networks' family, the Self-Organizing Map (SOM). The 188 loci selected revealed a very high variability between populations with a total polymorphism of 100% and a global coefficient of gene differentiation estimated by the Nei's index (*Gst*) of 0.238. Nevertheless, the largest part of variability was expressed among individuals within populations (AMOVA: 73.71% and mean polymorphism of 94% within populations). Genetic differentiation among the DBM populations did not reflect geographical distances between them. The two classification methods have given excellent results with less than 1.3% of misclassified individuals. The origin of the high genetic differentiation and efficiency of the two classification methods are discussed.
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*Keywords:* Decision-making tool; Inter Simple Sequence Repeat; Genetic differentiation; *Plutella xylostella*; Self-Organising Map; Pest management; Population analysis

## 1. Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera; Plutellidae), is the major cosmopolitan pest of brassica and other crucifer crops in many areas of the world. DBM can live under wide climatic conditions and is known to migrate across the world (Chu, 1986; Honda, 1990; Honda et al., 1992; Chapman et al., 2002; Coulson et al., 2002). DBM is a prolific species in tropical climates, where it can have more than 20 generations a year. DBM can cause more than 90% crop loss (Verkerk and Wright, 1996) and only few fourth stage larvae on a cabbage can make it unsaleable (Shelton et al., 1983; Maltais et al., 1998).

Extensive insecticide applications are used for its management which has led to a rapid increase in DBM resistance. Resistance to DDT appeared in 1953 (Ankersmit, 1953) and to *Bt* in 1980 (Tabashnik et al., 1990; Shelton et al., 1993a,b; Tabashnik, 1994). The cost of DBM populations control worldwide has been estimated as

☆ This study forms a part of O. Roux's PhD thesis on the relationship between DBM and a larval parasitoid *Cotesia plutellae*. The authors are part of a university laboratory which has for interest diversity and evolution in agro-ecosystem and part of an Agricultural Research Centre for International Development where some laboratories are implicated in IPM in tropical areas. M. Gevrey was developing and adapting neural network to molecular work. L. Arvanitakis and D. Bordat are IPM entomologists were providing us the background of knowledge on DBM, reared in laboratory all populations from fields. This work was directed by L. Legal and C. Gers who are both evolutionary ecologists.

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approximately one billion US $ annually (Talekar and Shelton, 1993).

Many authors have noticed differences in susceptibility to many insecticides between DBM strains (Díaz-Gomez et al., 2000; Gonzalez-Cabrera et al., 2001; Mohan and Gujar, 2002, 2003; Liu et al., 2003). However, migration capabilities of the pest cause difficulties for strains determination and delay the use of IPM programs. The development of some markers is necessary to identify and characterize DBM strains. Such markers have to be low cost and to give fast results.

This topic study explores the usefulness of Inter Simple Sequence Repeat (ISSR) markers to identify and discriminate several populations of DBM worldwide through genetic variations. The ISSR is known to evolve rapidly and consequently generate a large number of polymorphic bands at the intraspecific level. Bands are generated by a single-primer PCR reaction where the primer is a repetition of a di-, tri- or tetranucleotide and the amplified region is a portion of genome between two identical microsatellite primers with an opposite orientation on the DNA strand. These primer sequences are broadly distributed on the genome. Therefore, the ISSR-PCR technique permits to screen quickly a wide part of the genome without prior DNA sequence knowledge. As for RAPD (Random Amplification of Polymorphic DNA), ISSR bands are considered as dominant markers but have higher reproducibility (Fang and Roose, 1997; Nagaoka and Ogihara, 1997). The diallelique interpretation (presence/absence) may cause matters. Indeed the absence of a band can correspond to one or several divergences in primer site or to a chromosomal rearrangement (Wolfe and Liston, 1998) and presence of two bands with the same weight does not necessarily affirm similarity, as the variability is probably underestimated. Nevertheless, ISSR has already been used in numerous organisms for genetic characterization (Reddy et al., 1999; Sobhian et al., 2003; Cano et al., 2005), to assess genetic diversity (Qiu et al., 2004; Wang et al., 2005; Lu et al., 2006; Zhang et al., 2006), to identify genetic trait loci (Zietkiewicz et al., 1994; Ratnaparkhe et al., 1998; Blair et al., 1999; Arcade et al., 2000), and for understanding phylogenetic and/or interspecific relationships (Wolfe and Liston, 1998; Josh et al., 2000; Wolfe and Randle, 2001; Datwyler and Wolfe, 2004; Wu et al., 2005).

Several families of Lepidoptera have been investigated using ISSR markers; Noctuidae, Pyralidae, Pieridae and Sphingidae (Luque et al., 2002; Hundsdoerfer et al., 2005; Hundsdoerfer and Wink, 2005). An interesting element is that depending the type of population studied (open or close), variability and number of informative bands varies from 50% for the localized species: *Diarsia brunnea* (Noctuidae) (Luque et al., 2002) to 100% for a quasi cosmopolitan species such as *Pieris rapae* (Pieridae) (Hundsdoerfer and Wink, 2005).

The Self-Organizing Map (SOM) (Kohonen, 1982), which is an advanced statistical method of Artificial Neural Networks' family, is an efficient method when complex non-linear relationships are present in the analysed system to classify complex data (Lek et al., 1996; Lek and Guégan, 2000; Park et al., 2003a). SOM provides an alternative to traditional statistical methods as Principal Component Analysis, Polar Ordination, Correspondence Analysis and Multidimensional Scaling (Foody, 1999; Giraudel and Lek, 2001; Brosse et al., 2001). SOM's are used widely for knowledge discovery, pattern recognition, clustering and visualisation of large multi-dimensional datasets (Ferran and Ferrara, 1992; Chon et al., 1996; Park et al., 2003b; Gevrey et al., 2004). To our knowledge only a few recent studies have used SOM with genetic data (Giraudel et al., 2000; Zhao et al., 2006), but it has been successfully used over the last few decades in biology (Lek and Guégan, 2000; Recknagel, 2003; Lek et al., 2005). This study attempts to associate a molecular marker and a classification statistical method to provide a complete decision-making tool in DBM invasion management.

## 2. Materials and methods

### 2.1. Plants and insects

DBM populations native to 16 countries and 19 different localities given in Table 1 were collected on cabbage, *Brassica oleracea* var. *capitata*. DBM females laid on Indian mustard, *Brassica juncea*, in Plexiglas 50 × 50 × 50 cm cages. Water and honey were provided as food *ad libitum*. All larval stages were reared on *B. oleracea* var. *capitata*. DBM populations were maintained in climatic rooms at 25 ± 1 °C, 40–50% RH and a 12L:12D photoperiod. All adults used to this study were spring from the first rearing generation.

For molecular analysis, DBM adults were killed in liquid nitrogen and conserved to −80 °C until DNA extraction.

### 2.2. DNA extraction

Abdomens were cut from dead and frozen DBM males and incubated 12 h at 50 °C in 350 μL of lyses buffer B (10 mM Tris, pH 7.5, 25mM EDTA, and 75 mM NaCl) with 500 μg of Proteinase K and 20 μL of 20% SDS. Proteins and residues were precipitated with 200 μL of saturated NaCl solution and centrifuged at 1400 rpm for 30 min. DNA from supernatant was saved and precipitated with 400 μL of cold isopropanol and centrifuged at 1400 rpm for 40 min at 2 °C. The isopropanol was eliminated and the precipitate was washed with 500 μL of 70% ethanol, centrifugated at 1400 rpm for 10 min at 2 °C, dried and redissolved in 100 μL of TE buffer and conserved at −28 °C until utilization.

### 2.3. ISSR-PCR and electrophoresis

Inter Simple Sequence Repeat (ISSR) analysis was performed using seven different primers listed in Table 2.

Table 1
Location and date of collection of the 19 DBM populations

| Country | Site | Abbreviation code | Site coordinate | | Date of collection |
|---|---|---|---|---|---|
| | | | Latitude | Longitude | |
| Canada | Beaverlodge | Bea | 55°13′N | 119°25′O | 01-2002 |
| United States | Geneva, NY | Gen | 42°52′N | 76°59′O | 09-1998 |
| Brazil | Brasilia | Bra | 15°47′S | 47°53′O | 09-2000 |
| Martinique Island | Le Carbet | Car | 14°41′N | 61°11′O | 02-2001 |
| France | Montpellier | Mon | 43°37′N | 03°52′E | 02-1999 |
| Romania | Iasi | Ias | 47°10′N | 27°35′E | 10-2003 |
| Austria | Seibersdorf | Sei | 47°58′N | 16°31′E | 12-2002 |
| Uzbekistan | Tashkent | Tas | 41°17′N | 69°16′E | 07-1998 |
| Egypt | El Fayoun | ElF | 29°19′N | 30°50′E | 04-2000 |
| Benin | Cotonou | Cot | 06°22′N | 02°26′E | 02-2001 |
| South Africa | Pretoria | Pre | 25°44′S | 28°12′E | 09-1999 |
| Réunion island | Piton Hyacinthe | P.Hy | 21°13′S | 55°31′E | 11-1999 |
| Hong Kong | Hong Kong | HK | 22°23′N | 114°13′E | 12-1998 |
| Laos | Vientiane | Vie | 17°57′N | 102°37′E | 02-1999 |
| Japan | Okayama | Oka | 34°40′N | 133°55′E | 09-1999 |
| Australia | Adelaide | Ade | 34°57′S | 138°34′E | 06-1998 |
| | Melbourne | Mel | 37°51′S | 144°57′E | 09-1999 |
| | Brisbane | Bri | 27°27′S | 153°30′E | 06-1998 |
| | Sydney | Syd | 33°52′S | 151°12′E | 06-1999 |

Table 2
SSR primer screened for ISSR-PCR and their annealing temperature

| Primer code | Primer sequence (5′ → 3′) | Primer abbreviation | Annealing *T*°C |
|---|---|---|---|
| CA | CACACACACACACA | $(CA)_7$ | 47 |
| CA+ | CACACACACACACARY | $(CA)_7RY$ | 50 |
| +CA | RYCACACACACACACA | $RY(CA)_7$ | 50 |
| +ACA | BDBACAACAACAACAACA | $BDB(ACA)_5$ | 50 |
| ACA+ | ACAACAACAACAACABDB | $(ACA)_5BDB$ | 50 |
| +GACA | WBGACAGACAGACAGACA | $WB(GACA)_4$ | 58 |
| GACA+ | GACAGACAGACAGACAWB | $(GACA)_4WB$ | 58 |

With B = T, C or G; D = A,T or G; R = A or G; W = A or T; Y = C or T.

