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“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

Introduction générale

Les Brassicacées sont parmi les plus importantes cultures alimentaires de la planète, développées sur une superficie de trente millions d'hectares, avec une production annuelle de 105 millions de tonnes (pour les choux, les choux-fleurs et le colza) (FAO 2000). Cette famille botanique est l'une des plus importantes économiquement (Feeny 1977). Le continent asiatique constitue la plus grande zone de production de ces cultures (Grzywacz et al. 2010) Elle comprend 350 genres et 3500 espèces cultivées et sauvages (Warwick et al. 2003). Parmi ces espèces cultivées, les choux constituent une importante source alimentaire et de revenus pour les populations rurales et urbaines en termes de production, de commercialisation et de transformation (Grzywacz et al. 2010). Ils contribuent aujourd'hui à plus de 26 milliards $ US dans l'économie mondiale (FAOSTAT 2012). En Afrique de l'Ouest, les choux sont cultivés sur 13900 hectares avec une production annuelle estimée à 140500 tonnes (FAOSTAT 2003).

Toutefois, la production des choux est sérieusement affectée par un insecte ravageur communément appelé la "Teigne des Crucifères", *Plutella xylostella* L. (Lepidoptera: Plutellidae) qui peut causer des pertes énormes estimées à plus de 90% de la production totale (Talekar & Shelton 1993; Verkerk & Wright 1996; Shelton 2004; Sarfraz et al. 2005). Au Sénégal, ce taux est estimé entre 51 et 94 % selon la direction de l'horticulture. En zone tropicale, on peut observer plus de 25 générations par an (Rowell et al. 2005; Grzywacz et al. 2010) rendant ainsi sa gestion difficile. Cette espèce oligophage se nourrit exclusivement des plantes de la famille des Brassicacées (ex : Crucifères). L'insecte est attiré par les composés soufrés (essentiellement des glucosinolates : sinigrine, sinalbine, myrosine, isothiocyanate d'allyle) que contiennent ces espèces végétales. Ces composés constituent des phagostimulants pour les chenilles et des stimulants de l'oviposition chez les femelles (Gupta & Thornsteinson 1960 ; Justus & Mitchel 1996 ; Spencer 1996).

L'emploi des insecticides organiques de synthèse constitue la principale méthode de lutte contre cet insecte (Kibata 1996). Cependant, ces pesticides peuvent entrainer plusieurs problèmes environnementaux et sanitaires tels que l'intoxication des producteurs et des consommateurs, l'élimination des ennemis naturels de la teigne, l'augmentation du coût de production et l'apparition de souches résistantes (Hooks & Johnson 2003 ; Macharia et al. 2005 ; Sarfraz & Keddie 2005 ; Shelton et al. 2007 ; Huang et al. 2010). *Plutella xylostella* est aussi le premier insecte ravageur à développer une résistance aux biopesticides à base de *Bacillus thuringiensis* Berliner au champ (Tabashnik et al. 1990; Iqbal et al. 1996). Le phénomène de résistance aux insecticides est un sérieux problème en zones tropicales dont en

Afrique Sub-Sahara (Kibata 1997; Sereda et al. 1997), mais aussi dans les zones tempérées (Talekar & Yang 1993).

Il devient donc nécessaire de trouver des méthodes alternatives permettant de maintenir le contrôle des populations de *P. xylostella* sans présenter les problèmes environnementaux des insecticides (Tabashnik et al. 1987). La tendance est actuellement à la lutte intégrée qui associe la lutte chimique utilisée de façon raisonnée à d'autres types de lutte (variétale, agronomique, biologique…). Elle permet de lutter contre ce ravageur en abaissant sa population à un niveau inférieur au seuil économique tout en tenant compte des facteurs extérieurs (environnement, entomofaune). Aujourd'hui, les programmes de gestion intègrée des populations de *P. xylostella* sont basés essentiellement sur la lutte biologique qui constitue une alternative durable à la lutte chimique classique (Hill & Foster 2003).

La lutte biologique utilise des entomopathogènes, des parasitoïdes et des prédateurs ; elle est la méthode la plus efficace contre les populations du ravageur résistantes aux insecticides (Lim 1992). Les produits à base de *Bacillus thuringiensis* (Bt) et d'insecticides naturels dont les extraits de Neem (*Azadirachta indica*) constituent actuellement des alternatives efficaces et respectueuses de l'environnement (Charleston et al. 2006 ; Ling et al. 2008 ; 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.

En effet, la lutte biologique utilisant les parasitoïdes peut être améliorée considérablement grâce à la connaissance de la biologie et de l'écologie de ces ennemis naturels (Martínez-Castillo et al. 2002). L'étude de la biologie et du comportement des parasitoïdes a d'abord été motivée par leur intérêt en tant qu'auxiliaires dans les programmes de lutte biologique (Van Alphen & Jervis 1996). Parmi ces ennemis naturels, *Oomyzus sokolowskii* Kurdjumov (Hymenoptera : Eulophidae), constitue un potentiel agent de lutte biologique contre la "Teigne des Crucifères" (Fitton & Walker 1992). Cette espèce est un endoparasitoïde naturel de la teigne à travers le monde (Wang et al. 1999 ; Ferreira et al. 2003) et en particulier au Sénégal (Sall-Sy 2005 ; Sow 2007).

Il est important d'étudier la compatibilité des approches intégrées, telles que la lutte biologique avec l'application de pesticides naturels pour une gestion efficiente et durable des populations de *P. xylostella*. En outre, l'insuffisance de données sur cet insecte d'importance économique et ses auxiliaires dans notre pays justifie le choix de notre thème d'étude.

L'objectif général de cette thèse est de contribuer à la sécurité alimentaire et la réduction des dégats causés par la "Teigne des Crucifères" grâce à une approche de gestion intégrée et durable du ravageur dans le respect de l'environnement par la réduction de l'utilisation des insecticides chimiques de synthèse.

Les objectifs spécifiques sont les suivants :

- Evaluer l'impact des facteurs climatiques (saison, pluviométrie, température) et de la plante hôte sur la dynamique des populations de *P. xylostella* et les parasitoïdes endémiques dans la zone des Niayes.
- Etudier la biologie du parasitoïde *Oomyzus sokolowskii* en conditions de laboratoire
- Evaluer la performance du parasitoïde *O. sokolowskii* sur son hôte *P. xylostella* au laboratoire.
- Evaluer l'efficacité de l'entomopathogène *Bacillus thuringiensis*, des extraits de neem et du méthamidophos sur des larves de *P. xylostella* in vitro.
- Evaluer l'effet d'un traitement alterné *Bacillus thuringiensis* et Neem sur *P. xylostella* et ses ennemis naturels au champ.
- Déterminer l'effet du traitement alterné *Bacillus thuringiensis* et Neem sur les paramètres agronomiques du chou.

Nos travaux sont présentés sous forme d'articles scientifiques et ce mémoire comporte quatre chapitres.

Le chapitre 1 dresse un bilan synthétique des connaissances sur le ravageur *Plutella xylostella*, ses plantes hôtes, les méthodes de lutte et les insectes parasitoïdes.

Après ce chapitre de présentation générale du systéme tritrophique plante hôte-ravageur-parasitoides, le chapitre 2 relate les interrelations entre *Plutella xylostella*, les facteurs climatiques, la plante hôte et les ennemis naturels du ravageur en zone tropicale (Article 1). Dans le chapitre 3, nous aborderons l'impact des entomophages: Exemple d'*Oomyzus sokolowskii* Kurdjumov (Hymenoptera : Eulophidae). Il comprend deux articles. Dans l'article 2, nous étudierons les traits d'histoire de vie d'*Oomyzus sokolowskii* Kurdjumov (Hymenoptera : Eulophidae), parasitoïde de la "Teigne des Crucifères". L'article 3 est consacré à l'étude de la performance du parasitoïde *Oomyzus sokolowskii* Kurdjumov (Hymenoptera : Eulophidae) sur son hôte *Plutella xylostella* (Lepidoptera : Plutellidae) en conditions de laboratoire.

Le chapitre 4 sera consacré à l'étude de l'effet des applications de solution biologique et naturelle sur la "Teigne des Crucifères" et ses ennemis naturels et comprend trois articles. L'article 4 est consacré à l'étude de la toxicité de *Bacillus thuringiensis* Berliner, de l'huile de Neem et du méthamodophos sur des stades larvaires de *P. xylostella* en conditions de laboratoire. Dans l'article 5, nous aborderons l'effet d'un traitement alterné *Bacillus thuringiensis* et Neem sur *P. xylostella* et ses effets sur les ennemis naturels de la teigne. L'article 6 traite de l'effet d'un traitement alterné *Bacillus thuringiensis* et Neem sur les paramètres agronomiques du chou.

Enfin, une conclusion générale et des perspectives de recherche pouvant être développées à la suite de ce travail seront également présentées.

Chapitre 1

Synthèse bibliographique

I. Le ravageur : *Plutella xylostella* (L.)

1. Systématique

L'espèce *Plutella xylostella* (L.) est communément appelée la "Teigne des Brassicacées" ou "Teigne des Crucifères". Elle a été décrite pour la première fois par Linné en 1758. Elle est parfois classée dans la famille des Yponomeutidae. Ayant subi plusieurs changements de nom, elle a longtemps été appelée *Plutella maculipennis* (Curtis 1832) avant d'acquérir son nom actuel (Moriuti 1986). Le genre comprend plus d'une douzaine d'espèces dont cinq sont d'importance économique.

La position systématique de *Plutella xylostella* (L.) est actuellement :

Embranchement : Arthropoda

Classe : Insecta

Ordre: Lepidoptera

Famille : Plutellidae

Genre: *Plutella*

Espèce : *xylostella*

2. Origine et répartition géographique

Son origine exacte est sujette à controverse. D'après Hardy (1938), Balachowsky (1966) et Talekar & Shelton (1993) *Plutella xylostella* serait originaire de la région méditerranéenne de l'Europe occidentale, zone d'origine du chou. De plus, de nombreuses espèces de parasitoïdes et de Brassicacées ont été recensées dans cette zone (Pichon 2004). Mais, plus récemment Kfir (1998) situe l'origine de cette espèce en Afrique du sud, en s'appuyant également sur la présence d'un grand nombre d'espèces de parasitoïdes et de Brassicacées endémiques dans cette région.

La répartition des populations de *Plutella xylostella* est mondiale (Chu 1986 ; Talekar & Shelton 1993). L'espèce est devenue cosmopolite suite au développement de la culture des Brassicacées dans le monde entier et elle se retrouve de nos jours dans 128 pays répartis sur cinq continents (Birot 1998). En Afrique, elle a été rencontrée pour la première fois en

Gambie (Afrique de l'Ouest) et au Cap (Afrique du sud) en 1880 puis en Ethiopie en 1902, en Tanzanie en 1906 et au Zaïre (actuel RDC) en 1935 (Ngouembe 1990).

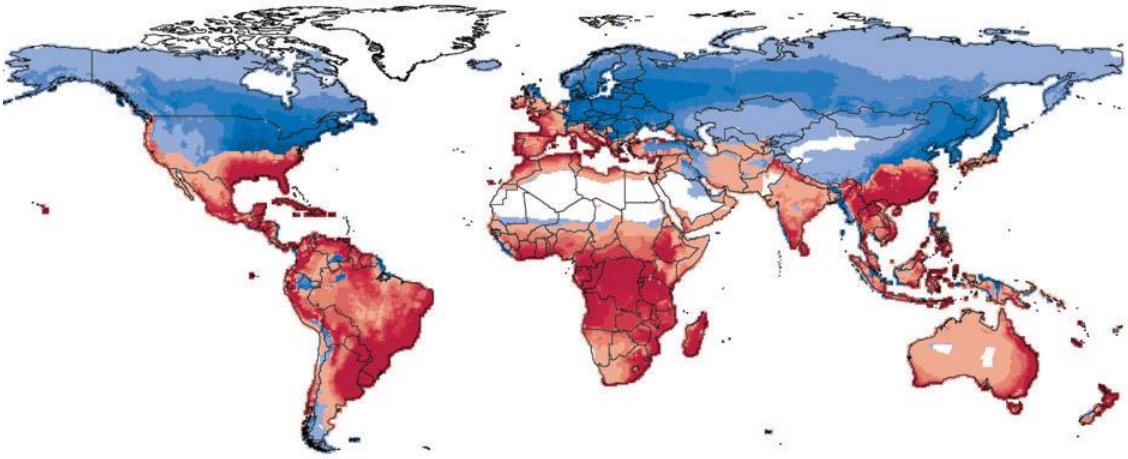


Figure 1: Carte de répartition de *Plutella xylostella* d'après Zalucki & Furlong (2011)

NB : Les zones en rouge indiquent la présence de *P. xylostella* toute l'année et en bleu, les zones où le ravageur ne persiste pas toute l'année.

3. Migrations

La migration d'une espèce d'insecte est un des facteurs importants dans l'extension de sa distribution (Pichon 1999). Grand migrateur ou accidentellement introduit, ce ravageur est devenu cosmopolite (Kfir 1998 ; Sorribas 1999). La propagation de *P. xylostella* dans le monde est due à l'extension des cultures de Brassicacées, mais aussi à la capacité de déplacement de l'espèce (Pichon 2004). 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 dehors d'une propagation liée à celle des plantes hôtes, *P. xylostella* est un très grand migrateur, capable de franchir plus de 3000 km d'une traite à l'aide des vents, traversant ainsi de grandes étendues marines (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 tous les printemps (Harcourt 1957 ; Smith & Sears 1982 ; Honda 1992 ; Honda et al.1992).

Tableau 1: Migrations de *Plutella xylostella* (Shirai 1995)

Localités	**Latitude**	**Saisons**
Royaume-Uni	55˚ N	Fin Juin, 1958
	50-55˚ N	Fin Juin à début Juillet, 1958
	55-60˚ N	Mi-Juin, 1966
	60˚ N	Début Juin et fin Juillet, 1980
Norvège	80˚ N	Fin Juin, 1978
U.S.A	40˚ N	Fin Avril, 1936
Canada	50-60˚ N	Fin Mai, 1955
	45-50˚ N	Mai et Juin, 1979 à 1981
Japon	35˚ N	Début Juin, 1962
	40˚ N	Début Mai, 1982 et 1983 Fin Août, 1984 Mi à fin Mai, 1984 à 1987
Océan Pacifique	29˚ N	Fin Juin et fin Août, 1968
Mer Est de Chine	31˚ N	Début et mi Juillet, 1985 et 1987 ; Fin Juin, 1991

4. Biologie

4.1 .Morphologie et Ethologie

4.1.1. L'adulte

L'adulte est un papillon brunâtre de 15 mm d'envergure. Les ailes antérieures sont allongées, étroites, arrondies à l'apex et de couleur jaune brun ponctué de taches plus foncées. Leur bord postérieur est frangé. Les ailes postérieures sont beaucoup plus courtes, lancéolées, aigues, d'une couleur gris foncé, très longuement frangées. La tête est rougeâtre ; et les antennes striées de noires et de blancs sont dirigées vers l'avant (Balachowsky 1966). Chez le mâle, il existe un contraste prononcé entre le jaune du centre des ailes antérieures et le brun de leur extrémité (Dommee 1999).

En position de repos, les ailes se joignent en forme de toit et se caractérisent par une bande longitudinale blanche et ondulée sur le dos (Balachowsky 1966). Les adultes, nectarivores, présentent une activité de vol plus intense au couché du soleil (Harcourt 1986).

4.1.2. L'œuf

L'œuf est de forme ovale assez allongé, de petite taille et aplatie sur la face qui est en contact avec la feuille (Talekar & Shelton 1993). Il mesure environ 0,5mm x 0,25mm. Sa coloration est jaune pâle et devient plus sombre à l'approche de l'éclosion. En laboratoire, les œufs, pour la plupart, sont déposés sur la face supérieure des feuilles (Chua & Lim 1979), alors que dans la nature, ils sont majoritairement répartis sur la face inférieure (Robertson 1939 ; Balachowsky 1966). La durée de l'incubation dépend de la température (Balachowsky 1966 ; Talekar & Shelton 1993).

4.1.3. La chenille

Le développement des chenilles passe par quatre stades larvaires (Robertson 1939 ; Talekar & Shelton 1993). La durée des quatre stades larvaires varie en fonction de la température (Bhala & Dubey 1986 ; Sarrthoy et al. 1989).

Stade 1 : Après l'éclosion, la chenille reste mineuse durant le premier stade. La larve néonate est claire peu mobile, très petite (inférieur à 1mm), s'enfonce dans l'épiderme foliaire où elle creuse une galerie allongée (Bourdouxhe 1982). Dans les tissus mésophylles de la feuille, elle laisse apparaître des virgules blanches (Talekar & Shelton 1993). Le stade L1 dure 3 à 4 jours (Birot 1998).

Stade 2 : Après la première mue, la chenille mesure 2 à 2,5 mm de long. Elle est reconnaissable par sa capsule céphalique noire (Talekar & Shelton 1993). A ce stade la larve quitte la galerie pour vivre à l'extérieur surtout à la face inférieure de la feuille de la plante hôte. Elle se nourrit alors du limbe en ne laissant qu'un seul épiderme. Il apparaît ainsi dans la feuille des plages translucides appelées fenêtres (Talekar & Shelton 1993).

Stade 3 : La chenille est de couleur verte foncé. Elle présente une capsule céphalique beige. Ce stade larvaire s'étend sur deux jours selon Birot (1998). Elle provoque d'importants dégâts sur les cultures de Brassicacées.

Stade 4 : Ce dernier stade larvaire dure 3 à 4 jours à 25°C. Sa coloration est vert pâle tirant sur le grisâtre. La chenille L4 est de forme allongée (11 à 12 mm de long), amincie aux extrémités. Elle possède une large capsule céphalique beige.

Un dimorphisme sexuel est observable à ce stade larvaire. Ainsi, les chenilles qui donneront des mâles présentent une tâche blanche sur la face dorsale visible par transparence. Elle correspond aux gonades mâles ou testicules (Ngouembe 1990 ; Lui & Tabashnik 1997). A la fin du dernier stade larvaire la chenille tisse un cocon autour d'elle (Talekar & Shelton 1993).

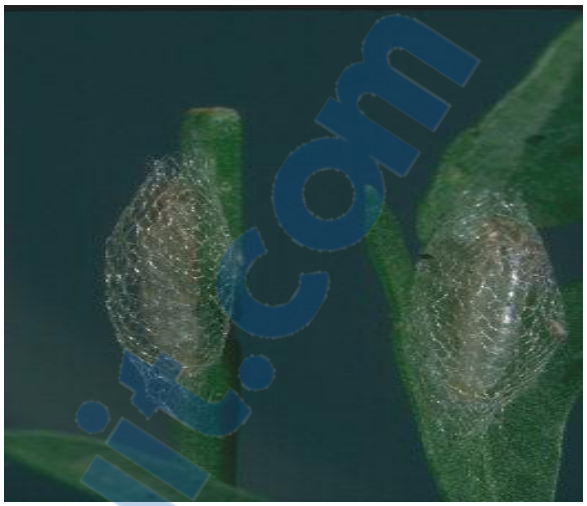
4.1.4. La nymphe

La chenille se transforme d'abord en prénymphe puis en nymphe. La nymphe est fusiforme et mesure 8 mm de longueur environ. Elle présente d'abord une coloration verdâtre et pâle, qui vire au brun foncé à l'approche de l'émergence de l'adulte (Balachowsky 1966 ; Talekar & Shelton 1993). Elle est logée dans un cocon fusiforme constitué de soie étroit et translucide à mailles très lâches. Le cocon mesure environ 7 à 10 mm de long. Les cocons restent fixés aux nervures et surtout, sur la face inférieure de la feuille de chou. La nymphose est aérienne et dure environ 4 jours à 25°C (Birot 1998).

Adulte de *P. xylostella*

Œufs de *P. xylostella*

Chenille (L4) de *P. xylostella*



Nymphes de *P. xylostella*

Figure 2: Différents stades immatures de *P. xylostella*

4.2. Cycle de développement

Le cycle de développement de *P. xylostella* (L) dépend de la température, il dure une à deux semaines selon les conditions climatiques. Au niveau des tropiques, le cycle est plus court que dans les régions tempérées. A des températures de 25°C, le cycle de développement complet peut durer 16 jours : 3 jours pour l'éclosion des œufs, 9 jours pour le développement larvaire et 4 jours pour la nymphose (Pichon 2004). En effet, la chaleur et l'humidité favorisent une croissance plus rapide de la population de ce lépidoptère (Dommee 1999). La durée de vie varie aussi selon le sexe. Elle est, en moyenne de 10,4 jours pour les mâles et de 12,1 jours pour la femelle (Patil & Pokharkar 1971).

Le nombre de génération de l'espèce varie non seulement d'un pays á l'autre, mais aussi dans un même pays suivant les conditions climatiques de l'année ou de la région (Balachowsky 1966). Dans les régions tropicales, *P. xylostella* (L) peut avoir 13 à 14 générations dans l'année (Chelliah & Sinivassan 1986) ; alors que dans les zones tempérées le nombre maximum de générations est de 3 à 4 (Hardy 1938).

Tableau 2: Durée du cycle de développement de *Plutella xylostella* élevé sous différentes températures (Koshihara 1986)

Température (˚C)	**Durée du cycle de développement en jours**		
	Œuf	**Larve**	**Nymphe**
30,0	2,4 ± 0,1	7,8 ± 0,2	3,2 ± 0,2
27,5	2,5 ± 0,1	8,3 ± 0,1	3,5 ± 0,1
25,0	3,0 ± 0,1	9,2 ± 0,2	3,8 ± 0,2
22,5	3,1 ± 0,1	11,7 ± 0,5	4,8 ± 0,2
20,0	4,1 ± 0,1	13,5 ± 0,2	5,4 ± 0,3
17,5	5,7 ± 0,1	19,1 ± 0,4	9,6 ± 0,3

4.3. La diapause

C'est un état physiologique d'arrêt de la croissance ou de la reproduction induit par un changement de la longueur des jours et par des températures extrêmes (Hance et al. 2007). En effet, aucune période de diapause n'a été mise en évidence chez les populations de la "Teigne des Crucifères" (Harcourt & Cass 1966 ; Yamada & Umeya 1972). Du fait de l'absence de diapause, *P. xylostella* est un ravageur important dans les pays d'Asie du Sud-Est et d'Afrique. Cependant, sa présence dans de nombreuses régions du Canada, des Etats-Unis et d'Europe n'entraine que des dégâts d'importance réduite (Lim 1986).

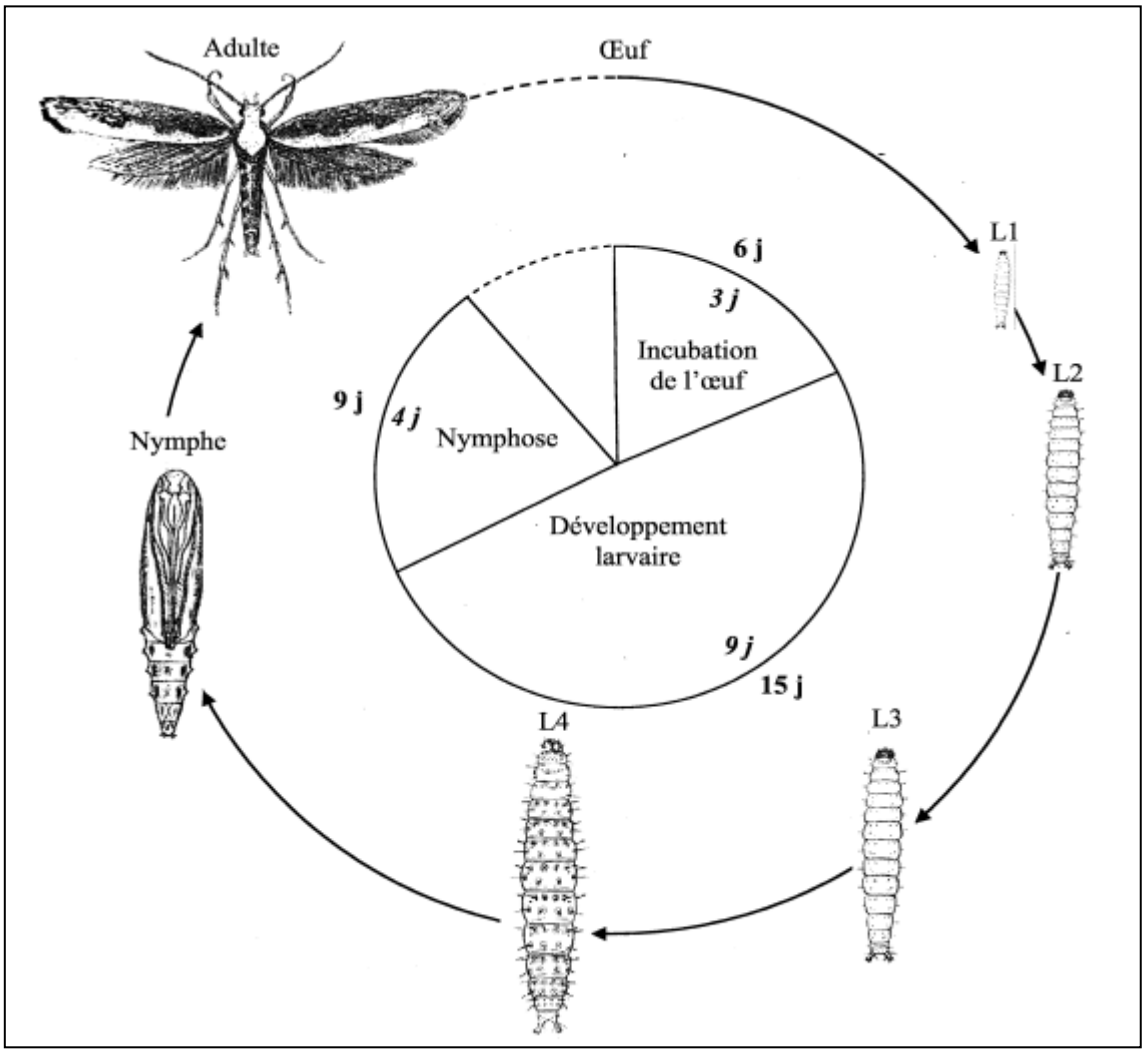


Figure 3: Cycle de développement de *P. xylostella* à 20°C (à l'extérieur du cercle) et à 25°C (à l'intérieur du cercle) (valeurs en jours, Salinas, 1986) (dessin : Carpenter, 2005)

4.4. L'accouplement et la ponte

Les adultes de *P. xylostella* s'accouplent dés leur émergence. Chez la "Teigne des Brassicacées", l'accouplement se fait dos à dos (Figure 4). Un même mâle peut s'accoupler trois fois alors que la femelle n'acceptera qu'un seul mâle (Balachowsky 1966). L'accouplement s'effectue surtout au coucher du soleil (Poelking 1990). Au Canada, par exemple, les meilleures conditions d'accouplement sont une température d'environ 20°C et une faible vitesse des vents (Harcourt 1986).

