The influence of cabbage cultivars on the fitness of *Plutella xylostella* (Linnaeus 1758) (Lepidoptera: Plutellidae) and its biological control agent *Cotesia vestalis* (Haliday 1834) (Hymenoptera: Braconidae)

by

PHOPHI DZIVHULUWANI NETHONONDA

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SUPERVISOR: DR. R.S. NOFEMELA

CO-SUPERVISOR: PROF. D.M. MODISE

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Declaration

I, Phophi Dzivhuluwani Nethononda, declare that this thesis submitted to the Department of Agriculture and Animal Health at the University of South Africa for Master of Science (Agriculture) is my original work that has never been submitted by me for a degree at this or any other tertiary institution. All sources used or quoted herein are properly acknowledged.

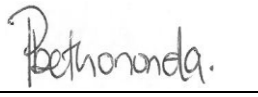
Signature: 

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Table of Contents

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Dedication

I dedicate this thesis to my beautiful daughter Vhulenda Precious Nethononda and in the memory of my beloved late uncle Rembuluwani, Edward, Joseph, "Teacher', Mundalamo, Netswinga, Tshishonga. Thank you God almighty for such a wonderful soul.

List of plates

List of figures

List of tables

ABSTRACT

The diamondback moth, *Plutella xylostella* (Linnaeus 1758.) (Lepidoptera: Plutellidae), is a major insect pest of *Brassica* crops in many parts of the world leading to economic losses amounting to an estimated US$ 4-5 billion. Although parasitoids (biological control agents) play a major role in suppressing the pest populations during November – May in South Africa, the pest reaches outbreak status during September and October due to low impact of parasitoids, which has necessitated regular application of insecticides. However, insecticide applications have often resulted in the pest developing resistance, and when coupled with the negative effects of several insecticides on parasitoids, integration of the two pest control strategies for effective management of *P. xylostella* population density has been difficult to achieve. One approach that has received little attention is integration of host plant resistance (bottom-up effect) and biological control (top-down effect) for effective management of *P. xylostella*. However, the interaction between host plants, the insect pest, and parasitoids is not simple and straight forward, as strong negative impact of host plants on fitness of the insect pest can be cascaded up the food chain and have a negative impact on a given parasitoid, which in turn may reduce the desired complementary effect between the two pest control strategies. To identify optimal interactions between cabbage (*Brassica oleracea* L. var. *capitata*, Brassicaceae), *P. xylostella* and its larval parasitoid *Cotesia vestalis* (Haliday 1834) (Hymenoptera: Braconidae), this study investigated (i) the effects of seven cabbage cultivars (Empowa, Hollywood F1, Megaton, Leano, Menzania, Beverley Hills and Karabo) on fitness parameters (survival, developmental time, pupal weights, longevity without food and oviposition rates) of *P. xylostella*; (ii) the influence of the same host plant cultivars on fitness parameters (developmental time, pupal weights, longevity

without food, fecundity, emergence rate and sex ratio) of *C. vestalis.* Furthermore, net reproductive rates and the intrinsic rates of natural increase were calculated for *C. vestalis* that emerged from hosts fed on each of the cultivars. All experiments were conducted in climate-controlled laboratory rooms maintained at 22 ± 1 ºC (mean ± S.D.), 60 ± 5 % RH and 16L: 8D photoperiod.

Under the no choice test, overall survival of *P. xylostella* immature stages was highest on Karabo (67.26%) and lowest on Megaton (44.92%). The larval and pupal developmental period, and generation time was prolonged on Empowa (18.48 days), Karabo (14.64 days) and Beverly Hills (17.48 days), while developmental period on Hollywood F1 (13.79 days) was shortest. Male and female *P. xylostella* pupal weights were lighter from larvae that fed on Megaton (4.13 and 4.65 mg), Menzania (4.53 and 4.91 mg), and Hollywood F1 (4.11 and 5.08 mg), whereas pupal weights from Karabo (6.0 and 6.82 mg) were the heaviest. Unfed female moths originally reared on Beverley Hills had the highest longevity (5.05 days), whereas those reared on Leano (3.54 days) and Megaton (3.89 days) had the shortest life span. Under the choice-test, *P. xylostella* moth laid significantly more eggs on Empowa (48.8%) and Hollywood F1 (45.6%) and least on Menzania (11.8%) and Leano (10.6%). Megaton was more resistant to *P. xylostella* due to lower survival rates of immature stages, lower pupal weights and moth longevity.

The generation time of *C. vestalis* was shortest on Karabo (10.10 days) and Leano (10.38 days), and longest on Megaton (12.57 days) and Empowa (12.80 days). The highest pupal weight of *C. vestalis* was obtained from parasitoids reared from *P. xylostella* fed Menzania (5.4 mg), Megaton (5.25 mg) and Beverly Hills (4.85 mg) and the lightest on Karabo (3.8 mg). Parasitoids reared on larvae that fed on Hollywood F1 lived the longest (2.28 days) followed by Menzania (1.94 days) and Beverly Hills (1.8 days),

whereas those whose hosts fed on Leano had shortest life span (0.83 days). Despite the parasitoids from Megaton hosts being heavier, their fecundity and number of female progeny per female (16.87 and 3.60, respectively) were lowest. *Cotesia vestalis* fecundity and daughters produced per female were highest on hosts fed on Menzania (38.00 and 9.13, respectively) and Beverly Hills (32.87 and 9.07, respectively). As a consequence, the net reproductive rate (R_0) and intrinsic rate of increase (r) were higher on Menzania (7.87 and 0.58, respectively) and Beverly Hills (8.29 and 0.62, respectively).

As survival and overall fitness of *P. xylostella* was lower on Megaton, this cultivar can play a major role in restricting population growth of this pest and thus generational number of eggs deposited on it during September and October. However, this strong bottom-up effect of Megaton on *P. xylostella* was cascaded up the food chain, as overall fitness of *C. vestalis* was lower on hosts developing on it. In contrast, the overall fitness of *C. vestalis* was higher on hosts that developed on Menzania and Beverly Hills. As these cultivars showed potential to sustain population density of *C. vestalis* at higher levels, it is also assumed that the period required for the parasitoid to reach the critical density to suppress the host population at a lower average density will be reached quicker than on other cultivars. Thus, their cultivation may improve biological control of *P. xylostella* during November–May in South Africa.

Chapter 1: General introduction

Nearly all plant species around the world are attacked by insect herbivores (Futuyma, 2000), and this relationship is known to have evolved more than 350 million years ago (War *et al*., 2012). However, plants are not necessarily helpless victims to their predators, as they have demonstrated an ability to defend themselves against herbivory (Karban and Baldwin, 1997). The defensive mechanisms used by plants are either direct, (are always present on a plant), or indirect, (are induced by herbivory) (Mithöfer and Boland, 2012).

1.1 Direct plant defence

Direct plant defence includes plant attributes that have a negative effect on insect preference such as host plant selection, oviposition, and feeding behaviour or its performance, such as growth rate, developmental rate and reproduction success (War *et al*., 2012). These plant attributes may include plant morphological traits such as thorns, spines, trichomes (hairs), epicular wax films, tissue toughness and chemical defences, such as secondary metabolites, digestibility reducing proteins and anti-nutritive enzymes (Chen, 2008). Direct plant defences are exhibited on a plant by one or a combination of mechanisms such as antixenosis, antibiosis and tolerance (Stout, 2013). Antixenosis refers to plant properties (morphological traits such as plant colour, odour, and texture, such as waxiness and trichomes) that make it unacceptable or unattractive for oviposition by insect herbivores. Thus, antixenosis has a direct influence on insect behaviour (Andrahennadi and Gillott, 1998; Stout, 2013). For an example, glandular trichomes exhibit both morphological and chemical resistance factors as glands produce substances which may act as repellents or feeding deterrents or prevent mobility on the leaf surface

(Chen, 2008). For an example, Jyoti *et al*. (2001) found that among five Brassicaceae species tested (*B. oleracea* variety *Italica* L., 'Green Comet'; *B. oleracea* L., 'Rapid Cycling'(Crucifer Genetics Cooperative 3-1); *B. oleracea* variety *botrytis* L, 'a standard cauliflower cultivar'Amazing; *B. carinata* L., and *Sinapis alba* L., Cornell Alt 543) *Sinapis alba* was the only species which exhibited antixenosis factors due to its trichomes around the stems that act as physical barriers to oviposition by the cabbage maggot, *Delia radicum* (L.). Leaf toughness affects the penetration of plant tissues by mouthparts of piercing–sucking insects and increases mandibular wear (fractures) in biting-chewing herbivores (Schoonhoven *et al*., 2005 pp 29).

Antibiosis refers to plant properties such as secondary plant compounds that affect life history parameters such as growth and development of insect herbivores (Stout, 2013). Antibiosis may be as a result of both chemical and morphological plant attributes which may cause death of young larvae or other chronic effects on the herbivore that may cause mortality of larvae, pupae, or adults. Antibiosis results in small body size, low body weight, prolonged development of herbivore immature stages, low fecundity and survival of adults (Jyoti *et al*., 2001). Chen (2008) categorised direct plant defence in the form of antibiosis as anti-nutrition and toxicity. Anti-nutrition includes plant defence mechanisms that limit food supply before food is ingested and mechanisms that reduce nutritive value after food is ingested. Toxicity includes the disruption of physical structure of the herbivore or inhibiting chemical pathways in the insect (Chen, 2008). Plants can limit herbivore food supply during pre-ingestion when plants use mechanisms such as physical barriers, cell wall fortification, hypersensitive reactions, anti-manipulation and the production of insect repellents that prevent its accessibility to herbivore feeding (Chen, 2008). During post-ingestion, plants may be responsible for the reduction of nutritional value by removing essential nutrients and/or inhibiting digestion through some plant

enzymes that remain active in the herbivore mid-gut after ingestion that destroy nutrients that are supposed to be used by insect herbivore or through the production of chemicals that are responsible for disrupting herbivore physical structures (Chen *et al*., 2007). For an example, maize (*Zea mays* L.) resistant line prevents feeding by Lepidopterans species such as fall armyworm, *Spodoptera frugiperda* by rapidly mobilizing a unique 33 kDa cysteine protease which causes damages to the peritrophic matrix, (a semi-permeable, non-cellular structure which surrounds the food bolus in an organism's midgut) which assist in digestion and protects the midgut from physical and chemical damage (Pechan *et al*., 2000). Thus the disruption of insect peritrophic matrix may result in the inhibition of insect growth and development (Pechan *et al*., 2000). In other instances, some herbivores have the ability to sequester plant allelochemicals in their haemolymph and use them to defend themselves against their natural enemies (English–Loeb *et al*., 1993; Opitz and Müller, 2009).

Plant can tolerate herbivore damage by modification of plant proteins, increased oxidative enzyme activity and altering resource reallocation (Eickhoff *et al*., 2008). Tolerance refers to the differential ability of plants to compensate for herbivory by regrowth (Strauss and Agrawal, 1999). For an example, Sarfraz *et al*. (2007) indicated that *B. napus* Q2, *B napus* Conquest, *B. rapa* and *S. alba* were able to tolerate feeding damage by *P. xylostella* by increasing their root mass.

1.2 Indirect plant defence

Plants can also have an indirect defence mechanism of protecting themselves against herbivores. Indirect plant defence is the release of volatile plant signals which attracts natural enemies (Degenhardt, 2009). This kind of plant defence enhances biological

control which is the use of natural enemies (parasitoids and predators) to suppress herbivore population density and thus damage on a plant (Degenhardt, 2009). Plants emit volatile organic compounds known as Herbivore-Induced Plant Volatiles (HIPVs) in response to herbivory attack which attract natural enemies of insect pests (see Plate 1.1) to their host habitat and thus also to their prey or hosts (Dicke and Takabayashi, 1999; Gols *et al*., 2009). These HIPVs differ in both quality and quantity depending on the plant species, plant genotype and herbivore species feeding on a plant (Dicke and Baldwin, 2010). HIPVs can consist of hundreds of compounds made up of terpenoids, green leaf volatiles and benzenoids which are responsible for repelling or attracting herbivores and their natural enemies (Dicke and Baldwin, 2010). The composition of HIPVs both qualities and quantities are specific for attracting a particular enemy to the plant damaged by the herbivore (Sabelis *et al*., 2007; Arimura *et al*., 2009). For example, *Cotesia vestalis* (Haliday 1834) [= *C. plutellae* (Kurdjumov) (Hymenoptera: Braconidae) a specialist endoparasitoid of the diamondback moth, *Plutella xylostella* (Linnaeus) [= *P. maculipennis* (Curtis)] (Lepidoptera: Plutellidae) larvae is attracted to a blend of sabinene, *n*-heptanal, α-pinene, and (Z)-3 hexenyl acetate at ratios 1.8:1.3:2.0:3.0 (Uefune *et al.,* 2012).