The reaction mixture (25 μL) contained 50 ng of DNA template, 50 μM of primer, 5 mM of PCR nucleotide Mix (C144H, Promega), 2.5 μL of 10× PCR buffer (10 mM Tris–HCl, pH 9, 50 mM KCl, and 0.1% Triton® X-100), 3.5 μL of $MgCl_2$ (25 mM) and 2 units *Taq* polymerase (M166A, Promega). PCRs were carried out on a T3 Thermocycler Biometra. The cycling conditions were: initial denaturation of 4 min at 94 °C, 39 cycles of 45 s at 94 °C, 45 s at 50 or 58 °C and 2 min at 72 °C, one last step of 10 min at 72 °C, followed by 4 °C storage.

Primers were first tested in order to identify those producing a clear amplified product. Seven micro-litres of amplified products were mixed with 3 μL of bromophenol blue and electrophoresis was performed on a 2% agarose gel using 1× Tris acetate EDTA buffer at 130 V and on 10 cm, detected with ethidium bromide under UV light and digitized.

### 2.4. Data analysis

Smeared and weak bands obtained with certain primers were excluded. Reproducible bands were scored as 1 (presence) or 0 (absence) for individuals, and matrices generated by each primer were assembled.

The binary matrix was used under the Hardy–Weinberg equilibrium to calculate the percentage of polymorphism (*P*), Nei's gene diversity (*H*), Shannon's information index (*i*), total gene diversity (*Ht*), within population gene diversity (*Hs*), between population gene diversity (*Dst*), coefficient of gene differentiation (*Gst*), the level of gene flow (*Nm*), Nei (1972) genetic identity (*I*) and genetic distance (*D*) using POPGEN 1.32 (Yeh et al., 1997). In order to describe genetic structure and variability among and between populations, the non-parametric Analysis of Molecular Variance (AMOVA) was performed using Arlequin software (version 2.000, Schneider et al., 2000) with 10,000 permutations and Euclidian distances.

#### 2.4.1. Classical method

Most of time the UPGMA distance method was used to treat the band data. However, this method does not fully take into account evolutionary patterns and is not searching for optimal tree. Therefore a heuristic search for an optimal tree was carried out by TBR (Tree–bisection–reconnection) branch swapping. Distance (minimum evolution) measure uses the mean character difference. Distance analysis was performed using PAUP version

4.0b10 (Swofford, 2001). Negative branch lengths were allowed, but set to zero for tree-score calculation. Steepest descent options were not in effect. Starting tree(s) were obtained via neighbour joining and no out-group was used.

### 2.4.2. SOM method

An unusual statistical method for ISSR data analysis was also used to evaluate differentiation of populations, the Self-Organizing Map (SOM) (Kohonen, 1982). Its classification potential using such data was assessed. This artificial neural network method is powerful and adaptive. It used an unsupervised learning algorithm that is efficient in modelling complex non-linear relationships. SOM performs a non-linear projection of the multi-dimensional data space onto 2D space. Two layers (input and output) of elements called neurons constitute this artificial network. The input layer is associated to the *Real Vectors* (*RV*) represented by the samples of the data, previously randomly mixed. There are as many neurons in this layer as element in the samples. The output layer is often represented by a map or a rectangular grid with $l$ by $m$ neurons, laid out in a hexagonal lattice in order to not favour horizontal and vertical directions (Kohonen, 2001). Each neuron of this layer is associated with a *Virtual Vector* (*VV*) composed of as many elements as neurons in the input layer. The neurons of both layers are connected by links that are called weights.

The SOM algorithm can be summarised as follows:

- The virtual vectors ($VV_j$, $1 \leqslant j \leqslant c$) are initialised with a random sample.
- The virtual vectors are updated in an iterative way.
  - A real vector ($RV_k$) is chosen as an input vector.
  - The Euclidean distance between this $RV_k$ and each $VV$ (each output neuron) is computed.
  - The $VV$ closest to the $RV_k$ is selected and called "best matching unit" (BMU).
  - The BMU and its neighbours are moved slightly towards the $RV_k$ using this rule:

$$VV_j(t+1) = VV_j(t) + \eta(t) \cdot N(t,r)(RV_k(t) - VV_j(t))$$

where $t$ is the number of iterations, and $RV_k(t)$ is a real vector. In other words, $RV_k(t)$ is a vector of the values of the input neurons at iteration $t$, $VV_j(t)$ is a virtual vector that represents the weights between a neuron $j$ of the output layer and all the neurons of the input layer at iteration $t$, $\eta(t)$ is the learning rate that is a decreasing function of iteration $t$ and $N(t,r)$ is the neighbourhood function with $r$ representing the distance in the map between the winning neuron and its neighbouring neurons. This function defines the size of the neighbourhood of the winning neuron (BMU) to be updated during the learning process. The learning algorithm is broken down into two parts: (i) the $VV$ are widely modified in a large neighbourhood of the BMU (large values of $\eta$), the ordering phase; (ii) only the $VV$ adjacent of the BMU are modified ($t$ much larger than the former phase and $\eta$ decreasing very slowly toward 0), the tuning phase.

More details of the SOM algorithm could be found in Kohonen (2001) and Chon et al. (1996). Giraudel and Lek (2001) or Park et al. (2003b) are example studies that also detailed this method.

The aim of this method is an organisation and visualisation of the real vectors according to their similarities by arranging the distribution to the samples onto a 2D space represented by the map. The samples placed in the same output neuron are considered similar. Moreover, the samples that are neighbours on the map are also expected to be more similar to each other and then belong to the same cluster. A cluster analysis is then applied to the virtual vectors to define the cluster boundaries on the map, which are the groups of similar output neurons. Commonly a hierarchical cluster analysis with a Ward linkage method is applied. We used the functions implemented in the SOM toolbox for Matlab (MathWorks, 2001).

## 2.5. Validation test

To check the validity of the results, cluster and map, a special validation procedure was established for this study. For each of the 19 DBM populations, new individuals were created (Fig. 1). Fifteen individuals from each population were extracted randomly to build five new individuals per

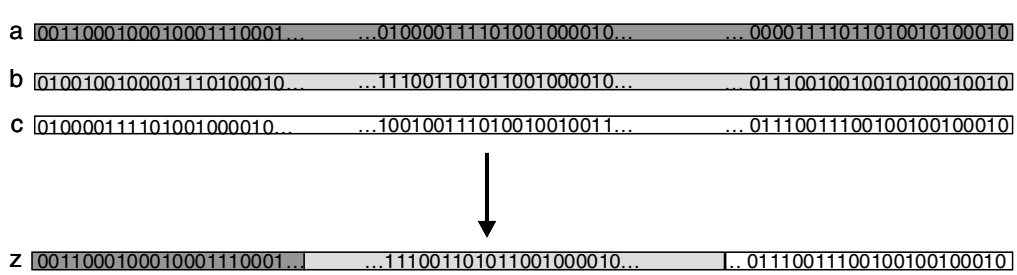


Fig. 1. Creation of the "new" individual for the validation of methods. One part of the sequence of 188 bands was selected in three individuals (a–c) of the same population. Each part was complementary such that when they were joined, they formed a "new" individual (z). Limits between each part were randomly selected for all "new" individuals.

population. The 95 new individuals were then introduced in the matrix data set of PAUP and into the calibrated SOM map. If the obtained results are powerful, these individuals have to be classified in the clusters and neurons where individuals of the same population have been classified.

## 3. Results

### 3.1. ISSR profile

Seven ISSR primers were initially tested on the 19 DBM populations. All gave amplified products but the three lesser specific primers (CA, +CA and CA+) produced smears. Only anchored tri-nucleotide SSR primers produced clear and reproducible fragments. From these, a total of 188 scorable ISSR loci (bands) were selected in the 539 DBM individuals screened from the 19 populations. Within populations, the number of loci amplified per primer ranged from 28 to 49 (Table 3), with an average of 42.37 loci. Each of the 539 individuals presented a unique ISSR genotype, indicating extensive genetic variation within populations.

### 3.2. Genetic structure and diversity

At the species level (all populations), the total polymorphism was maximal (100%). The total gene diversity (*Ht*) was $0.347 \pm 0.016$, gene diversity within populations (*Hs*) was $0.265 \pm 0.009$ and between populations was $0.082 \pm 0.007$. The global coefficient of gene differentiation (*Gst*) was 0.238.

AMOVA indicated a highly significant genetic difference among populations (26.29%, df = 18, $P < 10^{-5}$). Nevertheless, the largest variability was expressed among individuals within populations (73.71%, df = 520, $P < 10^{-5}$).