La ponte débute immédiatement après l'accouplement (Balachowsky 1966). Elle est généralement élevée, ainsi la femelle pond en moyenne 160 œufs au cours de sa vie (Balachowsky 1966 ; Talekar & Shelton 1993). D'autres auteurs font état de valeurs différentes entre 81 et 379 œufs (Ho et al. 1983) ou entre 124 et 414 œufs avec une moyenne

de 288 œufs (Ooi & Kelderman 1979). La ponte dépend de nombreux facteurs tels que la température, la qualité de la nourriture de la femelle pendant les stades larvaires ou de la densité des populations (Guilloux 2000). La ponte totale des femelles ne semble pas significativement différente d'une population de *P. xylostella* à une autre. Cependant, chaque population possède son propre comportement de ponte. Ainsi, certaines populations du ravageur pondent rapidement leurs œufs dès les premiers jours de l'émergence de la femelle, alors que d'autres ont tendance à l'étaler dans le temps. Ceci est lié aux conditions environnementales auxquelles sont soumises les populations de *P. xylostella* (Pichon 1999).

Figure 4: Accouplement de *P. xylostella* (mâle à droite, femelle à gauche)

Tableau 3: La ponte chez diverses populations de *Plutella xylostella* (Guilloux 2000)

Pays	**Nombre d'œufs pondus par femelle**		**Référence**
	Moyennes	Extrêmes	
Taiwan	75	-	Lu & Lee 1984
Pakistan	190	-	Abro et al.1992
Corée	-	50-240	Kim & Lee 1991
Canada	160	-	Harcourt 1986
USA	140	55-220	Salinas 1986
Vénézuela	160	-	Salinas 1986
Grande-Gretagne	250	100-600	Hardy 1938
France	205*	-	Pichon, 1999
Australie	188*	-	
Benin	184*	-	
Brésil	167*	-	
Philippines	145*	-	

NB : * : Au laboratoire

5. Symptômes et Dégâts

Les attaques par les chenilles de *P. xylostella* peuvent commencer en pépinière sur les jeunes plantes. Cependant, les chenilles phyllophages préfèrent les jeunes feuilles situées au cœur de la plante hôte (Ooi 1986). Sur les plantes plus âgées, elles dévorent surtout la face inférieure des feuilles en laissant le côté opposé intact, ce qui fait apparaître des taches translucides ou fenêtres. Elles peuvent consommer entièrement le limbe provoquant l'apparition de trous au niveau des feuilles. Si l'attaque est très forte, seules les nervures vont subsister, les plantes ont alors l'aspect d'un squelette et le champ de choux prend un aspect grisâtre (Graf et al. 2000).

Au Sénégal, la "Teigne des Crucifères" peut détruire totalement de nombreuses cultures de choux, ce qui pousse beaucoup de maraîchers à abandonner cette spéculation. Les dégâts varient fortement d'une région et d'une période à l'autre. La presqu'île du Cap Vert, le cordon littoral et la région de Thiès sont généralement les zones les plus affectées par ce ravageur.

Dans la région de Dakar, les dégâts causés par *P. xylostella* sont très importants en saison sèche et chaude (Mars, Avril, Mai). Pendant cette période, aucune récolte de choux n'est possible sans applications phytosanitaires. Les ravages sont nettement moins graves en saison chaude et humide ou pendant l'hivernage (Bourdouxhe 1982).

Figure 5: Dégâts sur choux

6. Impact des facteurs environnementaux

Certains facteurs climatiques peuvent agir sur la densité des populations de *P. xylostella*. En effet, la température, l'humidité relative, la pluviométrie et la photopériode influencent le développement des différents stades de *P. xylostella* (Tableau 4).

Tableau 4: Impact de différents facteurs climatiques sur *P. xylostella* (Ngouembe 1990)

Facteur	Stade affecté	Impact
Photopériode	Femelles adultes	Corrélation positive entre photopériode et fécondité
Luminosité	Adultes	Temps nuageux réduisant leur activité
Altitude	Œuf	Corrélation positive entre altitude et durée d'incubation
	Chenille	Corrélation positive entre altitude et durée du développement
Pluviométrie	Chenilles L1, L2	Facteur principal de mortalité (même effet dans les zones d'accumulation d'humidité) Effet combiné forte intensité pluvieuse-basse température
	Chenilles et adultes	Epidémies de champignons (persistance d'humidité)
	Adultes	Reproduction et dépôt des œufs perturbés
Température	Tous	Corrélation positive entre température et durée de développement

7. Plantes hôtes

L'espèce *P. xyostella* vit presque exclusivement sur les Brassicacées (Patil & Pokharkar 1971 ; Rahn 1983). En effet, l'une des caractéristiques des Brassicacées est la production de métabolites secondaires soufrés appelés glucosinolates qui 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). L'hydrolyse de ces glucosinolates est à l'origine de la formation de l'isothiocyanate dont l'odeur est caractéristique des Brassicacées (Roux 2006).

Par ailleurs, l'espèce aurait été observée exceptionnellement sur d'autres familles botaniques telles que l'oignon, la capucine, l'amarante (Chelliah & Sinivassan 1986). Ces végétaux possèdent dans leurs tissus des glucosides identiques à ceux des Brassicacées : la sinigrine, la sinalbine et la myrosine (Gupta & Thorsteinson 1960).

Bien que ce lépidoptère préfère les Brassicacées cultivées plus nourrissantes, il peut se retrouver sur des Brassicacées sauvages servant de réservoir durant les périodes où les cultures ne sont pas disponibles (Muhammad et al. 1994).

Parmi les Brassicacées, les espèces du genre *Brassica* (chou pommé, Chou-fleur…) sont les plus attractives et particulièrement appétantes (Monnerat 1995). Elles comprennent un nombre important d'espèces, environ 3000, réparties sur toute l'étendue du globe. Cependant, elles sont plus importantes dans l'hémisphère nord (Guignard 1998). Elles sont utilisées aussi bien dans l'alimentation, la médecine, l'industrie que dans l'ornementation et représentent parfois la source alimentaire principale de certaines populations asiatiques (Sall-Sy 2005).

Dans les pays en développement des régions tropicales et subtropicales, la production des Brassicacées est caractérisée par de petites surfaces cultivées et une utilisation intensive de la terre et des pesticides. Les champs sont généralement dans les zones périurbaines et assurent l'approvisionnement des grandes villes en choux frais (Pichon 2004). Le chou (*Brassica oleracea*) en particulier, originaire de la région méditerranéenne autour de la Sicile (Raimondo 1997), est surtout une culture de saison sèche et fraîche. Il préfère les sols humides et riches en matières organiques (Thiam 2001). Cependant ses variétés adaptées aux conditions de saison de pluies donnent des rendements plus faibles.

II. Lutte contre *Plutella xylostella*

Le coût de la lutte contre les chenilles de *P. xylostella* est estimé à un milliard de dollar US (Chilcutt & Tabashnik 1997). Face aux dégâts causés par la "Teigne des Crucifères", plusieurs méthodes de lutte ont été testées en vue de réduire les populations de chenilles de ce ravageur et d'augmenter les rendements.

1. Lutte chimique

1.1. Utilisation des insecticides chimiques

Les traitements chimiques restent actuellement le moyen de lutte le plus employé pour réduire les populations de *Plutella xylostella* et autres ravageurs des Brassicacées.

Le premier essai de contrôle de *P. xylostella* a été effectué en Russie en 1914. Il consistait à intercaler des rangées de choux avec des rangées de tomates (Vostrikov 1915). L'efficacité limitée de cette méthode notamment aux climats plus chauds comme l'Inde (Chelliah & Srinivasan 1986), les Philippines (Magallona 1986) et la Malaisie (Sivapragasan & Saito 1986) a conduit à l'apparition de nouveaux produits à base d'insecticides de synthèse.

Parmi ces produits utilisés contre ce ravageur, figurent des composés minéraux (arsenicaux et fluorures), des fumigants (avec ajouts d'huile et d'hydrocarbures) et quelques composés végétaux : extraits de tabac (produit actif : nicotine), de derris (roténone) ou de pyrèthres (pyréthrines) (Huckett 1934). Trop toxiques ou peu efficaces, ils sont supplantés à partir de 1945 par les insecticides de synthèse, d'une efficacité très supérieure, le premier étant le DDT rapidement suivi du lindane et autres organochlorés, puis des organophosphorés et d'autres composés par la suite (Greaves 1945 ; Harcourt & Cass 1955).

Actuellement, un retour à des insecticides d'origine biologique, extraits de végétaux, est tenté. Le plus efficace et le moins nocif pour la faune des auxiliaires semble être les extraits de neem issus de l'*Azadirachta indica* (Meliaceae) dont le principe actif est l'azadirachtine (Morallo-Rejesus 1986 ; Schmutterer 1992 ; Hermawan et al. 1998 ; Goudegnon et al. 2000 ; Löhr & Kfir 2004).

Au Sénégal, ce sont les pyrèthrinoides de synthèse et les organophosphorés qui sont les plus utilisés dans la lutte contre *P. xylostella* (Sall-Sy 2005 ; Sow 2007). Ces insecticides synthétiques sont souvent appliqués avec des pulvérisateurs à main et sans mesure de sécurité pour l'utilisateur. Par ailleurs, les restrictions concernant leur utilisation ne sont généralement pas respectées. Tous ces facteurs contribuent à une totale dépendance vis-à-vis des fournisseurs des produits pour contrôler les ravageurs. Pour contourner les difficultés rencontrées, certains producteurs mélangent les formulations et traitent plus fréquemment, ce qui entraine de nombreuses conséquences telles que l'augmentation des coûts de production, la présence de résidus sur les plantes consommées et dans le sol, l'apparition dans les populations de *P. xylostella* de phénomènes de résistance.

1.2. Résistance

Actuellement, l'utilisation régulière et répétée des insecticides a entraîné l'apparition de populations de *P. xylostella* résistantes. Ainsi, une étude réalisée, en 1989 dans 14 pays, a montré que des populations de *P. xylostella* sont résistantes à 51 insecticides (Guilloux 2000). La résistance correspond à la capacité de survivre après contact avec des doses de pesticides antérieurement mortelles. L'acquisition de cette résistance aux pesticides chez les ravageurs contribue largement au phénomène de résurgence. Le ravageur réapparaît après les traitements pesticides, principalement les chenilles mineuses peu exposées aux insecticides de contact (Sall-Sy 2005). La résistance aux insecticides est un phénomène qui évolue, due à une sélection intensive des individus après des traitements massifs et continus. Il y a aussi l'apparition de résistances croisées, c'est-à-dire de résistances à des composés autres que ceux par lequel ils ont été sélectionnés (Miyata et al. 1986). Les plus forts niveaux de résistance se trouvent dans les régions où la culture de choux est intensive, en particulier lorsqu'elle est maintenue toute l'année (Cheng et al.1986).

Le contrôle des populations de *P. xylostella* reste difficile car il a développé des résistances à tous les pesticides de synthèse utilisés y compris ceux à base de la bactérie *Bacillus thuringiensis* (Berliner) (McGaughey & Whalon 1992 ; Sanchis et al. 1995 ; Tabashnik et al. 1997).

Tableau 5: Exemples de cas de résistance aux insecticides chez *Plutella xylostella* (Guilloux 2000)

Apparition de la résistance	Pays d'apparition	Produits concernés	Références
1951	Indonésie	DDT	Ankersmith 1953
1956	Malaisie	DDT, Lindane, Dieldrine	Ooi 1997
1959	Indonésie	Organochlorés	Mo 1959
1964	Indonésie	Malathion	Cheng 1986
1965	Thailande	Carbaryl	Rushtapakornchai & Vattanatanguen 1986
1966	Inde	DDT, Parathion	Raju 1996
1974	Philippines	EPN, Mevinphos	Talekar & Shelton 1993
1978	Inde	Diazinon	Raju 1996
1981	Japon	Methomyl	Cheng 1986
1981	USA : Hawaii	Tous les produits autorisés	Tabashnik et al. 1987
1981	Taiwan	Pyréthrinoides, DDT, Organophosphorés, carbamates, Cartap	Lui et al.1981 Lui et al.1982
1982-1982	Thailande	Prothiophos, Profenophos	Rushtapakornchai & Vattanatanguen 1986
1986	Taiwan	Organophosphorés, Carbamates	Chen & Sun 1986
1986	Japon	Cartap	Hama et al. 1992
1987	USA : Hawaii	Pyréthrinoides,	Tabashnik et al.1987
1988	Malaisie	IGR, Abamectine	Fauziah et al. 1992
1988	Thailande	IGR (Benzolphenylurées)	Talekar & Shelton 1993
1986-1988	Japon	Endotoxines « Bt »	Hama et al. 1992
1989	Inde	Pyréthrinoides	Raju 1996
1989	Honduras	Cyperméthrine, Méthomyl, Méthamidophos	Ovalle & Cave 1989
1989-1990	USA : Hawaii	Endotoxines « Bt »	Tabashnik et al. 1997
1990	Malaisie	IGR (Benzolphenylurées)	Ooi 1997
1990	USA : Pennsylvanie	Endotoxines « Bt »	Tabashnik et al. 1997
1990	USA : Floride, New York	Endotoxines « Bt »	Shelton & Wyman 1992
1993	Philippines	Endotoxines « Bt »	Tabashnik et al. 1997
1999	(Laboratoire)	Plantes transgéniques« Bt »	Tang et al.1999

2. Lutte biologique

La lutte biologique est définie comme la suppression, le contrôle ou la régulation des populations de ravageurs à l'aide de prédateurs, parasitoïdes, pathogènes et/ou herbivores (Debach & Rossen 1991 ; Hawkins & Cornell 1999). Elle représente une alternative durable pour contrôler les populations de la "Teigne des Brassicacées", actuellement résistantes aux insecticides de synthèse. En effet, elle reste la technique la plus employée, en remplacement de la lutte chimique si *P. xylostella* est vraiment trop résistante, ou en association (lutte intégrée) quand c'est possible, pour ralentir l'apparition de telles résistances et limiter la quantité de pesticides libérés dans l'environnement (Guilloux 2000).

Il existe trois méthodes principales de lutte biologique: par introduction, conservation ou augmentation (van Driesche & Bellows 1996).

La lutte par introduction ou lutte biologique classique est utilisée pour lutter contre des ravageurs exotiques. Elle consiste à rechercher des ennemis naturels de ces ravageurs dans leur zone géographique d'origine et à les introduire dans le pays que le ravageur a envahi.

La lutte biologique par conservation vise à assurer des conditions favorables à la présence, la survie et la reproduction des ennemis naturels indigènes. Elle consiste à rendre disponibles, au sein ou à proximité des cultures à protéger, des ressources qui contribuent à leur efficacité telles que des refuges physiques, des hôtes alternatifs, des sources de nourriture ou des signaux attractifs. Sa mise en place nécessite que des ennemis naturels efficaces pour contrôler les populations de ravageurs soient déjà présents localement.

La lutte biologique par augmentation consiste à sélectionner un parasitoïde efficace, l'élever en masse et le lâcher dans la culture à protéger au moment le plus opportun. Elle est utilisée lorsque les parasitoïdes indigènes arrivent trop tard ou sont trop peu nombreux dans la culture pour assurer un contrôle efficace du ravageur.

Une diversité d'agents est utilisée comme moyen de lutte biologique contre *P. xylostella*. Il s'agit essentiellement des parasitoïdes, des micro-organismes entomopathogènes et des prédateurs. Ces organismes, ennemis naturels du ravageur, peuvent être indigènes ou exotiques.

2.1. Utilisation de parasitoïdes

Les parasitoïdes sont des organismes qui se développent sur ou dans un autre organisme, leur hôte, en tirent leur subsistance et le tuent comme résultat direct ou indirect de leur développement (Eggleton & Gaston 1990). Ils sont les plus étudiés, les plus utilisés et les plus efficaces. Ils sont présents partout, mais individuellement, chacune des espèces possède une aire de répartition plus réduite que celle de leur hôte (Roux 2006). Plus de 90 espèces de parasitoïdes de *P. xylostella* ont ainsi été répertoriées (Goodwin 1979). Parmi les plus importantes, on trouve 6 espèces parasites d'œufs, 38 de chenilles et 13 de nymphes (Lim 1986).

En Afrique Sub-Saharienne, de nombreux parasitoïdes associés à *P. xylostella* ont été répertoriés notamment par Lohr & Kfir (2004). Il s'agit de parasitoïdes indigènes constitués d'espèces appartenant aux familles des Braconidae, Chalcididae, Ceraphronidae, Eulophidae, Eurytomidae, Ichneumonidae, Perilampidae, Pteromalidae, Tachinidae et Trichogrammatidae.

Au Sénégal, des espèces d'hyménoptères parasitoïdes indigènes ont été recensées sur *P. xylostella*. Il s'agit de *Oomyzus sokolowskii* (Kurdjumov), *Apanteles litae* (Nixon), *Cotesia plutellae* (Kurdjumov), *Brachymeria citrea* (Steffan), *Hockeria* sp (Walker) (Bourdouxhe 1983 ; Ndiaye 1995 ; Camara 1999 ; Sall-Sy 2005 ; Sow 2007).

Tableau 6: Parasitoïdes de *Plutella xylostella* d'après Lim (1985)

Stade concerné	Parasitoïdes
Œuf	*Trichogramma brasiliensis* (Ashm) *T. minutum* Riley *T. pretiosum* Riley *Trichogrammatoidea armigera* Nagaraja
Larve	*Antrocephalus* sp *Apanteles aciculatus* (Ashm) *A.albipennis* Nees *A. fuliginosus* Wetm *A. halfordi* Ullyett *A. ippeus* Nixon *A. laevigatus* (groupe) *A. limbatus* Marsh *A.* (=*Cotesia*) *plutellae* Kurdjumov *A. ruficrus* Hal *Apanteles* sp (groupe glomeratus) *Brachymeria phyta* (Walk) *B. sidnica* Hlgr *Chelonus ritchiei* Wlksn *Compoletis* sp *Diadegma armillata* Grav *D. eucerophaga* Horstm *D. fenestralis* Holmgren *D. insularis* (Cresson) *D. neceorophaga* Horstm *D. plutellae* Viereck *D. rapi* (Cambridge) *D. varuna* Gupta *Diadegma* sp (proche de *lateralis* Grav) *Diadromus erythrostomus* (Cameron) *Habrocytus* sp *Itoplectis* sp *Macrobracon hebetor* Say *Macromalon orientale* Kerrich *Microplitis plutellae* (Meus) *Spilochalcis hirtifemora* (Ashmead) *Stictopisthus* sp *Cadurcia plutellae* Van Emden *Tetrastichus* sp
Nymphe	*Diadromus plutellae* (Ashmead) *D. subtilicornis* Grav *Dibrachys cavus* (Walkr) *Euptromalus viridescens* (Walsh) *Celis tenellus* (Say) *Habrocytus* sp (proche de *phycidis* Ashm) *Itoplectis maculator* Fab *Phaeogenes* sp *Spilochalcis albifrons* (Walsh) *Stomatoceras* sp *Tetrastichus ayyarai* Rohw *Tetrastichus* (=*Oomyzus*) *sokolowskii* Kurdj. *Thyraella collaris* Grav.

2.2. Utilisation de micro-organismes entomopathogènes

Des micro-organismes entomopathogènes appartenant à différents groupes tels que les champignons, les virus, les bactéries, les microsporidies et les nématodes, ont été étudiés, même si la plupart restent encore à l'état expérimental.

Les champignons entomopathogènes tels que *Beauveria bassiana* (Hyphomycète : Deuteromycotinae) et *Zoophtora radicans* (Zygomycète : Entomophtoracae) attaquent généralement les chenilles et parfois les nymphes de *P. xylostella* (Wilding 1986). Les larves infectées par *Z. radicans* consomment moins de tissus foliaires et arrêtent leur alimentation deux jours après l'infection (Pichon 2004). Cependant, les champignons sont assez peu utilisés car nécessitent une atmosphère très humide qui favorise l'apparition de champignons pathogènes pour les cultures, et seulement sous de fortes densités de populations de chenilles, donc lorsque les dégâts sont déjà importants (Rietmacher et al. 1992). De plus, ils peuvent réduire l'efficacité des espèces de parasitoïdes (Furlong & Pell 1996).

De même, les virus entomopathogènes comme les Bacculovirus semblent avoir une certaine efficacité contre les chenilles de la "Teigne des Crucifères" (Simmonds 1971). Les granulovirus semblent être très pathogènes, pouvant provoquer jusqu'à 90% de mortalité chez les larves de premier et second stades (Roux 2006). Cependant, toutes les souches de *P. xylostella* n'ont pas la même sensibilité. Les nucléopolyhedrovirus, par exemple, perdent très vite leur virulence au fil des générations de *P. xylostella* (Sarfraz et al. 2005).

Le bacille *B. thuringiensis* est la principale bactérie entomopathogène utilisée pour le contrôle des populations de chenilles de *P. xylostella* (Pichon 2004). Les endotoxines protéiques produites par la bactérie tuent les chenilles en se fixant sur la membrane de l'intestin et en y créant des pores (Gill et al. 1992). Ses avantages liés à sa grande spécificité et les faibles risques sur la santé et l'environnement en ont fait un important outil de lutte contre les ravageurs de culture (Roux 2006). De même, les toxines de *B. thuringiensis* n'affectent pas la faune de parasitoïdes (Flexner et al. 1986 ; Lim, 1992). Cependant, des cas de résistances à ces toxines sont apparus en laboratoire (McGaughey 1985) et en milieu naturel (Wright et al. 1997 ; Tabashnik et al. 1997).

L'utilisation des microsporidies et des nématodes entomophathogènes reste au stade expérimental. Les premières peuvent interférer avec les parasitoïdes en réduisant considérablement leur efficacité (Bordat et al. 1994 ; Gruarin 1998) tandis que les secondes

ont un coût de production élevé et elles sont sensibles à la lumière, aux températures extrêmes et à la sécheresse (Lello et al. 1996 ; Baur et al. 1997).

2.3. Utilisation de prédateurs

Les espèces prédatrices de *Plutella xylostella* appartiennent principalement à l'ordre des Diptères (Sirphidae, Drosophilidae et Lauxaniidae), des Coléoptères (Carabidae, Staphilinidae), des Dermaptères (*Euborellia annulipes*) et des araignées (Delobel 1978). Selon Ibrahim (2004), *Podisus maculiventris* (Hemiptera, Pentatomidae) est efficace dans le contrôle de la "Teigne des Crucifères".