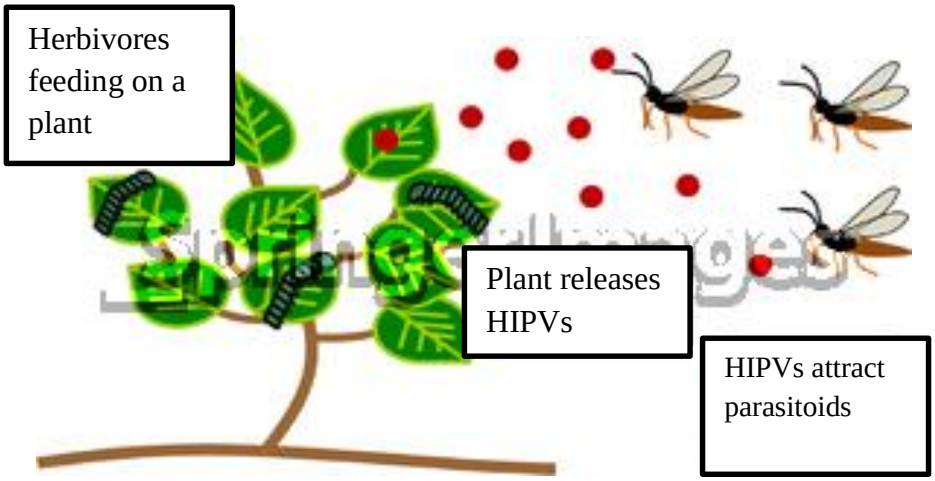

Plate 1.1 Plants emit Herbivore Induced Plant Volatiles in response to herbivore damage which attracts natural enemies**.** (Taken from Prince and Pohnert (2009) for illustration purposes).

1.3 The tritrophic system studied

1.3.1 The plant

Cabbage, *Brassica oleracea* L. var. *capitata*, is a vegetable crop grown for its large leafy head (Singh *et al*., 2006). It was used for medicinal purposes to cure diseases such as headaches, gout, diarrhoea and peptic ulcers (Singh *et al*., 2006). Cabbage is also known to have cancer preventing properties (Brooks *et al*., 2001), and is rich in Vitamin C, β-carotene, lutein, DL-α-tocopherol and phenolics (Singh *et al*., 2006). Apart from having medicinal benefits, cabbage is grown in all South African provinces mainly in Western Cape, Kwazulu-Natal, Eastern Cape, Gauteng, Free State and North West (DAFF, 2013). Fresh cabbages are sold in fresh produce market, restaurants, processors, hawkers and supermarkets (DAFF, 2013). The crop can be eaten raw in salads such as coleslaw and atchaar or cooked. In many rural and peri-urban areas, it is mainly grown for subsistence (Kfir, 1997). It is estimated that about R186.2 million worth of cabbage was sold in the South African fresh produce markets during 2011 (DAFF, 2013). Success

of the US$ 26 billion worth cabbage market per annum worldwide (FAO STAT, 2015) is threatened by a variety of diseases, and insect pests of which flea beetles (*Phyllotreta cruciferae*), cabbage looper (*Trichoplusia ni* (Hübner), aphids (*Brevicoryne brassicae*) and the diamondback moth (*Plutella xylostella*) are regarded as the most important (Talekar and Shelton, 1993).

1.3.2 The pest

Plutella xylostella is a major pest of *Brassica* crops worldwide (Talekar and Shelton, 1993; Sarfraz *et al*., 2005; Furlong *et al.*, 2013). In warmer parts of the world, it occurs in overlapping generations of up to 20 per year (Talekar and Shelton, 1993; Nofemela, 2010). Its ability to develop resistance to all classes of insecticides has increased its pest status in many parts of the world (Talekar and Shelton, 1993; Sarfraz *et al*., 2005). In addition, the lack of effective alternative control measures in many areas are contributing to the total cost of US$ 4 and 5 billion annually for controlling *P. xylostella* and the direct damage it causes on produce (Zalucki *et al*., 2012). It was believed to have originated from the Mediterranean region of Europe and later spread throughout the world with its host plants (Hardy, 1938; Talekar and Shelton, 1993) but, Kfir (1998) is of the view that it may have originated from southern Africa on the basis of its natural enemy diversity (21 species of parasitoids) and richness of wild species of host plants (175 species, of which only 32 species are exotic), which may indicate a long association between the insect pest, its host plants and natural enemies. However, Pichon *et al*. (2006) reported that there is a large genetic variation among *P. xylostella* populations in different parts of the world, which makes it hard to pin its origins to a single point.

Plutella xylostella is an oligophagous pest, which feeds only on plants in the family Brassicaceae that contains mustard oils and glucosinolates such as canola (*Brassica napus*), mustard (*B. juncea*), cabbage (*B. oleracea* var. *capitata*), Chinese cabbage (*B. rapa. cv. gr pekinesis*), broccoli (*B oleracea* var. *italica*), radish (*Raphanus sativus*), cauliflower (*B. oleracea* var. *botrytis*), kale (*B. oleracea var. alboglaboratoryra*), Brussels sprout (*B. oleracea* var. *gemifera*), collard (*B. oleracea* var. *acephala*), watercress (*N. officinale*) and some wild weedy *Brassica* species such as sond (*R. raphanistrum* L.), roth (*Rorippa micrantha*), yellow rocket (*Barbarea vulgaris*) and jonsell (*Rorippa nudiuscula*) (Talekar and Shelton, 1993; Kahuthia-Gathu, 2008). Glucosinolates are a group of defensive secondary compounds containing nitrogen and sulphur (Fahey *et al*., 2001), and the composition and/or quantities of glucosinolates is specific to each species, and may also differ among accessions of the same species, individuals, different developmental stages and plant parts due to abiotic and biotic environmental factors (Brown *et al*., 2003). When a plant is damaged by an insect herbivore, the glucosinolates come into contact with the plant myrosinase, a β-thioglucosidase. The myrosinase removes the β-glucose moiety from glucosinolates and forms unstable toxic breakdown products called isothiocyanates, nitriles, thiocyanates (Ratzka *et al*., 2002). These compounds act as toxins, growth inhibitors and feeding deterrents to generalist herbivores (Ratzka *et al*., 2002). However, crucifer specialists use glucosinolates and their by-products isothiocyanates as positive cues for host location. Isothiocyanates serve as volatile cues that attract specialist herbivores to their host plants and glucosinolates are responsible for triggering oviposition or feeding after the insect has located and landed on the plant (Renwick and Chew, 1994; Ratzka *et al*., 2002; Renwick *et al*., 2002). Specialist herbivores overcome the toxic glucosinolates and its hydrolysis products by rapid excretion, hydrolysis of the glucosides, inhibition of

hydrolysis, and by sequestering of the glucosinolates (Schoonhoven *et al*., 1998). For example, *P*. *xylostella* uses glucosinolates for host location, oviposition and as a feeding stimulant (Renwick and Chew, 1994). *Plutella xylostella* has glucosinolates sulfatase accumulated in the gut of its larvae which is responsible for converting glucosinolates ingested with plant materials into dusulfo-glucosinolates which prevents the hydrolysis of glucosinolates into toxic isothiocyanates (Figure 1.1) (Ratzka *et al*., 2002). However, this detoxifying mechanism does not seem effective on all potential host plants. For instance, presence of saponin in some varieties of *B. vulgaris* leads to low survival of *P. xylostella*, making them potentially good trap or dead end plants (Shinoda *et al*., 2002; Agerbirk *et al*, 2003).

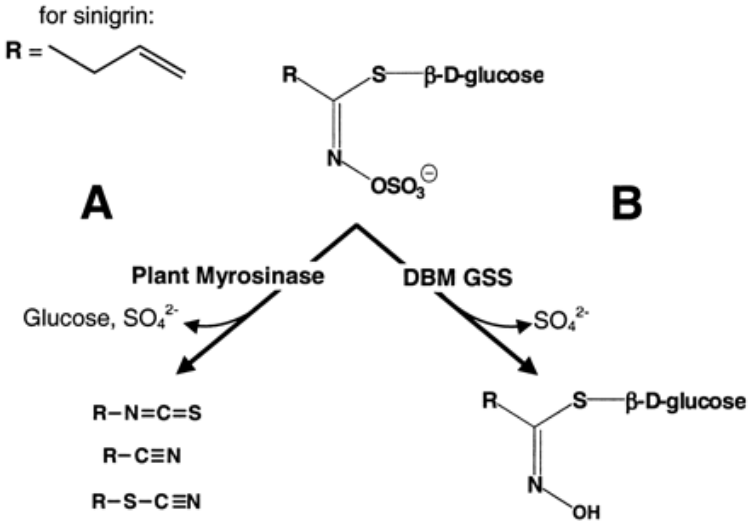

Figure 1.1 Reactions catalyzed by plant myrosinase and diamondback moth Glucosinolate Sulfatase (GSS) *(A)* Myrosinase removes glucose from glucosinolates leading to the formation of toxic hydrolysis products (isothiocyanates, nitriles, thiocyanates, (*Bottom Left*). (*B)* DBM GSS forms dusulfo-glucosinolates (*Bottom right*). (Taken from Ratzka *et al.* (2002) for illustration purposes).

Plutella xylostella damages the leaves of its host plants from the seedlings until harvest period, but only the larval stage is herbivorous (Amit *et al*., 2001). The first instar larvae are very small, and they feed by mining leaves (Plate 1.2 A). Mines are so small that they are usually not noticed at low infestation levels. The larvae emerge from the mines to feed on the underside of the leaf. From the 2nd instar through 3rd and 4th instars, the larvae feed on the leaf surface (Nofemela, 2013a). The larvae strip away the bottom side of leaf between major veins except for the upper epidermis, which results in a characteristic window-like damage. The window-like areas soon turn into irregular holes as the leaves grow and expand (Plate 1.2 B). Serious economic damage is caused by high numbers of larvae per plant. When *P. xylostella* larvae feed on small cabbages or under the loose protective leaves, a major yield loss is expected because such plants do not produce economically viable heads (Plate 1.2.C).

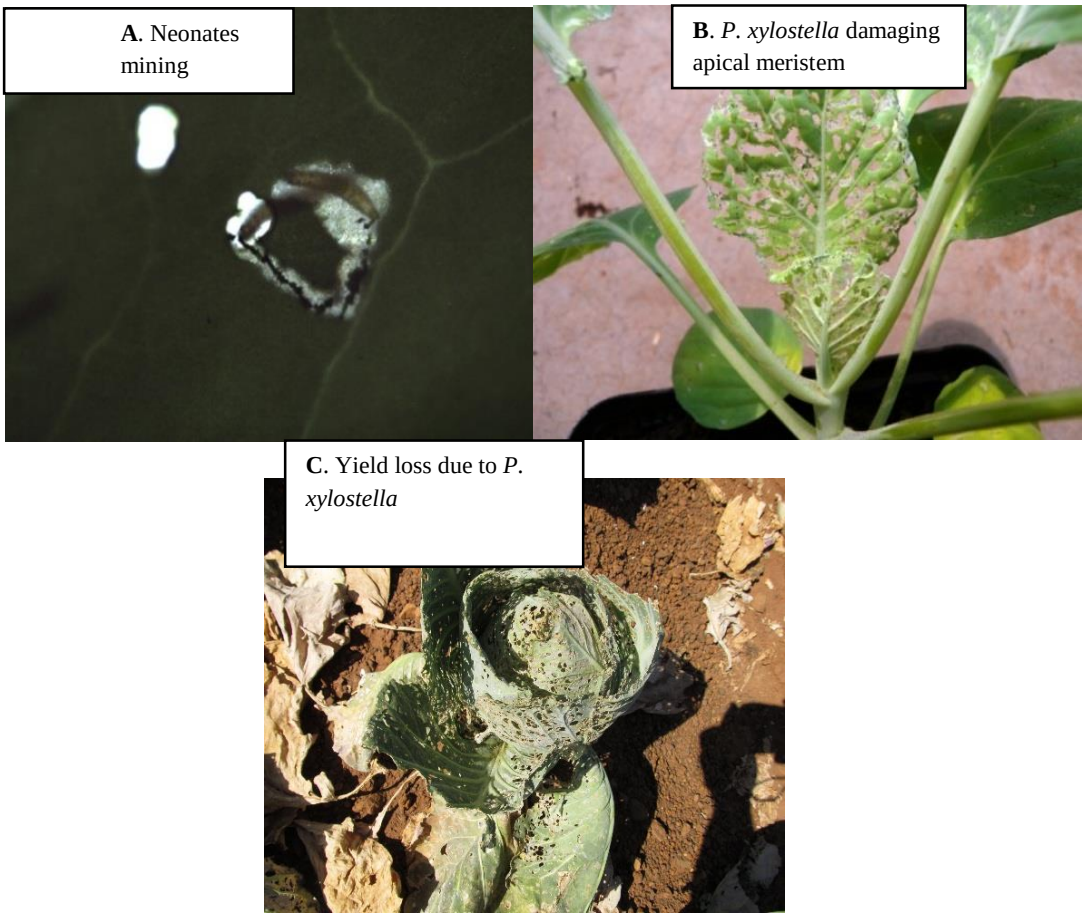

Plate 1.2 Characteristic *Plutella xylostella* damage on a cabbage plant © Nethononda P.D. 10/07/2013

1.3.2.1 The life cycle of *Plutella xylostella*

Plutella xylostella has a holometabolous life cycle (Plate. 1.3), which consist of egg, larval (four instars), pupal and adult stages. The moths are small (about 7 to 8 mm long), slender, and greyish to brown in colour with pronounced antennae which are always held perfectly straight forward when the moth is at rest (Talekar and Shelton, 1993). *Plutella xylostella* moth has a broad cream or light brown band along the back. The wings form a paler upper area and a darker lower area. The band is sometimes constricted to form light-coloured diamonds on the back, hence its common name – the diamondback moth (Gunn, 1917; Ullyett, 1947). When viewed from the side, the tips of the wings can be seen to turn

upward slightly (Gunn, 1917). The moths have a high reproductive potential, and each female can lay up to 300 eggs during its short lifespan of 14 days in summer and 18 to 21 days in winter (Gunn, 1917; Ullyett, 1947).