The mean percentage of polymorphic loci (*P*) within populations was 94%, ranging from 84% in Seibersdorf and Okayama populations to 99% in the Beaverlodge population. The Shannon's index (*i*), Nei's gene diversity (*H*) and the percentage of polymorphism (*P*) within each population are summarized in Table 3. The coefficient of gene differentiation (*Gst*) and the gene flow (*Nm*) between populations by pair is given in Table 4. The Okayama population seems to be the most differentiated with a mean *Gst* of 0.235. Genetic identity (*I*) among populations ranged from 0.795 to 0.950, with an average of $0.888 \pm 0.032$, and the genetic distance (*D*) between populations varied from 0.051 between Tashkent and Iasi to 0.230 between Cotonou and Okayama with an average of $0.119 \pm 0.036$.

### 3.3. Population's classification and cluster analysis

#### 3.3.1. Classical distance analysis

Unrooted tree obtained by distance analysis provided a clear discrimination of the 19 populations. For each population, individuals were regrouped in a spindle-shape except for one individual of the Iasi population (Iasi 09) which was inserted between Australian populations of Brisbane and Sydney (Fig. 2a). Branches that support OTU (Operational Terminal Unit) i.e. individuals, were longer than branches that separate populations. The Okayama population had the longest branch (Fig. 2b) but also the shortest individual branches (Fig. 2a) that show a great differentiation with other populations on the one hand but a relative similarity of individuals within the population on the other. At the

Table 3
Genetic variability parameters of the 19 DBM populations for each usable SSR primer

| | +ACA | | ACA+ | | +GACA | | GACA+ | | *P* mean | *He* ± SD | *i* ± SD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *N* | *P* | *N* | *P* | *N* | *P* | *N* | *P* | | | |
| Beaverlodge (26)[a] | 47 | 100 | 49 | 98 | 42 | 100 | 43 | 100 | 99 | 0.319 ± 0.147 | 0.482 ± 0.189 |
| Geneva (30) | 47 | 91 | 49 | 96 | 40 | 98 | 35 | 97 | 96 | 0.273 ± 0.170 | 0.417 ± 0.232 |
| Brasilia (30) | 47 | 100 | 48 | 96 | 42 | 100 | 48 | 94 | 97 | 0.354 ± 0.145 | 0.524 ± 0.186 |
| Le Carbet (30) | 45 | 98 | 48 | 96 | 41 | 98 | 42 | 88 | 95 | 0.303 ± 0.179 | 0.452 ± 0.239 |
| Montpellier (30) | 45 | 96 | 45 | 96 | 40 | 100 | 40 | 95 | 97 | 0.252 ± 0.178 | 0.387 ± 0.240 |
| Iasi (26) | 39 | 95 | 45 | 93 | 41 | 100 | 43 | 91 | 95 | 0.244 ± 0.168 | 0.379 ± 0.232 |
| Seibersdorf (27) | 47 | 89 | 44 | 98 | 40 | 98 | 28 | 50 | 84 | 0.226 ± 0.184 | 0.346 ± 0.260 |
| Tashkent (28) | 44 | 86 | 46 | 89 | 41 | 100 | 40 | 95 | 93 | 0.267 ± 0.174 | 0.407 ± 0.239 |
| El Fayoun (30) | 39 | 79 | 44 | 95 | 42 | 100 | 45 | 98 | 93 | 0.252 ± 0.167 | 0.390 ± 0.231 |
| Cotonou (27) | 44 | 93 | 47 | 96 | 41 | 98 | 43 | 91 | 94 | 0.297 ± 0.178 | 0.444 ± 0.240 |
| Pretoria (30) | 44 | 89 | 47 | 96 | 39 | 97 | 44 | 93 | 94 | 0.256 ± 0.169 | 0.396 ± 0.230 |
| Piton Hyacinthe (25) | 45 | 91 | 48 | 98 | 42 | 100 | 45 | 89 | 94 | 0.284 ± 0.156 | 0.436 ± 0.210 |
| Hong Kong (30) | 47 | 100 | 45 | 98 | 40 | 100 | 46 | 87 | 96 | 0.306 ± 0.162 | 0.461 ± 0.215 |
| Vientiane (26) | 46 | 98 | 47 | 96 | 40 | 98 | 41 | 93 | 96 | 0.268 ± 0.168 | 0.413 ± 0.226 |
| Okayama (29) | 32 | 81 | 42 | 86 | 34 | 88 | 33 | 82 | 84 | 0.192 ± 0.196 | 0.292 ± 0.276 |
| Adelaide (29) | 40 | 93 | 45 | 96 | 42 | 100 | 44 | 98 | 96 | 0.249 ± 0.170 | 0.387 ± 0.231 |
| Brisbane (26) | 30 | 80 | 44 | 89 | 37 | 95 | 39 | 92 | 89 | 0.216 ± 0.185 | 0.332 ± 0.262 |
| Melbourne (30) | 41 | 98 | 48 | 94 | 39 | 92 | 46 | 96 | 95 | 0.273 ± 0.181 | 0.414 ± 0.242 |
| Sydney (30) | 29 | 86 | 41 | 88 | 38 | 100 | 44 | 91 | 91 | 0.197 ± 0.180 | 0.308 ± 0.253 |
| Mean | 42 | 92 | 46 | 94 | 40 | 98 | 42 | 90 | – | – | – |

[a] Number of individual; *N*: Number of loci (bands); *P*: percentage of polymorphism; He: Nei's gene diversity; *i*: Shannon's information index; SD: standard deviation.

Table 4
Coefficient of gene differentiation (*Gst*) below the diagonal and estimated gene flow per generation (*Nm*) above the diagonal between pairs of populations

| | Bea | Gen | Bra | Car | Mon | Iasi | Sei | Tas | EIF | Cot | Pre | P.Hy | HK | Vie | Oka | Ade | Bris | Mel | Syd |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bea | **** | 5.593 | 5.619 | 4.221 | 5.235 | 4.564 | 2.580 | 5.296 | 3.736 | 3.982 | 4.717 | 5.408 | 4.907 | 5.633 | 1.964 | 5.758 | 2.990 | 3.094 | 3.117 |
| Gen | 0.082 | **** | 3.771 | 2.929 | 3.064 | 4.564 | 2.713 | 5.195 | 3.670 | 4.254 | 3.502 | 4.276 | 3.712 | 3.926 | 1.549 | 4.611 | 2.646 | 2.464 | 2.580 |
| Bra | 0.082 | 0.117 | **** | 4.056 | 3.615 | 3.192 | 2.524 | 3.825 | 3.203 | 4.157 | 3.962 | 3.722 | 4.972 | 3.931 | 2.274 | 4.223 | 2.491 | 3.510 | 2.974 |
| Car | 0.106 | 0.146 | 0.110 | **** | 3.597 | 3.250 | 2.522 | 3.444 | 3.087 | 3.268 | 3.093 | 4.129 | 5.663 | 3.865 | 1.931 | 2.882 | 2.569 | 3.388 | 2.368 |
| Mon | 0.087 | 0.140 | 0.122 | 0.122 | **** | 2.997 | 2.136 | 3.453 | 2.655 | 3.069 | 2.853 | 3.743 | 4.434 | 3.542 | 1.722 | 3.082 | 1.996 | 2.886 | 2.172 |
| Iasi | 0.099 | 0.099 | 0.135 | 0.133 | 0.143 | **** | 2.997 | 6.131 | 3.806 | 3.657 | 3.498 | 5.180 | 4.208 | 4.359 | 1.469 | 4.663 | 3.466 | 3.005 | 3.125 |
| Sei | 0.162 | 0.156 | 0.165 | 0.165 | 0.190 | 0.143 | **** | 2.631 | 2.442 | 2.599 | 2.313 | 2.994 | 2.763 | 3.160 | 1.278 | 2.174 | 1.833 | 1.991 | 2.080 |
| Tas | 0.086 | 0.088 | 0.112 | 0.127 | 0.127 | 0.075 | 0.160 | **** | 3.481 | 4.951 | 3.943 | 4.218 | 3.814 | 4.251 | 1.526 | 5.287 | 3.002 | 2.936 | 2.881 |
| EIF | 0.118 | 0.120 | 0.135 | 0.139 | 0.159 | 0.116 | 0.170 | 0.126 | **** | 3.054 | 2.910 | 3.694 | 3.772 | 3.654 | 1.411 | 2.873 | 2.101 | 2.334 | 1.840 |
| Cot | 0.112 | 0.105 | 0.107 | 0.133 | 0.140 | 0.120 | 0.161 | 0.092 | 0.141 | **** | 4.103 | 3.947 | 3.709 | 3.158 | 1.523 | 3.527 | 2.708 | 3.004 | 2.819 |
| Pre | 0.096 | 0.125 | 0.112 | 0.139 | 0.149 | 0.125 | 0.178 | 0.113 | 0.150 | 0.109 | **** | 3.865 | 3.450 | 4.014 | 1.406 | 3.343 | 2.850 | 2.160 | 2.757 |
| P.Hy | 0.085 | 0.105 | 0.118 | 0.108 | 0.118 | 0.088 | 0.143 | 0.106 | 0.119 | 0.112 | 0.125 | **** | 6.214 | 5.514 | 1.732 | 3.924 | 3.162 | 2.881 | 3.102 |
| HK | 0.093 | 0.119 | 0.091 | 0.081 | 0.101 | 0.106 | 0.153 | 0.116 | 0.117 | 0.119 | 0.127 | 0.075 | **** | 5.799 | 2.102 | 3.269 | 2.553 | 4.342 | 2.584 |
| Vie | 0.082 | 0.113 | 0.113 | 0.115 | 0.124 | 0.103 | 0.137 | 0.105 | 0.120 | 0.137 | 0.111 | 0.083 | 0.079 | **** | 1.690 | 3.302 | 2.912 | 3.065 | 2.803 |
| Oka | 0.203 | 0.244 | 0.180 | 0.206 | 0.225 | 0.254 | 0.281 | 0.247 | 0.262 | 0.247 | 0.262 | 0.224 | 0.192 | 0.228 | **** | 1.597 | 1.446 | 1.925 | 1.379 |
| Ade | 0.080 | 0.098 | 0.106 | 0.148 | 0.140 | 0.098 | 0.187 | 0.086 | 0.148 | 0.124 | 0.130 | 0.113 | 0.133 | 0.132 | 0.238 | **** | 3.071 | 2.841 | 3.333 |
| Bris | 0.143 | 0.159 | 0.167 | 0.163 | 0.200 | 0.126 | 0.214 | 0.143 | 0.192 | 0.156 | 0.149 | 0.137 | 0.164 | 0.147 | 0.257 | 0.140 | **** | 2.709 | 2.816 |
| Mel | 0.139 | 0.169 | 0.125 | 0.129 | 0.148 | 0.143 | 0.201 | 0.146 | 0.177 | 0.143 | 0.188 | 0.148 | 0.103 | 0.140 | 0.206 | 0.150 | 0.156 | **** | 2.303 |
| Syd | 0.138 | 0.162 | 0.144 | 0.174 | 0.187 | 0.138 | 0.194 | 0.148 | 0.214 | 0.151 | 0.154 | 0.139 | 0.162 | 0.151 | 0.266 | 0.131 | 0.151 | 0.178 | **** |