Tableau 7: Prédateurs de *P. xylostella* (Alam 1990 ; Muckenfuss et al. 1990)

Classe	Famille	Espèces
Arachnida	Erigonidae	*Eperigone fradeorum* (Berland)
	Linyphiidae	*Florinda coccinea* (Hentz)
	Lycosidae	*Pardosa milvina* (Hentz) *P. pauxilla* (Montgomery) *P. deliculata* (Gertsch & Wallace)
Insecta	Anthocoridae	Inconnue
	Carabidae	*Calosoma sayi*
	Chrysopidae	*Ceraeochrysa claveri* (Navas)
	Coccinellidae	*Coccinella septempunctata* L. *Hippodamia convergens* (Guerin-Meneville) *Coleomegilla maculata* (De Geer) *Scymnus* spp. *Cycloneda sanguinea* L.
	Formicidae	*Solenopsis invicta*
	Hemerobiidae	Inconnue
	Labiduridae	Inconnue
	Lygaeidae	*Geocoris punctipes* (Say) *G. uliginosus* (Say)
	Nabidae	*Nabis americoferus* (Carayon)
	Pentatomidae	*Podisus maculiventris* (Say)
	Reduviidae	Inconnue
	Staphylinidae	*Belonuchus gagates* (Erichson)
	Syrphidae	*Toxomerus dispar* (Fab) *Toxomerus watsoni* (Curran) *Pseudodoros clavatus* (Fab)
	Vespidae	*Polistes* spp.

3. Utilisation des phéromones sexuelles

Les phéromones sexuelles sont des substances chimiques qui, lorsqu'elles sont secrétées par les espèces animales et libérées dans l'environnement à très faibles doses, induisent généralement un changement de comportement chez les animaux de sexe opposé de la même espèce. Elles sont principalement utilisées dans les études de dynamique des populations mais aussi comme moyen de destruction massive de tous les mâles en les attirant dans des pièges ou piégeage sexuel. En effet, la densité des populations est un paramètre

important qui affecte le succès de lutte avec les phéromones sexuelles, les bas niveaux de populations étant corrélés avec une bonne réduction (Regnault-Roger 2005).

La confusion sexuelle est aussi une technique qui consiste à disperser dans la culture des morceaux de plastique imprégnés de phéromones pour tromper les mâles et perturber l'accouplement en saturant l'atmosphère de phéromones, ce qui neutralise leur effet en empêchant les mâles de localiser les vraies femelles et donc de s'accoupler (FAO 1988). Elle agit sur le comportement de l'insecte, et elle est parfois qualifiée de lutte éthologique. La technique est un excellent palliatif, en cas de résistance des ravageurs aux insecticides (Regnault-Roger 2005).

Cependant, l'utilisation fiable des phéromones de synthèse en protection intégrée des cultures ne peut s'envisager sans une connaissance parfaite du comportement des insectes, de leurs écosystèmes, de leurs déplacements et de la variabilité populationnelle (Regnault-Roger 2005).

4. Lutte culturale

La lutte culturale désigne toute modification des pratiques agronomiques visant à réduire les dégâts occasionnés par les ravageurs ou donnant des résultats similaires même si elle a été appliquée à d'autres fins. Parmi ces pratiques, on peut citer : la sélection variétale, les cultures intercalaires, l'utilisation de plantes pièges, la rotation culturale.

La sélection culturale consiste à utiliser des variétés de choux résistants donc moins sensibles aux attaques de chenilles. Des tests de sélection variétale pour lutter contre *P. xylostella* ont été menés sur le colza, importante culture en Amérique du Nord mais l'emploi de la transgénèse s'avère plus intéressant pour créer des variétés de chou résistantes.

Le principe des cultures intercalaires consiste à cultiver des rangs alternés de choux et d'une autre plante comme la tomate ou l'ail, dont l'odeur repousse l'insecte (Guilloux 2000). Cependant, la seconde plante peut s'avérer peu intéressante pour le maraicher car n'étant pas d'un aussi bon rapport économique que le chou. De plus, il peut y avoir des problèmes de compétition entre les deux plantes, ce qui réduit alors le rendement (Schellhorn & Sork 1997).

Des essais effectués en Inde avec la moutarde ont montré que celle-ci est une plante piège qui protège le chou contre les insectes déprédateurs. En effet, les papillons déposent

préférentiellement leurs œufs sur la moutarde épargnant ainsi les choux. Cette technique se développe en Inde (Talekar & Shelton 1993) et en Afrique du Sud (Charleston & Kfir 2000).

La rotation des cultures a pour but d'interrompre les séries de générations chevauchantes en région tropicale. En effet, la pérennité de la culture de choux au cours de l'année, assure la présence constante de nourriture pour *P. xylostella* et lui permet donc de se reproduire sans contrainte de temps (Talekar & Shelton 1993).

L'irrigation de manière intermittente par aspersion réduit de 7 fois le nombre d'œufs pondus par *P. xylostella* sur ses plantes hôtes (Tabashnik & Mau 1986).

5. Lutte intégrée

La lutte intégrée est un système de gestion des populations de ravageurs qui, dans le contexte de l'environnement associé et des dynamiques des populations des espèces nuisibles, met en œuvre toutes les techniques appropriées, d'une manière aussi compatible que possible, pour les maintenir à des niveaux inférieurs à ceux causant des dommages d'importance économique (FAO 1967). La protection intégrée a montré des résultats encourageants en combinant l'utilisation de plusieurs pratiques compatibles entre elles et avec l'environnement, telles que l'amélioration génétique des variétés de choux (Eigenbrode et al. 1990 ; Eigenbrode & Shelton 1992), la culture intercalaire avec des plante-pièges ou des cultures alternatives qui repoussent le ravageur (Chelliah & Srinivasan 1986 ; Talekar et al. 1986), la rotation des cultures (Talekar & Shelton 1993), la confusion ou le piégeage sexuels (Reddy & Urs 1997), et la lutte biologique à l'aide d'antagonistes.

Les programmes de lutte intégrée (IPM) se basent sur l'écologie et la biologie des ravageurs et des agents de lutte biologique, au sein d'un système agricole particulier (Rincon 2006). Ils se déroulent en plusieurs phases : définition du problème, recherche et développement et mise en œuvre du projet. L'évaluation des ennemis naturels et la sélection d'insecticides biologiques et sélectifs sont effectuées par des bioessais et des tests en conditions naturelles. Les résultats obtenus sur différents paramètres, en relation avec la lutte intégrée, sont utilisés pour développer des programmes de lutte au niveau des cultures et testés aux champs (Pichon 2004). L'application de tels programmes a permis la réduction de plus de 80% du coût lié aux insecticides et de d'accroitre les productions en Malaisie, en Thaïlande, à Hawaii et à Singapour (Nakahara et al. 1986 ; Ng et al. 2002).

III. Les insectes parasitoïdes

1. Le parasitoïdisme

Le parasitoïdisme est un mode de vie qui se trouve 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 propre développement. Cependant, seul les stades pré-imaginaux nécessitent un tel mode de vie d'où leur appellation aussi d'insectes parasites protéliens (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 subsistance de ces hôtes et les tuent en résultat direct ou indirect de leur développement (Eggleton & Gaston 1990).

Cependant, il constitue un mode de vie moins répandu. Il est tout de même l'apanage de 10 à 20% des insectes (Godfray 1994). Sept ordres d'insectes présentent des espèces parasitoïdes : les Hyménoptères, les Diptères, les Coléoptères, les Neuroptères, les Lépidoptères, les Trichoptères et Strepsiptères (Quicke 1997). Les Hyménoptères parasitoïdes sont nettement le groupe d'organisme le plus important en lutte biologique et il est responsable de la majorité des succès tant du point de vue économique qu'environnemental (LaSalle 1993)

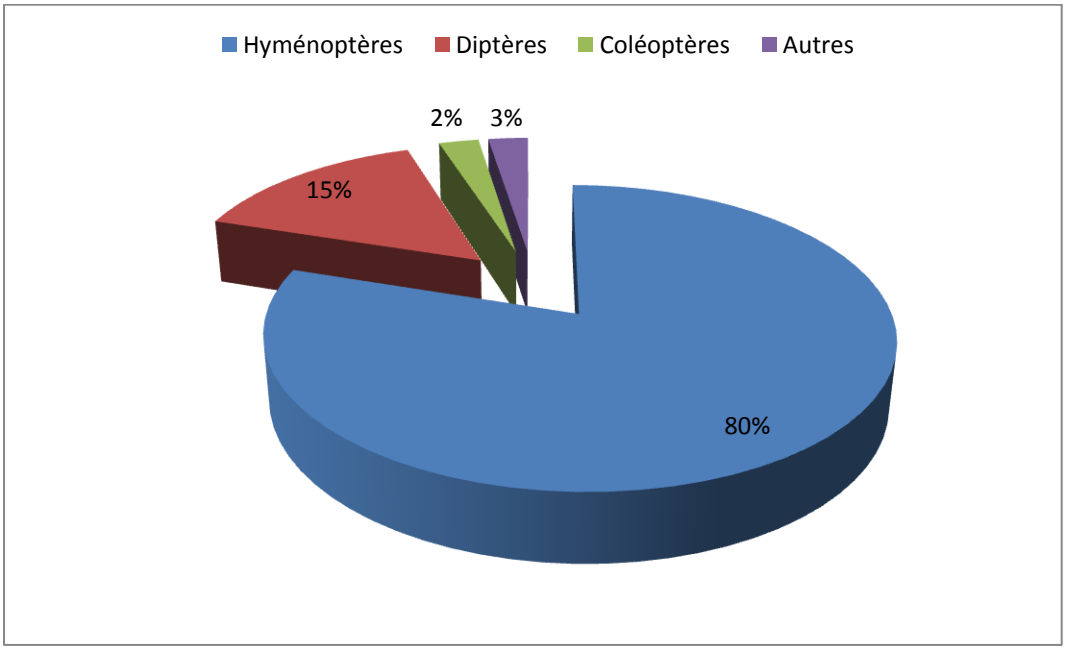


Figure 6: Répartition des différents ordres d'insectes parmi les parasitoïdes. (Quick 1997, modifiée)

2. Caractéristiques biologiques des parasitoïdes

Les différentes espèces de parasitoïdes s'attaquent à une très grande variété d'hôtes appartenant également à de nombreux ordres d'insectes. Il existe des espèces spécialistes qui attaquent une seule espèce hôte et des espèces généralistes s'attaquant à plusieurs espèces hôtes mais qui sont le plus souvent phylogénétiquement ou écologiquement proches. Tous les stades des hôtes peuvent être parasités, avec un degré plus ou moins important de spécialisation : les œufs, les larves, les nymphes et les imagos.

Les parasitoïdes peuvent se développer à l'extérieur (ectoparasitoïdes) ou à l'intérieur (endoparasitoïdes) de leur hôte et dans les deux cas, ils se développent soit en solitaire (un seul parasitoïde émerge par hôte parasité) ou en situation grégaire (plusieurs individus émergent du même hôte) (Grandgirard 2003).

Les parasitoïdes peuvent également avoir des modes de développement différents (koïnobionte ou idiobionte). Les koïnobiontes maintiennent leur hôte en vie jusqu'à la fin de leur développement alors que les idiobiontes tuent ou paralysent leur hôte au moment du parasitisme (Quicke 1997). Souvent, les premiers attaquent les jeunes stades alors que les seconds attaquent les stades plus âgés de l'hôte (Godfray 1994).

Des études comparatives sur un large nombre d'espèces ont démontré que le mode de développement des parasitoïdes était effectivement corrélé à leurs traits d'histoire de vie (Tableau 8) (Blackburn 1991 ; Mayhew & Blackburn 1999 ; Jervis et al. 2001).

3. Relations hôte-parasitoide

L'acte de parasitisme au sens strict est précédé par un ensemble de séquences comportementales du parasitoïde, qui le rapproche de son hôte. D'après Vinson (1976), on distingue les étapes suivantes : la localisation de l'habitat de l'hôte, la localisation de l'hôte, l'acceptation et l'identification de l'hôte, l'adéquation de l'hôte (âge, état parasitaire et physiologique) et enfin la régulation de l'hôte.

Dans la relation hôte-parasitoïde, il intervient un facteur écologique très important : le biotope de l'hôte. Il est représenté par la plante qui constitue non seulement l'habitat et la ressource alimentaire pour le développement de l'insecte phytophage, mais c'est elle qui sert de signal aux ennemis naturels de l'hôte (Roux 2006).

Chez les parasitoïdes, il existe également des signaux intrinsèques aux individus qui vont refléter leur état interne et donc affecter leurs stratégies de ponte. La décision de pondre dans un hôte parasité peut être alors fonction de l'espérance de vie de la femelle parasitoïde (par exemple de son âge au moment de la ponte), du nombre d'œufs restant à pondre ou de ses réserves énergétiques (Roitberg et al. 1992 ; Roitberg et al. 1993 ; Houston & McNamara 1999 ; Clark & Mangel 2000 ; Wajnberg et al. 2006). Les antennes sont aussi le support de nombreux récepteurs sensoriels ou sensilles (excroissances cuticulaires) impliqués dans la recherche et la localisation de l'habitat, dans la reconnaissance spécifique de l'hôte et dans le processus de discrimination de cet hôte (Vet et al. 1995).

Tableau 8: Traits d'histoire de vie associés aux stratégies koïnobionte et idiobionte

	Mode de vie	**Koïnobionte**	**Idiobionte**	**Références**
Traits d'histoire de vie	Développement larvaire	Endoparasitoïde	Ectoparasitoïde / Endoparasitoïde	Askew & Shaw 1986 ; Mayhew & Blackburn 1999
	Fécondité	Elevée	Faible	Mayhew & Blackburn 1999
	Taille des œufs	Petite	Grande	Mayhew & Blackburn 1999
	Type d'œufs	Hydropique	Anhydropique	Jervis et al. 2001
	Taille du corps	Grande	Petite	Traynor & Mayhew 2005
	Durée du développement	Longue	Courte	Blackburn 1991 ; Mayhew & Blackburn 1999
	Durée de vie des adultes	Courte	Longue	Blackburn 1991 ; Mayhew & Blackburn 1999
	Spectre d'hôtes	Etroit (spécialiste)	Large (généraliste)	Askew & Shaw 1986
	Indice d'ovigénie	Elevé (proovigénie)	Faible (synovigénie)	Jervis et al. 2001
	Piqûres nutritionnelles sur l'hôte	Absentes	Présentes	Jervis et al. 2001 ; Jervis et al. 2008
	Fenêtre de parasitisme	Courte	Longue	Mayhew & Blackburn 1999

4. Biotaxonomie de quelques parasitoïdes de *P. xylostella*

Les parasitoïdes naturels de *P.xylostella* ont des efficacités et des répartitions très variables selon les régions. Au Sénégal, trois espèces de parasitoïdes sont majoritairement recensées sur *P. xylostella*. Il s'agit de : *O. sokolowskii*, *C. plutellae* et d' *A. litae*.

4.1. *Oomyzus sokolowskii*

4.1.1. Systématique

Embranchement : Arthropoda

Classe : Insecta

Ordre : Hymenoptera
Super-famille : Chalcidoïdea
Famille : Eulophidae
Genre : *Oomyzus*
Espèce : *sokolowskii* (Kurdjumov)

4.1.2. Cycle de développement et morphologie

a. L'adulte

Il est de très petite taille entre 1 à 2 mm. Son corps est de couleur noir brillant avec des reflets métalliques. Un dimorphisme sexuel assez marqué nous permet de distinguer le mâle de la femelle :

- ✓ Le mâle a un abdomen cylindrique, de même diamètre que le thorax. Il possède des antennes de grande taille pourvues de quatre articles portant de 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.

b. L'œuf

Il 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 de l'hôte bien que la femelle n'ait pas de site de ponte préférentiel (Birot 1998). 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 1 à 2 jours à 25°C. La segmentation de l'embryon est visible par transparence dans l'œuf.

Figure 7: Chenille et nymphe parasitées par *O. sokolowskii* (à gauche) et nymphe saine (à droite)

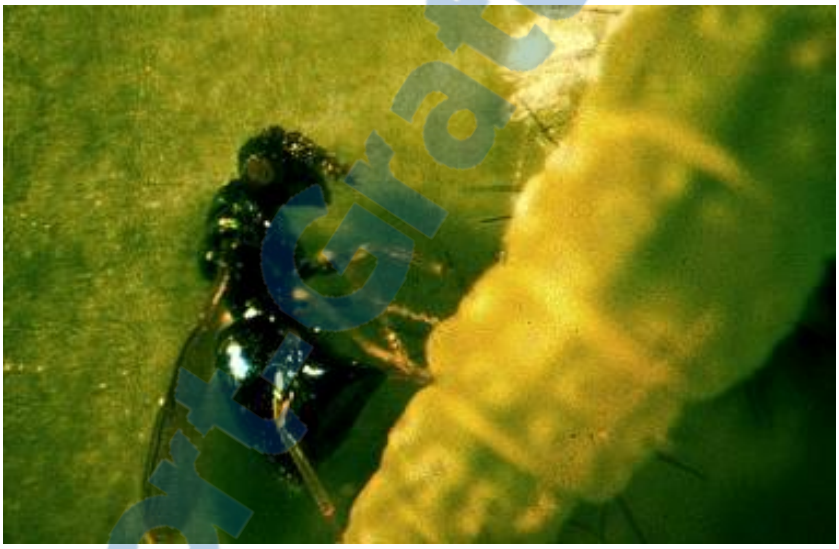


Figure 8: Femelle d'*O. sokolowskii* en oviposition

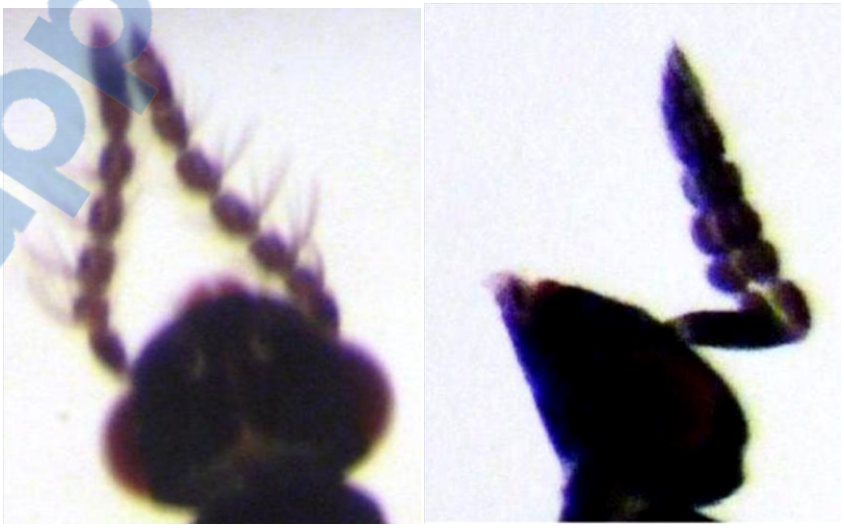


Figure 9: Antennes d'*O. sokolowskii* : femelle (à gauche) et mâle (à droite)

c. Le stade larvaire

La recherche bibliographique ne nous a pas permis de préciser le nombre de stades larvaires. La larve est de forme vermiforme, arrondie aux extrémités, quasi transparente et possède un tube digestif sur toute sa longueur. Les larves sont de taille variable dans la chrysalide parasitée. Au fur et à mesure de leur développement, les plus grosses colonisent la totalité de la nymphe, tandis que la compétition qui s'instaure entre elles provoque une diminution sensible de leur nombre dont les plus petites. En fin de développement, les larves d'*O. sokolowskii* ont vidé la chrysalide de *P. xylostella* de son contenu. La durée du stade larvaire varie de 8 à 12 jours à la température de 26-27°C (Birot 1998).

d. Le stade nymphal

La nymphose se déroule en 4 étapes : la larve donne d'abord une prénymphe de couleur blanche où aucun organe du futur adulte n'est visible, la nymphe aux yeux rouges lorsque les yeux et les ocelles se colorent en rouge, ensuite la nymphe brune et enfin la nymphe noire où de nombreux organes ou structures commencent à apparaitre. Ce stade se déroule sur 6 à 10 jours à la température de 26-27°C (Birot 1998).

4.1.3. Caractéristiques bio-écologiques

C'est un endoparasitoïde dont la femelle pond dans les chenilles de *P. xylostella* à tous les stades, avec une nette préférence pour les chenilles L3 et L4 où les taux de parasitisme sont respectivement 70% et 61% (Talekar & Hu 1996). Cependant, certains auteurs le considèrent comme un parasitoïde larvaire (Ooi 1988 ; Alam 1990; Talekar & Hu 1996 ; Kfir 1997), mais d'autres comme un parasitoïde nymphal (Chelliah & Srinivason 1986 ; Waterhouse & Norris 1987 ; Wakisaka et al. 1992 ; Noyes 1994). Les larves du parasitoïde continueront leur développement dans la nymphe de *P. xylostella* d'où émergeront les adultes. L'accouplement, qui a lieu dés l'émergence, stimule fortement les capacités de parasitisme de la femelle (Birot 1998). La durée du cycle biologique varie en fonction de la température (Tableau 9). A la température de 26-27°C, Birot (1998) a trouvé des durées de cycles pouvant aller de 17 à 24 jours. La durée de vie moyenne d'un adulte est de 7 jours (Hirashima et al. 1990).

Comme chez la plupart des hyménoptères, il existe une reproduction sexuée, avec fécondation des ovules par les spermatozoïdes. La descendance, issue d'œufs fécondés, se

compose de femelles diploïdes. La reproduction par parthénogénèse arrhénotoque est aussi très fréquente. Dans ce cas, le développement embryonnaire des œufs non fécondés donne une descendance uniquement composée de mâles haploïdes (Birot 1998). La femelle gravide décide du 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).

Cette espèce est actuellement présente dans les cinq continents, ce qui prouve sa grande capacité d'adaptation face à des conditions climatiques variées, qualité nécessaire pour une lutte biologique efficace (Gruarin 1998). *Oomyzus sokolowskii* est une espèce protélienne (seules les larves sont parasites, les adultes étant libres) et grégaire (plusieurs adultes issus d'œufs pondus par une même femelle émergent de l'hôte). Son spectre d'hôte se limite à une seule espèce, elle est à spécificité oïoxène (Birot 1998), mais cet hyménoptère peut aussi agir comme un hyperparasitoïde facultatif ou parasitoïde secondaire de *Cotesia plutellae* (Garnier 1996).

Figure 10: Couple d'*O. sokolowskii* (Femelle à gauche et Mâle à droite)

Tableau 9: Durée du cycle biologique d'*Oomyzus sokolowskii* sous différentes températures (Wang et al 1999)

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

4.2. *Cotesia plutellae*

4.2.1. Systématique

Embranchement : Arthropoda
Classe : Insecta
Ordre : Hymenoptera
Super famille : Ichneumonoidea
Famille : Braconidae
Sous-famille : Microgastrinae
Genre : *Cotesia*
Espèce : *plutellae* (Kurdjumov)

4.2.2. Cycle de développement et morphologie

a. L'adulte

Il mesure environ 3 mm de long. Le corps est de couleur marron-noir. Les ailes sont transparentes. La paire antérieure porte une tache le long de la nervure costale. Il existe un dimorphisme sexuel apparent chez cette espèce :

- ✓ Chez le mâle : On distingue de grandes antennes, plus longues que le corps. Il possède un abdomen arrondi à son extrémité. Comme les autres hyménoptères à reproduction sexuée, il est haploïde, trait caractéristique de la reproduction haplodiploïde (Guilloux, 2000).

- ✓ Chez la femelle : Elle est légèrement plus massive, avec des antennes plus courtes. Son abdomen est terminé par un court ovipositeur ou tarière. Elle est dipoïde (Guilloux 2000).