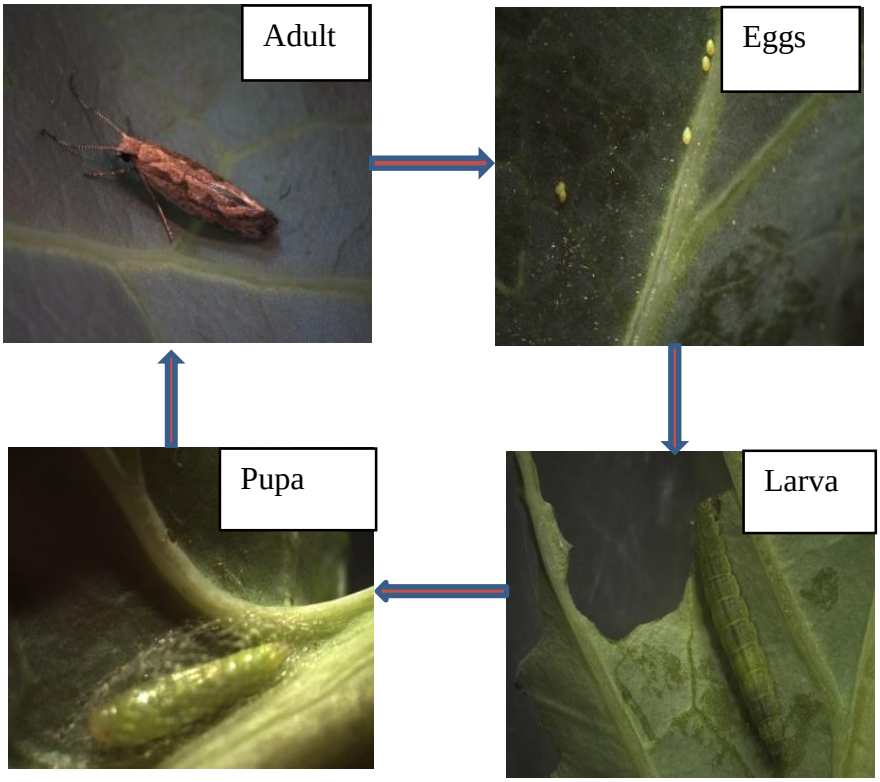

Plate 1.3 Life cycle of *Plutella xylostella* © Nethononda P.D. 12/09/2013

The eggs are laid singly or in small clusters of 2 to 8 along the midrib (Talekar and Shelton, 1993). Eggs are laid on the upper and lower leaf surfaces, stems and petioles (Talekar and Shelton, 1993). The eggs are oval, flattened and yellow to pale green in colour. The eggs darken before they hatch (Ho, 1965). It takes about 3 days for eggs to hatch during warm weather, whereas they take 4 to 5 days to hatch in cold weather (Gunn, 1917). The neonates are translucent, very tiny (<0.5 mm) and they feed on the spongy mesophyll by mining into the leaves (Ullyett, 1947). After 2 to 4 days, they emerge from

the mines to feed on the surface and moult. The fully grown fourth instar larvae are about 12 mm long (Ullyett, 1947). The larvae produce a silken thread that they use to hang on from the plant when disturbed. The larval stage takes approximately 9 to 22 days to complete at temperatures above 25 ºC and when food is not a limiting factor (Smith, 2004). On completion of the larval stages, they spin a loose silken cocoon in which they pupate. Pupation occurs on the underside of leaves or the stem near the ground (Ullyett, 1947), and the pupae are 7 to 8 mm long (Talekar and Shelton, 1993). The pupa changes in colour as it ages from green, yellowish then brownish colour before the adult moth appears 5 to 15 days later depending on ambient temperature (Ullyett, 1947).

1.3.3 The parasitoid

Cotesia vestalis (Haliday 1834) (Hymenoptera: Braconidae) is a solitary, koinobiont, larval endoparasitoid of *P. xylostella* that is widely used in biological control programmes against this pest worldwide (Shaw, 2003; Nofemela and Kfir, 2008). It is believed that *C. vestalis* originated from Europe and later spread throughout the world with its host, *P. xylostella* (Lim, 1982; Waterhouse and Norris, 1987; Talekar and Shelton, 1993). Besides some morphological differences between populations (Rincon *et al*., 2002), it was also established that populations from different far-flung regions are reproductively isolated (Rincon *et al.*, 2006). This partly explains very variable level of suppression that *C. vestalis* exerts on *P. xylostella* throughout its range (Rincon *et al.*, 2006; Nofemela and Kfir, 2008). In South Africa, it is the most efficient species often accounting for more than 80 % of total parasitism levels of *P. xylostella* (Nofemela and Kfir, 2005, 2008; Nofemela, 2013b; Bopape *et al*., 2014).

1.3.3.1 The life cycle of *Cotesia vestalis*

Cotesia vestalis has a holometabolous life cycles that includes egg, larval (three instars), pupal and adult stages (Yu *et al*., 2008). It develops inside a host larva that continues to grow and feed after being parasitized, and it is a koinobiont (Yu *et al*., 2008). Although *C. vestalis* can parasitizes all *P. xylostella* instars, it has a high preference for 2nd and 3rd instar larvae (Talekar and Yang, 1991; Shi *et al.*, 2002; Nofemela, 2004).

Egg. Female *C. vestalis* can lay up to 42.13 ± 12.2 (mean ± SD) eggs over a life span of 5.23 ± 2.7 days with the majority of eggs laid within the first two days of eclosion (Nofemela, 2004). The eggs are spindle shaped and transparent, with a narrow pedicel at the end (Yu *et al*., 2008). The newly laid eggs adhere firmly to the host tissue, mainly the gut (Yu *et al*., 2008). The developing embryo has three membrane layers. A group of cells grow from both anterior and posterior poles of the egg and spread all over the embryo surface to form a complete outer membrane called serosal membrane (Yu *et al*., 2008). The serosal membrane is comprised of a syncytium in the abdominal region and discrete polar cells which forms teratocytes (Yu *et al*., 2008). Teratocytes helps in absorption of nutrients and production of proteins that are stored inside the cells or released into the host haemolymph (Firlej *et al*., 2007). Teratocytes growth pattern is a physiological factor which reflect the suitability of the host, as their number or absence may result in insufficient food supply for the developing parasitoid which is reflected by prolonged parasitoid development (Firlej *et al*., 2007). Studies have shown that reduced successful parasitism is caused by a reduced number of teratocytes during the first day of parasitoid larval development (Alleyne *et al*., 2001; Barratt and Sutherland, 2001)

First instar. It takes about two days for an egg to hatch into a 1st instar larva at 25 ± 1 °C (Yu *et al*., 2008). During hatching, the chorion raptures and the discrete polar teratocytes dissociate into host's haemolymph (Yu *et al*., 2008). The 1st instar larva is

transparent and caudate-mandibulate with a large head, and is about 0.07 ± 0.01 mm long (Yu *et al*., 2008). On the first day of hatching, the segmentation of the larval body is invisible because the serosal membrane is still attached (Yu *et al*., 2008). On the 2nd day, 13 segments which include, 3 thoracic and 10 on the abdomen become visible (Alizadeh *et al*., 2011). It takes about 3.25 ± 0.05 days to complete the first stadium at 25 ± 1ºC (Alizadeh *et al*., 2011).

Second instar. The 2nd instar larva is referred to as a vesiculate form because its horn tail in the first instar is replaced by vesiculate structure (anal vesicle) which is attached to the midgut with a visible constriction (Alizadeh *et al*., 2011). The 2nd instar larva is transparent with visible segments and gut. The head capsule remains the same size as in the 1st instar. The 2nd instar larva changes colour several times during its development. For instance, after three days it becomes opaque with a cream colour and a light green gut, and after a further two days it becomes yellow, and the 1st segments of the abdomen become dark yellow (Yu *et al*., 2008). The tracheal system is visible at this stage and the anal vesicle begins to shrink back as it moults to become the third instar (Yu *et al*., 2008). It takes about 2.78 ± 0.10 days to complete the 2nd instar stadium at 25 ± 1ºC (Alizadeh *et al*., 2011).

Third instar. The third instar larva is vermiform and yellowish green, with a slightly curved body (Yu *et al*., 2008). This instar is referred to as a hymenopteriform due to its tapered anterior with distinct segmentation and the absence of vesicle and mouthparts (Yu *et al*., 2008). The mature 3rd instar larva is white with a light yellow tint. After nine days of oviposition, the matured larva emerges from the side of 4th instar host larva at 25 ± 1ºC (Yu *et al*., 2008). It takes about 4 to 5 minutes for the parasitoid larva to exit from the host (Yu *et al*., 2008) to form a cocoon (Plate 1.4 B). The host larvae from which it egressed can live for a day or two (Yu *et al*., 2008).

Cocoon. After emergence from the host body, the *C. vestalis* larva immediately spins a creamy-white silken cocoon in which it pupates (Plate 1.4 C).

Pre-pupa. The pre-pupa is the stage between cocoon formation and shedding of the 3rd instar exuvium and it has a primrose head and thorax with an ivory-white abdomen with 13 segments and orange red eyes (Yu *et al*., 2008). Mouth structures such as mandibles, labium, labial palpi and maxillary palpi become visible at this stage. The prepupal stage last for about 1.9 ± 0.006 days at 25 ± 1ºC (Alizadeh *et al*., 2011).

Pupation. After pupation the body become yellow and meconia (undigested food) become clearly visible on the midgut (Yu *et al*., 2008). After two days in the pupal stage, the pupa becomes bright yellow and slightly transparent, two red-dorsal ocelli, legs, wings and antennae become visible. After three days of pupation, the head and thorax becomes black until the whole body become black (Yu *et al*., 2008). The pupal stage last for about 4.03 ± 0.11 days at 25 ± 1ºC (Alizadeh *et al*., 2011).

Adult. The adult emerges 6 days after cocoon formation at 25 ± 1ºC (Yu *et al*., 2008), and is a small wasps of about 3 to 7 mm long with a black body, yellowish colour on the legs and the sternites and tergites (Plate. 1.4 A) (Yu *et al*., 2008). The antennae are black antennae with 16 segments with uniform slender shape (Alizadeh *et al*., 2011). There is sexual dimorphism in body length with females being longer than males (Alizadeh *et al*., 2011).

Plate 1.4 A) Adult *Cotesia vestalis*; B) Parasitoid larva coming out of the *Plutella xylostella* larva; C) *C. vestalis* cocoon next to a dead fourth instar *P. xylostella* larva © Nethononda P.D. 15/06/2013

1.3.3.2 *Cotesia vestalis* feeding and overcoming of host immune defence

The developing larva feeds freely on the host haemolymph, and thus avoids feeding directly on the host vital organs until it is ready to pupate (Nofemela, 2004). The female injects a polydnavirus (PDV), which is a dsDNA virus with a poly-disperse genome which contains a series of different circular DNAs (Turnbull and Webb, 2002), venom and teratocytes in order to overcome its host defences during oviposition (Shi *et al*., 2008). Yu *et al*. (2007) demonstrated that the calyx fluid in *C. vestalis* suppresses the host immune system and its venom has a limited effect on haemocytes and probably synergizes the effects of calyx fluid on *C. vestalis* polydnavirus (CvBV).

1.4 Problem statement

Although several cabbage cultivars are commercially available in South Africa (DAFF, 2014), growers cultivate them without any knowledge on their susceptibility to *P. xylostella.* Cultivars resistant to *P. xylostella* will help growers reduce production costs by reducing insecticide applications and all risks associated with their use. Plant resistance mechanisms such as antibiosis and antixenosis have the ability to reduce pest load on plants and may allow biological control agents such as parasitoids to be more

effective in controlling insect pest (Stout, 2013). Some studies have indicated that plant resistance and biological control can be compatible (Kalule and Wright, 2002; Schmale *et al*., 2003). For an example, Fathi *et al*. (2011) indicated that *P. xylostella* infestation among six canola cultivars (Zarfam, Elite, Okapi, Option 500, Hyola 401 and Opera) was higher in all cultivars except Opera which shows some resistance towards *P. xylostella* and in addition parasitism rate by *Diadegma majale* was highest on that cultivar. However, others have shown that host plants can negatively affect parasitoid fitness (Gassmann and Hare, 2005; Simmons and Gurr, 2005). As herbivores obtain their food directly from plants, parasitoids also obtain their nutrition indirectly from the plants and as a consequence, parasitoids may be negatively affected when they encounter allelochemicals from their herbivore host (Bottrell *et al*., 1998). Thus, there is a need to identify optimal interactions between plant resistance and biological control.