See Table 1 for abbreviations.

other extreme, Brasilia and Beaverlodge populations have very long OTU branches that revealed a great differentiation of individuals in these populations. Genetic distances observed on the tree do not reflect geographic distance between populations. Only three Australian populations (Brisbane, Sydney and Adelaide) were grouped in the same cluster and have a geographic proximity.

### 3.3.2. SOM

The SOM neural network was constituted of 188 neurons (one for each band) in the input layer linked to the 539 DBM individuals such that the representation of the presence/absence of the 188 bands for each individual formed 539 real vectors. The output layer comprised 456 neurons organised in an array with 24 rows and 19 columns. Each neuron of the output layer is linked to the input neurons (i.e. 188) by weights forming a virtual vector. During the learning process, a virtual individual is then computed in each neuron. Several maps were created using different sizes. We used the topographic and the quantization errors (Kohonen, 2001) to determine final map size. For the final map size (456 neurons), these errors were sufficiently low, 0.0019 and 4.943 respectively. The convergence was mostly reached in 725 iterations with a learning rate beginning from 0.5 for the ordering phase and for the tuning phase, from a learning rate of 0.05, the maximum number of iterations was 2765. Each individual was classified in a neuron of the output layer (the map). In general, the individuals coming from the same population are in the same neuron or in neighbour neurons. The Pretoria population is the most concentrated with all the individuals classified into 5 neighbouring neurons. The Beaverlodge population is the most widespread with its individuals classified into 16 neurons (Fig. 3a).

A hierarchical cluster analysis was applied to the 456 virtual vectors of this map. As many clusters as populations were chosen, i.e. 19, to test the quality of the classification method (Fig. 3b). Individuals are relatively well classified into the 19 clusters according to their origin population. However, few individuals were considered as badly classified, i.e. when these individuals were found in neuron/cluster mainly occupied by individuals of a different population. One Adelaide individual was classified in the cluster of Iasi, two individuals of Piton Hyacinthe were classified in the Le Carbet and in Vientiane clusters, two individuals from Iasi were classified in the cluster of Brisbane and Vientiane, while one Vientiane individual was classified in the Tashkent cluster and one Beaverlodge individual in the Cotonou cluster.

## 3.4. Validation

A special procedure has been used to validate the quality of the cluster and the map using new individuals created randomly (see Fig. 1 and "validation test" Section 2). Ninety five new individuals were introduced into the matrix data set of PAUP and into the SOM calibrated model, i.e.

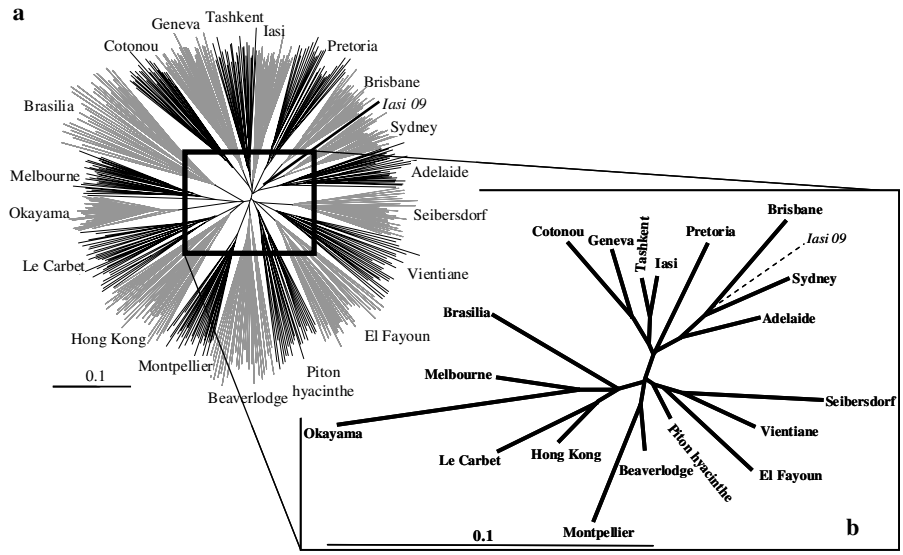


Fig. 2. Unrooted tree obtained by distance analyses of the 19 DBM populations. (a) Complete unrooted tree; (b) unrooted tree simplified and enlarged on population separation part.

the map. All 95 were correctly classified into the cluster and neurons of their origin population. For example, the five new individuals of Brisbane have been correctly classified into the Brisbane cluster using either PAUP or SOM. The new tree, obtained by a completely new computation of distances with PAUP, was not different from global arrangement of population clusters from the previous tree.

## 4. Discussion

### 4.1. Origin of genetic diversity

Most studies of population genetic characterisation on DBM have used allozymes (Caprio and Tabashnik, 1992; Kim et al., 1999), RAPDs (Heckel et al., 1995), mitochondrial gene encoding cytochrome oxidase I (COI) (Chang et al., 1997; Pichon, 2004), enzyme electrophoresis (Arvanitakis et al., 2002; Pichon et al., 2002, 2006), oviposition behaviour (Arvanitakis et al., 2002), morphology (Chacko and Narayanasamy, 2002) and microsatellites (Endersby et al., 2002, 2005, 2006) with mixed results. The best results have been found with highly variable markers, such as RAPDs, microsatellites and enzymes despite the fact that morphological characters on Indian populations (Chacko and Narayanasamy, 2002) and behavioural characters on populations of Benin (Arvanitakis et al., 2002) have also given good results in terms of population discrimination.

Despite a very high variability at the intra-population level, ISSR seems to permit differentiation of each of the studied populations very easily. All populations had high coefficients of genetic differentiation (*Gst*). That was not surprising given the large scale covered. Nevertheless, the resolution of ISSR patterns allowed genetic differentiation ($Gst = 0.150$) of Australian populations separated by only 620 km (distance between Melbourne and Sydney), at a scale where Endersby et al. (2006) found no differentiation with microsatellites. Beside geographic distances, this level of divergence between and within populations can be attributed to other things. First, biological characteristics of DBM may be involved. In tropical areas, there may be more than 20 generations a year. This results in an increase in appearance of mutations and therefore increases divergence between individuals within populations. Secondly, massive uses of insecticides create bottlenecks in populations increasing divergence between populations by selecting different haplotypes. All chemical contaminants and insecticides are known for their high mutagen power, this also increases the number of mutations in resistant individuals. ISSR markers are mainly constituted of non-codant DNA that preserves all mutations. In the long term, these successive increases and massive disappearances of haplotypes, occurs independently in each population, and have a negative effect on the conservation of phylogenetic information increasing the population genetic differentiation. Such effects are increased if populations are separated in time without sufficient genetic flow.

DBM shows an overall observed polymorphism of 100%. Similar to that of *Pieris rapae* (Hundsdoerfer and Wink, 2005). It seems that crop pests, where man has an important influence in their distributions are loosing their geographical population genetic structure. Nonetheless, another type of population genetic structure is generated and accessible for analysis using ISSR markers. ISSRs can be used as tools to evaluate human influence on natural pest populations and their level of dispersal.