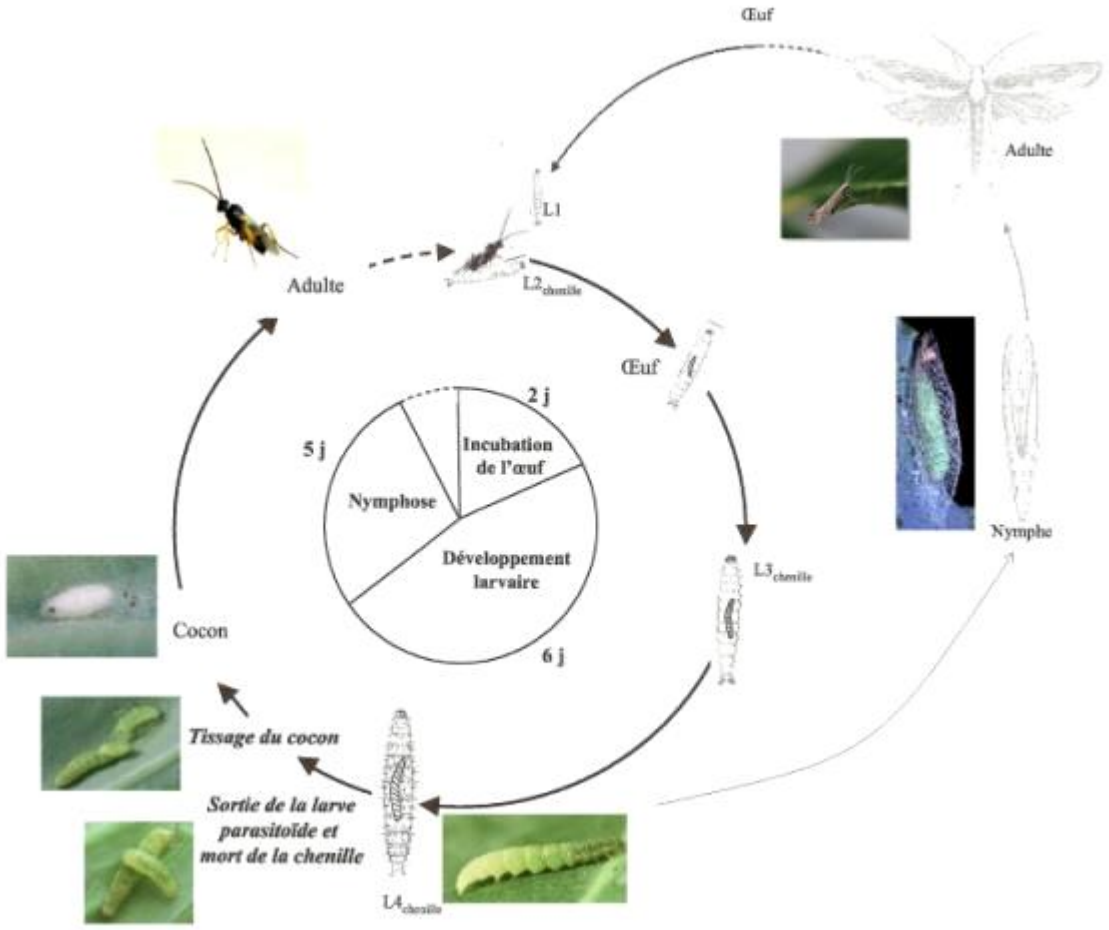


Figure 11: Cycle de développement de *C. plutellae* à 20°C (Delucchi et al. 1954, modifiée)

b. L'œuf

L'œuf est de couleur blanchâtre et en forme de croissant. Il mesure 0,3 mm de long pour 0,1 mm de diamètre. Celui-ci est pondu sans préférence de localisation dans le corps des chenilles de *P. xylostella* (Roux 2006).

c. Le stade larvaire :

Le nombre et l'aspect des phases du développement larvaire de *C. plutellae* sont controversés par plusieurs auteurs. Cependant, Guilloux (2000) a trouvé deux stades larvaires :

- La larve L1 est caractérisée par une grosse tête munie d'un prolongement caudiforme avec une vésicule caudale. Elle est ornée de spinules tergaux et dispose de mandibules simples et pointues, qui peuvent lui servir à lutter contre les autres larves en cas de superparasitisme de la chenille-hôte (Lloy 1940).
- La larve L2 possède une petite tête faiblement chitinisée avec des mandibules légèrement dentées. Les tergites sont ponctués, sans spinules (Guilloux 2000). Elle se nourrit des fluides de son hôte, certains nutriments pouvant être captés par osmotrophie (Quicke 1997).

d. Le stade nymphal :

La larve de *C. plutellae* émerge en perforant le tégument de la chenille-hôte et tisse son cocon juste à coté d'elle. Le cocon est de couleur blanc-jaune à aspect soyeux. La nymphe se colore et se chitinise progressivement, acec séparation des appendices qui deviennent bien visibles, plaqués contre le corps (Guilloux 2000).

4.2.3. Caractéristiques bio-écologiques

Son cycle biologique varie en fonction de la température. Il est de 11 à 14 jours à 25-27°C (Chua & Ooi 1986). L'accouplement peut se produire dès l'émergence des adultes. La femelle commence à pondre au cours des 24 heures qui suivent l'émergence (Guilloux 2000). Elle peut parasiter tous les stades larvaires de *P. xylostella*, sauf le premier, mais parait avoir une préférence pour les chenilles L2 et L3 (Talekar & Yang 1991 ; Fouilhe 1996 ; Shi et al. 2002). Son taux de parasitisme peut atteindre 75% en laboratoire (Fouilhe 1996) comme au champ (Alam 1990).

Cet hyménoptère est endoparasitoïde du stade larvaire de *P. xylostella*. Il est une espèce solitaire car d'une chenille hôte, ne sortira qu'une seule larve de *C. plutellae* même si plusieurs œufs y ont été pondus (Bach & Tabashnik 1990 ; Gruarin 1998). C'est une espèce koïnobionte : la chenille parasitée continue son développement jusqu'à la nymphose d'où sortira le parasitoïde ayant terminé son développement, ce qui accroît alors les ressources nutritives détournées (Guilloux 2000).

Dans la nature, la femelle de *C. plutellae* repère les choux attaqués par l'odeur caractéristique qu'ils émettent (Potting et al. 1999 ; Liu & Jiang 2003). De même, le superparasitisme est fréquent chez *C. plutellae*, car les femelles ne font pas la différence entre

les chenilles déjà parasitées et chenilles non attaquées (Lloyd 1940). Comme de nombreux endoparasitoïdes, *C. plutellae* injecte lors de l'oviposition un polydnavirus qui permet d'éviter l'encapsulation de l'œuf (Roux 2006).

4.3. *Apanteles litae*

4.3.1. Systématique

Embranchement : Arthropoda
Classe : Insecta
Ordre : Hymenoptera
Super famille : Ichneumonoidea
Famille : Braconidae
Sous-famille : Microgastrinae
Genre : *Apanteles*
Espèce : *litae* (Nixon)

4.3.2. Cycle de développement et morphologie

a. L'adulte

L'adulte mesure environ 2,8 mm de long. Les mâles comme les femelles sont de couleur noire avec un thorax ponctué. Leurs pattes sont grêles et terminées par de petits crochets. Ils ont des ailes transparentes dont la paire antérieure présente de petits crochets, les hamulis, sur le bord supérieur.

L'espèce présente un dimorphisme sexuel :

- ✓ Le mâle présente des antennes aussi longues que son corps mais beaucoup plus longues que celles de la femelle. Il a une taille généralement plus petite.
- ✓ La femelle possède un long ovipositeur apical, caractère qui le distingue sur le terrain de la femelle de *C. plutellae* à ovipositeur court et situé ventralement. Elle a aussi un abdomen plus effilé.

b. L'œuf

L'œuf est de couleur blanche en forme de concombre. Il est terminé par un appendice. Les œufs sont déposés dans l'hémolymphe de la chenille parasitée pour une durée

d'incubation d'environ 24 heures. Au terme de ce délai et après éclosion, apparait une larve de type 1.

c. Le stade larvaire

Selon Jeamblu (1995), on distingue des larves de type 1 et de type 2 :

- La larve de type 1 : Elle est apode et transparente avec un appendice caudal. Elle présente une tête et des pièces buccales très apparentes.
- La larve de type 2 : Elle est apode et vermiforme. La tête est réduite avec des mandibules dentées. Une vésicule anale se substitue à l'appendice caudal. Cette vésicule apparait en fin de premier stade puis disparait avant la sortie de la larve de son hôte. La larve est de couleur claire avec une partie longitudinale au centre plus foncée, représentant le tube digestif dont l'aboutissement est la vésicule anale. Le rôle de celle-ci est de recueillir les déjections du parasitoïde, afin d'éviter qu'il n'intoxique son propre milieu alimentaire.

d. Le stade nymphal

La larve de type 2 émerge de l'hôte par une déchirure de la cuticule. Cette sortie est suivie de la mue de la larve qui tisse un cocon à l'intérieur de celui de l'hôte. Les cocons d'*Apanteles* sont blancs à aspect parcheminé (Guilloux 2000). La nymphose dure environ 6 jours à 25°C (Jeamblu 1995).

Figure 12: *Apanteles litae* (cocon et adulte)

4.3.3. Caractéristiques bio-écologiques

Cet hyménoptère est un endoparasitoïde solitaire comme *C. plutellae* avec qui, il présente quelques similitudes. C'est aussi un parasitoïde très spécifique de *P. xylostella*.
Comme la plupart des hyménoptères, *A. litae* présente une reproduction sexuée et une reproduction à parthénogénèse arrhénotoque, c'est-à-dire que les œufs fécondés donneront des mâles et des femelles, par contre, les œufs non fécondées ne produiront que des mâles. C'est un parasitoïde koïnobionte, c'est-à-dire que l'hôte poursuit son développement même après le parasitisme, ce qui offre donc au parasitoïde des ressources croissantes. Cependant, la sortie de la larve provoque la mort instantanée de l'hôte qui présente alors un aspect noirâtre et desséché (Jeamblu 1995).

Chapitre 2

Interrelations entre la "Teigne des Crucifères", les facteurs climatiques, les ennemis naturels et la plante hôte

Article 1

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

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Résumé

En vue d'étudier les interactions entre *Plutella xylostella* (L.), (Lepidoptera : Plutellidae), les conditions climatiques (saison, température, pluie), la plante hôte et la faune auxiliaire associée, des essais ont été conduits au champ dans les Niayes de Dakar. Les échantillonnages ont été effectués en hivernage et en saison sèche, dans des parcelles non traitées sur deux années (2009-2011). Dans chacun des plants de chou, des prélèvements aléatoires de 10 choux ont été réalisés tous les 10 jours. Sont recueillis : le nombre de *P. xylostella* (tous les stades à l'exception des stades L1 mineuses), les cocons des parasitoïdes, sur le chou (âge et diamètre du plant) et les données climatiques (température, pluviométrie). Les résultats ont montré que les populations larvaires et nymphales de *P. xylostella* étaient plus importantes dans les plants de chou en saison sèche qu'en hivernage. Une corrélation négative entre les populations du ravageur et la température a été trouvée. La corrélation était significative entre la population de la teigne et l'âge de la plante hôte. La population de *P. xylostella* augmentait avec le développement de la surface foliaire. Les stades immatures (L2 et L3) se sont développés préférentiellement chez les jeunes plants de chou contrairement aux larves de stades L4 et aux nymphes qui se développent chez les plants plus âgés. Les populations de parasitoïdes ont été plus importantes pendant la saison séche. Les fortes températures étaient corrélées à une baisse de la densité de *P. xylostella* et du taux de parasitisme. La pluviomètrie a un effet minime sur le ravageur, ses auxiliaires et la plante hôte. Une corrélation positive a été trouvée entre la population du ravageur et le parasitisme. Quatre espèces de parasitoïdes ont été trouvées : *Cotesia plutellae, Apanteles litae, Oomyzus sokolowskii* et *Brachymeria citrae*. Mais, elles n'ont pas été efficaces pour contrôler les populations de *P. xylostella*. Ces résultats sont importants pour comprendre les facteurs favorisant ou inhibant les populations de *P. xylostella* et leurs enemis naturels, et donc indispensables pour une protection efficace des cultures.

Mots clés : lutte biologique, parasitoïde, plante hôte, *Plutella xylostella*, pluviométrie, température

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 (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* (Vandermer 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* cv. 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 5kg 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 10min 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 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, $\alpha = 0.05$ was considered significant.

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; Table 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) (Figure 1).

Table 1. Overall relationship between 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 ± 71.4a	29.1± 9.2b
Parasitism (%)	5.5 ± 1.6a	0.4 ± 0.1b
Oomyzus sokolowskii	7.7 ± 1.5a	0.9 ± 0.3b
Apanteles litae	10.3 ± 2.3a	0.6 ± 0.2b
Cotesia plutellae	3.9 ± 2.1a	1.1 ± 0.7a
Brachymeria citrae	0 a	0.1 ± 0.1a
Mean temperature (°C)	23.8 ± 1.5b	29.7 ± 2.6a
Total rainfall (mm)	0 b	498.0 ± 0.0a

Means in rows followed by the same letters are not significantly different by Student – Newman – Keuls test at p = 0.05

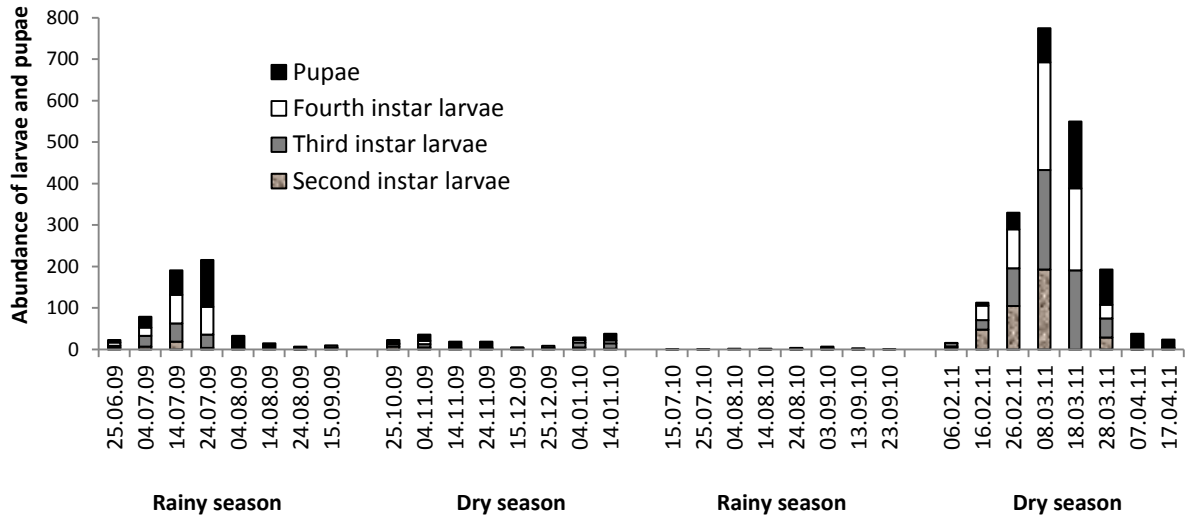

Figure 1. Abundance of *P. xylostella* larvae and pupae on unsprayed cabbage fields during the rainy and dry seasons from June 2009 to April 2011

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; Table 1). Rainfall was higher during the rainy season than the dry season (Figure 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; Table 1). Temperatures were higher (30 to 42°C) during the rainy season than the dry season (Figure 2).

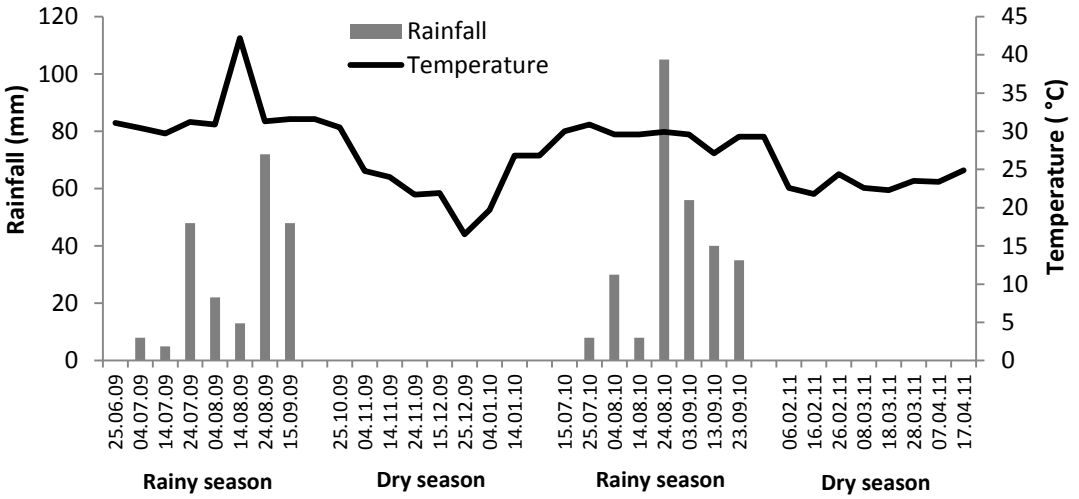

Figure 2. Total rainfall and mean temperature recorded at Malika (Senegal) during the rainy and dry seasons from June 2009 to April 2011

Relationships 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 with the age of the cabbage ($r = 0.44$; $p = 0.007$) (Figures 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.

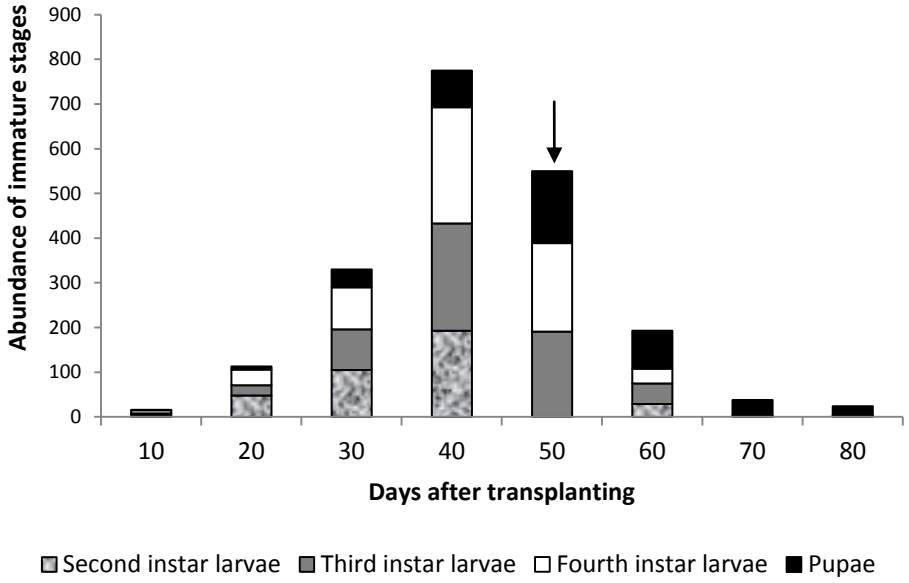

Figure 3. Relationship between the abundance of immature stages of *P. xylostella* and the age of the cabbage in the dry season. Arrow indicates the beginning of hearting (cabbage maturation)

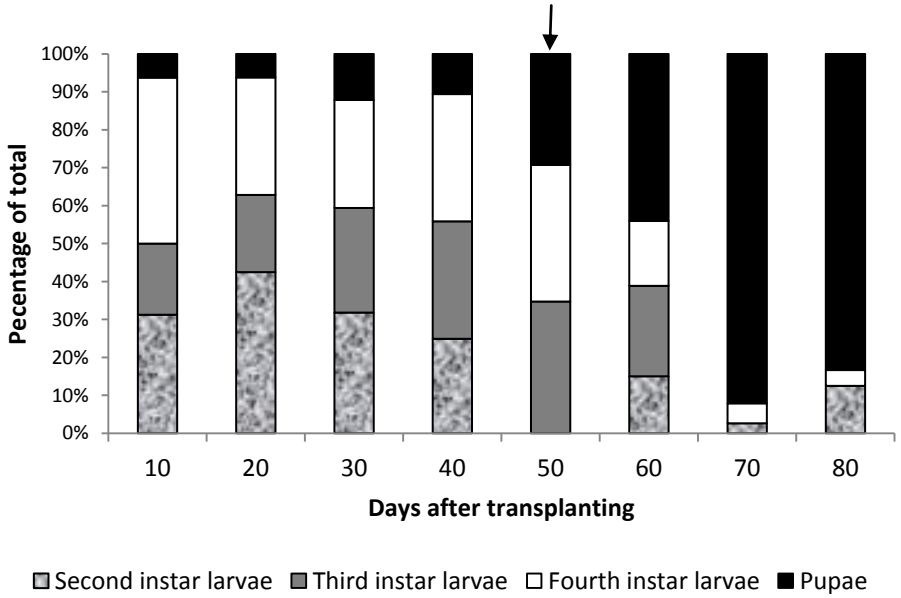

Figure 4. Relationship between the relative abundance of immature stages of *P. xylostella* and the age of the cabbage in the dry season. Arrow indicates the beginning of hearting (cabbage maturation)

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 (Table 1).

Four indigenous parasitic Hymenoptera were found in the pest populations (Figure 5). *Oomyzus sokolowskii* Kurdjumov (Eulophidae), a larval-pupal, was active throughout the year and dominated the parasitoid complex (Figure 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; Table 1). *Apanteles litae* Nixon (Braconidae), a larval parasitoid, was most predominant in the dry season (F = 7.55; p = 0.009; Table 1). *Cotesia plutellae* Kurdjumov (Braconidae), a larval parasitoid, was also recorded throughout the year, but its activity was sporadic (Figure 5). There was no significant difference between seasons (F = 1.71; p = 0.19; Table 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; Table 1). Hyperparasitoids were not recorded.

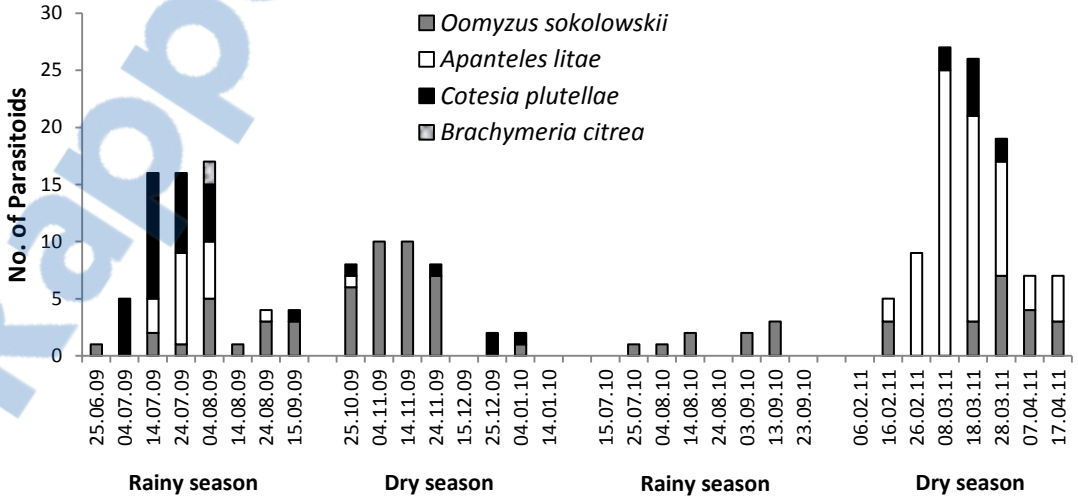

Figure 5. Abundance of parasitoids associated with *P. xylostella* on unsprayed cabbage fields during the rainy and dry seasons from June 2009 to April 2011

Effects of rainfall and temperature

No significant correlation was found between rainfall and parasitoid populations (r = -0.27; p = 0.1) (Figure 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; Figure 6). There was a negative correlation between cabbage age and *O. sokolowskii* (r = -0.44; p = 0.007; Figure 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).

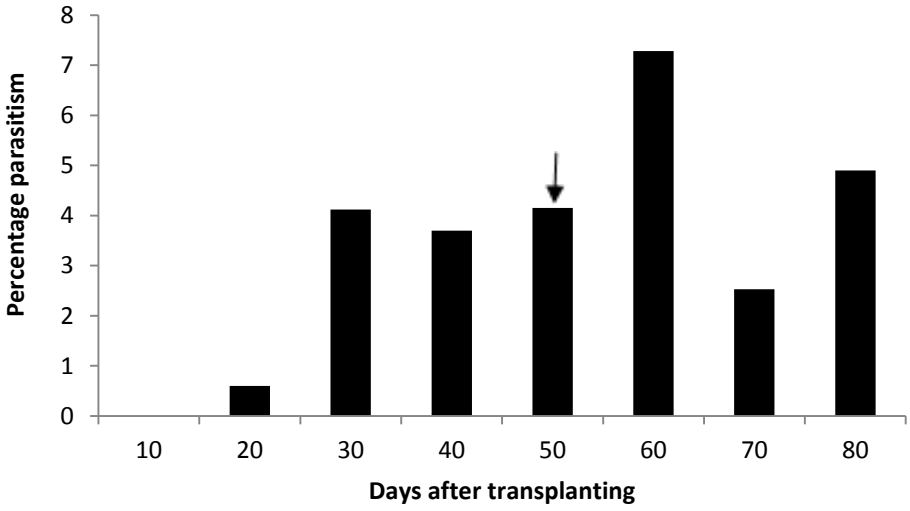

Figure 6. Relationship between *P. xylostella* parasitism and the age of the cabbage in the dry season. Arrow indicates the beginning of hearting (cabbage maturation)

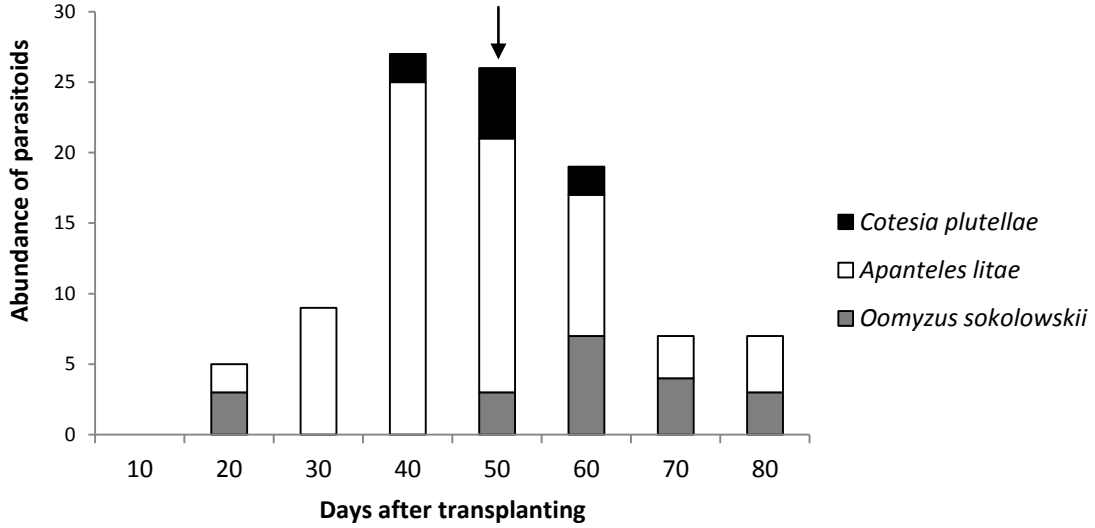

Figure 7. Relationship between the abundance of natural enemies and the age of the cabbage in the dry season. Arrow indicates the beginning of hearting (cabbage maturation)

Relationships between *P. xylostella* populations and natural enemies

The correlation between pest populations and total parasitism was positive (r = 0.36; p = 0.003) (Figures 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 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 (Hopking 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.