1.5 Hypothesis

1. Female moths oviposit more on cabbage cultivars on which fitness of the offspring is highest.
2. The fitness of *Cotesia vestalis* is higher on cabbage cultivars on which performance of *Plutella xylostella* is optimal.

1.6 Aim and objectives of the study

The aim of the study was to investigate the influence of different cultivars on interactions between organisms of the second and third trophic levels in a tritrophic system involving *Brassica oleracea* L. var. *capitata – P. xylostella – C. vestalis*.

Specific objectives of the study are:

- To determine *P. xylostella* fitness on the seven different cabbage cultivars.
- To determine *C. vestalis* fitness when its hosts are reared on different cultivars.

1.7 Thesis outline

After Chapter 1 of introduction, Chapter 2 determines the effect of seven cabbage cultivars on the fitness of *P. xylostella* while chapter 3 determines the indirect effect of the cabbage cultivars on the fitness of *C. vestalis*. Chapter 4 is the general discussion

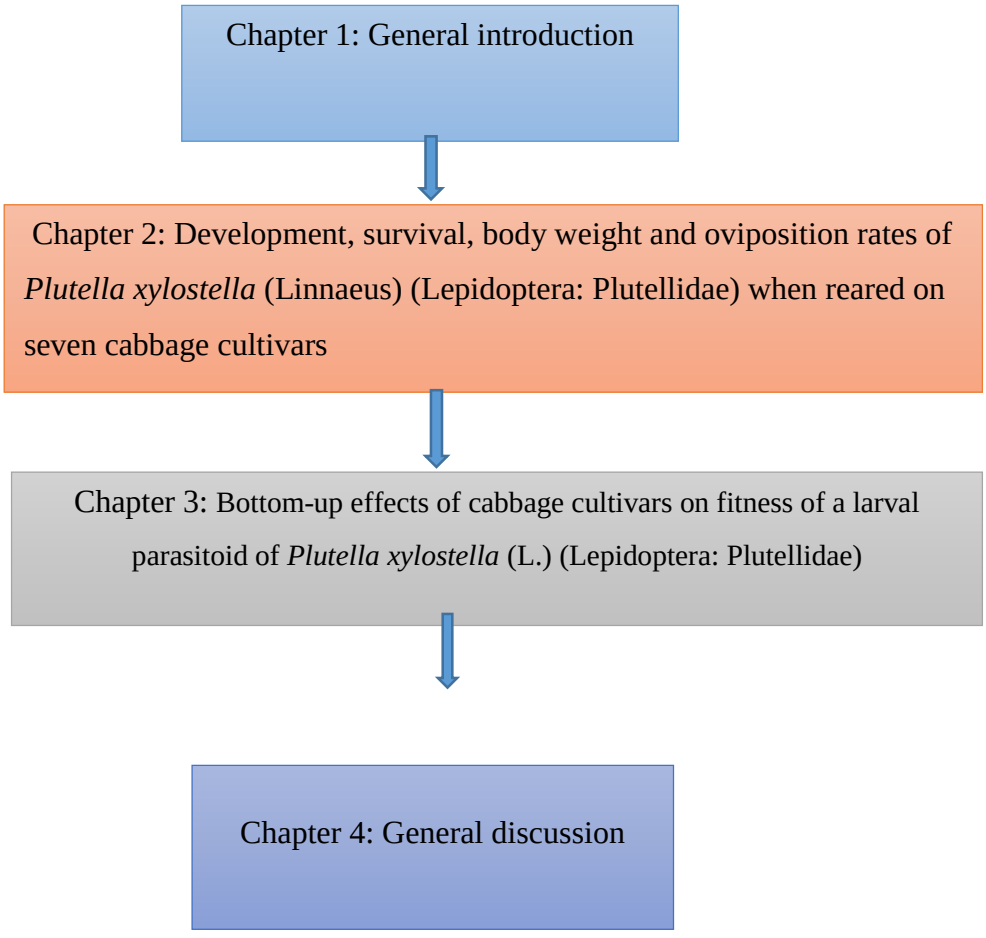

Plate 1.5 Structure of the thesis

Chapter 2: Development, survival, body weight and oviposition rates of *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) when reared on seven cabbage cultivars

2.1 Abstract

Plant cultivars that negatively influence fitness of target phytophagous insects can be an important component of integrated pest management when they substantially restrict population growth of the target pest. In this study, the effects of seven cabbage (*Brassica oleracea* var. *capitata* L.) cultivars on survival and development of immature stages, pupal weights, moth longevity and oviposition rates of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), were evaluated in the laboratory. Under the no choice test, overall survival of *P. xylostella* immature stages was highest on Karabo (67.26 %) and lowest on Megaton (44.92 %). The larval and pupal developmental period, and thus generation time took longer on Empowa (18.48 days), Karabo (14.64 days) and Beverly Hills (17.48 days), while development on Hollywood F1 (13.79 days) was the fastest. Male and female *P. xylostella* pupal weights were lower in larvae that fed on Megaton (4.13 and 4.65 mg), Menzania (4.53 and 4.91 mg), and Hollywood F1 (4.11 and 5.08 mg), whereas pupal weights from Karabo (6.0 and 6.82 mg) were the heaviest. Unfed female moths reared on Beverley Hills lived the longest (5.05 days), whereas those reared on Leano (3.54 days) and Megaton (3.89 days) lived for a shortest period. Under the choice-test, *P. xylostella* moth laid significantly more eggs on Empowa (48.8 %) and Hollywood F1 (45.6 %) and least on Menzania (11.8 %) and Leano (10.6 %). Although these results show differential impact of the cultivars on the fitness parameters studied, low survival rate of offspring on a crop is the primary target for using plant resistance as a pest management tactic. The significant reduction in survival rates, pupal weights, adult

longevity, fecundity and negative impact on developmental period of *P. xylostella* reared on Megaton indicate that this cultivar has higher level of resistance against *P. xylostella*. The results of this study show that Megaton can play a major role in restricting population growth of *P. xylostella* and generational number of eggs deposited on it.

Key words: diamondback moth; oviposition preference; offspring performance; plant resistance; integrated pest management

2.2 Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is an oligophagous pest of plants in the family Brassicaceae (Furlong *et al*., 2013). Since the larvae are forced to feed, and develop if possible, on plants on which they hatch (Löhr and Gathu, 2002; Zalucki *et al*., 2002; Sarfraz *et al*., 2010), *P. xylostella* is a perfect target for investigating the impact of different *Brassica* species and/or cultivars on offspring fitness. Although Brassicaceae are well known for their glucosinolate-myrosinase defensive system which is largely efficient against generalist insect herbivores and microorganisms (Müller and Sieling, 2006), specialist herbivores like *P. xylostella* use glucosinolates as oviposition and feeding stimulants, and their volatile breakdown products for host location (Renwick *et al*., 2006). However, the composition of glucosinolates and/or quantity varies between species, cultivars and organs of the same plant (Harvey *et al.,* 2007a; Martin and Müller, 2007; van Leur *et al*., 2008). High content of glucosinolates (>0.6 μmol/g of fresh weight) and associated myrosinase has been found to be detrimental to *P. xylostella* offspring fitness, which suggests that the counter-adaptation to glucosinolate hydrolysis by the pest may be dose-dependent (Siemens and

Mitchell-Olds, 1996; Li *et al*., 2000; Agerbirk *et al*., 2003). Other studies show that presence of saponin, a feeding deterrent, is a major reason for the low survival of *P. xylostella* on *Barbarea vulgaris* (Shinoda *et al*., 2002; Agerbirk *et al*., 2003). The high attraction to gravid moths and low survival of larvae is observed on several species of wild host plants. As a consequence, several studies have investigated the potential of using wild host plants as trap crops (Charleston and Kfir, 2000; Badenes-Perez *et al*., 2004; Lu *et al*., 2004), but *P. xylostella* can survive fairly well on some species (Kahuthia-Gathu *et al*., 2008). Where the bottom-up effect of a trap crop is sufficiently strong, the cost of controlling the target pest can be substantially reduced (Badenes-Perez *et al*., 2005). However, adoption of trap cropping is often hampered by: 1) unwillingness of growers to cultivate, in limited farmland space, plants they do not have a market for (Khan *et al*., 2007), 2) trap crop harbouring other pests of the main crop (Nofemela, 2008), 3) variable success of the strategy (Shelton and Badenes-Perez, 2006).

Although high or induced glucosinolate and myrosinase content can reduce herbivory and increase plant fitness (Agrawal, 1999), breeding crops for high expression has so far led to reduced crop yield (Stowe and Marquis, 2011). As growers prefer to cultivate crops with better agronomic traits, it is important to investigate among the currently available crops the ones possessing resistance against *P. xylostella* (Hamilton *et al*., 2005). Several studies have shown that *P. xylostella* fitness differs among *Brassica* crops (Golizadeh *et al*., 2009; Niu *et al*., 2013) and even among cultivars (Ebrahimi *et al*., 2008; Soufbaf *et al*., 2010; Fathi *et al*., 2011). The resistance mechanisms range from antibiosis resistance as reflected by lower survival and longer developmental rates of larvae (Golizadeh *et al*., 2009; Soufbaf *et al*., 2010; Fathi *et al*., 2011), antixenosis as reflected by variable oviposition preference (Ebrahimi *et al*., 2008) to plant tolerance (Sarfraz *et al*., 2007). Thus, these studies emphasise the need to further investigate the influence of intrinsic

factors already present in the various *Brassica* crops that make them resistant to *P. xylostella.* Although reduced oviposition can be considered the first line of reducing infestations by a target pest (Hamilton *et al*., 2005), it is unlikely to work in mono-cropping as gravid moths will lay eggs regardless, and thus selection of cultivar with reduced survival of larvae becomes a critical factor to the cost of controlling the pest. This aspect is very important for South Africa where *P. xylostella* enters a population outbreak phase in spring (Nofemela, 2010).

Over 132,600 tons of cabbage (*Brassica oleracea* var. *capitata* L.) and other *Brassica* crops are estimated to have been harvested from 2,314 ha during 2013 in South Africa (FAO STAT, 2015), the bulk of which is consumed mainly by rural and peri-urban communities to supplement the largely maize-based diet. As of November 2014, 51 cabbage cultivars were registered for commercial production in South Africa, excluding red cabbage hybrids (DAFF, 2014). However, no attempt was ever made to investigate the fitness of *P. xylostella* on the different cabbage cultivars in South Africa. In an attempt to provide baseline data on fitness of *P. xylostella* on different cabbage cultivars, the study presented here investigated several life history parameters (developmental time, survival rates of immature stages, pupal weight, and longevity of the moths without food) of the offspring on seven cultivars. The ovipositional preference for either of the seven cultivars by the moths was also investigated. The results of this study are particularly useful in identifying cultivars that on which *P. xylostella* performs badly with the view of recommending those cultivars for integrated pest management.

2.3 Material and methods

2.3.1 Insect culture

The larvae and pupae of *P. xylostella* were originally collected from cabbage fields at Brits Agricultural Research Station (23°25'33"S, 27°76'67"E, altitude 1,102 m), North-West Province, South Africa (Nofemela 2004), and at Baviaanspoort Correctional Services Centre (25º 38'S 28º 30'E, alt. 1164 m) in Pretoria, Gauteng Province, South Africa (Bopape *et al*., 2014). The moth culture was reared on week old *Brassica napus* L. seedlings following the method described in Nofemela (2004) in rearing cages (43L×30B×33H cm) made of wood, glass and gauze. At monthly intervals, the culture was bolstered with field-collected individuals.

2.3.2 Host plants

Seven cabbage cultivars: Empowa, Beverley Hills, Karabo, Leano, Hollywood Fl, Megaton and Menzania, were used for this study. Each cultivar was raised from seeds in a greenhouse, and the seedlings were transplanted into 12.5 cm plastic pots filled with a mixture of compost, soil and vermiculite at 2:1:1 ratio at the four- leaf stage. The plants were then taken to the laboratory at six- to eight-leaf stage to conduct experiments. The plants were taken care of by irrigating, removing weeds and physical control of insect pests when necessary.

2.3.3 Experiments

All experiments were conducted in climate-controlled rooms maintained at 22 ± 1 °C (mean ± SD), 60 ± 5 % RH and 16L: 8D photoperiod at Agricultural Research Council – Plant Protection Research Institute, Rietondale Research station, Pretoria, South Africa.

2.3.3.1. No-choice test

One plant from each cultivar was placed singly in a cage made from a 3.7 litre transparent rectangular plastic container with large holes cut on opposite sides and replaced with gauze for ventilation. The lids of the containers were cut to fit the mouth of the 12.5 cm plastic pot with a cabbage plant, and the container was then inverted over the plant. Each of the seven cabbage cultivars had 5 replicates. Two pairs of newly emerged (<16 hours old) male and female moths from the culture that were mating were placed in each cage with a plant. The female moths were allowed to oviposit on the plants for 48 hours.