### 4.2. Gene flow and long range migrations

Although massive annual DBM migration has been described in several parts of the world (Smith and Sears,

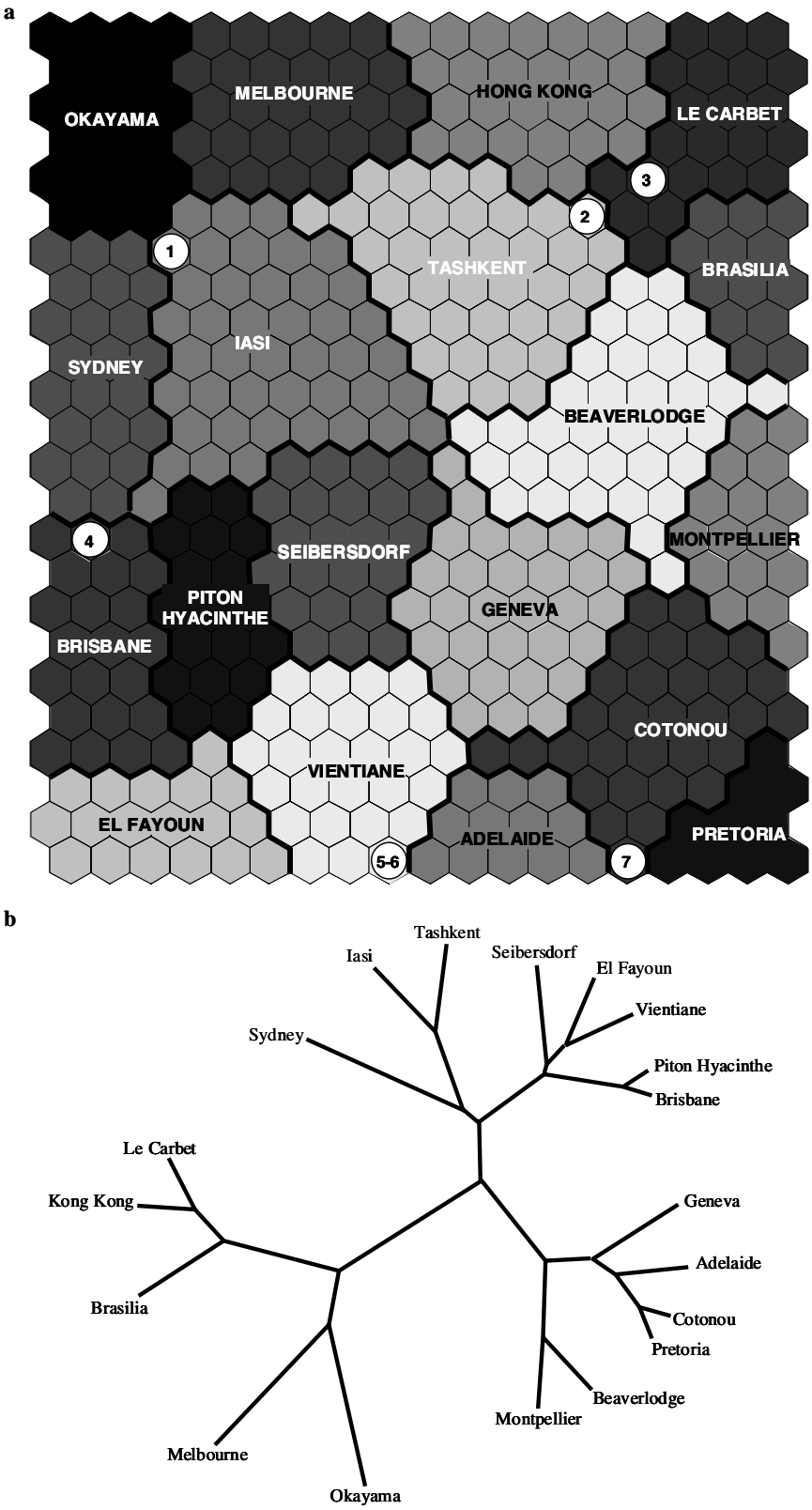


Fig. 3. Classification of 539 DBM individuals from 19 populations using Self-Organising Map (SOM) method. (a) The patterned SOM map. Based on similarities between ISSR bands, the individuals are ordinated into the map. Similar individuals are in the same or neighbour neurons. The 19 clusters issued from b are differentiated by bold lines and gradient of grey colour. Individuals misclassified are numbered 1–7: with individual 1 from Adelaide, 2 from Vientiane, 3 and 6 from Piton Hyacinthe, 4 and 5 from Iasi, and 7 from Beaverlodge. (b) Hierarchical classification of the SOM map. The unrooted tree has been simplified in the figure and only the 19 clusters are represented.

1982; Chu, 1986; Honda et al., 1992; Chapman et al., 2002), very long range migrations (at the scale of our sampling) are probably irregular or anecdotal elsewhere. In our study, despite values greater than one successful migrant per generation, the gene flow (*Nm*) seems to be insufficient to effectively homogenise populations as Slatkin (1987) predicted, probably because of interactions between characteristics of DBM and ISSR fragments described above. Very limited migrations have been described in Hawaiians populations of DBM that were separated by less than ten kilometres (Tabashnik et al., 1987). Mo et al. (2003) have also shown that dispersal of DBM rarely occurs beyond 200 meters in healthy cabbage patch exploitation. When growing conditions are favourable, moths do not migrate. Moreover, in areas free of massive annual migration commercial and cultural cabbage movement probably has more impact on DBM genetic structure than natural gene flows. The absence of correlation between genetic and geographic distances found here is not unusual in crop pest populations (Chang et al., 1997) and has already been observed in DBM (Arvanitakis et al., 2002; Pichon et al., 2006). Our study suggests no phylogenetic inference occurred.

### 4.3. Classification methods

The observed branch length on the dendrogram from the classical distance analysis shows that distances between individuals are longer than between populations (Fig. 2a). This reflects an acceleration of diversification of non-coding DNA in recent times, without exchanges between populations. Greater distances between individuals over populations probably reflect the massive and recurrent use of insecticides. Such evolutionary change is of concern because it favours insecticide resistance. It would not occur with the use of a biological agent to control the pest.

The discrimination power of this analysis was excellent with only one individual misclassified. Nevertheless, the computation time was very long with more than 20 h on a modern desktop computer. The validation test of the method required are computation of results and gave a new tree for distance analysis with PAUP. We think that the insertion of a completely new population will probably produce a new cluster.

After the iterative learning phase in the SOM analysis, each of the 539 DBM individuals was associated with an output neuron. Moreover, mapping individuals according to their ISSR band similarities into the SOM map revealed that some individuals were associated either with the same neuron or in the same cluster of similar neurons. SOM appeared to have correctly organised individuals such that the individuals from the same population were associated. Only seven individuals were classified in the wrong cluster.

To assess this more rigorously, the SOM output was validated. The validation procedure shows the capacity of the method to correctly classify new individuals. However the new "created" individuals all came from known (by the model) individuals. Had a completely new individual been introduced in the model, it would be classified in a neuron of the map to the neuron closest to it, based on band similarity. If the individual came from a new population however, the model nonetheless is obliged to classify it. This is the drawback of this method. It can however be avoid using a recent algorithm, the Evolving Self-Organizing Map, which creates a new neuron and evolves the map accordingly (Deng and Kasabov, 2003).

Foody (1999) compared SOM with three other classification methods (K-means, Hierarchical clustering and fuzzy c-means) and with ordination method (Principal component analysis (PCA)) in community data analysis. Giraudel and Lek (2001) compared SOM with Polar ordination, PCA, correspondence analysis and multidimensional scaling for ecological community ordination. Finally Brosse et al. (2001) used SOM and PCA to study fish assemblages. They all showed that SOM can be successfully applied to complex data and constitute a useful alternative to common multivariate statistical analysis. In this paper, SOM is for the first time compared to a genetic classification method. The worst misclassification individual rate was only of 1.3%. Classical distances and SOM analyses gave similar results for population discrimination but produced different clusters between populations. This difference comes probably from their respective algorithms. With the heuristic method, individuals are compared between them, while in the SOM, they are compared to virtual individuals which are modified progressively during the training.

## 5. Conclusion

With this study, we present a complete decision-making tool. The ISSR-PCR technique combines the two qualities required for routine analyses i.e. low cost and fast processing with minimum equipment requirements. This study has shown that the population genetic of DBM (described by ISSR bands) can be mapped by the SOM artificial neural networks method; faster than other methods. The high variability obtained with ISSR markers can also allows assessment at low geographic scales.

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# DISCUSSION GENERALE & PERSPECTIVES

Les questions de recherche développées dans le présent travail peuvent être réunies sous une même thématique, celle des relations tritrophiques en milieu tropical, avec comme modèle le chou - la teigne des Brassicacées (*Plutella xylostella*) - et les auxiliaires (en particulier *Oomyzus sokolowskii* et *Cotesia vestalis*) dans deux pays d'Afrique de l'Ouest, le Sénégal et le Bénin.

L'intensification de la culture du chou en zone tropicale, plus particulièrement en Afrique, n'est pas très ancienne. Dans les années 1970, cette espèce était cultivée mais souffrait durant sa culture des conditions climatiques tropicales (fortes chaleurs et forte humidité), non adaptées à sa croissance, les variétés utilisées à l'époque étant des cultivars européens. Depuis une trentaine d'années, l'arrivée sur le marché de cultivars "tropicalisés" d'origine japonaise a fait prendre un essor important à cette espèce légumière, à tel point qu'elle entre maintenant de façon courante dans l'alimentation locale des familles de ces deux pays. Il faut savoir également que les africains, particulièrement les béninois, sont de grands consommateurs de "légumes feuilles", dont le chou fait partie et dont les pommes récoltées se conservent très bien lors des transports routiers effectués sur les pistes, contrairement à d'autres légumes plus fragiles. Les surfaces plantées en choux ont donc augmenté et il n'est pas rare actuellement de voir chez les producteurs des parcelles cultivées dépassant 5 000 $m^2$ et cela tout au long de l'année.

Malheureusement, cette multiplication des surfaces cultivées de façon continue tout au long de l'année (présence au même moment de choux à tous les stades de la culture) offre à son principal ravageur, le lépidoptère défoliateur *Plutella xylostella*, l'occasion de se multiplier de façon exponentielle, dérégulant ainsi certains agrosystèmes précédemment en équilibre.

Au niveau mondial, pour tenter de contrôler les populations de chenilles de ce ravageur, les cultivars de chou pommé ont fait l'objet d'une sélection variétale basée sur des changements de la structure des cires épicuticulaires dont l'effet recherché était de diminuer l'appétence du chou pour les chenilles de *P. xylostella*. Cependant, ces variétés n'ont pas été exploitées car les modifications induites favorisent d'autres ravageurs et donnent un aspect brillant aux feuilles (glossy leaves), ce qui est peu apprécié des consommateurs.