Conclusion

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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Chapitre 3

Impact des entomophages: Exemple d'*Oomyzus sokolowskii* Kurdjumov (Hymenoptera : Eulophidae)

Article 2

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

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Résumé

Oomyzus sokolowskii (Kurdjumov) (Hymenoptera : Eulophidae) est un parasitoïde de *Plutella xylostella* (L.) (Lepidoptera : Plutellidae) majoritairement rencontré au Sénégal. L'objectif de ce travail est d'étudier quelques traits d'histoire de vie du parasitoide tels que le cycle de développement, le dimorphisme sexuel, le mode de reproduction, le comportement de ponte et de recherche de l'hôte en conditions de laboratoire. Les résultats ont montré que la durée moyenne de développement du parasitoïde est de 15,6 jours à 25°C. Il existe un dimorphisme sexuel lié à la taille chez *O. sokolowskii*. Les femelles sont significativement plus grandes que les mâles. Le taux de parasitisme est différent entre les femelles accouplées et les femelles non accouplées. Les femelles accouplées ont produit une descendance normale composée de mâles et de femelles alors que les femelles vierges ont donné seulement des mâles. Cette espèce synovogénique peut aussi parasiter tous les stades larvaires et les prénymphes de *P. xylostella*. Cependant, le taux de parasitisme est plus élevé chez les larves L4. Dans la recherche de l'hôte, la femelle d'*O. sokolowskii* semble plus agressive vis à vis des chenilles quand elle se trouve dans un pondoir de 7 cm^3 de volume, où le taux de parasitisme est plus important que dans un pondoir plus grand ou plus petit. Les résultats obtenus permettent une meilleure connaissance de la biologie d'*O. sokolowskii* et de rendre plus efficace son utilisation lors de la mise en place de programmes de gestion des populations de *P. xylostella* basés sur la libération d'ennemis naturels.

Mots clés : *Plutella xylostella*, dimorphisme sexuel, ovogénie, parthénogenèse, koinobionte, comportement, lutte biologique

Abstract

In this study, we characterize the life-history of *Oomyzus sokolowskii* (Kurdjimov), a parasitoid of the diamondback moth (DBM) *Plutella xylostella* (L.). We studied the life cycle, adult size, fecundity, ovigeny, parthenogenesis, host age preference, host-searching behaviour by parasitoid females under laboratory conditions. The *O. sokolowskii* 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 imply that mating stimulates the behaviour of parasitism. Thelythokous parthenogenesis was not recorded. Females can parasitize all larval stages and prepupae, but the parasitism rate was higher in the fourth larval stages of DBM. The host-seeking behaviour was influenced by volume; *O. sokolowskii* females were more efficient when they were placed in a 7 cm^3 oviposition box.

Keywords: *Plutella xylostella*, sexual dimorphism, ovigeny, parthenogenesis, koinobiont, behavior, biological control

Introduction

Parasitoids are insects whose females lay their eggs in or on other invertebrates (mostly other insects) and whose larvae feed on the host and kill it (Jervis *et al.* 2001). The study of parasitoid biology and behaviour was first motivated by their interest as auxiliaries in biological control programs (van Alphen & Jervis 1996). The life history traits are directly related to the organism fitness, hence to their reproductive success and survival (Le Lann *et al.* 2011). Parasitoids fitness is generally measured by the life history traits (Roitberg *et al.* 2001). 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, parasitoids whose larvae are associated with the development of their hosts and emerge at the end of their development, and idiobiont, which kill or paralyze their host at the time of parasitism and use the resources available at the time of oviposition (Quicke 1997). 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 thelytokous 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 that have their 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 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; Godfray 1994). For this reason, knowledge of parasitoid foraging behaviour can promote implementation of a successful biological control program (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 a significant parasitoid and potential biocontrol agent of the diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), a 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 (Sy-Sall *et al.* 2004).

We studied life history traits of *O. sokolowskii* under laboratory conditions: the parasitoid development cycle, adult size, fecundity, ovigeny, parthenogenesis, host age preference and foraging behaviour of females. This knowledge will be used to inform development of biocontrol programs against DBM in tropical areas.

Material and methods

Insect rearing

The study was conducted at the Laboratory of Entomology for International Cooperation in Agronomic Research for Development Center (CIRAD) in Montpellier (France). The parasitoid population was obtained from parasitized pupae of DBM collected in 2011 from cabbage crops (*Brassica oleracea* var. *capitata*) in the "Niayes" area, situated in North West of Senegal (12°54' 44''N; 12°8' 84''NW, and 189 m altitude).

Cultures of DBM were maintained by allow adult females to oviposit on brown mustard plants (*Brassica juncea* L. Czern.) in oviposition boxes (50cm×50cm×50cm). Egg clutches were collected every 24hours. At hatching, larvae were placed on fresh leaves of cauliflower (*Brassica oleracea* var. *botrytis* L.). Mature larvae were transferred to new leaves in a large plastic box (28 cm × 27 cm × 8cm) 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 in height and 8 cm in diameter). After 24 hours exposure, all larvae were removed and placed in an identical container. Parasitoid adults 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 24h-old parasitoid females were daily put with thirty fourth-instar DBM larvae in a clear plastic container (5 cm in height and 8 cm in diameter). After 24 hours exposure, all pupae were recovered and placed individually in clear plastic pill bottles (1cm in heigh and 3 cm in diameter). Each day, some pupae were dissected and observed under the microscope. Development time of the parasitoid was measured from oviposition to adult eclosion, in days.

Adult size and ovigeny

Oomyzus sokolowskii adult size was assessed by measuring the length of the hind tibiae of 30 24h-old males and 30 24h-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×3cm). Upon emergence, 30 virgin females were dissected and the number of eggs in each was counted. Maximum potential lifetime fecundity was determined by presenting thirty 24h-old mated parasitoid females 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 (1cm in heigh and 3 cm in 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) were calculated and compared for 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 parasitoid females in a clear plastic container (5 cm in height and 8 cm in diameter). After 24 hours 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, female number laid, 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 analyzed using one-way ANOVA. Female productivity, parasitism rate, sex ratio, and offspring development time from the three oviposition-boxes were analyzed 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 (± 0.20 SE) days. The eggs were aggregated to each other forming one or more clusters of 5 to 20 units. Most eggs gathered in the back of the larvae and the presence of isolated eggs was sometimes observed. Three days after parasitism, young vermiform larvae hatched. These larvae grow until eventually occupying almost the entire body of the pupae. The duration of the larval stage ranged from 4 to 7 days (mean 4.6 ± 0.24 SE). The parasitoid prepupae were white with red eyes, and the pupae were black. The nymphal stage lasts 7.8 days in average. Adults emerge from the pupae parasitized by drilling several holes through the cuticle. Males and females of the parasitoid emerged simultaneously from DBM pupae. The development time of *O. sokolowskii* from egg to adult was 15.6 (±0.40 SE) days (Figure 1).

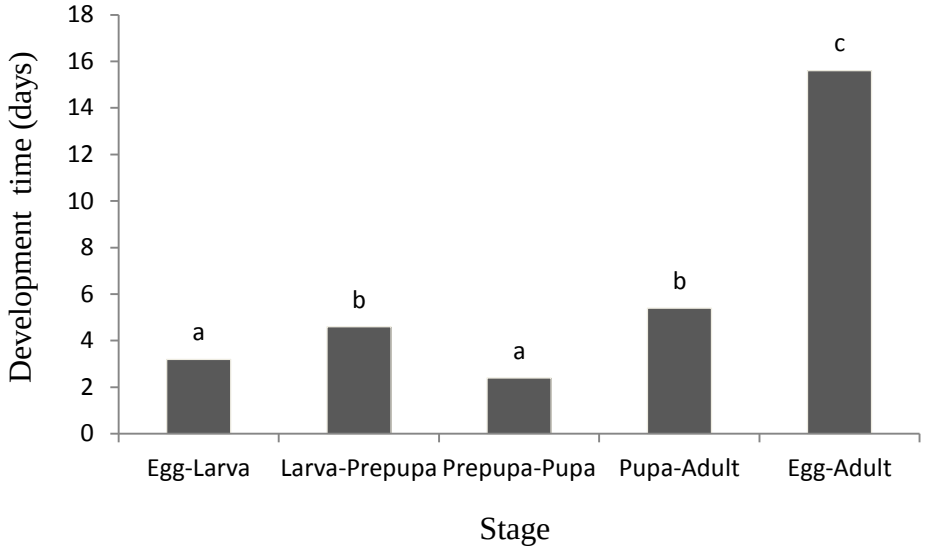

Figure1: Development time in days of *O. sokolowskii* from egg to adult at 25 °C. Different letters indicate significant differences (SNK; $P < 0.05$)

Adult size and ovigeny

Females were significantly larger than males (Table 1), and the size difference was significant (t = -8.71; df = 58; P<0.0001). After the emergence, *O. sokolowskii* female had an average initial egg load of 14 eggs. The average potential fecundity was 4 times larger. The ovigeny index of this species was 0.3 (Table 1).

Table 1: Sexual dimorphism, fecundity and and ovigeny index (mean ± SE) of *O. 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).

Parthenogenesis

The parasitism rate was significantly different between unmated and mated females (t = 6.39; df = 6; P = 0.0007). The mated females produced normal sexual offspring (male and female) while unmated females have produced only males (Table 2).

Table 2: Offspring productivity, parasitism rate and sex ratio (mean ± SE) between mated and unmated *O. sokolowskii* female

	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)

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. It was not significantly different between L2 and L3 stage (Fisher, P > 0.05). The parasitism rate was significantly lower in prepupae and zero in pupae (Table 3).

Table 3: Host age preference (immature DBM stages) of *O. sokolowskii* females

Host age (instars)	Parasitism (%)	Range (%)
2nd	39.9 ± 7.57 b	23.3 - 63.3
3rd	54.7 ± 8.71 b	23.3 - 73.3
4th	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 (± SE) in columns followed by the same letters are not significantly different by the Student-Newman-Keuls test.

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 the box B (Fisher, P <0.05) than in the boxes A and C. Ten females laid in B, whereas with A and C, respectively 3 and 1. Male and female offspring number in box B was significantly different than in boxes A and C ($F_{2.18}$ = 5.87; P = 0.008 and $F_{2.18}$ = 10.00; P = 0.001, respectively). Similarly, 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 in box C, not statistically different in A and B ($F_{2.18}$ = 9.01; P = 0.004) (Table 4).

Table 4: Oviposition - box volume effect on the parasitism percentage and the *Oomyzus sokolowskii* female production (mean ± SE)

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 ± 3.01a	6.6 ± 3.43a	14.3 ± 0.35a	89.5 ± 0.84a
7 (B)	85.5 ± 7.60 c	10	2.1 ± 0.63b	13.6 ± 1.70b	15.7 ± 2.04b	15.7 ± 0.30a	86.8 ± 2.36a
40 (C)	5.0 ± 3.93 a	1	0.2 ± 0.12a	0.8 ± 0.54a	1.0 ± 0.61a	18.0 ± 0.04b	80.0 ± 0.02a

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

Discussion

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

According to La Barbera (1989), size sexual dimorphism is commonly observed in the animal world 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 instance Bokonon-Ganta *et al.* (1995) and Aruna & Manjunath (2010) showed that females of *Anagyrus mangicola* (Hymenoptera: Encyrtidae) and *Nesolynx thumus* (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; males emerge first thus, they are smaller than females even though there are 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 the 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) produced the same number of offspring.

After emergence, *O. sokolowskii* females have fewer eggs to lay. However, they do not seem to be limited in number of eggs for the first attack. Our results show that the female continues to mature eggs throughout her 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 life and the behavioural manipulation of the rate of egg laying. The work of Ellers & Jervis (2003) on parasitic wasp 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 1; thus, it is a synovigenic species according to the classification of Jervis *et al.* (2001). However, the average egg corresponding to the *O. sokolowskii* lifetime seems relatively low. Indeed, 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 related to the mode of development of the parasitoid. Koinobiont parasitoids tend to have an ovigeny index on average higher than idiobiont species. *Oomyzus sokolowskii* is a koinobiont gregarious larval-pupal parasitoid. According to Talekar & Hu (1996) and Wang *et al.* (1999), *O. sokolowskii* parasite 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 behaviour (Slansky 1986), handling the host development (Vinson & Iwantsch 1980) and the control of host immune responses (Strand & Pech 1995). This mode allows parasitoid development to increase diverted food resources. Koinobiont species can manipulate the physiology and feeding behaviour of their host to their advantage (Harvey *et al.* 1999). Strand (2000) found that koinobiont parasitoids have developed strategies making 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 increased resources in the larger tracks. 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 as 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 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). The olfactory sensilla located on antennae and ovipositor are the chemoreceptors (van Baaren & Nenon 1996). These receptors can be quickly saturated when the parasitoid is on a patch, and then there is a decrease in sensitivity to chemical signals from the host, therefore reducing the search success of the host and oviposition. *Oomyzus sokolowskii* female seem more sensitive to the presence of the host in the $7cm^3$ box. In the $3cm^3$ 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 spawning, resulting in lower female productivity. The behavioural process of host-seeking by females of

O. sokolowskii is an important factor that determined the number of offspring of a female; therefore, it is directly linked to the success of the reproduction and to the fitness (Godfray 1994).

The results presented in this study provide valuable information on some life history traits of *O. sokolowskii*, a major natural enemy of DBM, pest of Brassicaceae. This information leads to a better understanding of the biology of this species and more efficient use of it in population management of DBM.

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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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Résumé

Oomyzus sokolowskii (Kurdjumov) est un parasitoïde larvo-nymphal et grégaire de la "Teigne des Crucifères" *Plutella xylostella* (L.). L'objectif de cette étude est d'étudier en conditions de laboratoire, les interactions entre le parasitoïde et son hôte, en examinant l'effet des facteurs tels que le grégarisme, l'origine et les stades de l'hôte, et l'âge de la femelle du parasitoïde sur le taux de parasitisme, le nombre de descendants, la durée de développement et la sex-ratio de la descendance d'*O. sokolowskii*. Le pourcentage de parasitisme et le nombre de descendants produits augmentent avec le nombre de femelles d'*O. sokolowskii* mis en présence des chenilles hôtes. *Oomyzus sokolowskii* préférait les larves de quatrième stade (L4) que les autres stades larvaires. Le taux de parasitisme et la production de la descendance d'*O. sokolowskii* décroit avec l'âge du parasitoïde. Cependant, la durée de développement et la sex-ratio de la progéniture n'ont pas été significativement différentes. Nos résultats confirment que le grégarisme stimule la capacité de parasitisme et la production des femelles du parasitoïde et de la préférence larvaire d'*O. sokolowskii*. L'étude a aussi montré que l'origine géographique de l'hôte est un paramètre important qui affecte la performance du parasitoïde. Ces résultats peuvent aider à une meilleure connaissance de l'espèce dans la perspective de conceptualisation de programmes de lutte biologique pour le contrôle des populations de *P. xylostella*.

Mots clés : parasitoïde, *Plutella xylostella*, chou, élevage, taux de parasitisme, stade hôte, lutte biologique

Abstract

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

Keywords: Parasitoids; *Plutella xylostella*; cabbage; rearing; parasitism rate; host-stage; biological control

Introduction

Cabbage is an important agricultural crop which 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 loss 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 $ one billion (Grzywacz et al., 2010) exclusively for synthetic insecticides application. Complete dependence on chemical based strategy for crop protection is however, not sustainable. Also, insects have developed resistance to many synthetic chemicals and biopesticides including *Bacillus thuringiensis* (Berliner) formulations (Lui 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 (IPM) systems.

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

Efficient biological control strategy is only possible through knowledge of the biology and ecology of both the pest and its natural enemies (Andow et al., 1997; Martínez-Castillo et al., 2002). Diamondback moth populations from different localities present differences in biological behaviour (Kirk et al., 2001; Arvanitakis et al., 2002). Previous studies have demonstrated the existence of genetic variations between various DBM populations (Roux et al., 2007; Pichon et al., 2001). The performance of a parasitoid can be affected by its host, especially in gregarious species (Silva-Torres et al., 2009b). 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 ecological conservation programs against 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 the gregarious behaviour, host origin and stages, female parasitoid age on the parasitism rate, development time, number of offspring, and sex ratio of *O. sokolowskii*.

Materials and Methods

Study site

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

Insect rearing

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

The *Oomyzus sokolowskii* colony was obtained from DBM pupae parasitized 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 cm × 5 cm) for oviposition for 24 hours. 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 plastic box and fed with honey drops deposited on the mesh cover.

The climatic conditions of all insect rearing were maintained at 25 ± 1°C temperature, 60 ± 5% relative humidity and 12L/12D photoperiod.

Gregariousness of O. sokolowskii

24 h-old females of *O. sokolowskii* were used in this experiment; 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 hours. The number of host larvae was increased to avoid the risk of superparasitism. Larvae were then transferred individually into transparent plastic pill boxes (3.5 cm ×1 cm) with a leaf disc of cabbage as food supply and monitored daily until emergence of adult parasitoids. The parasitism rate was calculated from the number of parasitized larvae and

pupae. The developmental time (from egg to adult), the number of offspring and sex ratio were also compared in each cohort. The experiment was replicated seven times.

Effect of host origin on the performance of O. sokolowskii

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; 61°10'33''W and 1200 m elevation). For each population, the size of pupae was measured using a dissecting microscope equipped with an ocular micrometer. Thirty (30) 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 hours of exposure, all larvae were collected and placed individually in pill boxes and followed up until emergence of adult parasitoids. The parasitism rate was calculated from the number of parasitized larvae and pupae of each population. Developmental time (from egg to adult), number and sex ratio (% females) of offspring were calculated. Bioassays were replicated seven times.

Effect of DBM stages on the performance of O. sokolowskii

Thirty (30) DBM larvae (2^{nd}, 3^{rd}, 4^{th}), prepupae and pupae were exposed to fifteen (15) *O. sokolowskii* females in a transparent plastic box for 24hours. The DBM population in this experiment was native to Senegal. Honey drips were deposited on the cover a 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 emergence of parasitoid adults. The parasitism rate for each DBM stage was calculated from the number of parasitized pupae. Developmental time (from egg to adult), number of offspring and sex ratio (% female) of parasitoids, were compared among the different host-stages. The experiment was replicated ten times for each DBM stage.

Effect of female parasitoid age

Fifteen (15) 1, 5, 15 and 28-days-old *O. sokolowskii* females were respectively exposed to 30 fourth-instar DBM larvae for 24hours. Larvae were collected and transferred individually into pill boxes. They were monitored until emergence of parasitoids. The parasitism rate,

developmental time, number of offspring and 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). The parasitism rate, adult offspring total number, development 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). The 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. (2009b) formula as the proportion of females. In all statistical analyses, the level of significance was kept a 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 difference between 5, 10, 15 and 20 females ($P < 0.05$). There were significant differences between the number of female parasitoids on the number of adults ($F_{(4,24)} = 17.80$; $P < 0.0001$), 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).

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

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

Means in columns followed by the same letters are not significantly different by the Student-Newman-Keuls test.

Effect of host origin on the performance of O. sokolowskii

The difference in size between DBM pupae from Senegal and DBM pupae from Martinique was significant ($t = 6.091$; $df = 58$; $P < 0.0001$). 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 male in the offspring was significantly different between the two populations ($t = -2.68$; $df = 12$; $P = 0.02$). There was no significant differences 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 2: Mean size of DBM pupae (mean ±SE) from different geographic origins

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

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

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

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

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

Effect of DBM stages on the performance of O. sokolowskii

The parasitism rate varied significantly according to host stages ($F_{(4,36)} = 26.23$; $P < 0.0001$). It was higher in L4 larvae and lower in pre-pupae respectively 75.9% and 15.3%. However, there were no significant differences between L2 and L3 larval stages (Table 4). There were no significant differences in the total production of adult ($F_{(3,27)} = 0.50$; $P = 0.68$) 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).

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

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

Means in columns followed by the same letters are not significantly different by the Student-Newman-Keuls test.

Effect of female parasitoid age

The parasitism rate was significantly affected by the age of female *O. sokolowskii* ($F_{(3,12)}$ = 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-days-old female parasitoids. The number of adult also was 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 parasitoid ($F_{(3,12)}$ = 1.01; P = 0.42 and $F_{(3, 12)}$ = 1.55; P = 0.25, respectively).

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

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

Means in columns followed by the same letters are not significantly different by the Student-Newman-Keuls test.

Discussion

The knowledge of the biology and ecology of biocontrol agents is of paramount importance in the management of pests such as DBM. Our results suggested that gregariousness promotes increased parasitism rates and increased number of offspring per female. In fact, gregariousness is related to superparasitism, which in return favors higher number of offspring (Gu et al., 2003; Silva-Torres and Matthews, 2003; Keasar et al., 2006). According to Silva-Torres et al. (2009b), gregariousness and superparasitism can adversely affect parasitoid fitness. These behaviors 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 favorable 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).

DBM population from Senegal seemed to be of higher fitness than the population from Martinique; according to Chown et al. (2009), insects with a bigger size have a higher chance of survival and reproduction. In this 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. The effect of host size has been reported by Edwards (1954), who discovered that most female parasitoids, especially in gregarious species, first estimated 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). The same assertion was made by Zaviezo and Mills (2000); the offspring production in gregarious parasitoids is often correlated with host size. In our results, DBM pupae from Senegal are larger than DBM from Martinique, which would imply that the female parasitoid would oviposit more in Senegalese larvae. DBM individuals from Senegal, which were bigger 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; this implies that *O. sokolowskii* could be a more efficient biocontrol 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 performance of *O. sokolowski* (Shelly et al., 2003); it has been shown that distributions of both host and their parasitoids are influenced by altitude (Sivinski et al., 2000, 2004), which presumably is due in turn 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 been also shown that the quality of the host affects the fitness of offspring in parasitoids (Godfray, 1994). Our results indicated 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*. According to Harvey et al. (2004), host stages of bigger sizes contain more resources for the parasitoid larval stages. These observations are similar to previous work reported by Talekar and Hu (1996).

There were no significant differences in number of offspring, development time and *O. sokolowskii* progeny sex ratio in relation to the age of host stages. These observations are similar to those reported by Wang et al. (1999). The explanation of these results could be due to the gregariousness and superparasitism behaviour of *O. sokolowskii*. Although there were differences in parasitism rate between stages of DBM, potential competition for resources between larval parasitoid could affect the number of offspring. According to Silva-Torres et al., (2009b), gregariousness and superparasitism can adversely affect the number offspring. However, the consequences of such behaviour affect more 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. Our results did not corroborate his findings.

The success of the parasitism depends on the age of the female parasitoid (Medeiros et al., 2000); generally, parasitism rate decreased with age as depicted in our study; 5-day-old females lay more eggs in their host than older females. The decrease in productivity could be explained by a decrease in physiological activity (Giron and Casas, 2003). Silva-Torres et al. (2009a) showed that the parasitism rate was higher in 2-4 days old females of *O. sokolowskii*. According to Rajapakse (1992), the ideal age *Cotesia marginiventris* (Cresson) ranged from 48 to 96 hours to properly parasitize larvae of *Spodoptera frugiperda* (Smith), whereas *Ceratogramma etiennei* (Delvare), the ideal age of parasitism varied from one to two days post emergence (Amalin et al., 2005). Our results are similar to the work of Silva-Torres et al. (2009a) 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) argue that offspring production from females of *Trichogramma minitum* Riley (Hymenoptera: Trichogrammatidae) decreases with age. However, 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 ioles* (Hymenoptera: Mymaridae) does not affect the sex ratio of the parasitoid.
The parasitism rate tended to decrease drastically in the five-days-old females, suggesting that beyond a certain period of maturity, female *O. sokolowskii* 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), other methods need to be envisaged.