Thereafter, the moths were removed and the numbers of eggs laid on each plant were recorded with the aid of a magnifying glass. Hatching of the eggs was monitored every day at 09h00 and 15h00. As neonate larvae mine the leaves on hatching (Ullyett, 1947), the number of first instar larvae was recorded as they leave the mines to feed on the leaf surface. Thus, the duration of the first instar stadium was determined as the difference between larval appearance on the leaf surface and the day of egg hatching. The durations of development in each of the subsequent larval instars was also monitored every day on each plant. The duration of pupal stage was determined as the difference from the day a larva forms pupa and the day of moth emergence.

The survival rates of the all larval instars were recorded. The number of pupae that were formed on each plant was recorded, as well as duration of the pupal stage. Survival of pupae, as determined by proportion of *P. xylostella* moths that emerged out of the total number of pupae, was also recorded. Pupal weights were measured using a Sartorius GMBH Supermicro® scale (Sartorius GMBH, Gottingen, Germany). On emergence, the moths were separated based on gender and placed individually in Petri dishes. The days

lived by each moth were recorded at 09h00 and 15h00 to determine longevity of unfed adults. Identification of gender in the pupal stage is difficult, therefore males and females were separated at 4th larval stage. The male larva have a clearly visible white dot on the 5th abdominal segment from the caudal end, whereas females have none (Liu and Tabashnik, 1997).

2.3.3.2. Choice test

The ovipositional preference of the moths was tested by placing all seven cabbage cultivars in a large (27L×27B×40H cm) gauze-covered cage. This experiment also enables one to establish if female moths choose to oviposit mainly on cabbage cultivars that provide the highest fitness gain for the progeny as determined from the no-choice test. Four pairs of mating moths from the culture were introduced into the cage and the females were allowed to oviposit on the plants for 48 hours. Thereafter, the plants were removed from the cage and the numbers of eggs laid on each plant were recorded with the aid of a magnifying glass. The cages were cleaned with soap and water after each trial, and thereafter left in the sun to dry. The position of each cultivar in the cage was randomised in each of the 5 replicates.

2.4 Data analysis

The data on duration of development, survival rates, generation time, pupal weights, longevity, and oviposition rates were subjected to a one-way analysis of variance (ANOVA). Prior to ANOVA, Shapiro-Wilk test was performed on the standardised residuals to test for deviations from normality. In cases where there was significant deviation from normality, the outliers were removed until the data set was normally

distributed. Where significant differences were detected with ANOVA, the means were separated using Student's t-Least Significant Difference (LSD) test. All data analyses were performed at 5 % level of significance (SAS, 1999).

2.5 Results

2.5.1. Duration of development

There was a significant difference ($F_{6, 441} = 3.33$; $P = 0.0157$) in the durations of development of 1st instars (Table 2.1) as they fed on the different host plant cultivars. The duration of the 1st instar was shortest on Hollywood F1 and longest on Beverley Hills (Table 2.1). There were no significant differences in the durations of development among 2nd instar ($F_{6, 434} = 1.13$; $P = 0.377$) and 3rd instar ($F_{6, 415} = 1.33$; $P = 0.2839$) larvae on the different cabbage cultivars (Table 2.1). The duration of the 4th instar was significantly different ($F_{6, 348} = 2.56$; $P = 0.0461$) among the cultivars. The shortest period was observed on Karabo, Megaton, Hollywood F1, Menzania and the longest developmental time was on Empowa (Table 2.1). The developmental rates of pupae were significantly different ($F_{6, 248} = 12.46$; $P<0.001$) among the seven cabbage cultivars (Table 2.1). The shortest development was observed on Leano and the longest on Beverley Hills, Empowa, Karabo and Menzania (Table 2.1). The generational times (i.e., duration of egg-adult development) of *P. xylostella* were significantly different ($F_{6, 248} = 11.81$; $P<0.001$) among the cabbage cultivars (Table 2.1). The generation time was shortest on Hollywood F1 and longest on Empowa (Table 2.1).

Table 2.1 The durations of development (days) of *Plutella xylostella* life stages (mean ± SD) when reared on seven cabbage cultivars in the laboratory.

Cultivar	1st instar	2nd instar	3rd instar	4th instar	Pupa	Generational time
Beverley Hills	4.27±1.23a	2.0±0.77 a	1.91±0.78a	3.18±1.19ab	5.90±1.27a	17.48±1.36ab
Empowa	3.99±1.00ab	1.49±0.42a	1.47±0.43a	3.62±1.22a	5.80±0.96a	18.48±1.38a
Karabo	3.52± 0.38ab	1.54±0.33a	1.47±0.40a	2.60±0.84b	5.43±0.58a	17.64± 0.81ab
Megaton	3.24±1.06abc	1.53±0.62a	2.11±0.40a	2.87±1.17b	3.81±0.81bc	16.78±0.66bc
Menzania	3.22± 0.79abc	1.52±0.52a	1.93±0.71a	2.72±1.26b	5.17±1.44a	16.81±2.16bc
Leano	3.06±0.75bc	1.84±0.75a	1.78±0.78a	3.27±1.24ab	3.33±0.50c	15.43±0.72c
Hollywood F1	2.17± 0.53c	1.29±0.31a	1.47±0.34a	2.63±0.63b	4.25±0.91b	13.80±1.03d

Means with the same letters are not significantly different at $P = 0.05$ Student's t-LSD test.

2.5.2 Pupal weights

There was a significant difference ($F_{1, 523}$ =59.18; P<0.001) in pupal weights of male (5.13 ± 1.12 mg) (mean ± SD) and females (5.71 ± 1.2 mg) from the various cultivars (Table 2.2). The male pupal weights were also significantly different ($F_{6, 253}$ =30.29; P<0.001) among the cultivars (Table 2.2). The pupal weight was significantly higher on Karabo than the rest of cultivars. The second heaviest pupae were obtained from Beverley Hills, but did not differ significantly from Empowa and Leano. The lowest weight came from Hollywood, Menzania and Megaton (Table 2.2).

Table 2.2 Pupal weights (mg) (mean ± SD) of *P. xylostella* reared on different cabbage cultivars.

Cultivar	Male	Female
Karabo	6.0 ± 0.88a	6.82 ± 0.98a
Beverley Hills	5.88 ±1.01ab	6.09 ± 1.22b
Empowa	5.46 ± 0.81b	6.07 ± 0.88b
Leano	4.87 ± 0.76c	6.0 ± 0.88b
Hollywood F1	4.11 ± 0.7d	5.08 ± 1.06c
Menzania	4.53 ± 0.61cd	4.91 ± 0.79c
Megaton	4.13 ± 1.29d	4.65 ± 0.99c

Means with the same letters are not significantly different at P =0.05 Student's t-LSD test.

Like with males, the female pupal weights also differed significantly ($F_{6, 269}$ =24.92; P<0.001) among the seven cabbage cultivars (Table 2.2). Female pupae that developed on Karabo were significantly heavier than those developed on all other cultivars. Again the second highest pupal weights occurred on Beverley Hills but did not vary significantly from Empowa and Leano. The lowest pupal weights came from Hollywood F1, Menzania and Megaton (Table 2.2).

2.5.3 Longevity without food

The longevity of unfed male *P. xylostella* moths was significantly different ($F_{6, 139}$ = 10.76; P<0.001) among the cabbage cultivars (Table 2.3). Male *P. xylostella* moths reared on Beverley Hills had the longest lifespan and those that developed on Leano had the shortest lifespan (Table 2.3).

The longevity of female *P. xylostella* moths was significantly different ($F_{6, 139}$ = 15.47; P<0.001 from the different cabbage cultivars (Table 2.3). Females that developed on Beverley Hills had a longer lifespan without food whereas those that developed on Leano had the shortest life span (Table 2.3).

Table 2.3 Longevity (days) of unfed adult *P. xylostella* when reared on seven cabbage cultivars.

Cultivar	Male	Female
Beverley Hills	4.56 ± 0.89a	5.05 ± 0.69a
Empowa	4.36 ± 0.61ab	4.55 ± 0.71bc
Menzania	3.96 ± 0.52bc	4.65 ± 0.54b
Karabo	3.93 ± 0.78c	4.2 ± 0.70cd
Megaton	3.74 ± 0.72c	3.89 ± 0.45de
Hollywood F1	3.61 ± 0.59c	3.99 ± 0.624d
Leano	3.11 ± 0.34d	3.54 ± 0.22e

Means with the same letters are not significantly different at P = 0.05 Student's t-LSD test.

2.5.4 Oviposition preference

The numbers of eggs deposited on the cabbage cultivars was significantly different ($F_{6, 34}$ = 2.68; P = 0.0391) (Figure 2.1). *Plutella xylostella* moths laid most eggs on Empowa and the least on Menzania and Leano (Figure 2.1).

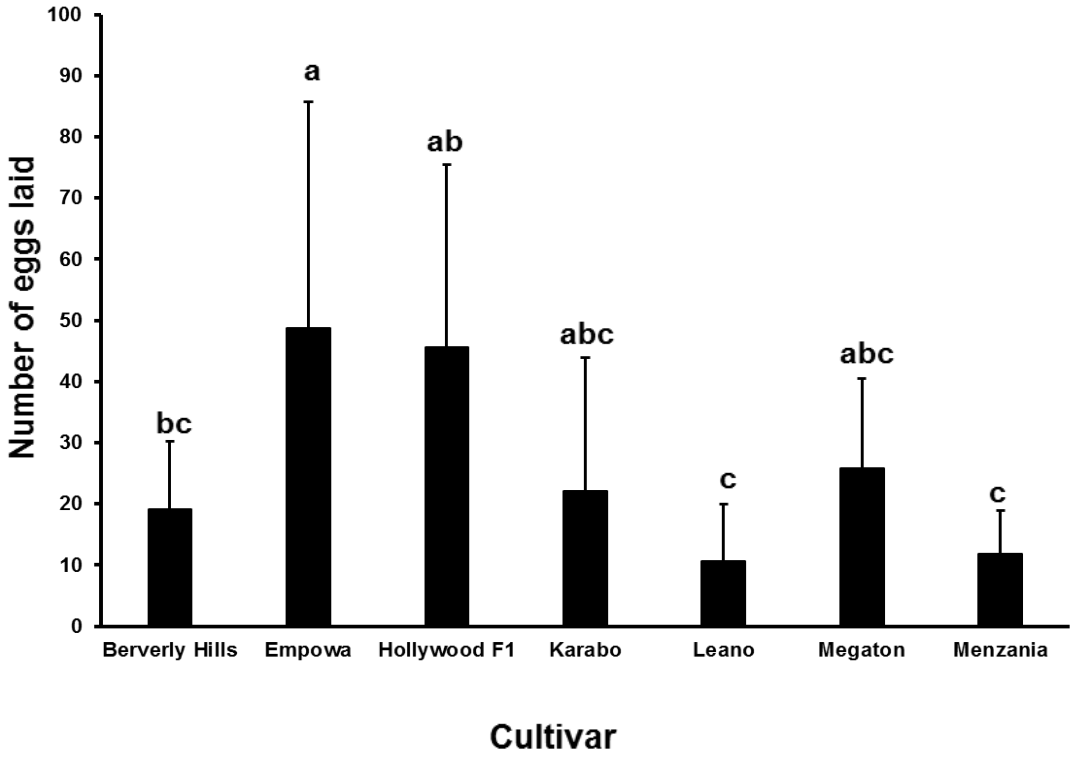

Figure 2.1 Oviposition preference of *Plutella xylostella* among seven cabbage cultivars. Means with the same letter are not significantly different at $P = 0.05$ Student's t-LSD test.

2.5.5 Survival rates

As 1st instar larvae leave the mines in order to moult into 2nd instar (Ullyett 1947), for practical purposes, the starting point for investigating survival rates of larvae on the different cabbage cultivars is the 1st instar larvae that had left the mines. There was no significant difference ($F_{6, 34} = 0.80$; $P = 0.5795$) in the survival rates of 2nd instar larvae among the cabbage cultivars (Table 2.4). There was 100% survival rate through the 2nd instar for larvae that developed on Beverley Hills, Megaton, Hollywood F1, Karabo and Leano, whereas 98.88% were still alive on Empowa and Menzania at the end of the 2nd instar (Table 2.4). The survival rates of the larvae through the 3rd instar were significantly different ($F_{6, 34} = 4.21$; $P = 0.005$) among the cabbage cultivars (Table 2.4). The highest survival rate of 3rd instars was observed on Beverly Hills, Empowa, Hollywood F1, Karabo and Leano, and survival was lowest on Megaton (Table 2.4). The survival rates

of the 4th instar larvae on the different cabbage cultivars were significantly different ($F_{6, 34}$= 2.64; *P* = 0.0414). The highest survival rate was observed on *P. xylostella* that developed on Empowa, Hollywood F1, Karabo and Leano and the least on Megaton (Table 2.4). There was no significant difference ($F_{6, 24}$ = 1.98; *P* = 0.1079) in survival rates of *P. xylostella* pupae on the different cabbage cultivars. Overall survival of *P. xylostella* immature stages was highest on Karabo (67.26%) and lowest on Megaton (44.92%) (Table 2.4).

Table 2.4 Survival rates (%) of *P. xylostella* immature stages on the different cabbage cultivars.