La teigne du chou est recensée en plus ou moins grand nombre sur tous les continents et à quasiment toutes les latitudes, partout où il y a des cultures de Brassicacées (Shelton 2004). Dans les conditions tropicales, les populations de *P. xylostella* sont très importantes en nombre d'individus et de ce fait difficilement contrôlables. En effet, les températures élevées et l'humidité de ces zones favorisent la multiplication des populations qui effectuent entre 20

et 24 générations par an suivant les endroits, alors qu'elles n'en ont que trois en zones tempérés.

Du fait de sa répartition mondiale, les populations de ce ravageur subissent des pressions locales de sélection dépendant du climat, des ennemis naturels présents, des systèmes de culture des agriculteurs, etc.

La question fondamentale qui se pose est la suivante : ces populations sont-elles identiques au niveau de leurs "performances" en tant que ravageur ? Il est impossible de mettre au point une lutte "générique" dans tous les pays où sévit la teigne et les différentes formes de luttes mises en place pour contrôler les populations de chenilles ne sont pas efficaces partout : (1) la lutte chimique intensive entraîne dans de nombreux pays l'apparition de populations résistantes. Elle est cependant très employée et encore efficace dans certains pays, du fait de l'apparition des nouvelles molécules mises sur le marché ; (2) la lutte variétale est inefficace ; (3) la lutte biologique de conservation semble être actuellement la plus employée au niveau mondial, mais sa réussite dépend de l'efficacité des espèces locales d'auxiliaires présentes.

Des études en laboratoire (marqueurs isozymes) ont montré qu'au niveau mondial les populations de *P. xylostella* semblent être structurées génétiquement en plusieurs groupes homogènes, comme c'est le cas pour l'Australie et le Japon, au contraire des autres populations originaires d'Europe, d'Afrique et du continent américain qui sont nettement différenciées. En analysant ces groupes, nous nous sommes aperçus que toutes les populations provenant d'une localité tropicale (zone de basses latitudes comprises entre 0 et 23°) étaient regroupées dans un même ensemble, alors que celles natives de zones non tropicales (zone de moyennes et hautes latitudes entre 24 et 63°) constituaient un autre groupe bien séparé. Des groupes structurés ont été mis en évidence de la même manière lors de notre étude avec les ISSR. Chacun de ces deux marqueurs a montré de fortes divergences entre les populations étudiées, permettant ainsi de les différencier. D'un marqueur à l'autre, les distances génétiques calculées entre les populations ne sont pas équivalentes, probablement en raison de l'évolution indépendante de ces deux marqueurs mais surtout parce que les isozymes, considérés comme marqueurs neutres, peuvent quand même être soumis à une pression de sélection alors que les ISSR ne le sont pas. Dans les deux cas, l'information apportée par ces marqueurs n'est pas corrélée avec les distances géographiques, principalement en raison des caractéristiques de chacun d'eux.

Récemment, suite à des identifications d'individus de *P. xylostella* récoltés sur des choux, des chercheurs australiens utilisant le "Barcoding" ont identifié des individus d'une

autre espèce, différente de *P. xylostella*. Il s'est avéré que les génitalias des mâles et des femelles n'étaient pas identiques à celles de *P. xylostella*, alors que la couleur et la morphométrie étaient rigoureusement semblables. Ils ont décrit cette nouvelle espèce comme *P. australiana* (Landry & Hebert 2013). Cette information est capitale car *P. xylostella* est peut-être constitué d'un complexe d'espèces ou de biotypes équivalent au complexe de la "Mouche blanche" *Bemisia tabaci* (Gennadius) (Hemiptera : Aleyrodidae), non encore mises en évidence, ce qui pourrait expliquer les différences observées dans le comportement et le contrôle du ravageur.

Au delà de ces différences observées au niveau génétique entre les populations, avec la possibilité d'espèces cryptiques en mélange, les essais au laboratoire ont montré de grandes différences dans le nombre d'œufs pondus par les femelles de *P. xylostella* et dans la stratégie de ponte entre les populations originaires de cinq localités du Bénin (Arvanitakis et al. 2004). Cependant, il n'y a pas de corrélation entre les différences génétiques et les différences biologiques. Cela nous conforte dans l'idée que les populations de la teigne ne sont pas identiques entre elles et que leur contrôle ne peut se faire de façon générique, mais de manière spécifique au niveau de chaque population présente dans son environnement naturel.

Nos résultats ont montré l'importance des auxiliaires entomophages qui, sans être d'une efficacité totale dans le contrôle des populations de la teigne, excepté en Afrique du Sud (Kfir 2011), permettent cependant de réduire les populations du ravageur. La lutte biologique par conservation montre là toute sa nécessité et devient donc d'une grande utilité.

L'efficacité des parasitoïdes dépend également des pressions de sélection par leur environnement. Par exemple, *O. sokolowskii*, endoparasite grégaire larvo-nymphal, peut conduire à plus de 80% de parasitisme en conditions de laboratoire (conditions *optimum*), alors que ce taux ne dépasse pas 10% dans les conditions naturelles au Sénégal (Sow et al. 2013). Le pourcentage de parasitisme par cette espèce est de l'ordre de 40 à 50 % en Asie du Sud-Est (Srinivasan & Moorthy 1992).

Cette différence aperçue dans le pourcentage de parasitisme peut être due à plusieurs causes : (1) les conditions de laboratoire sont très favorables à une bonne croissance des populations des parasitoïdes (pas de recherche de l'hôte, pas de concurrence pour la ressource, absence d'antagonistes, conditions climatiques optimales), ce qui n'est pas le cas en milieu naturel ; (2) comme pour l'hôte, les capacités biologiques peuvent être différentes suivant leur origine ; (3) ils peuvent être porteurs de bactéries endosymbiotiques qui pourraient perturber leur efficacité.

En effet, de nombreuses espèces d'Hyménoptères sont naturellement infectées par des bactéries endosymbiotiques du genre *Wolbachia* (Werren et al. 1995). Ces endosymbiotes manipulent la reproduction de leurs hôtes, soit en biaisant la sex-ratio, soit en empêchant certains types de croisements, pouvant ainsi induire jusqu'à une différenciation des populations (Bordenstein et al. 2001). Ceci pourrait entrainer une baisse d'efficacité parasitaire chez les parasitoïdes infectés. C'est effectivement le cas de notre population d'*O. sokolowskii* en provenance du Sénégal qui était infectée par *Wolbachia.*

L'espèce *C. vestalis* est un endoparasite larvaire solitaire qui possède pratiquement la même répartition géographique que *P. xylostella*, ce qui en fait le principal ennemi naturel de ce ravageur. Dans ce cas encore, des différences importantes apparaissent dans son potentiel d'efficacité envers les populations de chenilles de la teigne. Au cours d'essais effectués au laboratoire sur sa réponse fonctionnelle, des femelles de ce parasitoïde provenant du Bénin pouvaient parasiter en 24 heures 80 chenilles sur 120 présentées, alors que des femelles originaires de Martinique n'en parasitaient que 40. Nous noterons ici que les femelles de l'hyménoptère issues de la population des Antilles étaient infectées par *Wolbackia* (Rincon et al. 2006) et de ce fait elles étaient moins performantes par rapport à celles du Bénin qui étaient saines.

Au Sénégal, en condition de plein champ, *C. vestalis* est peu présente, mis à part en période d'hivernage où les températures sont assez élevées (30°C en moyenne). Son taux de parasitisme n'excède cependant pas 5% (Sow et al. 2013). Par contre cette espèce peut aboutir à près de 90% de parasitisme dans la zone maritime du Bénin où, malgré ce taux extrêmement élevé, elle ne contrôle pas les populations de chenilles de *P. xylostella* (Arvanitakis et al. 2013, soumis à *BioControl*). Comme pour son hôte *P. xylostella* et le parasitoïde *O. sokolowskii*, les populations de *C. vestalis* sont hétérogènes au niveau génétique. Des études effectuées avec des marqueurs isozymes sur des individus de populations d'origines géographiques différentes (Ile de la Réunion, Taïwan, Bénin, Afrique du Sud et Martinique) ont montré un éloignement génétique entre les populations, ce qui pourrait influencer l'efficacité des différentes femelles sur leurs hôtes respectifs. Nous noterons également que le climat "doux" de la région de Dakar n'est peut être pas très favorable à *C. vestalis*, celui-ci préférant les environnements chauds et humides présents dans la zone maritime de Cotonou, où il est fortement présent sur les chenilles de *P. xylostella.*

Nous noterons également que les choux cultivés dans la zone maritime de Cotonou sont fortement infestés par *P. xylostella*. Il n'est pas rare de dénombrer plus de 100 chenilles par pied de chou. Le climat ne subissant que très peu de différences au cours de l'année tant

au niveau de la température que de l'humidité, celui-ci est très favorable à la croissance des chenilles de *P. xylostella*. Au Sénégal les différences de températures et d'humidité dues aux deux saisons (sèche et humide) ne sont pas très favorables au ravageur, ce qui entraîne des populations de chenilles beaucoup moins importantes qu'au Bénin.

Une co-évolution pourrait être survenue au Bénin où la sélection naturelle aurait favorisé les femelles de *C. vestalis* les plus performantes et éliminé les moins efficaces. La présence de *Wolbachia* dans les adultes, qui peut influencer sur son aptitude au parasitisme, ne peut être mise en cause puisque les populations du Sénégal et du Bénin ne sont pas infectées.

En zones tropicales, le comportement imprévisible des infestations de ce ravageur au niveau des parcelles cultivées (Zalucki & Furlong 2011 ; Wei et al. 2013) et surtout le système de culture des producteurs, qui favorisent la croissance des populations de la teigne dans les parcelles, sont des freins au maintien à un niveau faible des populations de *P. xylostella*. En effet, les agriculteurs laissent les pieds de choux en place lors de la récolte des pommes, ce qui favorise le redémarrage des repousses favorables au développement des chenilles. Ils se désintéressent également des foyers de chenilles présents sur le feuillage des planches cultivées en navet (ils ne vendent que les racines), qui sont souvent proches des planches de choux.