Conclusion

The present study confirms the importance of *O. sokolowskii* as a promising biocontrol 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. Age of female parasitoid could be a limiting factor for parasitoid production; therefore other parameters should be combined during DBM management. The results of this study showed 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 4

Application de solution biologique et naturelle

Article 4

Laboratory evaluation of toxicity of *Bacillus thuringiensis*, neem oil and methamidophos against *Plutella xylostella* L. (Lepidoptera: Plutellidae) larvae

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Soumis à International Journal of Biological and Chemical Sciences

Résumé

L'objectif de ce travail vise à évaluer en conditions de laboratoire la toxicité de *Bacillus thuringiensis,* l'huile de neem et du métamidophos à différentes doses sur des larves de *Plutella xylostella*. Pour chaque traitement, trois doses (faible, moyenne et forte) ont appliquées sur des feuilles de chou présentées à des larves L3 de *P. xylostella.* Les comptages des larves mortes en fonction des doses de chaque traitement et le témoin ont été réalisés chaque 24h pendant une période de huit jours. La mortalité larvaire est différente selon les traitements et les doses appliquées. Les trois doses du Biobit ont été toxiques sur les larves comparées au témoin. Le plus fort taux de mortalité (100%) en un temps court de 5j est enregistré avec le Biobit à forte dose. Concernant, l'huile de neem, les doses fortes et moyennes ont été les plus toxiques sur les larves avec des taux de mortalité moyenne respectivement de 70 et 61,5%. Le métamidophos a été moins toxique comparé au Biobit et à l'huile de neem avec des taux de mortalité moyenne de 52,5 et 51% respectivement à forte et moyenne dose. Aux doses forte et moyenne, le Biobit et l'huile de neem ont été plus toxiques sur les stades larvaires. Cependant, le métamidophos est plus toxique que l'huile de neem à faible dose. Le Biobit est plus efficace que l'huile de Neem et le Métamidophos contre les larves de *P. xylostella* quelles que soient les doses appliquées. Ces résultats montrent que les biopesticides à base de Bt et d'extraits de neem offrent de meilleures possibilités pour contrôler efficacement les populations de *P. xylostella* au Sénégal.

Mots clés : Teigne des Crucifères, biopesticide, *Azadirachta indica*, méthamidophos, chou, bioessai

Abstract

Studies were conducted to evaluate the toxicity of *Bacillus thuringiensis* (*Bt*), neem oil and methamidophos on larvae of *P. xylostella*, under laboratory conditions. Leaf-dip bioassay for DBM larvae was used to assess mortality. Larval mortality was performed every 24 hours for a period of 8 days among the treatments and doses applied. Three doses of Biobit were toxic on larvae compared with the control. The highest mortality rate (100%) was recorded with the high dose of Biobit after 5days of exposure. High and medium doses of neem oil were more toxic on larvae with average mortality rates respectively 70 and 61.5%. Methamidophos was less toxic compared to Biobit and neem oil with average mortality rate of 52.5 and 51% respectively at high and medium doses. High and medium doses of Biobit and neem oil were more toxic on larvae. At low dose, Biobit and methamidophos were more toxic than neem oil. Biobit was more effective than neem oil and methamidophos against larvae of *P. xylostella* whatever the dose. These results showed that *Bt*-based biopesticides and neem extracts offer greater opportunities to control effectively populations of *P. xylostella*.

Key words: Diamondback moth, biopesticide, *Azadirachta indica*, methamidophos, cabbage, bioassay.

Introduction

Diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is the most important pest of crucifers worldwide (Talekar and Shelton, 1993; Sarfraz et al., 2005). It can cause up to 90% crop loss (Verkerk and Wright, 1996; Iqbal et al., 1996). In Senegal, this rate is estimated between 51 and 94% according to the direction of horticulture. The use of synthetic organic insecticides is the main method of control against this pest (Kibata, 1996). These insecticides can cause several consequences such as the elimination of natural enemies of the DBM, the increased cost of production and the emergence of resistant strains (Hooks and Johnson, 2003; Macharia et al., 2005; Sarfraz and Keddie, 2005). Today, the use of *B. thuringiensis* in integrated programs management (IPM) is an effective alternative and respectful of the environment (Grzywacz et al., 2010). Several studies have demonstrated the efficacy of formulations *B. thuringiensis* against *P. xylostella* (Monnerat et al., 2000; Grzywacz et al., 2010). Moreover, natural insecticides whose extracts of neem, *Azadirachta indica* A. Juss (Meliaceae) have also shown efficacy against *P. xylostella* (Goudegnon et al., 2000; Charleston et al., 2006, Ling et al., 2008). With increasing resistance, environmental and health problems caused by chemical insecticides, entomopathogens and plant extracts are alternative control methods against insect pests (Ling et al., 2008; Patil and Goud, 2003).The effect of insecticides is generally evaluated by their toxicity and field efficacy (Prasad et al., 2007). However, it becomes necessary to conduct laboratory tests before being validated in the field.

The objective of this study was to evaluate the toxicity of *Bacillus thuringiensis* (Biobit), neem extracts (*Azadirachta indica*) and methamidophos, a chemical insecticide on DBM larvae under laboratory conditions. These data will be useful in the development of a pest management approach that integrates the use of *B. thuringiensis* and neem extracts to control diamondback moth in cabbage.

Material and Methods

Host plants

In this study, two varieties of cabbage were used: *Brassica oleracea* var. *capitata* and *Brassica oleracea* var. *botrytis*. Thirty-day old seedlings were transplanted on table (2m×1m) containing a mixture of peanut hull, laterite and rice straw. No insecticides were used. Irrigation was performed before and after transplanting. Plants were fertilized with a solution of macronutrients (Na, K, Ca, Mg, P) of concentration 5 ml / l and a solution of microelements (Fe, Zn, Cu, Mn, I) of concentration 2 ml / l. Each week, leaching is carried out.

DBM rearing

DBM population was collected at Malika situated in the suburban area of Dakar (N: 14°47'552; W: 17°19'818 and 189 m altitude). Larvae and pupae collected were isolated in cylindrical plastic boxes (3cm x 7cm) with lids pierced with small holes for ventilation. Larvae are fed with the leaves of the host plant (*Brassica oleracea* var. *capitata*) and then followed until emergence of moths. DBM adults emerged were recovered and introduced into a cubic cage 500mm side. The eggs are collected daily on a cauliflower plant which achieves larval development. In the fourth stadium (L4), larvae are transferred on fresh leaves settled to the bottom of a large plastic box (28cm × 27cm) where they perform their pupation. The nymphs are collected daily. At emergence, adults were placed in the nest cage and fed with water and honey. The DBM rearing was conducted in a room with controlled climatic conditions: 25 ° C, 75% RH and 12L/12D. Newly emerged third instar larvae were used in bioassay studies.

Treatments and doses

Three (3) treatments (Biobit, Neem oil and methamidophos) were tested at different doses. For each treatment, three doses (low, medium and high) were prepared as follows: With Biobit (*Bacillus thuringiensis* 1% WP) 5 mg, 7 mg and 10 mg were weighed and mixed in 10 ml of distilled water. For Neem (*Azadirachta indica* 3% EC; Meliaceae), three volumes of 0.3 ml, 0.6 ml and 1 ml were collected from each stock solution and mixed in 100 ml of distilled water prepare for the three doses. Concerning methamidophos (Metofos 600 EC; Organophosphorus), three doses were prepared from the volumes of 0.3 ml, 0.6 ml and 1 ml

and mixed in 250 ml of distilled water. The control is applied with distilled water. A wetting agent was added to different treatments.

Toxicity bioassays

The toxicity bioassay was a leaf dip method similar to that used by Tabashnik et al. (1990). For toxicity bioassay experiment, third instar larvae were treated by oral application through cauliflower leaf discs. Leaf tissue (6cm in diameter) was cut from uninfested cabbage plants raised in the table. Individual leaves were immersed in the prepared insecticide solution for 10 s and hung vertically to air dry for 2 h. Control leaves were treated similarly with tap water. Ten larvae from DBM rearing were placed in each petri dish (6 cm × 1.5 cm) containing a leaf disc. Larval mortality was recorded every 24 h up to 8 days of treatment. Larvae were considered dead if they did not move when lightly prodded with forceps (Hill and Foster, 2000). After 24 h of contact, larvae were continuously maintained on untreated fresh cauliflower leaves. Five replicates were maintained for each treatment.

Statistical analysis

The data were first normalized and then analyzed with the software XLSTAT version 2012.1.01. The analysis of variance (ANOVA) was performed after correction of mortality by Abbott's formula (Abbott, 1925). Means were separated using Students Newman Keuls test. In all statistical analyses p-values < 0.05 were considered significant.

Results

Effect of doses Biobit on larval mortality

Among the doses of Biobit, larval mortality rates were significantly different (F = 10.50; df = 3, 12; P <0.0001; Table 1). At high dose, Biobit caused 100% mortality after 5 days of exposure. At medium dose, the percentage of larval mortality was constant from 5 to 7 days and was 96%. The maximum rate of 100% mortality was reached at 8 days. At low dose, the percentage of larval mortality was the highest recorded at 7 days and was 92%. In the control, the maximum mortality rate was 48% after 8 days of exposure (Figure 1). Three doses of Biobit were toxic to larvae of *P. xylostella* compared with the control. However, there are no significant differences between three doses tested. Mortality was significantly lower in the control (Table 1).

Table 1: Mean larval mortality of *P. xylostella* among treatments

Treatments	**Biobit**	**Neem**	**Methamidophos**
High dose	84 a	70 a	52,5 a
Medium dose	82 a	61,5 a	51 a
Low dose	67,5 a	24 b	36 ab
Control	23,5 b	23,5 b	23,5 b
ANOVA F	10,50	8,94	3,98
P	<0,0001	0,0003	0,018

Means in columns followed by the same letters are not significantly different by Student – Newman – Keuls test

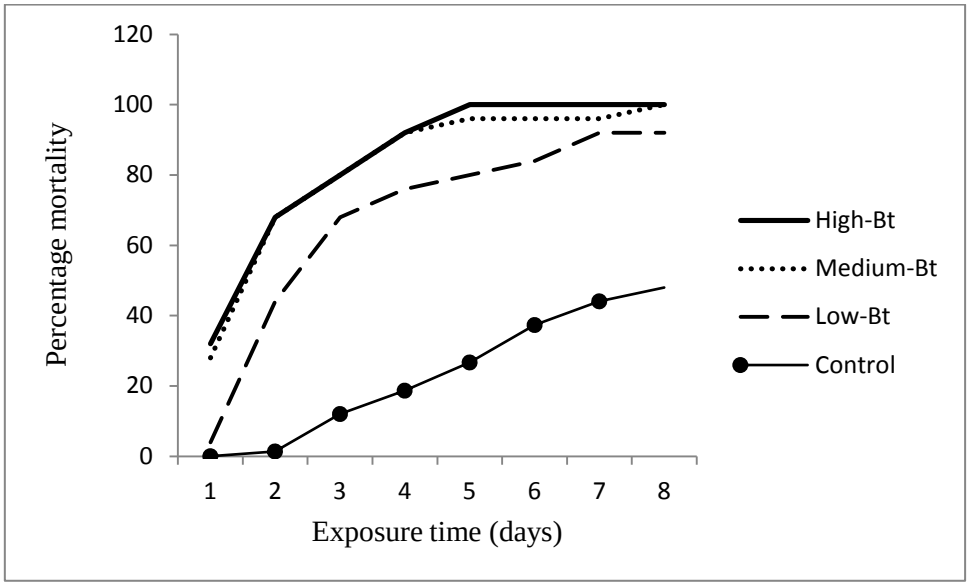

Figure 1: Percentage mortality of *P. xylostella* larvae in terms of the exposure time and doses of Biobit (Bt)

Effect of doses neem oil on larval mortality

Larval mortality rates were significantly different among the doses tested (F = 8.94; df = 3, 12; P = 0.0003; Table 1). However, there were no significant differences between high and medium doses, which were more toxic on larvae compared with the low dose and control. There were no significant differences between the low dose and the control (Table 1). At high dose, neem oil showed a percentage of larval mortality up to 92% after 7 days of exposure. At medium dose, the percentage of larval mortality reached to 88% after 8 days of exposure. At low dose, the maximum larval mortality rate was 40% between 6 and 8 days of exposure. This percentage was lower compared to control where it increased from 37 to 48% over the same period (Figure 2).

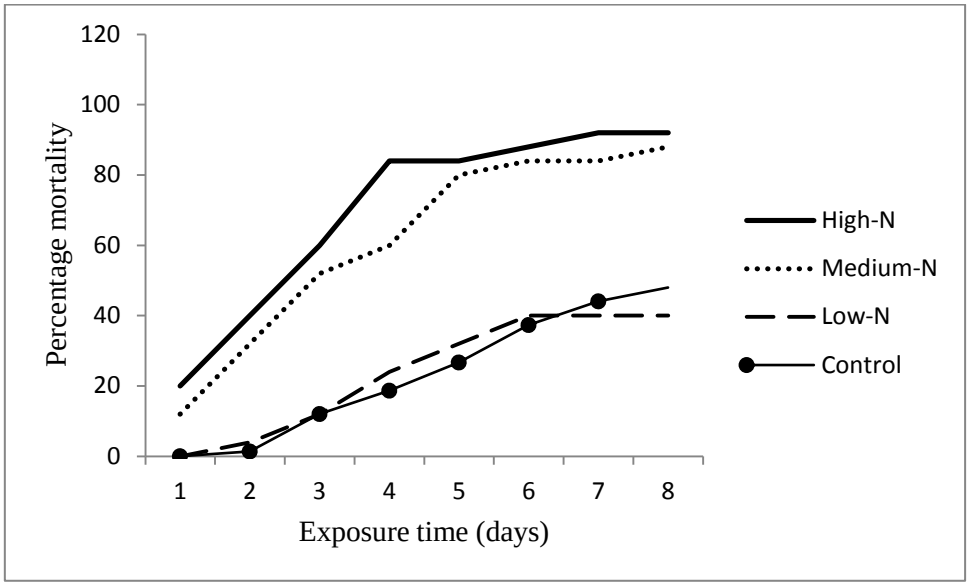

Figure 2: Percentage mortality of *P. xylostella* larvae in terms of the exposure time and doses of Neem (N)

Effect of doses methamidophos on larval mortality

Among the doses tested, larval mortality rates were significantly different (F = 3.98; df = 3, 12; P = 0.018; Table 1). However, there were no significant differences between high and medium doses that were more toxic on larvae. The low dose and control were not significantly different, they were less effective. The percentage of larval mortality recorded at high and medium doses had the same evolution. They have caused 68% mortality after 5 days of exposure while in the same period; the mortality rate obtained with low dose ranged from 44 to 52%. This rate was lower in control with a maximum of 48% mortality after 8 days of exposure (Figure 3).

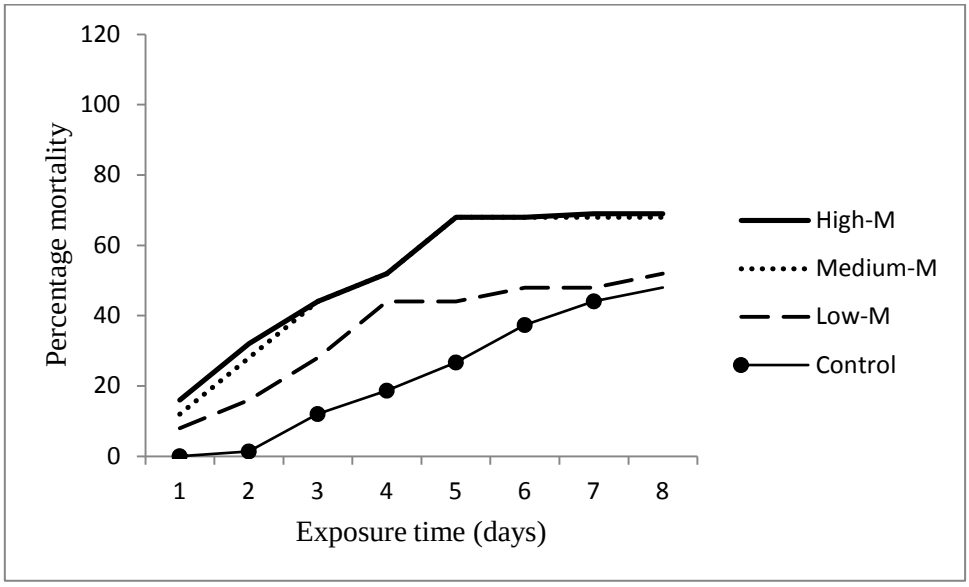

Figure 3: Percentage mortality of *P. xylostella* larvae in terms of the exposure time and doses of methamidophos (M)

Effect of treatments at high dose on larval mortality

The larval mortality was significantly different among treatments at high dose (F = 10.49; df = 3, 12; P <0.0001; Table 2). At high doses, Biobit caused 100% larval mortality after 5 days of exposure. Over the same period and at the same dose, we noted a mortality rate of 84% for neem oil with a maximum rate of 92% at 7 days, 68% for methamidophos and 27% in control. At high dose, Biobit was more toxic than neem oil and methamidophos (Figure 4).

Table 2: Mean larval mortality of *P. xylostella* among doses tested

Doses		**High dose**	**Medium dose**	**Low dose**
Biobit		84 a	82 a	67,5 a
Neem		70 ab	61,5 a	24 b
Methamidophos		52,3 bc	51 ab	36 b
Control		23,5 c	23,5 b	23,5 b
ANOVA	F	10,49	8,69	7,60
	P	<0,0001	0,0003	0,0007

Means in columns followed by the same letters are not significantly different by Student – Newman – Keuls test

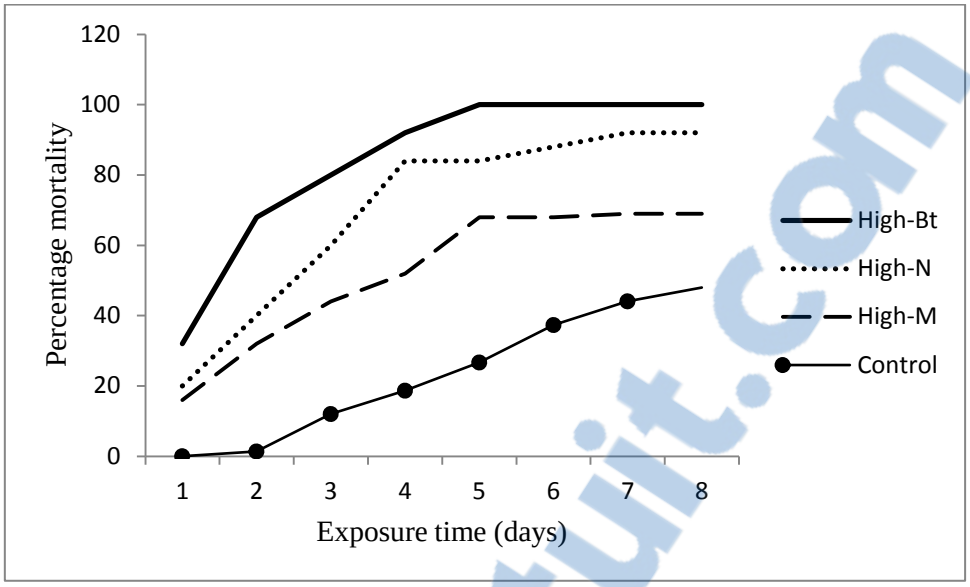

Figure 4: Percentage mortality of *P. xylostella* larvae depending on the exposure time and treatments at high dose (Bt= Biobit; N= Neem; M= Methamidophos)

Effect of treatments at medium dose on larval mortality

The larval mortality was significantly different among treatments at medium dose (F = 8.69; df = 3, 12; P = 0.0003; Table 2). At medium dose, the mortality rate for Biobit ranged from 96 to 100% between 5 and 8 days of exposure while neem oil over the same period, the rate varied from 80 to 88% and for methamidophos, it reached 68% mortality. At medium dose, Biobit and neem oil were more toxic than methamidophos (Figure 5).

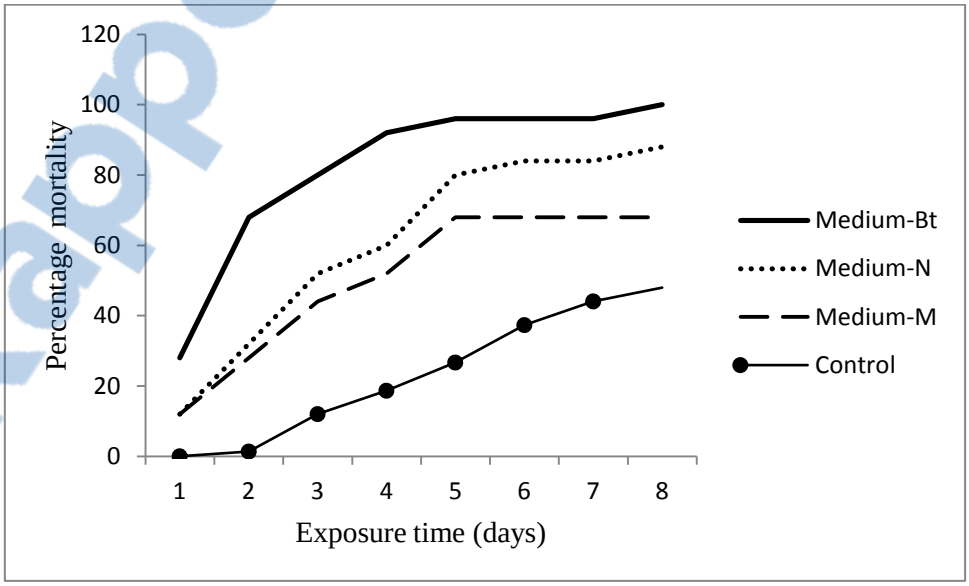

Figure 5: Percentage mortality of *P. xylostella* larvae depending on the exposure time and treatments at medium dose (Bt= Biobit; N= Neem; M= Methamidophos)

Effect of treatments at low dose on larval mortality

The larval mortality was significantly different among treatments at low dose (F = 7.60; df = 3, 12; P = 0.0007; Table 2). At low dose, the maximum mortality recorded after 7 days of exposure was 92% for Biobit. For Neem oil, the percentage mortality reached 40% after 6 days and a mortality rate of 52% obtained for methamidophos after 8 days of exposure. However, there were no significant differences in mortality occurred among the neem oil, methamidophos and control (Figure 6). At low dose, Biobit was more effective than neem oil and methamidophos.

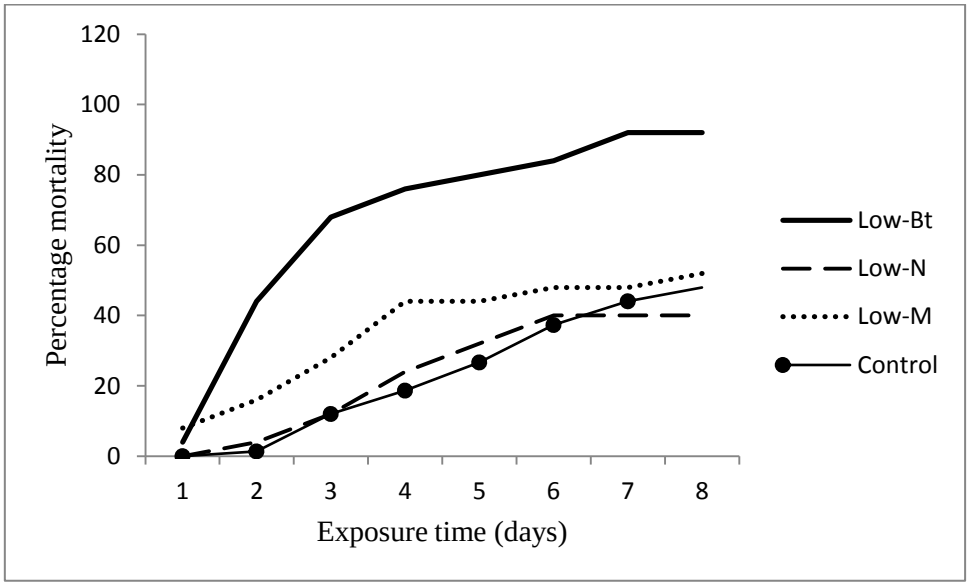

Figure 6: Percentage mortality of *P. xylostella* larvae depending on the exposure time and treatments at low dose (Bt= Biobit; N= Neem; M= Methamidophos)

Discussion

Our results showed that the toxicity of insecticides depends on types of formulation and doses tested. According to Ketoh et al., 2004, toxic products to insects are those that cause high mortality in the population at low concentrations. In our study, the Biobit was more toxic than the other insecticides. The toxicity of *B. thuringiensis* is primarily due to the presence of delta-endotoxin (Lereclus et al., 1993). The biopesticide has proven more effective on DBM larvae and this irrespective of the dose tested. González-Cabrera et al. (2010) have shown the efficacy of *B. thuringiensis* in the laboratory control of the tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). According to Regnault-Roger (2005), the effectiveness of this biopesticide is due to its rapid mode of action, the perfect control of its production on an industrial scale and the discovery of new strains for the expansion of its spectrum activity.