Cultivar	2nd instar	3rd instar	4th instar	Pupa
Beverley Hills	100 ± 0.00a	100±0.00a	81.46 ± 16.38ab	50.48 ± 35.97a
Empowa	98.88 ± 2.5a	98.88 ± 2.5a	93.88 ± 10.83a	83.22 ± 16.01a
Hollywood F1	100 ± 0.00a	96.66 ± 7.47a	90 ± 14.9a	88.66 ± 14.05a
Karabo	100 ± 0.00a	95.52 ± 8.74a	95.04 ± 8.67a	95.04 ± 8.66a
Leano	100 ± 0.00a	100 ± 0.00a	96 ± 8.94a	80.84 ± 34.69a
Megaton	100 ± 0.00a	66.30 ± 32.7b	60.96 ± 29.65b	60.96 ± 29.66a
Menzania	98.88 ± 2.5a	98.88 ± 2.5a	85.82 ± 18.84a	85.82 ± 18.84a

Means with the same letters are not significantly different at *P* = 0.05 Student's t-LSD test.

2.6 Discussion

In terms of the preference-performance hypothesis, females are expected to preferentially choose hosts on which the fitness of their offspring will be better (Mayhew,

1997). When given a choice among the seven cultivars, *P. xylostella* laid the most eggs on Empowa and oviposited the least on Leano and Menzania in the present study. Furthermore, the fitness of *P. xylostella* offspring as measured by survival rates of immature stages, their developmental rates, pupal weights and longevity of moths without food differed significantly among cabbage cultivars. Survival rates of offspring are an important parameter in pest management, as they determine expected pest load on the crop. Overall survival of *P. xylostella* immature stages was lowest on Megaton, while survival rates on Empowa, Leano and Menzania were similar. This result indicated that *P. xylostella* oviposited the least number of eggs on Leano and Menzania even though survival rates of offspring were as high on Empowa. In contrast, the survival rates of the offspring were lowest on Megaton overall, despite this cultivar having received the intermediate number of eggs from the female moths. Thus, it is expected that Megaton will have lower infestations in the field due to its lower suitability for development of *P. xylostella.* The inability of *P. xylostella* to accurately choose to oviposit on host plants that support the highest survival of its offspring is widely reported (Lu *et al*., 2004; Sarfraz *et al*., 2006, 2007; Ebrahimi *et al*., 2008; Marchioro and Foerster, 2014). However, Zhang *et al*. (2012) found a positive relationship between oviposition preference and host suitability for offspring development.

As *P. xylostella* is dependent entirely on resources acquired during larval development for egg production, its realised fecundity is correlated with lifespan (Kahuthia-Gathu *et al*., 2008). In the field, *P. xylostella* moths may not have access to adequate carbohydrate resource which in turn may influence their longevity and the number of eggs laid in a lifetime (Winkler *et al*., 2009). If adults do not have access to carbohydrates, they utilise nutrients derived from larval reserves for maintenance (Strand and Casas, 2007). Thus, the amount and quality of nutrients obtained during the larval stage influences longevity

in cases where moths do not have access to supplemental food. In the present study, longevity of unfed female moths was longer on Beverly Hills and shortest on Leano and Megaton. Although longevity of unfed male moths on Empowa was similar to Beverly Hills, female longevity was significantly shorter on Empowa. Reduced adult longevity is known to have an additional effect on population density (Asaro and Berisford, 2001) in addition to survival rates of immature stages (Nofemela, 2010). As far as results on female longevity show, lower incidence of *P. xylostella* on Megaton is expected, as survival of immature stages was also lower.

For many insects, positive relationships between adult longevity, fecundity and body size was established (Tammaru *et al.*, 2002) including *P. xylostella* (Sarfraz *et al.*, 2007; Kahuthia-Gathu *et al.*, 2008). In many instances, the weight of pupae is used as a proxy for adult body size (Tammaru *et al.*, 2002). In this study, the pupae from larvae that fed on Megaton, Menzania and Hollywood F1 were the smallest, whereas pupae from Karabo were the heaviest. Thus, the low lifespan of the moths from Megaton is positively related to the low weight of pupae.

As smaller individuals are more prone to faster depletion of nutrient reserves in periods of starvation than larger individuals (Gotthard, 2001; Stoks *et al.*, 2006), there is benefit for being large. However, it may require a longer period of development to achieve a large body size (Heisswolf *et al.*, 2005). This can be a fitness cost for species that suffer heavy mortality from natural enemies during the immature stages (Williams, 1999). Parasitoids are an important natural mortality factor of *P. xylostella* larvae and pupae during November–May in South Africa, and longer developmental time will extend exposure of hosts to these natural enemies (Kfir, 1997; Nofemela, 2010). The durations of larval and pupal periods, and thus generation time, took longer on Empowa, Karabo and Beverly Hills. The shorted duration of development was observed on Hollywood F1.

The clear linear relationship between duration of development and pupal weight of *P. xylostella* was observed on Hollywood F1 and Karabo.

In conclusion, the results of this study add to the existing body of knowledge that different host plants or cultivars have a differential impact on fitness of *P. xylostella* (see Verkerk and Wright, 1994; Sarfraz *et al*., 2007). Although the fitness parameters measured for *P. xylostella* in this study are all useful for evaluating cultivar effects on phytophagous insects (Moreau *et al*., 2006), low survival rate of offspring on a crop is the primary target for using plant resistance as a pest management tactic. While plant resistance to *P. xylostella* remains relatively underexplored as a pest control tactic in South Africa, the results of this study shows that Megaton can play a major role in restricting population growth of this pest and generational number of eggs deposited on it. This is especially important for the spring period when *P. xylostella* density is high due to lower efficacy of parasitoids (Nofemela, 2010). The additional fitness costs in terms of reduced body weight of females and their longevity (and fecundity) make Megaton an attractive option for integrated pest management for *P. xylostella*.

Chapter 3: Bottom-up effects of cabbage cultivars on fitness of a larval parasitoid of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

3.1 Abstract

As parasitoid development is dependent on resources provided by a single insect host, the quality and quantity of resources its herbivore host obtains from different host plants can influence parasitoid fitness. Laboratory studies were conducted on several fitness parameters (developmental time, pupal weights, longevity, fecundity and sex ratio) of a larval parasitoid *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae) when its host *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) developed on seven cabbage cultivars. In addition, population increase parameters of the parasitoid were compared on the various cultivars. The generation time of the parasitoid was shortest on Karabo (10.10 days) and Leano (10.38 days), and longest on Megaton (12.57 days) and Empowa (12.80 days). The heaviest *C. vestalis* pupae were recorded from Menzania (5.4 mg), Megaton (5.25 mg) and Beverly Hills (4.85 mg), and the lightest from Karabo (3.8 mg). Parasitoids reared on larvae that fed on Hollywood F1 lived the longest (2.28 days) followed by Menzania (1.94 days) and Beverly Hills (1.8 days), whereas those whose hosts fed on Leano lived the shortest period (0.83 days). Despite the parasitoids from Megaton hosts being bigger, their fecundity and number of female progeny per female (16.87 and 3.60, respectively) were lowest. *Cotesia vestalis* fecundity and daughters produced per female were highest on hosts fed on Menzania (38.00 and 9.13, respectively) and Beverly Hills (32.87 and 9.07, respectively). As a consequence, the net reproductive rate (R_0) and intrinsic rate of increase (r) were higher on Menzania (7.87 and 0.58, respectively) and Beverly Hills (8.29 and 0.62, respectively). Not only do these results suggest that the overall fitness of *C. vestalis* is higher on hosts developing on Menzania and Beverly Hills,

the critical density of the parasitoid required to suppress the host population at a lower average density will be reached quicker on these two cultivars than on others tested.

Key words: diamondback moth, *Cotesia vestalis*, Braconidae, biological control, plant resistance, trophic cascade, integrated pest management

3.2 Introduction

As they are free-living, female parasitoids actively search for host insects in/on which to lay eggs, and the offspring develop by feeding on haemolymph or tissues leading to host death (Harvey and Strand, 2002; Harvey, 2005). As parasitoid development is dependent on resources provided by a single host, parasitoid fitness is constrained by host quality, i.e., quality and quantity of resources available to the developing parasitoid (Godfray, 1994; Bottrell *et al*., 1998; Harvey, 2005). Parasitoid life history traits such as longevity, fecundity, developmental rate and survival rate are often used as currencies to determine overall fitness of parasitoids (Roitberg *et al*., 2001). As herbivorous insects obtain their nutrients from plants, then their parasitoids get their nutrition indirectly from plants (Bottrell *et al*., 1998). The relationship between the three trophic levels (plant-herbivore-parasitoid) is referred to as a tritrophic interaction (Kester and Barbosa, 1991). Several studies have shown that the fitness of parasitoids varies with the range of host plants utilised by their host (Kester and Barbosa, 1991; Bottrell *et al*., 1998; Kruse and Raffa, 1999; Caron *et al*., 2008). The negative bottom-up effects of host plants on parasitoid fitness include low survival rates, small body size, and longer development time of the offspring (Idris and Grafius, 1996; Schuler *et al.*, 2003; Sarfraz *et al.*, 2008; Gols *et al*., 2009; Bukovinszky *et al*., 2012). In some cases, herbivores sequester

secondary plant compounds to reduce their suitability for parasitoid development (Barbosa *et al.*, 1991; Kester and Barbosa, 1991; Gauld *et al*., 1992; Karowe and Schoonhoven, 1992; English–Loeb *et al*., 1993; Harvey *et al*., 2007b). As parasitoids are important natural enemies of insect pests in many agroecosystems and are widely used in biological control programmes, low fitness of (female) parasitoids can reduce ability to regulate the pest population at a lower average density (Sagarra *et al*., 2001; Arakawa *et al*., 2004; Santolamazza-Carbone *et al*., 2014).

For the better part of a year (i.e., November–May) in South Africa, population density of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), on unsprayed cabbage (*Brassica oleracea* var. *capitata* L.) is maintained below 1 *P. xylostella* per plant in the field by the strong top-down impact of parasitoids (Nofemela, 2013c; Bopape *et al*., 2014). The larval endoparasitoid *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae) is the most dominant, often accounting for more than 80 % of total parasitism levels (Nofemela, 2013b; Bopape *et al*., 2014). During September and October, the pest population density increases to high damaging levels due to lower impact of parasitoids during this time (Nofemela 2013a; Bopape, 2013). However, there is a wide variation in peak infestation levels between years on unsprayed crop (Nofemela, 2013a), implying that there may be differences in survival rates of the pest on different cultivars and/or a longer delay in density-dependent response of parasitoids of some cultivars. Recently, it was shown that some cabbage cultivars grown in South Africa have a strong bottom-up effect on *P. xylostella* (Nethononda *et al*., in press). Thus, it is worth investigating if this strong bottom-up effects of host plants on the herbivore are cascaded up the food chain. If true, then the critical threshold density of female parasitoids required to regulate the pest population at a low average density (Murdoch, 1994) is expected to be reached at different time periods on different cabbage cultivars. This study tested the hypothesis that

fitness of *C. vestalis* is higher on cabbage cultivars on which bottom-up effects are weak. The fitness parameters studied were: durations of development, pupal weights, longevity without food, fecundity of the F1 generation, emergence rates and numbers of female progeny in F2 generation. Life tables were constructed to compare population increase of the parasitoid on different cultivars.

3.3 Materials and methods

3.3.1 Study site

All laboratory experiments were conducted in climate-controlled rooms maintained at 22 ± 1 °C (mean ± SD), 60 ± 5 % RH and 16L: 8D photoperiod at the Rietondale campus of the Agricultural Research Council – Plant Protection Research Institute, Pretoria, Gauteng Province, South Africa.

3.3.2 Host plants

Seven cabbage cultivars: Empowa, Beverley Hills, Karabo, Leano, Hollywood Fl, Megaton and Menzania were used for this study. Seeds of each cultivar were planted in seedling trays and raised in a greenhouse. They were transplanted six weeks after germination at four-leaf stage into 12.5 cm plastic pots filled with a mixture of compost, red soil and vermiculite at 2:1:1 ratio. Plants were taken to the laboratory for experiments at six- to eight-leaf stage. There was no fertilizer application.

3.3.3 Insect cultures

Both *P. xylostella* and *C. vestalis* cultures were established from individuals originally collected from cabbage at Baviaanspoort Correctional Services Centre (25º 38'S 28º 30'E, altitude 1,164 m) in Pretoria (Bopape *et al*., 2014). Both *P. xylostella* and *C. vestalis* cultures were bolstered with field collected individuals at monthly intervals in order to reduce effects of inbreeding. *Plutella xylostella* was reared on *Brassica napus* seedlings and *C. vestalis* on 2nd and 3rd instar larvae of *P. xylostella* according to the methods described in Nofemela (2004) in rearing cages (43L×30B×33H cm) made of wood, glass and gauze.