On connaît les risques pour la santé humaine et l'environnement découlant des applications anarchiques d'insecticides utilisés par des acteurs souvent mal informés, voire ignorants des effets néfastes de ces pesticides (Furlong et al. 2012). La mise en place de programmes de libération d'auxiliaires biologiques (entomophages, champignons, virus etc.) est très coûteuse et nécessite une compétente technique importante qui est malheureusement absente chez ces producteurs. La solution à ces problèmes serait : (1) de favoriser la lutte biologique par conservation de la faune utile, en remplaçant par exemple les applications d'insecticides de synthèse par des formulations à base de produits naturels (huile de "neem") ou biologiques (*B.thuringiensis*) ; (2) d'effectuer des recherches sur les effets que pourraient apporter l'utilisation des plantes compagnes en association avec les espèces maraîchères cultivées qui possèdent un effet piège (moutarde chinoise, Pak choï…), un effet répulsif (oignon, ail, basilic…), ou neutre (salade, carotte…), mais qui pourraient perturber les femelles de *P. xylostella* à la recherche de sites de ponte (Sheehan 1986). Ces espèces cultivées pourraient également apporter un supplément de revenus et la mise en place de cette stratégie serait également en phase avec les compétences horticoles que possèdent les producteurs.

En effet, la majorité des agriculteurs sénégalais sont illettrés et ils connaissent la technique de la culture des légumes par leurs ascendants qui eux-mêmes la connaissent de leurs parents, voire grands-parents. Ils n'ont aucune connaissance phytopharmaceutique et appliquent sur leurs cultures tous les produits disponibles sur le marché, en particulier ceux utilisés en cultures cotonnières et qui sont dangereux pour les utilisateurs et les consommateurs. La pollution de la nappe phréatique est également concernée par ces méthodes, d'autant plus, que l'eau de cette nappe est régulièrement réintroduite lors des arrosages journaliers des cultures. Ils ne connaissent pas l'existence de la "faune auxiliaire" et pratiquent des cultures associées non pas par souci « agroécologique » mais par manque d'espace au niveau de leurs planches. La mise en place de programmes basés sur l'association d'espèces végétales cultivées et non sauvages, car entrant en concurrence avec elles au niveau de l'eau, et qui perturberaient les femelles du ravageur à la recherche de sites de ponte, seraient facilement acceptée par les agriculteurs, la technique de culture étant en rapport avec leurs compétences.

Comme nous avons pu le constater au Sénégal et au Bénin, la teigne du chou n'est pas contrôlée par ses ennemis naturels. Bien que ces deux pays se situent en zone tropicale, le nombre d'espèces de parasitoïdes et les taux de parasitisme sont variables. En conditions de laboratoire, l'efficacité parasitaire des deux espèces étudiées, *O. sokolowskii* et *C. vestalis*, vis-à-vis de leur hôte n'est pas remise en cause puisqu'elles sont performantes. On peut donc penser que le problème peut venir, d'une part, des populations du ravageur puisque nous avons montré qu'elles étaient différentes et structurées génétiquement à l'échelle mondiale et, d'autre part, des conditions climatiques qui varient d'un pays à l'autre.

J'ai eu l'occasion de faire une expertise en Bretagne (France) auprès de producteurs qui produisaient des choux porte-graines sous serre. La teigne du chou est présente en Bretagne mais n'est pas considérée comme un ravageur à cause du climat. Les adultes provenant des champs avoisinants rentrent dans les serres et y trouvent des conditions plus clémentes (températures élevées) et donc favorables à leur développement. Les producteurs étaient infestés par la teigne malgré un vide sanitaire de deux mois entre deux périodes de cultures et l'application de 23 traitements chimiques sur une saison. Cet exemple permet de voir que si on « tropicalise » artificiellement un agrosystème, il devient favorable au développement de cette espèce qui devient alors un ravageur.

Il peut être intéressant d'essayer de modifier les agrosystèmes par des aménagements à l'échelle de la parcelle, voire du paysage. On s'est rendu compte par exemple que les choux sont moins attaqués quand on les cultive en association avec de la carotte ou de l'oignon, ou

bien quand ils sont dans des parcelles plus ombragées. Dans cet objectif d'action, une approche agroécologique est peut-être l'une des solutions pour contrôler la teigne du chou en régions tropicales.

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# ANNEXES

# Interactions entre *Plutella xylostella* (L.), Lepidoptera : Plutellidae et la température, la plante hôte et les parasitoïdes

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*DU fait de sa capacité de multiplication (> 20 générations/an), la lutte biologique contre* P. xylostella *n'est pas efficace en zones tropicales. Nous avons donc priorisé une approche basée sur la recherche d'interactions existantes entre le ravageur et son environnement (température, plante hôte et parasitoïdes).*

## Materiels et méthodes

Cette étude est réalisée à Malika (Dakar, Sénégal). La parcelle d'échantillonnage comporte 200 choux non traités. Tous les dix jours, 10 plantes prélevées au hasard sont décortiquées. Les chenilles de *P. xylostella* récupérées, sont maintenues en élevage jusqu'à l'apparition de l'adulte ou d'un parasitoïde. Avant chaque prélèvement, le diamètre du chou est mesuré. La température est relevée tous les jours.

## Résultats

La température n'influe pas sur la dynamique des populations du ravageur. Les populations semblent même augmenter quand la température chute (figure 1).

Il y a une forte corrélation entre les chenilles et le diamètre de la plante hôte (figure 2).

Il y a également une forte corrélation entre les stades jeunes des chenilles (L2 + L3) et l'age de la plante hôte. Le nombre de jeunes chenilles diminue lorsque la plante vieillie et les stades plus âgés (L4 + Nymphes) sont plus nombreux quand elle approche de la maturation (figure 3).

Trois espèces de parasitoïdes sont présentes sur les chenilles de *P. xylostella*. *Cotesia plutellae* (Kurdjumov) et *Apanteles litae* (Dixon), deux Braconidae endo-parasites larvaires et Oomyzus sokolowskii (Kurdjumov), Eulophidae, parasitoïde larvo-nymphal.

Le pourcentage de parasitisme naturel est faible au cours des trois cultures consécutives, respectivement 5,2 % pour la première, 34,9 % pour la seconde et 1,4 % pour la dernière (tableau 1). Ce pourcentage de parasitisme est principalement dû à *O. sokolowskii,* puis à *C. plutellae*. *A. litae* n'est que faiblement représenté (figure 4).

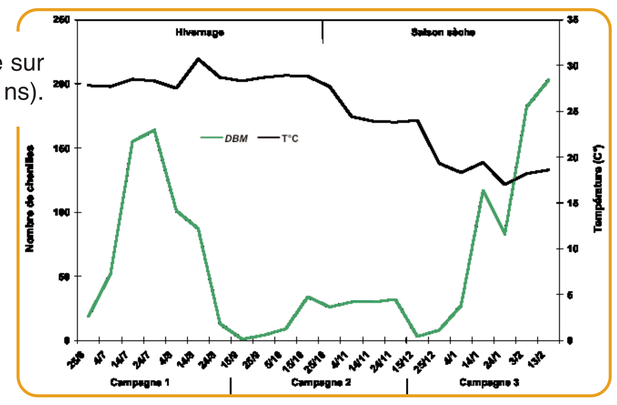


Figure 1. Influence de la température sur les populations de *P. xylostella* (r = - 0,294 ns).

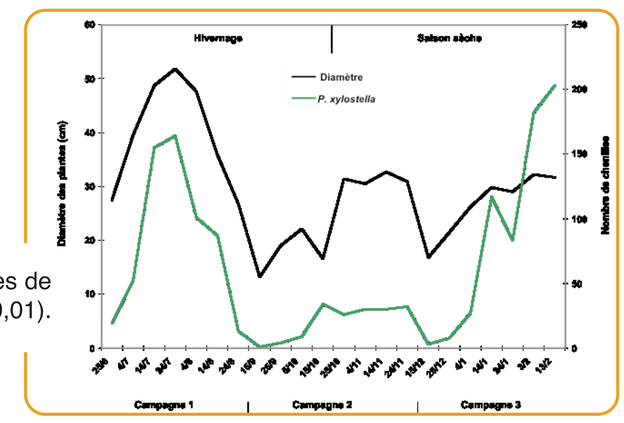


Figure 2. Interactions entre le nombre de chenilles de *P. xylostella* et le diamètre de la plante hôte (r = 0,661 ; P < 0,01).