However, Neem oil was effective at high and medium doses, whereas at low dose, it acts as methamidophos and control. According to Bouchikhi et al. (2010), the toxicity of insecticides of plant extracts varies with dose and duration of exposure. In our study, high and medium doses Neem oil were more toxic on larvae compared with the low dose where larval mortality was lower. Our results are similar with those of Charleston et al. (2005). Others works had demonstrated also the impact of neem on mortality of *P. xylostella* (Isman, 1995, Perera et al., 2000, Liang et al., 2003). Chen et al. (1996) showed that extracts of Syringa (Meliaceae) caused high mortality at different doses tested. However, less toxicity of neem oil in relation to Biobit could be explained by its effect mainly anti-palatable and repel insects (Mordue and Blackwell, 1993). Neem extracts have anti-palatable accompanied by a significant reduction in food consumption of the herbivorous insect (Liang et al., 2003). They contain an active ingredient, azadirachtin, which has anti appetizing, disgusting, sterile, inhibiting molting, growth and larval development (Dilawari et al., 1994; Schmutterer 1995). Several authors have demonstrated the efficacy of plant extracts against *P. xylostella* (Patil and Goud, 2003; Charleston et al., 2006, Ling et al., 2008; Lingathurai et al., 2011).

Methamidophos was less effective than Biobit and neem oil against the larvae of the diamondback moth. This could be explained by a decreased sensitivity of the pest to the insecticide. This phenomenon is probably due to resistance developed by the insect. The work of Sereda et al. (1997) and Shelton and Wyman (1992) demonstrated the resistance of *P. xylostella* to Methamidophos.

Our results showed that biopesticides based on *Bacillus thuringiensis* and Neem extracts were effective against *P. xylostella* compared to synthetic organic insecticides. They showed

greater toxicity on DBM larvae. The use of entomopathogenic and plant extracts capable of controlling insect pests in developing countries could provide an alternative approach to conventional insecticides. Further experiments are needed to clarify the nature of the compounds involved in their larvicidal activity to optimize the effective doses. Despite the results encouraging, the effectiveness of these treatments remains to be demonstrated in the field.

Acknowledgements

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Article 5

The use of *Bacillus thuringiensis* and Neem alternation on *Plutella xylostella* (Lepidoptera: Plutellidae) and its effects on natural enemies in cabbage production

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Soumis à Acta Horticulturae

Résumé

Plutella xylostella est un ravageur redoutable des cultures de chou au Sénégal. La lutte chimique est la plus utilisée dans la gestion de ce ravageur malgré ses revers environnementaux et sanitaires. Les produits à base de *Bacillus thuringiensis* (Bt) et de Neem sont actuellement considérés comme alternatives aux insecticides chimiques. 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. C'est pourquoi, l'objectif de ce travail est d'étudier l'impact de produits à base de Bt et de Neem et de leur alternance sur l'infestation de *P. xylostella* et la densité des parasitoïdes au champ. Il s'agit également d'évaluer l'effet de ces produits sur le taux de parasitisme de ces ennemis naturels de *P. xylostella*. Un dispositif en blocs complétement randomisés avec quatre traitements dont Bt, Neem, alternance Bt/Neem, diméthoate (témoin traité) et le témoin non traité, est utilisé et comprenant sept répétitions par traitement. Les résultats ont montré que bien qu'il n'y a pas de différences significatives entre les traitements Biobit, Bt/Neem et Neem, ces derniers ont réduit considérablement les populations de *P. xylostella* comparés au traitement avec le diméthoate et le témoin. Il y a une différence significative entre le témoin et le diméthoate ce qui suppose l'existence d'une résistance des populations de *P. xylostella* à l'insecticide chimique. Deux espèces de parasitoïdes ont été majoritairement répertoriées. Il s'agit d'*Oomyzus sokolowskii* et *Apanteles litae*. Le taux de parasitisme a été plus important dans les plants traités au Neem. Le pourcentage de parasitisme est fortement corrélé à l'abondance de *P. xylostella*. Cette corrélation a été observée dans tous les traitements excepté le diméthoate. Toutefois, cette corrélation est beaucoup plus forte dans le traitement Biobit/Neem et le Neem. Cette étude suggère que l'utilisation alternée Bt/Neem, après seulement quatre applications, est aussi efficace que les traitements solo dans la lutte contre la "Teigne des Crucifères" ; mais qu'en plus, cette pratique préserve les populations de parasitoïdes. Ce traitement est économiquement plus rentable donc peut être recommandé dans la production du chou.

Mots clés : parasitisme, chou, azadirachtine, Teigne des Crucifères, *Bacillus thuringiensis*

Abstract

The diamondback moth (DBM), *Plutella xylostella* (L.) is a major pest of cabbage in Senegal. Chemical control is the most commonly used control method despite its environmental and health issues. *Bacillus thuringiensis* (*Bt*) and Neem-based products are considered as relevant alternatives to synthetic chemical insecticides. The aim of this study was to assess the effect of the alternation of *Bt* and Neem (*Azadirachta indica*) on *P. xylostella* and its effect on parasitoids compared to sole applications of *Bt*, Neem and Dimethoate. Plants treated with Dimethoate recorded say three times more *P. xylostella* compared to applications of *Bt*, *Bt*/Neem and Neem. Results showed that although there were no significant differences between *Bt*, *Bt*/Neem and Neem, populations of *P. xylostella* were considerably reduced in these treatments as compared to Dimethoate and control. Four parasitoid species were recorded of which two species were important both in abundance and level of parasitism. These include *Oomyzus sokolowskii* and *Apanteles litae*. The parasitism rate was higher in the Neem treatment. The correlation between abundance of *P. xylostella* and parasitism rate was observed in all the treatments except that on Dimethoate and was stronger in *Bt*/Neem and Neem. The results demonstrated that in the absence of chemical insecticides, the impact of parasitoids was significant. This study suggests that the use of only four alternated applications of *Bt* and neem is as effective as sole treatments in the control of *P. xylostella* and is more cost effective to farmers.

Keywords: parasitism, cabbage, azadirachtin, diamondback moth, *Bacillus thuringiensis*

Introduction

Cabbage is an important crop in the world, it is as a source of income and food to many people (Grzywacz et al., 2010). In West Africa for instance, the annual production of cabbage is estimated at 140500 tonnes (FAOSTAT, 2003). Diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) has been considered to be the most important pest of cabbage and other Brassica crops (Talekar and Shelton, 1993; Shelton et al., 2007) causing over 90% of yield losses in the field (Grzywacz et al., 2010). Synthetic chemical pesticides which are expensive are the main control strategy (Kibata, 1996; Horowitz and Ishaaya, 1996). In addition to chemical control which is the most common method, other techniques such as biological control (Sarfraz and Keddie, 2005), cultural practices (Asman et al., 2001), the use of resistant varieties of cabbages and sex pheromone (Reddy and Urs, 1997) are suggested for DBM control. However, the use of synthetic chemicals is not safe due to environmental hazards and other human health threats. Moreover, DBM has developed resistance to many synthetic pesticides (Hooks and Johnson, 2003; Macharia et al., 2005; Shelton et al., 2007). Moreover, the management of DBM infestations cannot rely entirely on the existence of parasitoids because of the weak parasitism rate in area. Hence there is a crucial need to identify other environmental safe and friendly methods. Microorganisms such as *Bacillus thuringiensis* (*Bt*) and botanical pesticides such as neem (*Azadirachta indica*) are being promoted for the control of lepidopterian pests (Grzywacz et al., 2010; Reddy, 2011). Although these products have an advantage to be environmentally safe and less harmful to humans (Monnerat et al., 2000; Grzywacz et al., 2010), their side effects on biocontrol agents such as parasitoids is not clearly elucidated (Waiganjo et al., 2005-2007). The massive use of *Bt* is believed to induce resistance in DBM populations. In addition, the performance of alternated agrochemicals, their timely application for DBM control and their side effects on the complex of parasitoids is poorly understood. Therefore, the objective of this study was to determine the effect of the alternation of *B. thuringiensis* and neem-based agrochemicals on DBM populations and its repercussions on natural enemies abundance and their parasitism. This knowledge will be useful in the development of a pest management approach that integrates the use of *B. thuringiensis* and neem extracts to control diamondback moth in cabbage.

Materials 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 altitude). The area is characterized by long dry seasons from November to June with temperature range of 15-20 °C and short rainy seasons from July to October with temperatures ranging between 25 to 35 °C. The yearly precipitations do not exceed 500 mm between August and September. The experiments were conducted for the entire season of culture from 25 December 2010 until 17 April 2011.

Cabbage crops

Cabbage *Brassica oleracea* variety "*Marché de Copenhague*" which is drought tolerant was used in this experiment. In order to protect cultures from nematodes, Furadan was applied in the soil prior to planting. Poultry manure was applied as fertilizer 10 days later with intensive irrigation. Cabbage planting was effective a month later. Additional fertilizers NPK (10-10-20) and poultry manure were applied 15 days after planting. Crops were watered daily using sprinkler irrigation.

Phytosanitary applications and treatments

A randomized complete block design with seven replicate plots (e.g., 3 m × 4 m) per treatment was adopted for the experiments. Cabbage seedlings were planted at 40 cm × 50 cm spacing between rows and plants, respectively. Four (4) treatments were used: Biobit, *Bacillus thuringiensis* var. *kurstaki*, Crystal Chemical Company LTD (Europe), Neem (Suneem, *Azadirachta indica* 1% EC), alternation Biobit/Neem (in 10 days interval four times) and Dimethoate (Meteor 400 EC). An untreated control was also included in the experiment. Applications started 25 days after planting; crops were treated using manual sprayer every ten days. Biobit was applied at 1L for 100 L of water per hectare whereas for Neem, the application dose was 1L/ha. Dimethoate was applied at 1.5 L/ha. For the alternated treatment, four (4) applications of Biobit and Neem were used. Neem was applied first and the last application was Biobit. The alternated treatment was stopped 20 days before application of the other treatment.

Sampling methods

The samplings started 10 days after transplanting and were performed every ten days. Samples were collected randomly by selecting 10 cabbages in the central rows of each plot.

For each of the selected cabbage plant, the abundance of insects: second to fourth instar larvae, pupae of diamondback moth, cocoons of parasitoids and others insects pests within a cabbage were all collected and counted for each treatment. Eggs and larvae which were inside the leaves were not considered.

Statistical analysis

Data were normalized and transformed before subjecting them to analysis of variance (ANOVA). Means were separated using Students Newman Keuls Test. The percentage of parasitism was calculated using Mc Cutcheon (1987) formula:

$$\% \ Parasitism = \frac{[Number\ of\ parasitized\ mothes]}{[Total\ number\ of\ mothes - Number\ of\ dead\ mothes]} \times 100$$

Pearson correlation test was used to look at the level of correlation between variables. In all tests, the level of significance was kept at 5%.

Results

Effect of treatments on the abundance of *P. xylostella*

There was a significant difference between treatments on the abundance of *P. xylostella* (F = 60.07; df = 4, 24; P = 0.0001). Plots treated with Dimethoate hosted the highest number of DBM larvae (10 larvae / plant). Compared to plots treated with Dimethoate, plots treated with Biobit, Biobit/Neem and Neem recorded say three times lower number of DBM larvae. The least population of DBM larvae was recorded in plots treated with Biobit, Biobit/Neem and Neem. There were no significant differences between populations of *P. xylostella* in these treatments. However, there was a significant difference between the control and Dimethoate (Table 1).

Table 1: Mean abundances of *P xylostella* and parasitoids in cabbages treated with Biobit, Biobit/Neem, Neem and Dimethoate

Treatments	*P. xylostella*	*Parasitoids*	*O. sokolowskii*	*A. litae*	*C. plutellae*	*B. citrea*
Control	7.890b	0.188a	0.488a	0.189a	0.074a	0.000a
Biobit	2.597c	0.139ab	0.480a	0.077b	0.000b	0.000a
Biobit/Neem	2.928c	0.133ab	0.384a	0.143a	0.000b	0.005a
Neem	3.628c	0.105ab	0.232a	0.161a	0.026ab	0.003a
Dimethoate	9.997a	0.094b	0.189a	0.171a	0.013ab	0.003a

In column values bearing the same small letters are not significantly different in ANOVA, SNK at 5%.

Effect of treatments on the abundance of parasitoids

There were significant differences in the number of parasitoids recorded in the various treatments (F = 2.4; df = 4, 24; P = 0.05) (Table 1). The most abundant species found in field were *Oomyzus sokolowskii* (Hym., Eulophidae) and *Apanteles litae* (Hym., Braconidae). There were no significant differences between treatments on the abundance of *O. sokolowskii* (F = 2.35; df = 4, 24; P = 0.05). The abundance *A. litae* was significantly different between treatments (F = 3.8; df = 4, 24; P = 0.005). This parasitoid was less abundant in *Bt* treatment. However, there were no significant differences between the following treatments Neem, Biobit/Neem, Dimethoate and the control. The abundance *Cotesia plutellae* was significantly different between treatments (F = 2.8; df = 4, 24; P = 0.02). This species was more abundant in control than other treatments. There were no significant differences between Biobit,

Biobit/Neem, Neem and Dimethoate. There was no significant differences between treatments on the abundance of *Brachymeria citrea* (F = 0.9; df = 4, 24; P = 0.5) (Table 1).

Effect of treatment on the parasitism of *P. xylostella*

The total parasitism of parasitoids varied significantly between treatments (F = 2.6; df = 4, 24; P = 0.03) (Table 2). The percentage of parasitism was higher (9.8%) in the Neem treatment and lower (5.4%) in the Biobit treatment. However, there were no significance differences between Biobit, Biobit/Neem, Dimethoate and the control (P > 0.05). There were no significant differences in parasitism of the following parasitoids *O. sokolowskii, A. litae, B. citrae* between the treatments. The parasitism rate of *C. plutellae* was significantly different between treatments (F = 3.6; df = 4, 24; P = 0.006). The parasitism rate was higher in treatment with Neem. There were no significant differences between the following treatments Neem, Dimethoate and the control (Table 2).

Table 2: Mean percentage of parasitism of *O. sokolowskii, A. litae, C. plutellae* and *B. citrea* on *P. xylostella* in cabbages treated with Biobit, Biobit/Neem, Neem and Dimethoate

Treatments	Total	*O. sokolowskii*	*A. litae*	*C. plutellae*	*B. citrea*
Control	8.833ab	2.905a	5.513a	0.594ab	0.000a
Biobit	5.451b	1.382a	4.069a	0.000b	0.000a
Biobit/Neem	7.238ab	1.598a	5.448a	0.000b	0.192a
Neem	9.862a	1.467a	7.337a	0.995a	0.064a
Dimethoate	6.197ab	0.904a	5.238a	0.260ab	0.256a

In column values bearing the same small letters are not significantly different in ANOVA, SNK at 5%.

There was an overall correlation between the abundance of *P. xylostella* and the parasitism (r= 0.15; P < 0.0001) (Table 3). There was a significant correlation between the abundance of *P. xylostella* and parasitism in the treatments Biobit, Biobit/Neem, Neem and control. The correlation was stronger in the Biobit/Neem treatment. However, the correlation was not significant in the chemical treatment with Dimethoate (Table 3).

Table 3: Pearson's correlation coefficient test between *P. xylostella* abundance and the parasitism

	Biobit	**Biobit/Neem**	**Neem**	**Control**	**Dimethoate**
Observed value	0.144	0.323	0.287	0.181	0.096
Two-tailed p-value	0.004	< 0.0001	< 0.0001	0.000	0.057

Discussion

The least population of DBM was recorded in plots treated with *Bt, Bt*/Neem and Neem. There were no significant differences between these treatments on the abundance of *P. xyllostella*. The toxicity of *Bt* on Lepidoptera is known to be related to the presence of delta-endotoxins (Lereclus et al., 1993). These results on the use of *Bt* on *P. xylostella* have been reported by many authors (Roh et al., 2007; González-Cabrera et al., 2010). The effectiveness of Neem on *P. xylostella* has been observed in laboratory and Neem extracts are known to have anti-feeding, repellent, anti-moulting and growth regulator properties on *P. xylostella* (Liang et al., 2003; Patil and Goud, 2003).

It is believed that the massive use of *Bt* based-products induces resistance in insect populations (Monnerat et al., 2000). In that regard, the alternation of *Bt* and Neem was as effective as sole treatments on DBM populations as compared to Dimethoate and the control. The use of neem extracts have been recommended for the control of diamond-back and other pests resistant to *B. thuringiensis* (Ahmad, 1999; Senthil et al., 2004; Prasad et al., 2007). This would imply a synergistic effect between *B. thuringiensis* and Neem extract as suggested earlier by Sentil et al. (2004) on the rice leaf-folder *Cnaphalocrocis medinalis*. Our results suggest that the alternation of Biobit and Neem could offer better prospects to farmers as it reduces significantly the level of infestation of DBM after four applications only. Therefore, this technique can contribute substantially in the reduction of the occurrence of resistant strains among DBM populations (Reddy, 2011).

The abundance and the diversity of parasitoids populations varied significantly between treatments. The populations of parasitoids were higher in the control followed by the Biobit treatment. In this study, there were no significant differences between Biobit, Biobit/Neem and Neem which confirm the competiveness of the alternation in the control of DBM. These observations are similar to Charleston et al. (2006) who reported the importance of biopesticides in the conservation of natural enemies.

The low presence of parasitism in the Biobit treatment could be explained by the low level of infestation in DBM. For instance, Monnerat et al. (2000) showed that the level of parasitism of *P. xylostella* is highly related to the level of infestation in cabbage production. *Cotesia plutellae* was more abundant in the control, Neem and Dimethoate whereas in the Biobit and Biobit/Neem the abundance was very low. Atwood et al. (1997) reported in that regard that *Bt* has a negative effect on *C. plutellae* whereas (Chilcutt and Tabashnik, 1999) showed that the

toxins of *Bt* were not toxic to *C. plutellae*. The dynamic of DBM population seems to depend highly on *A. litae* and *O. sokolowskii*. These results confirm previous reports (Haseeb et al., 2004; Xu et al., 2004). Our results demonstrated that the level of parasitism was higher in plots treated with Neem are similar to previous findings of (Atwood et al., 1997; Charleston et al., 2006). Reddy (2011) demonstrated that the use of Neem in limited alternated treatments with synthetic chemical can result in more yield and cost effective outputs.

The absence of correlation between *P. xylostella* abundance and the parasitism in the chemical treatment show that the parasitoids were completely cleared and their impact on DBM was greatly reduced if not inexistent. These results have been reported by other scientists (Sarfraz et al., 2005; Sarfraz and Keddie, 2005). This suggests that in the absence of chemical application, parasitoids can contribute in the reduction of the pest populations to some extent (Noda et al., 2000; Braun et al., 2004).

These field results showed that alternation of Biobit and Neem with only four timely applications appears to be more promising for DBM management. Besides, the method is not harmful to parasitoids populations and their potentiality to reduce DBM populations. Alternation of *Bt* and Neem can be recommended in integrated pest management programs for DBM as it is also cost-effective and achievable by African farmers.

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Article 6

Effect of timely application of alternated treatments of *Bacillus thuringiensis* and neem on agronomical particulars of cabbage

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Résumé

La "Teigne des Crucifères" *Plutella xylostella* est un ravageur d'importance économique du chou. Les pesticides chimiques constituent le principal moyen de lutte contre cet insecte. Cependant, les pesticides botaniques et microbiens constituent une alternative aux insecticides chimiques conventionnels. L'objectif de cette étude est de comparer l'effet d'un traitement alterné *Bacillus thuringiensis* et Neem sur les paramètres agronomiques du chou par rapport à des applications en solo et chimiques. Un dispositif en blocs complétement randomisés avec quatre traitements dont Bt, Neem, alternance Bt/Neem, diméthoate (témoin traité) et le témoin non traité, est utilisé et comprenant sept répétitions par traitement. Les résultats ont montré que l'application alternée Bt/Neem est aussi efficace que les traitements en solo sur les insectes ravageurs dont *P. xylostella* que sur le chou. Les paramètres agronomiques ont été fortement liés au niveau d'infestation de *P. xylostella* et autres ravageurs. Le nombre de feuilles était plus élevé dans le témoin et le diméthoate en réponse aux dommages plus importantes, tandis que les diamètres des choux étaient plus élevés dans les traitements Biobit et Neem. Il n'y avait pas de différence significative entre le Biobit et le traitement alterné en termes de poids de choux. Le diamètre des choux traités avec le Bt seul était plus élevé que ceux traités avec l'alternance Bt/Neem. Cependant, il n'y a pas de différence significative entre le traitement alterné et le Neem. Le rendement a été plus important avec les plants de chou traités au Bt ; cependant, il n'ya pas de différence significative avec le traitement alterné et le Neem en solo. La corrélation a été significative entre les paramètres agronomiques et la présence des parasitoïdes. La corrélation était significativement plus élevée entre le nombre de feuilles, le diamètre et le poids de choux en présence d'*Oomyzus sokolowskii.* Ces résultats indiquent que le traitement alterné de *Bt* et Neem est économiquement durable par rapport au traitement solo et peut être adopté dans des programmes intégrés de gestion des ravageurs du chou.

Mots clés : *Plutella xylostella*, Biobit, Neem, chou, gestion intégrée des ravageurs, rendement

Abstract

Diamondback moth (DBM) *Plutella xylostella* is an economical pest of cabbage. Chemical pesticides constitute so far the major tool for pest management. However, the use of botanical pesticides and microbial is also considered. The objective of this study was to compare the effect of alternating treatments of *Bacillus thuringiensis* and Neem on agronomic particulars of cabbage as compared to solo and chemical applications. Results showed that the alternation of *B. thuringiensis* and Neem, performed as well as solo. Agronomic parameters were strongly related to the level of infestation of *P. xylostella* and other pests. The number of leaves was higher in the control and Dimethoate treatments depicting higher response to severe damages, whereas diameters of cabbageheads were higher in the Biobit and Neem treatments. There was no significant difference between the Biobit and the alternated treatment in terms of weight of cabbage. The diameter of cabbage treated with Biobit was higher than those treated with an alternated treatment. However, there was no significant difference between the alternated treatment and Neem. On the other hand, there was significant correlation between agronomic parameters and the presence of parasitoids. The correlation was significantly greater between the number of leaves, diameter and weight of cabbage in the presence of *Oomyzus sokolowskii*. These results indicate that timely application of alternated treatments of *Bacillus thuringiensis* and Neem can be more economically viable as compared to single treatments and should be adopted in integrated pest management programs for cabbage.

Keywords: Diamondback moth, Biobit, Neem, Cabbage, integrated pest management, yield

Introduction

Cabbage *Brassica oleracea* (Brassicaceae) is one of the most cultivated crops in the world; particularly in Africa where, it is a source of food and income to many communities living in the suburbs of West-African cities (FAOSTAT 2003). The production of cabbage is however, constrained by various insect pests among them *Plutella xylostella* (Lepidoptera: Plutellidae) the diamondback moth (DBM). This pest can cause severe crop damages (Collingwood et al. 1981, Talekar and Shelton 1993). Although it is difficult to estimate losses at small scale farming in Africa (Kibata 1996), Krishnamoorthy (2004) reported a 52% yield loss on cabbage which is beyond the economical thresholds.

The cost of pest control is estimated to cost US $ 1 billion each year (Grzywacz et al. 2010). Synthetic chemical pesticides are the main tools of pest management (Grzywacz et al. 2010). Cultural practice and the use of resistant varieties are often suggested (Asman et al. 2001). Due to their adverse effects on the environment and human health, the use of chemical pesticides is being superseded by biological control agents (Verkerk and Wright 1996, Wright 2002). In addition to that, synthetic chemical pesticides induce resistance among diamondback population (Eigenbrode and Shelton 1990). Their use is no longer economical in cabbage production.

On the other hand, DBM counts several natural enemies including parasitoids, predators and microorganisms (McCutcheon 1987, Rowell et al. 2005). Among the microorganisms, *Bacillus thuringiensis* was found to be very promising in the control of lepidopteran pests (Lereclus et al. 1993, González-Cabrera et al. 2010, Huang et al. 2010). Plant-derived pesticides such as Neem are also considered in Integrated Pest management (IPM) programs for the control of cabbage pests (Dilawari et al. 1994, Liang et al. 2003, Sarfraz et al. 2005, Charleston et al. 2006). However, reports showed that intensive use of *Bacillus thuringiensis* can induce resistance in Diamondback populations (Tabashnik et al. 1994, Meyer et al. 2001). Furthermore, the use of *Bacillus thuringiensis* for the control of DBM can also affect beneficials particularly the complex of natural enemies (Monnerat et al. 2000). The alternation of Neem and *Bacillus thuringiensis* is therefore expected to be a promising method for the control of DBM in cabbage (Ahmad 1999, Prasad et al. 2007, Roh et al. 2007). However, agronomical benefit of the use of such technique has not been well studied. Previous experiences have shown that most farmers adopt technologies after being exposed to concrete results of such an innovation.

The objective of this paper was to compare the effect of alternated treatments of *B. thuringiensis* and Neem on cabbage pest infestation especially DBM and its repercussions on the agronomical quality of cabbage yield in Senegal.

Materials and Methods

Study site

The study was conducted in Malika a district in the Niayes in Dakar, Senegal (N: 14°47'552; W: 17°19'818 and 189 m altitude). The area is characterized by long dry seasons from November to June with temperatures range of 15-20°C and short rainy seasons from July to October with temperatures ranging between 25 to 35°C. Yearly precipitations do not exceed 500 mm between August and September.