3.3.4 Fitness of *Cotesia vestalis* on host larvae reared on different cabbage cultivars

Experiment 1

One plant from each cultivar was placed in a cage made from a 3.7 litre transparent rectangular plastic container. Four pairs of newly emerged (<24 hours old) *P. xylostella* moths from the stock culture were introduced into each cage to oviposit for 48 hours. *Plutella xylostella* larvae were allowed to feed and develop normally until they reached third instar. Newly emerged (<24 hours old) *C. vestalis* females and males from the stock culture were introduced into glass vials (2.5 × 10 cm) at 2 male: 1 female ratio for 24 hours in order to ensure mating success (Nofemela, 2004). The parasitoids were provided with thin streaks of honey. To maximise parasitism rates of larvae from each cultivar, larvae were individually exposed to a mated female parasitoid in a small cage. Each parasitoid was allowed to parasitize one host from each of the seven cultivars. Nofemela (2004) reported that the parasitoid is regarded as parasitized its host when it inserts its ovipositor into the host larva and wriggles with it for a few seconds, as the larva tries to

escape. Once the parasitism behaviour outlined above was observed, the larva was removed and placed back on the plant it developed on. In total 50 larvae per cultivar were used for this experiment. Egg-pupal, pupal-adult developmental times (and thus generation time) of each parasitoid offspring was recorded. Pupal weights were recorded in batches of three, as individual pupae were not heavy enough to get a reading on the Sartorius GMBH Supermicro® scale (Sartorius GMBH, Gottingen, Germany). Twenty female parasitoids that emerged after the 09h00 observations from each treatment (cultivar) were placed separately in ventilated glass jars. As these parasitoids were not provided with honey, their lifespan was observed every day at 09h00 and at 15h00 to determine longevity without food.

Experiment 2

The F1 generation parasitoids that were provided with honey were used to determine whether fecundity is influenced by the host plant on which their hosts developed on. To ensure that host plant effects do not confound the objective of this experiment, all host larvae used were reared on canola. Fifteen female and 30 male parasitoids that developed on hosts that fed on one of the seven cabbage cultivars were used. To ensure that all females were mated prior to the experiment, each female was confined on the day of emergence with two males in a glass vial for 24 hours. To serve as food, thin steaks of honey were made on the vials. Fifteen cages (43L×30B×33H cm) made of wood, glass and gauze were used for each female parasitoid per cultivar. Thirty five 3rd instar *P. xylostella* larvae were placed on canola seedlings in each cage. A mated female parasitoid was introduced into each of the cages. The seedlings and larvae were removed daily from the cages and replaced with a fresh batch of larvae and canola until the death of the female

parasitoid. The larvae that were exposed to the female parasitoids were reared further on canola until they pupated or parasitoid pupae formed. The number of parasitoid pupae formed, emergence rate and number of female progeny were recorded.

Cotesia vestalis is able to parasitize hosts immediately after emergence, but its daily parasitism rate is limited as it continues to mature eggs post-emergence (Nofemela, 2004). Thus, *C. vestalis* is considered to be weakly 'synovigenic' (Jervis *et al*., 2001). In addition to establishing lifetime fecundity of *C. vestalis* from hosts fed on different cultivars, it is also important to establish if there are any differences in the timing of egg production. One tool that has proven important in this regard is the ovigeny index, defined as the proportion of egg laid on the first day to lifetime fecundity (Jervis *et al*., 2001).

3.3.5 Life table and population increase parameters

To determine which of the seven cultivars is likely to support high densities of *C. vestalis*, life tables were constructed. As population growth of the parasitoid population is dependent on the survival of females at each age class (l_x), and the number of daughters produced per female per age class (m_x), the contribution of each female to the next generation (R_0) is represented by the product of $l_x \times m_x$ (Price, 1997; Núñez-Campero *et al*., 2014). The period over which progeny are produced is represented by $T = \sum(x \times l_x \times m_x)/\sum (l_x \times m_x)$, and the intrinsic rate of increase by $r = log_e R0/T$ (Price, 1997).

3.4 Data analysis

The data on durations of development of immature parasitoids, pupal weights, fecundity and longevity were subjected to a one-way ANOVA to test for cultivar effects. Prior to ANOVA, Shapiro-Wilk test was performed on the standardised residuals to test

for deviations from normality. In cases where there was significant deviation from normality, the outliers were removed until the data set was normally distributed. Where significant differences were detected with ANOVA, the means were separated using student's t-Least Significant Difference test. All data analyses were performed at 5 % level of significance (SAS, 1999).

3.5 Results

3.5.1 Duration of development

The duration of egg–pupal development of immature *C vestalis* was significantly different ($F_{6, 333} = 51.63$; $P<0.001$) on host larvae reared on different cabbage cultivars (Table 3.1). The duration taken to reach the pupal stage was shortest on Karabo (6.17 days) and longest on Empowa (9.15 days) (Table 3.1). The time taken for pupae to reach adult stage was also significantly different ($F_{6, 263} = 8.45$; $P<0.001$) (Table 3.1). Pupal-adult developmental time was shortest on Beverly Hills (3.79 days) and longest on Megaton (4.71 days) (Table 3.1). The generation time (i.e., egg–adult development time) of *C. vestalis* were thus significantly different ($F_{6, 258} = 50.45$; $P<0.001$) when its hosts were reared on different cabbage cultivars (Table 3.1). Egg–adult developmental time was shortest for parasitoids on Karabo (10.10 days) and Leano (10.38 days), and longest on Megaton (12.57 days) and Empowa (12.80 days) (Table 3.1).

Table 3.1 The developmental time (days) of immature *Cotesia vestalis*, pupal weights (mg), longevity (days) of emergent adult parasitoids from host larvae reared on various cabbage cultivars.

Cultivar	Egg to pupa	Pupa to adult	Egg to Adult	Pupal weights	Longevity
Empowa	9.15 ± 1.31a	4.14 ± 0.57b	12.80 ±0 .72a	4.65 ± 0.49b	1.56 ± 0.06c
Megaton	8.36 ± 0.63b	4.71 ± 0.62a	12.57 ± 0.85a	5.25 ± 1.62a	1.49 ± 0.59cd
Hollywood F1	7.66 ± 1.46c	3.92 ± 0.42bc	10.54 ± 1.31c	4.55 ± 0.69b	2.28 ± 0.72a
Beverley Hills	7.22 ± 0.47d	3.79 ± 0.56c	10.98 ± 0.63b	4.85 ± 0.67ab	1.80 ± 0.48bc
Menzania	7.06 ± 1.06d	3.93 ± 0.60bc	10.61 ± 1.27bc	5.4 ± 0.82a	1.94 ± 0.72b
Leano	6.87 ± 0.63d	3.86 ± 0.95bc	10.38 ± 0.80cd	4.5 ± 1.00b	0.83 ± 0.34e
Karabo	6.17 ± 0.63e	4.05 ± 0.43bc	10.10 ± 0.86d	3.8 ± 0.62c	1.23 ± 0.38d

Means with the same letter are not significantly different at $P = 0.05$ Student's t-LSD test.

3.5.2 Pupal weights

There was a significant difference ($F_{6;\ 133} = 6.76$; $P<0.001$) in the weights of the parasitoid pupae from host larvae reared on different cultivars (Table 3.1). The heaviest *C. vestalis* pupae were observed on Menzania (5.4 mg), Megaton (5.25 mg) and Beverly Hills (4.85 mg) and the lightest on Karabo (3.8 mg) (Table 3.1).

3.5.3 Longevity without food

The longevity of unfed female *C. vestalis* reared from *P. xylostella* that fed on different cultivars differed significantly ($F_{6,\ 139} = 16.93$; $P<0.001$) (Table 3.1). Parasitoids reared on larvae that fed on Hollywood F1 lived the longest (2.28 days) followed by Menzania (1.94 days) and Beverly Hills (1.8 days), whereas those whose hosts fed on Leano lived the shortest period (0.83 days) (Table 3.1).

3.5.4 Fecundity

Although parasitoids successfully parasitized hosts throughout their life span, the rate of progeny production was highest on the first day post-emergence, and from then it declined as females aged (Figure 3.1). The number of progeny produced per parasitoid on the first day post-emergence was significantly different ($F_{6,\ 104} = 8.34$; $P<0.001$) (Table 3.2). Parasitoids that developed on hosts fed on Menzania produced the most offspring (17.60), whereas those from Megaton produced the least (7.67) (Table 3.2). The ovigeny index of *C. vestalis* was around 0.5 in most cultivars [Karabo (0.53), Empowa (0.50), Leano (0.48), Menzania (0.46), Megaton (0.46), Hollywood F1 (0.42) and Beverly Hills (0.37)], which indicates that the parasitoids invested more on reproduction on the first day (Table 3.2).

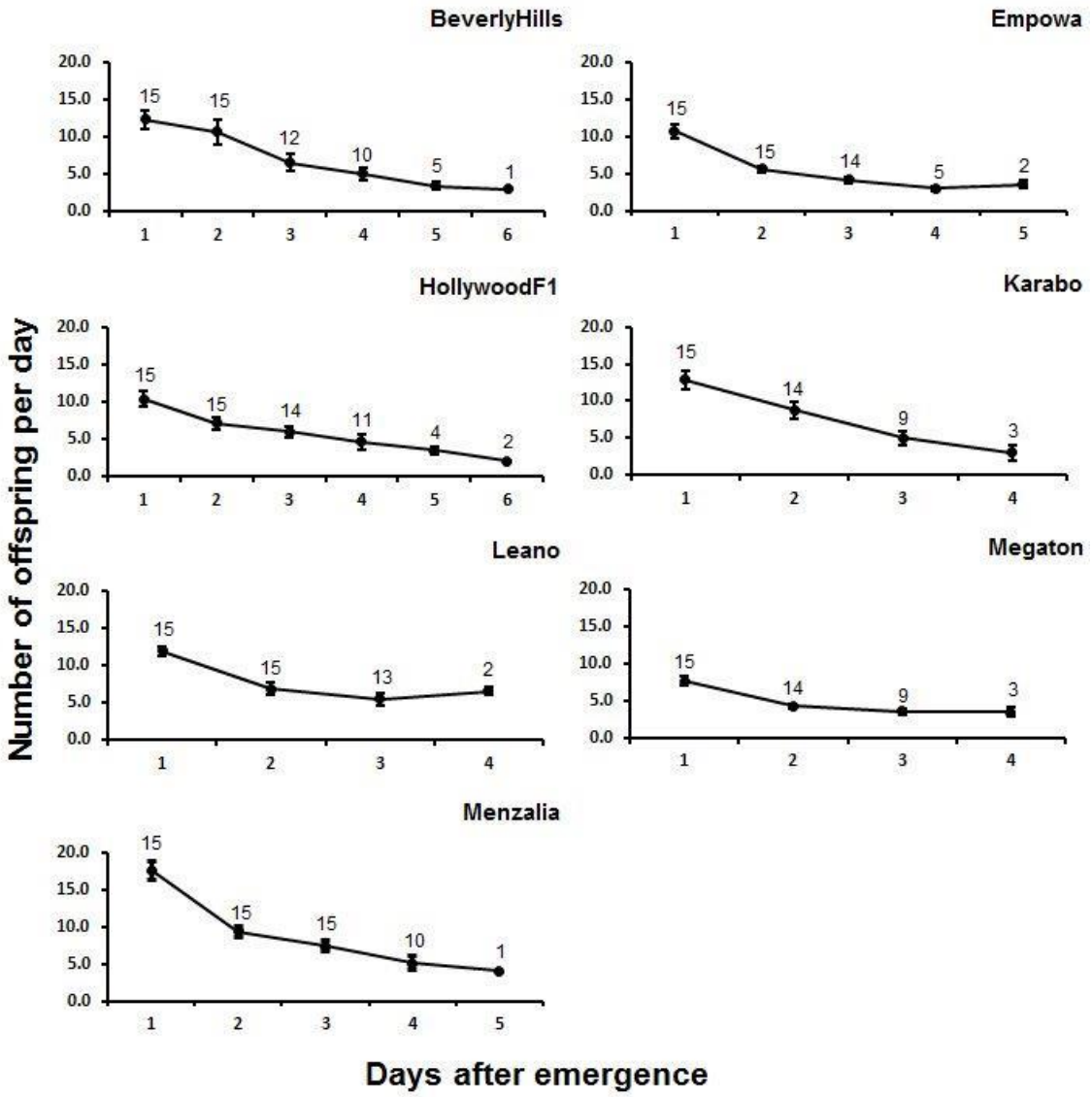

Figure 3.1 Daily rate of offspring production per female *Cotesia vestalis* whose hosts fed on different cultivars. The values in the middle of the graphs indicate the number of female parasitoids that were provided with hosts.

Progeny production on the second day was also significantly different ($F_{6, 102} = 5.51$; $P<0.001$) with parasitoids on Megaton hosts producing the least progeny (4.27) and those from Beverly Hills (10.67), Menzania (9.33) and Karabo (8.77) producing the most (Table 3.2). On the third day, progeny production per female parasitoid remained significantly different ($F_{6, 90} = 3.45$; $P = 0.0043$) (Table 3.2). Parasitoids that developed on Menzania hosts produced the most offspring (7.40), whereas those that developed on

Megaton hosts produced the least (3.53) (Table 3.2). Rate of progeny production on the fourth day declined sharply for all females, as a consequence no significant difference ($F_{6, 45} = 0.85$; $P = 0.5391$) in progeny produced per female was observed (Table 3.2). By the fifth day of the experiment, parasitoids from Megaton, Karabo and Leano hosts were dead (Figure 3.1). However, progeny production among the remaining females was not significantly different ($F_{3, 11} = 0.09$; $P = 0.9624$) (Table 3.2). Only three parasitoids were alive on the sixth day of the experiment, two from Hollywood F1 and one from Beverly Hills producing two and three offspring, respectively. The low number of treatments and the lack of replicates for Beverly Hills prevented performance of statistical analysis. Although the parasitoids reproduced until they died, the longer lifespan of parasitoids from Hollywood F1 and Beverly Hills hosts did not automatically result in high number of total progeny produced. Instead, it is parasitoids from Menzania and Beverly Hills hosts that reproduced significantly during the first three days of the experiment that produced a significantly high ($F_{6; 103} = 6.82$; $P<0.001$) total number of progeny at 38.00 and 32.87, respectively (Figure 3.1, Table 3.2).

