BASIS OF HOST PLANT RECOGNITION AND ACCEPTANCE BY *BUSSEOLA FUSCA* (FULLER) (LEPIDOPTERA: NOCTUIDAE) LARVAE

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Basis of host plant recognition and acceptance by *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) larvae

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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This thesis has been submitted for examination with our approval as university supervisors.

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DEDICATION

This piece of work is dedicated to my lovely mum, Kalasina, my dear wife Carolyn, my lovely children, Faith and Ken and my late brother Kenneth.

To mum and Kenneth: Thank you for your love, care, endurance and prayers

To Carolyn, Faith and Ken: You're the source of my inspiration.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AC/DC	Alternate current/ Direct current
ANOVA	Analysis of Variance
ARCU	Animal Rearing and Containment Unit (ARCU)
CNRS	National Centre of Scientific Research
Cyt b	Cytochrome b
DIMBOA	4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one
DNA	Deoxyribonucleic Acid
ECB	European corn borer
EDTA	Ethylenediaminetetraacetic acid
FRI	Feeding Response Index
GLM	General Linear Model
HF	Hydrofluoric acid
HCl	Hydrochloric acid
HPLC	High Pressure Liquid Chromatography
ICIPE	International Centre for Insect Physiology and Ecology
ICRAF	International Centre for Research in Agro-Forestry
IRD	Institut de Recherche pour le Développement
KI	Kenyan 1
KII	Kenyan II
L/D	Light/ Dark photoperiod proportion
LS	Lateral Styloconicum
MFO	Microsomal Function Oxidases
MS	Medial Styloconicum

NCPA	Nested Clade Phylogeographical Analysis
PCR	Polymerase Chain Reaction
PTFE	Polytetrafluoroethylene (Teflon)
PLSD	Protected Least Significant Difference.
PVP	Polyvinyl-pyrollidone
RGR	Relative Growth Rate
Rh	Relative humidity
SE	Standard Error
SD	Standard Deviation
SAS	Statistical Analysis Software
SDS	Sodium dodecyl sulphate
Taq	Thermus aquaticus
UV	Ultra-Violet

ABSTRACT

Busseola fusca (Fuller) (Lepidoptera: Noctuidae) is an important pest of maize and sorghum in sub-Saharan Africa whose larvae exhibits oligophagic than polyphagic feeding habits. The host plants for this species are primarily maize and sorghum though some populations appear to be restricted on wild sorghum. The purpose of this study was to determine the extent of oligophagy of larvae of this species based on (i) the sensory abilities to discriminate among different host plants (ii) feeding behaviour and growth on different Poaceae plant species present in its natural habitat (iii) plant stimuli that influence larval growth and feeding and (iv) the physiological adaptations to various host plant diets. The potential existence of genetically determined host-plant associated populations was also investigated. The results obtained from scanning microscopic preparations, selective silver nitrate staining and dose response electrophysiological experiments indicate that larval sensory structures present on the maxillae and the antennae are typical of other lepidopteran species and consist mainly of multiporous olfactory and uniporous gustatory sensilla. These sensorial equipments are thought to be involved in discriminating amongst chemical cues important for larval host recognition and selection. The gustatory role of both sensorial equipments was confirmed from the significant and positive dose-response electrophysiological tip recording tests, for the antennal sensillum ($F_{1,56}$ = 41.637, P<0.0001) and for the maxillary palp ($F_{1,58}$ = 32.124, P<0.0001) using increasing concentrations of sucrose. Moreover, this study demonstrated for the first time the presence of antennal taste receptors on a lepidopteran larva key to host choice, explaining the ability of larvae to quickly evaluate the phago-suitability of the host plant following landing on its surface.

Among the selected Poacaeae plant species used and which form the natural feeding repertoire of *B. fusca*, only *Zea mays* and *Sorghum arundinaceum* supported the highest larval performance. Endogenous silica which is thought to negatively influence feeding behaviour of many herbivorous insects, varied significantly among the plant species studied. The amount of silica found among the plant extracts studied ranged between 20µg/mg-55µg/mg of dry leaf weight following spectrophotometric determination of each of the plant leaf samples digested with dilute hydrofluoric acid. The silica levels in the plants's digests correlated negatively with both larval feeding and growth rates, hence confirming the importance of silica as a significant barrier to dietary adaptation by *B. fusca* larvae. All the polar and methanol-soluble plant extracts tested elicited feeding of third instar larva as compared to the non-polar hexane extracts both in choice and in non-choice bioassays. Methanol extracts of *Z. mays, S. arundinaceum* and *Arundo donax* were however the most phagostimulatory. Plant sugar content as identified by HPLC also varied among plants tested with sucrose contributing the highest (22µg/mg to 65µg/mg of dry leaf weight), which was an equivalent of between 25%-38% of the total sugar content of each plant leaf extract analysed. Larval feeding positively correlated with sucrose content but negatively with turanose, the most variable sugar fraction (5-36µg/mg dry leaf weight) of the plant extracts analysed. However, additional bioassays indicated that whereas turanose and sucrose play a phagodeterrent and phagostimulant roles respectively, a balance between the two (probably in the ratio of 3:1 respectively) appeared to be an important factor in host acceptance for larval feeding. Therefore, the level of silica and the balance between

the two sugars in the plant leaves seem to be key determinants of host plant choice and acceptance for feeding and growth by *B. fusca* larvae.