Cabbage crops

Cabbage *Brassica oleracea* var. "*Marché de Copenhague*" which, is drought tolerant was used in this experiment. In order to protect cultures from nematodes, Furadan was applied in the soil prior to planting. Poultry manure was applied as fertilizer, 10 days later with intensive water irrigation. After planting additional fertilizers with N-P-K in a ratio 10-10-20 and poultry manure were applied 2weeks after planting. Crops were watered daily using a sprinkler. The experimental design consisted in 35 plots of 2100 plants in a randomized bloc design. Cabbage crop were planted in 35 plots of 60 plants each, placed in six rows of 10 plants each. The spacing between rows was fixed at 40cm. Treatments were repeated five times.

Phytosanitary applications

Four (4) treatments were used: Biobit, *Bacillus thuringiensis* var. *kurstaki*, Crystal Chemical Company LTD (Europe), Neem (Suneem, *Azadirachta indica* 1% EC), alternation Biobit/Neem (in 10 days interval four times) and Dimethoate (Meteor 400 EC). An untreated control was also included in the experiment. Biobit was applied at 1L for 100 L of water per hectare. As for the Neem treatment, the dosage was 1L/ha. Dimethoate was applied at 1.5 L/ha. Applications started 25 days after planting; crops were treated using manual sprayer every ten days. For the alternated treatment Biobit/Neem, four timely applications were used: Neem was applied first and the last application was Biobit. These alternated applications were stopped 20 days before the other treatments.

Sampling methods

Samples were collected randomly by selecting 10 cabbageheads in the central row of each plot. The number of insects such as larva and pupae of *P. xylostella* and cocoons of parasitoids was recorded. Other insect pests including larvae of *Hellula undalis* Fabricius (Pyralidae), Aphididae and Aleyrodidae within a cabbage were all collected and counted in each treatment. Eggs and larvae of *P. xylostella* which, were inside the leaves were not considered.

In each treatment, the diameter of each cabbagehead plant was measured with a ruler. The cabbageheads were weighted at harvest with an electronic balance. The yield of cabbage was recorded for each treatment. The samplings started 10 days planting and were performed every ten days.

Data management

Data were normalized and subjected to ANOVA, and post-ANOVA comparisons of means were made using Student-Newman-Keuls test. The relations between agronomic features and pest infestation and the presence of parasitoids was determined using Pearson's correlation. The level of significance was kept at 5% in all data analysis.

Results

Interactions between agronomic parameters and insect pest infestations

The results show that agronomic features of cabbages such as the number of leaves, the weight and the diameter of cabbageheads were related to the level of pest (Table 1). However, there was no relation between the infestation of DBM and other pests (Table 1).

Table 1: Correlation between agronomic parameter and insect pest infestations in Cabbages

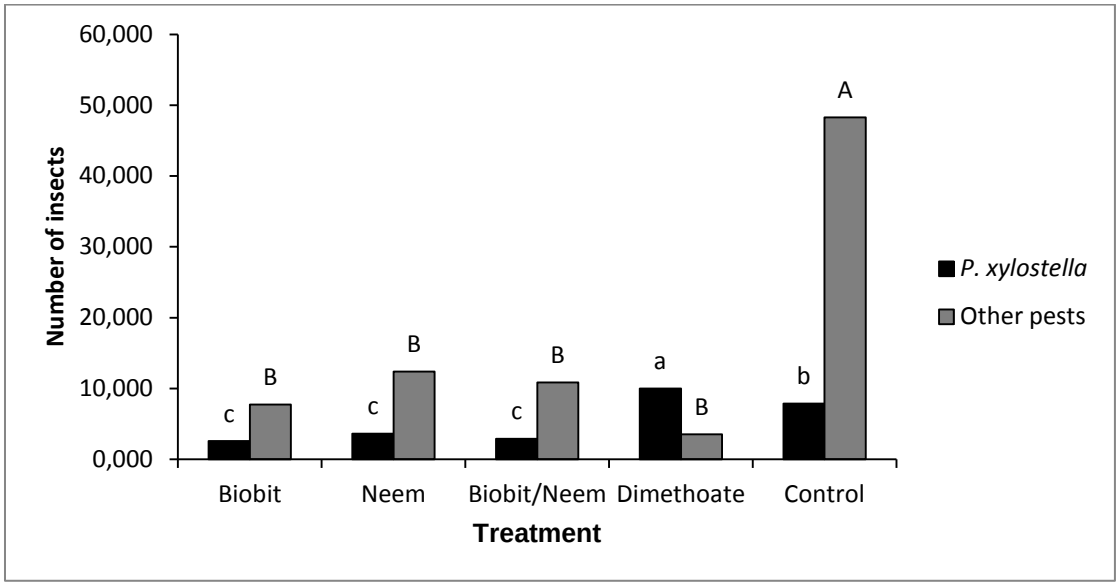
	N. of leaves	Diameter (cm)	Weight (g)	*P. xylostella*	Other Pests
N. of leaves					
Diameter (cm)	**0.527**				
Weight (g)	**0.201**	**0.545**			
P. xylostella	**0.221**	**0.366**	**0.134**		
Other Pests	**0.287**	**0.150**	**0.150**	0.008	

In bold, significant values (except diagonal) at the level of significance alpha=0.050 (two-tailed test)

Infestation levels of *Plutella xylostella* and other pests

There were significant differences between treatments on the infestation levels of *P. xylostella* ($F_{(4, 24)} = 63.14$; $P < 0.0001$) and other pests ($F_{(4, 24)} = 14.16$; $P < 0.0001$).

Plutella xylostella infestation was significantly higher in the chemical treatment and in the control. There were no significant differences between the treatments Biobit, Neem and Biobit/Neem. The infestation of other insect pests was higher in the control and was significantly different from the other treatments. However, there was no significant difference between the other treatments (Figure 1).



Means bearing the same small letters are not significantly different in ANOVA SNK. Means bearing the same capital letters are not significantly different in ANOVA SNK.

Figure 1: Infestation levels of *P. xylostella* and other pest on cabbage treated with Biobit, Biobit/Neem, Neem and Dimethoate

Effect of treatments on agronomic parameters and yield

There were significant differences between treatments on the weight ($F_{(4,24)}$ = 4.19; P = 0.002), the diameter ($F_{(4,24)}$ = 2.39; P = 0,049) and the number of leaves of cabbages ($F_{(4,24)}$ = 3.63; P = 0.006). The highest weights were recorded on the Biobit treatment and were not significantly different from Neem and the alternation Biobit/Neem. However, there were significant differences between Biobit and Dimethoate and the control. There were no significant differences between Neem, Biobit, Dimethoate and the control (Table 2).

The diameter of cabbage was higher in the Biobit treatment but was not significantly different from the Neem treatment. There were no significant differences between Neem and Biobit/Neem, Dimethoate and the control (Table 2).

In terms of number of leaves, there were no significant differences between Neem, Biobit/Neem, Dimethoate and the control. However, there were significant differences between Biobit and Dimethoate and between Biobit and the control (Table 2).

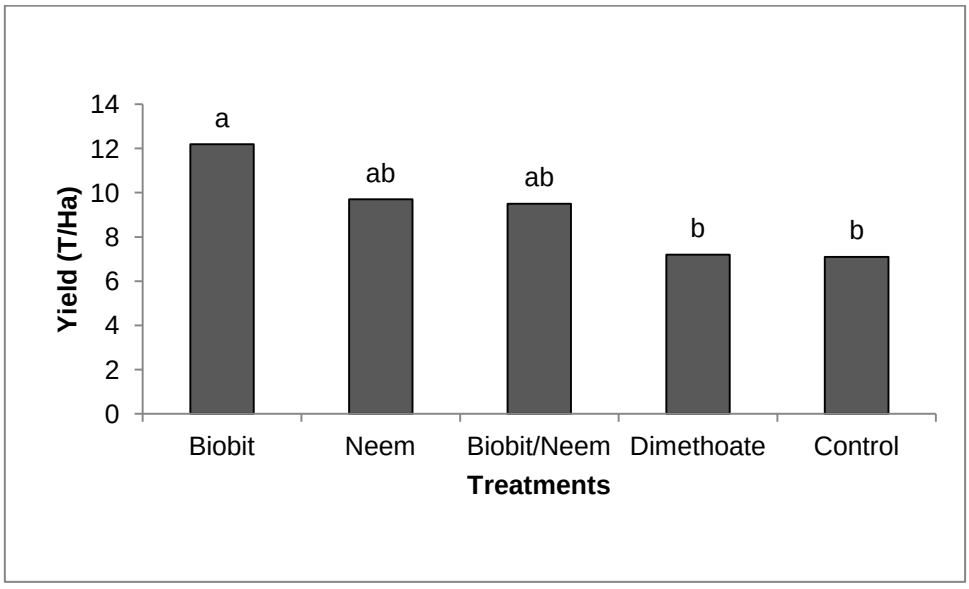
The yield of cabbage was significantly different between treatments ($F_{(4,24)}$ = 177.69; P < 0.0001). It was significantly higher in treatment Biobit with 12.2 t/ha. There were no significant differences between alternated treatment and the treatments Biobit and Neem

(P>0.05). However, the yield was significantly lower in plants treated with Dimethoate and in control; respectively yields of 7.2 t / ha and 7.1 t / ha (Figure 2).

Table 2: Agronomic features of cabbage treated with Biobit, Neem, Biobit/Neem and Dimethoate

Treatments	Weight (g)	Diameter (cm)	Number of leaves
Biobit	197.6a	31.9a	17.3b
Neem	158.2ab	30.3ab	19.6ab
Biobit/Neem	146.4ab	29.2b	18.8ab
Dimethoate	119.4b	30.2ab	20.2a
Control	118.9b	30.6ab	20.8a

Within columns, means bearing same small letters are not significantly different in ANOVA SNK



Means bearing the same letters are not significantly different in ANOVA SNK

Figure 2: Effect of Biobit, Biobit/Neem, Neem and Dimethoate application on the yield

Interactions between agronomic parameters and parasitoids

There was a significant relation between the agronomical characters of cabbages: weight, diameter and number of leaves and the presence of parasitoids. The weight, the diameters of cabbageheads and the number of leaves were significantly correlated to the presence of *O. sokolowski*. The presence *A. litae* was only correlated to the weight and the diameter of cabbages whereas *Cotesia plutellae* and *Brachimeria* sp. were only correlated to the weight (Table 3).

Table 3: Relation between agronomic features and the presence of parasitoids in cabbage plantation

Parasitoids	**Weight (g)**	**Diameter (cm)**	**N. of leaves**
Oomyzus skolowski	**0.105**	**0.137**	**0.085**
Apanteles litae	**0.099**	**0.135**	-0.009
Cotesia plutellae	**0.048**	0.043	-0.019
Brachymeria sp.	**0.060**	0.036	0.020

In bold, significant values (except diagonal) at the level of significance alpha=0.050 (two-tailed test)

Discussion

The level of pest infestation was significantly different between treatments however; *P. xylostella* infestation was not significantly different between the treatments Biobit, Neem and the alternation Biobit/Neem. Although significantly higher in the control, the level of infestation of other pests was not significantly different between Biobit, Neem, Biobit/Neem and Dimethoate. These results suggest that apart from damages caused by *P. xylostella*, the contribution of the other pests in the damage on cabbage is negligible.

The application of *Bacillus thuringiensis* against DBM and other lepidopteran pests has been recommended by many authors (Lereclus et al. 1993, Kibata 1996). *Bacillus thuringiensis* seems to present many advantages. Although, there were no significant differences between Biobit, Neem and the alternation, the application of *Bacillus thuringiensis* recorded the highest weights and the diameters of cabbageheads. However, there were no significant differences between Biobit, Neem and the alternation. This suggests that alternated treatment of Biobit and Neem which, is timely applied only on four occasions, could achieve similar results than solo treatment of Biobit and Neem. Similar findings have been demonstrated by many scientists (Verkerk and Wright 1996, Wright 2002, Prasad et al. 2007, Roh et al. 2007).

As for the number of leaves, results showed that the control recorded the highest values. The Biobit treatment was the lowest however, not significantly different from Neem and Biobit/Neem. The importance of the number of leaves could be considered as response to challenges or stresses causes by *P. xylostella* damages on the plants (Ayalew 2006). As larvae of DBM develop on cabbage leaves, they prevent physiological processes such as photosynthesis and respiration. As a response, more leaves are generated by the plant to bypass the stress (Verkerk and Wright 1996, Wojciechowska and Leja 1999, You and Yang 2001).

The higher yield observed in the treatments plants Biobit, Neem and alternating treatment could be explained by the low infestation levels of *P. xylostella*. The use of *B. thuringiensis* based formulations can increase yields (Huang et al. 2005; Cattaneo et al. 2006; Herdt, 2006). According to the Horticulture Development Centre (HRC), the standard yield of cabbage is estimated between 10 and 20 t / ha. Yield reductions are also due to damage *Hellula undalis*. The presence of other pests in the cabbages, particularly *Hellula undalis* (Lep., Pyralidae) whose larvae eat the terminal bud of newly planted cabbages, thus inducing growth of the axillary buds which produce unmarketable multiple heads at harvest (Goudegnon et al. 2000). In this study, there were no significant differences between treatments on the presence of

other pests. The low yields observed in the treatment with dimethoate and controls are primarily caused by the damage of *P. xylostella*. According to Ayalew (2006), the yield losses in cabbage may vary considerably depending on the levels of pest infestation.

The application of *B. thuringiensis* and Neem have been regarded as less harmful to beneficials such as parasitoid wasps and natural enemies as compared to chemical pesticides (Roh et al. 2007).

Results of this study showed that, there were significant relation between the presence of parasitoids and the agronomic features. The parasitoid *O. sokolowski* and *A. litae* seem to be more contributors to the agronomic features of the cabbage which is a considerable gain to the farmer. The study revealed that as far as cabbage production is concerned, it is better to rely on biocontrol agents than to apply synthetic chemical pesticides. This could be explained by negative effects of synthetic chemicals on the complex of natural enemies and the induction of resistance to pest populations. The use of alternated treatment can therefore be an opportunity to mitigate both pest infestation and manage the apparition of resistance. It has been demonstrated that uncontrolled application of *Bacillus thuringiensis* could be the source of resistance induction in DBM (Chilcutt and Tabashnik 1999, Monnerat et al. 2000).

As a conclusion, cabbage is one of the most difficult crops to grow and to sell in Africa particularly in Africa; this is due to heavy physical damages that occur on the leaves and discourage costumers. On the other hand, the use of high rates of synthetic chemicals can compromise the quality of cabbages exempted from damages. The study showed that by using four timely applications of Biobit and Neem, it is possible to achieve an efficient biological control against the DBM and to produce safe cabbage crops. The technique is cost-effective and therefore can be recommended to farmers in developing countries.

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Conclusion générale et perspectives

Ce travail se focalise sur la gestion intégrée et durable de la "Teigne des Crucifères", *Plutella xylostella* (L.), Lepidoptera ; Plutellidae. Au Sénégal, ce ravageur cause d'énormes pertes de production auprès des maraichers. Pour faire face aux dégâts, ils ont recours principalement aux traitements d'insecticides de synthèse. Cependant, ces pesticides sont à l'origine de nombreuses conséquences telles que, l'intoxication des producteurs et des consommateurs, l'élimination des ennemis naturels de la teigne, l'augmentation du coût de production et l'apparition de souches résistantes (Shelton et al. 2007 ; Huang et al. 2010). Il faut donc envisager la lutte intégrée comme une solution plus intéressante pour gérer à la fois les problèmes de résistance de *P. xylostella* et le manque d'efficacité de ses ennemis naturels, d'ailleurs parfois lié aux applications d'insecticides.

Les études de l'impact des facteurs abiotiques et biotiques (saison, température, pluviométrie, plante hôte et ennemis naturels) sur la dynamique des populations de *P. xylostella* au champ ont montré que :

Les facteurs climatiques influencent la dynamique des populations de *P. xylostella* et leur faune auxiliaire. Ainsi, les basses températures de la saison sèche semblent favoriser le développement du ravageur et ses auxiliaires.

Le parasitisme naturel reste faible dans la zone et n'exerce aucune influence sur le contrôle des populations locales de *P. xylostella*. D'après Ueno & Tanaka (1996), le taux de parasitisme est faible dans la nature. Cette faiblesse du taux de parasitisme pourrait s'expliquer par la succession élevée de générations du ravageur et de l'usage abusif des insecticides chimiques. Ces études sont importantes pour comprendre les facteurs qui favorisent ou inhibent les populations de ravageurs et leurs ennemis naturels, et par conséquent, essentielles à la protection efficace des cultures.

Parmi les ennemis naturels de la teigne, le parasitoïde larvo-nymphal *Oomyzus sokolowskii* (Kurdjumov), Hymenoptera ; Eulophidae, est considéré comme le plus important endoparasitoïde et agent de lutte biologique pour la gestion des populations de *P. xylostella* (Wang et al. 1999; Ferreira et al. 2003). Les études réalisées au laboratoire sur la biologie et la performance d'*O. sokolowskii* ont montré que :

Il existe un dimorphisme sexuel lié à la taille ; la femelle a une taille supérieure à celle du mâle. *Oomyzus sokolowskii* est un parasitoïde koïnobionte, c'est-à-dire que l'hôte poursuit son développement même après le parasitisme. C'est aussi un parasitoïde larvo-nympal et grégaire. Hormis la reproduction sexuée, les femelles d'*O. sokolowskii* sont capables de se reproduire par la parthénogenèse de type arrhénotoque. Les femelles pondent leur œuf tout au

long de leur vie reproductive : elle est synovogénique. Cette espèce parasite tous les stades larvaires et les prénymphes avec une nette préférence pour les stades larvaires L4 de l'hôte.

Ces études confirment l'importance du parasitoïde *O. sokolowskii* comme un agent biologique qui peut être utilisé dans une approche de lutte biologique par augmentation. Cependant, sa performance peut être affectée par l'origine géographique de l'hôte. L'âge du parasitoïde peut être aussi un facteur limitant dans la production d'*O. sokolowskii* ; donc d'autres paramètres doivent être considérés pour une gestion efficace des populations de *P. xylostella*. Ces résultats ont montré la nécessité de connaitre la période favorable pour asseoir un succès réel du parasitisme de la teigne par le parasitoïde.

Pour conclure, bien que ce travail s'inscrive clairement dans un contexte de recherche développement, les résultats obtenus pourraient aussi permettre la mise en place d'outils pour des applications dans la lutte biologique. Les résultats de ce travail permettent d'avoir une meilleure connaissance sur la biologie et le comportement d'*O. sokolowskii*, un des principaux ennemis naturels de *P. xylostella*, ce qui est nécessaire pour une meilleure utilisation de l'espèce en lutte biologique.

En dépit des quatre espèces de parasitoïdes rencontrés ; *O. sokolowskii, Apanteles litae* (Nixon), *Cotesia plutellae* (Kurdjumov), *Brachymeria citrae* (Steffan), les taux de parasitisme des stades immatures de *P. xylostella* produits ne peuvent contrôler efficacement les populations du ravageur d'où la nécessité de recours à d'autres méthodes de lutte additionnelles telles que le biopesticide à base de *Bacillus thuringiensis* (Bt) et le pesticide naturel, le Neem.

Ainsi, les études réalisées d'abord au laboratoire ont confirmé l'efficacité du Bt et du Neem sur les stades immatures de *P. xylostella* comparées aux insecticides chimiques largement utilisés dans la zone des Niayes.

Au champ, l'application d'un traitement alterné *B. thuringiensis* et Neem sur *P. xylostella* et ses ennemis naturels a montré sa plus grande efficacité dans la réduction des infestations du ravageur. Ces résultats obtenus au champ montrent que le traitement alterné du biopesticide et du produit à base de Neem paraît plus intéressant dans la gestion des populations de *P. xylostella* car réduisant considérablement la teigne après seulement quatre applications. En plus, ce traitement préserve efficacement les ennemis naturels de la "Teigne des Crucifères". Par ailleurs, ce traitement biologique et naturel a donné des poids et des rendements du chou plus importants. Ainsi, cette pratique doit être considérée dans les programmes de lutte intégrée et ceci d'autant plus qu'elle est plus lucrative pour l'agriculteur.

Les résultats présentés dans cette thèse ouvrent la voie à de nombreuses perspectives, notamment dans l'étude de l'implication des endosymbiontes dans les relations hôte-parasitoïde. Il sera important aussi d'étudier le mécanisme de la résistance de *P. xylostella* aux insecticides par une caractérisation de ce phénomène.

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Annexes

Annexe 1 : Les plantes hôtes de *Plutella xylostella* (Talekar & Shelton, 1993)

♦ Les crucifères cultivées

◊ Choux *Brassica oleracea var. capitata*

◊ Choux- fleurs *Brassica oleracea var. botrytis*

◊ Brocoli *Brassica oleracea var. italica*

◊ Radis *Raphanus sativus*

◊ Navet *Brassica rapa pekinenis*

◊ Choux de Bruxelles *Brassica oleracea var. gemmifera*

◊ Choux chinois *Brassica rapa*

◊ Cresson *Nasturtium officinale*

◊ Moutarde *Brassica juncea*

◊ Colza *Brassica napus*

♦ Les crucifères sauvages

Arabis glabra

Armoricia lapathifolia

Barbarea stricta

Barbarea vulgaris

Basela alba

Beta vulgaris

Brassica caulorapha

Brassica kaber

Brassica napobrassica

Bunias orientalis

Capsella bursa-pastoris

Cardamine amara

Cardamine cordifolia

Cardamine pratensis

Cheiranthus cheiri

Conringa orientalis

Descurainia sophia

Erysimum cheiranthoides

Galinsoga ciliata

Galinsoga parviflora

Hesperis matronalis

Iberis amara

Isatis tinctoria

Lepidium perfoliatum

Lepidium virginicum

Lobulria maritima

Mathiola incana

Norta altissima

Pringlea antiscorbutica

Raphanus raphanistrum

Rorippa amphibia

Rorippa islandica

Sinapsis alba

Sisymbrium austriacum

Sisymbrium officinale

Thlaspi arvense

Annexe 2 : Insectarium des populations de *Plutella xylostella* (Laboratoire de Biodiversité des agrosystèmes horticoles, Cirad)

Annexe 3 : Serre (Laboratoire de Biodiversité des agrosystèmes horticoles, Cirad)

Nom et prénoms du Candidat : Gallo SOW

Titre de la thèse : Gestion intégrée des populations de *Plutella xylostella* L. (Lepidoptera : Plutellidae), principal ravageur du chou au Sénégal

Date et lieu de soutenance : Le 16 Janvier 2013 à l'UCAD

Jury: Président: Bhen Sikina TOGUEBAYE, Professeur titulaire, FST/UCAD
Membres: Kandioura NOBA, Professeur titulaire, FST/UCAD
Dr. Dominique BORDAT, Chercheur HDR, CIRAD/Persyst
Dr. Emile Victor COLY, Directeur de recherche, ISRA/CDH
Dr. Therry BREVAULT, Chercheur, CIRAD/Persyst
Karamoko DIARRA, Professeur titulaire, FST/UCAD

Résumé :

La "Teigne des Crucifères", *Plutella xylostella* (L.) (Lepidoptera : Plutellidae) est un insecte ravageur redoutable des cultures de choux au Sénégal. L'utilisation des insecticides chimiques de synthèse reste la méthode la plus employée dans la gestion des populations de *P. xylostella* malgré ses revers environnementaux et sanitaires d'où la nécessité de trouver d'autres alternatives. Dans le but de mettre en œuvre une approche de gestion intégrée et durable des populations de *P. xylostella*, des études ont été conduites au champ et au laboratoire. Nous avons consacré une première partie de notre étude aux interactions entre la "Teigne des Crucifères", les conditions climatiques, la plante hôte et la faune auxiliaire. Les résultats ont montré que les populations de *P. xylostella* étaient plus importantes dans les plants de chou en saison sèche qu'en hivernage de même que la faune auxiliaire. Les basses températures de la saison sèche sont favorables à l'augmentation de la densité des stades immatures de *P. xylostella*. Les taux de parasitisme sont faibles pour le contrôle des populations du ravageur. Les résultats obtenus de l'étude des traits d'histoire de vie et de la performance du parasitoïde *Oomyzus sokolowskii* au laboratoire ont montré une préférence des stades larvaires L4 de l'hôte. Elle est une espèce synovogénique, koinobionte et capable de se reproduire par parthénogenèse de type arrhénotoque. Elle peut être un bon agent de lutte biologique pour le contrôle des populations de *P. xylostella*. En outre, nos résultats obtenus au champ indiquent que l'application alternée de *Bacillus thuringiensis* (Bt) et du Neem est efficace pour le contrôle de la "Teigne des Crucifères" et préserve la faune auxiliaire associée. Ce traitement est économiquement plus rentable et peut être recommandé dans la gestion durable des populations du ravageur.

Mots clés : *Plutella xylostella*, parasitoïde, chou, température, *Oomyzus sokolowskii*, traits d'histoire de vie, *Bacillus thuringiensis*, Neem, lutte biologique, Niayes