Table 3.2 Fecundity of F1 generation *Cotesia vestalis* females that developed on host larvae fed on different cabbage cultivars.

Cultivar	Fecundity						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Total
Menzania	17.60 ± 4.84a	9.33 ± 2.58ab	7.40 ± 3.04a	5.10 ± 2.92a	4.00	*	38.00 ± 10.94a
Empowa	10.73 ± 3.75b	5.60 ± 1.84cd	4.14 ± 1.75cd	3.00 ± 0.71a	3.50 ± 0.71a	*	21.67 ± 8.57b
Leano	11.93 ± 2.12b	6.87 ± 3.42bcd	5.46 ± 2.96abcd	6.50 ± 0.71a	*	*	24.40 ± 9.09b
Megaton	7.67 ± 2.38c	4.27 ± 1.16d	3.53 ± 1.55d	3.50 ± 1.38a	*	*	16.87 ± 6.27b
Karabo	12.87 ± 4.90b	8.77 ± 4.25ab	5.00 ± 2.67bcd	3.00 ± 1.14a	*	*	24.33 ± 10.79b
Hollywood F1	10.40 ± 4.34bc	7.13 ± 3.25bc	6.00 ± 2.77abc	4.64 ± 3.50a	3.50 ± 1.00	2.00 ± 0.00	24.64 ± 11.03b
Beverley Hills	12.33 ± 5.02b	10.67 ± 6.51a	6.50 ± 3.94ab	5.00 ± 2.65a	3.40 ± 1.14a	3.00	32.87 ± 14.78a

Means with the same letter are not significantly different at $P = 0.05$ Student's t-LSD test. The asterisk (*) indicates that all female parasitoids were dead.

3.5.5 Emergence rate

Emergence rates of the F2 generation parasitoids were significantly different ($F_{6; 97}$ = 3.72; P = 0.0023) (Table 3.3). The highest emergence rate was found on the Leano (88.95 %) and Megaton (80.13 %) (Table 3.3).

Table 3.3 Emergence rates (%) and female progeny per female from *Cotesia vestalis* mothers reared on hosts fed different cabbage cultivars.

Cultivar	% Emergence	Number of female progeny
Menzania	73.06 ± 12.85bc	9.13 ± 2.95a
Empowa	75.62 ± 16.45bc	5.47 ± 3.02bc
Leano	88.95 ± 13.38a	6.47 ± 1.41abc
Megaton	80.13 ± 15.29ab	3.60 ± 2.20c
Karabo	67.98 ± 13.02c	5.13 ± 2.77bc
Hollywood F1	69.66 ± 17.25bc	7.40 ± 8.23ab
Beverley Hills	67.82 ± 18.69c	9.07 ± 7.68a

Means with the same letter are not significantly different at P = 0.05 Student's t-LSD test.

3.5.6 Female progeny production

There number of female progeny produced per female parasitoid was significantly different ($F_{6, 104}$ = 2.82; P = 0.0142) when hosts were reared on different cabbage cultivars (Table 3.3). Females that developed on Menzania, Beverly Hills and Hollywood F1 hosts produced more female progeny in their lifetime, whereas those developing on Megaton, Karabo, Empowa and Leano hosts produced the least female progeny (Table 3.3).

Table 3.4 Population increase parameters of F1 generation *Cotesia vestalis* when *Plutella xylostella* larvae were reared on seven different cultivars.

Parameters	Menzania	Empowa	Leano	Megaton	Karabo	Hollywood F1	Beverly Hills
R_0 (Net reproductive rate)	7.87	5.09	5.80	3.28	4.49	6.70	8.29
r (Intrinsic rate of increase)	0.58	0.44	0.47	0.20	0.42	0.48	0.62
T (Mean generation time)	2.07	1.85	1.98	2.17	1.70	2.25	2.00

3.5.7 Population increase parameters

The net reproductive rate of the parasitoid was higher on Beverly Hills (8.29) and Menzania (7.87), whereas it was lowest on Megaton (3.28) (Table 3.4). This is despite the similarity of the mean generation times of the parasitoids on the three cultivars (Table 3.4). The intrinsic rate of increase of *C. vestalis* was much higher on Beverly Hills (0.62) and Menzania (0.58), and it was much lower on Megaton (0.20) (Table 3.4).

3.6 Discussion

The bottom-up effects of host plants on insect herbivores can have a significant influence on biological control of insect pests (Price, 1997; Bottrell *et al*., 1998; Cortesero *et al.*, 2000). As parasitoids are important natural enemies of insect pests in many crop systems (Hawkins *et al.*, 1999), a strong negative impact of a host plant on survival rates of a target herbivore may cause the latter to die before koinobiont parasitoid offspring can complete development (Schuler *et al*., 2003), which in turn may reduce availability of suitable hosts for these natural enemies. As koinobiont parasitoids kill their hosts after several days following parasitism, it is vital to plant crops with some level of resistance to the target herbivore (Brewer *et al*., 1999). However, the majority of studies in this regard have shown that cultivars that reduce consumption rate of herbivore larvae and thus increase their developmental time are ideally suited for integration with parasitoids than those that reduce survival rates of the pest (Verkerk *et al*., 1998; Groot and Dicke, 2002). Recently, it was shown that developmental time of *P. xylostella* is longer when feeding on cabbage cultivars Empowa, Beverly Hills and Karabo (Nethononda *et al.*, in press). While hosts feeding on these cultivars are likely to be exposed for longer period to parasitism, the influence of these cultivars on fitness of the parasitoid is as important

as parasitism rates to biological control. In this study, the developmental time of *C. vestalis* was longer on Empowa and Megaton, whereas parasitoids developing on hosts fed on Karabo and Leano developed faster. Although longer generation time of a parasitoid can lead to generational asynchrony between it and the host population (Godfray *et al*., 1994), the generation time of *C. vestalis* is shorter than that of *P. xylostella* on either cultivar (Nethononda *et al*., in press). While longer developmental time can increase vulnerability to hyperparasitism (Caron *et al*., 2008), there can be trade-off between developmental time and other fitness traits (Harvey, 2005). For example, a linear relationship between developmental time and adult body size has been observed in several species (Harvey, 2005). The longer developmental time of *C. vestalis* on Megaton resulted in heavier pupae, whereas shorter developmental time on Karabo yielded smaller pupae. However, there was no clear relationship between body size and longevity of the parasitoids on any cultivar.

Parasitoids that do not host-feed as adults like *C. vestalis*, are entirely dependent on resources acquired during larval development for egg production. Thus, the quantity and quality of resources obtained during larval stage can greatly influence lifetime reproductive success (Heimpel and Rosenheim, 1998; Lebreton *et al*., 2009). The number of eggs available for oviposition among female *C. vestalis* can be used to measure variation in nutritional status of hosts fed on different cultivars. The fecundity of *C. vestalis* was highest on hosts fed on Menzania and Beverly Hills, and lowest for parasitoids that developed on Megaton hosts. Although parasitoid pupae from Megaton hosts were heavier, their fecundity was lower. As fecundity alone does not determine efficacy of a parasitoid in suppressing a pest population, instead the number of daughters produced per female is vital for maintenance of the critical ratio of the parasitoid to host density. In this study, *C. vestalis* that developed on Menzania and Beverly Hills produced

the most female progeny, whereas those that developed on Megaton hosts produced the least daughters. As a consequence, the intrinsic rate of increase of the parasitoid was highest on Beverly Hills and Menzania hosts, and least on Megaton hosts. These findings support the hypothesis that fitness of *C. vestalis* is higher on cabbage cultivars on which bottom-up effects are weak.

In conclusion, the findings of this study suggest that fitness of *C. vestalis* and thus its performance is influenced by the cabbage cultivar on which *P. xylostella* developed. A lot more hosts were parasitized by *C. vestalis* females from Menzania and Beverly Hills hosts. These parasitoids did not only produce more offspring, but they also produced more daughters per females. As female offspring are only produced from fertilized eggs, the higher number of female progeny implies that the mothers carried more sperm. In addition, the intrinsic rate of increase of *C. vestalis* was higher when host larvae were fed on Menzania and Beverly Hills. When viewed in totality, these results suggest that cultivation of Menzania and Beverly Hills will sustain population density of *C. vestalis* at higher levels, which could result in *P. xylostella* pest population to be regulated at a low average density for longer periods during November – May in South Africa.

Chapter 4: General discussion

Notwithstanding the efficiency of pest control tactics such as biological control (Sarfraz *et al*., 2005; Gichini *et al*., 2008; Nofemela, 2010), and trap cropping (Shelton and Badenes-Perez, 2006), in suppressing crop infestations by *P. xylostella* in some areas, it is increasingly being recognised that its effective management in many areas requires more than one tactic in what is termed integrated pest management (Furlong *et al.*, 2013). Nowhere is this fact clearer than in tropical areas of South East Asia and Hawaii where *P. xylostella* develops resistance to insecticides within two years of application (Zhao *et al*., 2006; Furlong *et al*. 2013). *In October 1998, the United States Department of Agriculture and Environmental Protection Agency issued a joint statement that for integrated pest management (IPM) to be effective practiced, a farm should have a management strategy for "prevention, avoidance, monitoring, and suppression" of pests.* They went on further to say, to qualify as an IPM farm, the grower should have at least 3 of these tactics in place. Prevention refers to removal of pest sources through tillage, crop rotation or crop–free periods. Avoidance makes reference plant resistance, release of natural enemies or use of trap crops. Monitoring refers to regular scouting of the crop to determine the need for another control tactic. Suppression comes in when prevention and avoidance strategies have failed, and it often refers to application of an insecticide. This four-pronged management strategy may have motivated the IPM definition of Ehler and Bottrell (2000) "A comprehensive approach to dealing with pests that strives to reduce pest status to tolerable levels through the use of methods that maximise economic benefits, are environmentally sound and sustainable."

While the term IPM is used widely in the *P. xylostella* literature, Nofemela (2013a) reported that nowhere in the world it is truly practiced, as monitoring of the densities of

the pest and its natural enemies are often not done. The potential of estimating *P. xylostella* infestations through monitoring densities of male moths in synthetic sex pheromone traps was demonstrated, and action threshold of 8.52 moths per trap per week equivalent to 1 immature *P. xylostella* per plant was established for South Africa (Nofemela, 2010). Furthermore, a method of estimating background parasitism levels based on ratios of *C. vestalis* pupae to *P. xylostella* infestations was developed in which 20 % ratio is equivalent to 50 % parasitism level (Nofemela, 2013b). While the development of these methods for monitoring the pest and its natural enemy densities ought to have advanced integrated management of *P. xylostella* in South Africa in terms of insecticide applications, the lack of options besides biological control when it comes to the avoidance strategy has proven to be a stumbling block. For instance, the lower efficiency of the parasitoids during September and October enables the pest enter a population outbreak phase and cause serious economic damage on *Brassica* crops (Mosiane *et al.*, 2003; Nofemela, 2010, Bopape *et al*. 2014). The only viable option was to apply insecticides on a weekly basis in order to protect crop yield. However, this practice has proven detrimental to parasitoid population growth (Furlong *et al*., 2008; Bommarco *et al*., 2011). Even the application of a selective insecticide (*Bacillus thuringiensis* variety *kurstaki*) was found to negatively influence parasitoid diversity (Bopape *et al*., 2014), which is essential for stability of biological control efficiency in the presence of hyperparasitoids (Nofemela, 2013c). Efforts to introduce *Diadegma semiclausum* (Hellén) (Hymenoptera: Ichneumonidae), a parasitoid that is efficient at cool temperatures in order to prevent the population outbreak of the pest during spring have failed, due to lower virulence of the parasitoid on the South African population of *P. xylostella* (R.S. Nofemela, personal communication). Chapter 2 clearly shows that cultivation of Megaton during late winter and spring can play a major role in restricting

the population growth of *P. xylostella* and generational number of eggs deposited on it. Thus, it hoped that this finding can help reduce frequency of insecticide applications during spring while achieving the goal of keeping the pest population density low. However, *C. vestalis* did not perform very well on Megaton, but on Menzania and Beverly Hills (Chapter 3). As parasitoid efficiency is higher during November–May each year (Nofemela, 2010; Bopape *et al*., 2014), the cultivation of Menzania and Beverley Hills cultivars during this period has a potential to enhance biological control. Thus, study has managed to bring in aspects related to bottom-up effects of host plants in relation to integrated management of *P. xylostella* for the first time in South Africa.

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