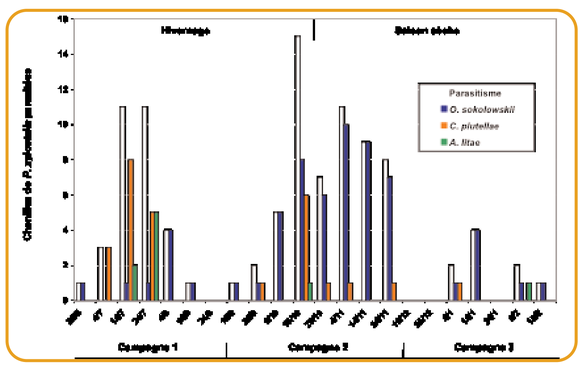


Tableau 1. Nombre d'adultes de parasitoïdes et pourcentage de parasitisme obtenus sur les populations de chenilles de *P. xylostella.*

| Cultures consécutives | Nombre de *P. xylostella* | Nombre de *C. plutellae* | Nombre d' *A. litae* | * Nombre d' *O. sokolowskii* | Nombre de parasitoïdes | % Parasitisme |
|---|---|---|---|---|---|---|
| 25/06-24/08/08 | 591 | 16 | 7 | 8 | 31 | 5,2 |
| 15/09-24/11/08 | 166 | 10 | 1 | 47 | 58 | 34,9 |
| 15/12-13/02/09 | 623 | 1 | 1 | 7 | 9 | 1,4 |

* Nombre de nymphes de *P. xylostella* parasitées (en moyenne 8 adultes de *O. sokolowskii* / nymphe)
*C. plutellae* = *Cotesia plutellae* (Kurdjumov) ; *A. litae* = *Apanteles litae* (Dixon) ;
*O. sokolowskii* = *Oomyzus sokolowskii* (Kurdjumov)

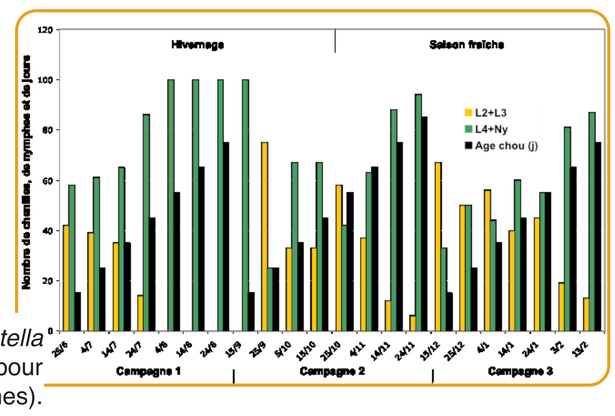


Figure 3. Interactions entre le nombre de chenilles de *P. xylostella* et l'age de la plante hôte (r = - 0,561 ; P < 0,01 pour les L2 + L3, r = - 0,561 ; P < 0,01 pour les L4 + Nymphes).

## Conclusion

Contrairement à de nombreuses zones tropicales, les populations de *P. xylostella* n'augmentent pas dès que la température s'élève. Ce fait est probablement dus au micro climat de Dakar (20-25°C), à l'exception des 4 mois d'hivernage où la température est de 30°C. L'espèce se serait adaptée à ces températures. Il faut noter que les surfaces cultivées en chou augmentent considérablement dès que la saison sèche arrive, ce qui favorise l'augmentation des populations du ravageur.

Pour les femelles de *P. xylostella,* la taille du plant semble favoriser la ponte, plus le diamètre du plant augmente plus le nombre de chenilles est important. L'attractivité des choux pour la femelle est due au ratio entre la taille du plant et les glucosinates produits par celui-ci, qui sont également favorables au développement des chenilles. Dès que le chou pomme, la production de glucosinate diminue et les femelles vont à la recherche d'autres plantes plus jeunes pour y pondre.

Le parasitisme naturel est faible (1,4 % à 34,9 %). La température ambiante de la zone de Dakar (20-25°C) favorise la multiplication des populations d'*O. sokolowskii,* mais elle pénalise celles de *C. plutellae* qui préfèrent des températures plus élevées. On trouve cette espèce en plus grand nombre en hivernage (30°C), où les populations d'*O. sokolowskii* sont moins nombreuses. Le climat de la zone de Dakar n'ait pas favorable à la multiplication d'*A. litae,* qui est plutôt présent dans les zones sahéliennes.


Conception : CIRAD, Martine Dupontel, Avril 2010 — Photos : © Dominique Bordat



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# Several life traits history of *Oomyzus sokolowskii* parasitoid of Diamondback moth.

*THE species* Oomyzus sokolowskii *(Kurdjumov), a major parasitoid of Diamondback moth (DBM)* Plutella xylostella *(L.) pest of Brassicaceae is a potential biological control agent against this species. These species are gregarious and cosmopolitan like its host (Fitton and Walker, 1992).*

*The aim of this work is to study under laboratory conditions some life history traits of this Hymenoptera species. The life-traits knowledge is very important to build entomophagous programs to control populations DBM in cabbage crops field farmers in tropical areas.*

Female in laying position on *Plutella xylostella* larva


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## Material and Methods

*O. sokolowskii* adults emergence.

- The parasitoid population was obtained from parasitized pupae of DBM collected in cabbage crop (*Brassica oleracea* var. capitata) in Fass Boye, in the "Niayes" area, situated in North West of Senegal. The population of DBM is native to the same locality.
- The rearing of the host and its parasitoid were conducted in climate rooms with the following conditions: 25 °C temperature, 60% relative humidity and 12L/12D photoperiod.
- Traits such as development stage cycle, reproductive mode, host age preference, foraging behaviour of the female were assessed. All tests performed in this study were realized in the laboratory of Entomology for International Cooperation in Agronomic Research for Development Center (CIRAD) in Montpellier (France).
- All data were analyzed with the software StatView 4.55.

## Results

- The duration of the larval stage is between 4 and 7 days. The pupal stage during 7 days. In our study conditions, the development time from egg to adult is 15 days. (Table 1)
- Parasitism rate was significantly different between unmated and mated females ($t = 6.391$, $df = 6$, $P = 0.0007$).The mated females produced normal sexual offspring (male and female) while unmated females have produced only males. (Table 2)
- The parasitism rate varies significantly with age of the host ($F = 26.23$, $df = 4.16$, $P < 0.0001$). This rate is significantly higher at the L4 larval stages. (Table 3)
- The parasitism rate was significantly different in the three laying-boxes ($F = 15.87$, $df = 2.18$, $P < 0.0001$). Male offspring number was significantly different among the three laying-boxes ($F = 5.87$, $df = 2.18$, $P = 0.008$). Female offspring number was significantly different among the three laying-boxes ($F = 10$, $df = 2.18$, $P = 0.001$). The offspring development time was significantly different between the laying-boxes ($F = 9.01$, $df = 2.18$, $P = 0.004$). (Table 4)

Table 1. Developmental stage of parasitism and *O. sokolowskii* biological cycle.

| | |
|---|---|
| J0 | Laying inside L4 larvae |
| J+1 | Eggs inside L4 or pupae |
| J+2 | Eggs inside pupae |
| J+3 | Hatching |
| J+4 | Young larvae, visible holes inside the pupae |
| J+5 | Idem |
| J+6 | Ponctued larvae visible trough the pupae |
| J+7 | Prepupae |
| J+8 | White pupae |
| J+9 | Idem |
| J+10 | Pupae eyes begin red |
| J+11 | All pupae eyes are red |
| J+12 | Pupae body become black |
| J+13 | All pupae body are black |
| J+14 | Idem |
| J+15 | Adults emergence |

Table 2. Offspring productivity, parasitism percentage and sex ratio (% female) between mated and unmated *O. sokolowskii* female.

| | Males | Females | Total progeny | Parasitism (%) | Sex ratio |
|---|---|---|---|---|---|
| Mated female | 1.80 ± 0.40 a | 8.40 ± 0.70 a | 10.20 ± 1.00 a | 45.60 ± 3.90 a | 83.00 ± 2.00 a |
| Unmated female | 10.30 ± 0.90 b | 0.00 ± 0.00 b | 10.30 ± 0.90 a | 12.20 ± 0.10 b | 0.00 ± 0.00 b |

Values are mean ± SE. Numbers in columns followed by the same letter are not significantly different (P > 0.05).

Table 3. Host age preference in *O. sokolowskii* females on immature DBM stage.

| Host age | Parasitism % | Min / Max |
|---|---|---|
| 2nd | 39,9 ± 7,6 b | 23,3 / 63,3 |
| 3rd | 54,7 ± 8,7 b | 23,3 / 73,3 |
| 4th | 75,9 ± 2,4 c | 70,0 / 83,3 |
| Prepupa | 15,3 ± 5,9 a | 0,0 / 36,7 |
| Pupa | 0,0 ± 0,0 a | 0 / 0 |

Values are mean ± SE. Numbers in columns followed by the same letter are not significantly different at P = 0.05 (ANOVA; Fisher). Min = Minimum; Max= Maximum.

Table 4. Laying box volume effect on the parasitism percentage and the *O. sokolowskii* female production.

| Laying box | Parasitism % | Females laid | Males | Females | Total adults | Cycles (days) | Sex-ratio |
|---|---|---|---|---|---|---|---|
| 3 (A) | 30,0 ± 15,3 b | 3 | 0,7 ± 0,3 a | 5,9 ± 3,0 a | 6,6 ± 3,4 a | 14,3 ± 0,3 a | 89,5 ± 0,8 a |
| 7 (B) | 85,5 ± 7,6 c | 10 | 2,1 ± 0,6 b | 13,6 ± 1,7 b | 15,7 ± 2,0 b | 15,7 ± 0,3 a | 86,8 ± 2,3 a |
| 40 (C) | 5,0 ± 5,0 a | 1 | 0,2 ± 0,1 a | 0,8 ± 0,5 a | 1,0 ± 0,6 a | 18,0 ± 0,0 b | 80,0 ± 0,0 a |

Values are mean ± SE. Numbers in columns followed by the same letter are not significantly different at P = 0.05 (ANOVA; Fisher).

## Conclusion

- The *O. sokolowskii* life cycle lasted 15 days. The parasitism rate is significantly different between mated and unmated females which imply that mating stimulates the behaviour of parasitism. Females can parasitize all larval stages including prepupae of DBM. However, the parasitism rate was higher in the fourth larval stages. The host-seeking behaviour is influenced by volume.
- The results presented in this study provide valuable information on some *O. sokolowskii* life traits history, a major natural enemies of DBM, pest of Brassicaceae. This information can help a better understanding on the biology of this species and allow more efficient use of this parasitoid in the programs of population management of DBM in the release of entomophagous.

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