Larvae fed on *Z. mays* and *S. arundinaceum;* plants that supported the highest larval survival and growth rates had pronounced levels of sugar than amino acid degrading enzymes, indicating the general over reliance of larvae on carbohydrates over proteins for survival. However, esterase enzymes were also highly induced in homogenates of larvae that consumed leaves of their least preferred host plants that included *P. maximum P. desteum* and *S. megaphylla.* These three plant species had also the highest total phenolic content in their leaf tissues following spectrophotometric determination of the same in their leave tissues. Therefore, the induction of esterase in larvae fed on the three high phenolic containing plants could possibly indicate the accompanying physiological responses of larvae to a specific or a number of deterrent phenolic glycosides in the particular plant leaf tissue. Finally, no conclusive evidence was deduced for host associated genetic differentiation among individuals of *B. fusca* larva found on wild plants (*A. donax*) or cultivated crops (*Z. mays*) as was inferred from genetic analysis of two fragments of the gene coding for cytochrome *b.*

CHAPTER ONE

1. 0: INTRODUCTION AND LITERATURE REVIEW

1. 1: General introduction

In sub-Saharan Africa, phytophagous lepidopteran stem borers are among the major field insect pests that cause extensive damage to cereal crops, particularly maize and sorghum (Ingram, 1958; Seshu Reddy, 1991). Yield losses caused by stemborer pests are reported to vary widely among regions depending on the pest population density and phenological stage of the crop at infestation. Estimates of crop losses due to stemborer damage in sub-Saharan Africa varies from 20 to 40% of the potential yield (Seshu Reddy and Walker, 1990; DeGroote, 2002), indicating the importance of phytophagous stem borers as a major limiting factor of cereal crops productivity in the region. Most cereal stem-borers of maize and sorghum are generally polyphagous and have several other cultivated and non-cultivated graminaceous host plants (Khan *et al*., 1991).

Busseola fusca (Fuller) (Lepidoptera: Noctuidae), a phytophagous noctuid moth indigenous and restricted to Africa, is among the most economically important and widely spread stemborer pest of cereals in the sub-Saharan Africa (Harris and Nwanze, 1992; Kfir *et al.,* 2002). Third instars larvae of *B. fusca* always bore and feed inside the stems of host plants (grasses and cereal crops) especially maize and sorghum and therefore cause major yield losses in subsistence cereal production throughout sub-Saharan Africa. The percentage loss of cultivated cereals accruing from damage by *B. fusca* alone is estimated to range between 10 and 39% in

southern Africa (van den Berg and Ebenebe, 2001) and 4-73% in eastern Africa (Seshu Reddy and Walker, 1990).

Larva which is the most destructive stage of this pest initially feeds on host plant leaves but later, during its third instar stage, penetrates the stems and cause extensive damage to the infested plants as a consequence of feeding and tunnelling into the plant stem.

B. fusca is primarily a pest in the dry savannah zone of Africa with its distribution status varying by region. It occurs throughout mainland Africa, south of the Sahara and has been formally recorded in West, East and Southern Africa (Harris & Nwanze, 1992). In West Africa, the species occurs from sea level to an altitude of in excess of 2000 M including the wetter parts of the tree savannah of Ghana (Tams & Bowden, 1953) and Burkina Faso (Nwanze, 1988). In Eastern and southern Africa, *B. fusca* is generally a pest of higher altitudes, occurring at altitudes of between 600 and 2700 M (Sithole, 1989). In Southern Africa, *B. fusca* is the dominant stemborer species at altitudes above 900 M but some populations have been found at a much lower altitudes in the same region, indicating the ability of this species to adapt to lowlands and warmer areas (Sithole, 1989). Similarly, some *B. fusca* populations have been recently recovered from the coastal areas of Kenya and Tanzania contradicting reports that previously indicated the absence of this species in coastal regions (Harris and Nwanze, 1992), but similarly suggesting the ability of the *B. fusca* to spread out in more areas than initially reported.

A Phylogenetic and Nested Clade Phylogeographical (NCPA) genetic analysis has separated *B. fusca* into three geographically isolated population units: one from Western Africa, and two from Eastern and Central Africa (Sezonlin *et al.*, 2006).

These three population units display ecological preferences along altitudinal gradients with the West African population primarily inhabiting the low altitudes of dry savannah zones (Harris and Nwanze, 1992). The East and central Africa populations predominantly inhabit the wet and cold zones of the highlands (Kfir *et al.,* 2002). The intra and inter-population distance observed among the populations however suggest that these three population units were isolated at the same period into three different refuges of sub-Saharan Africa prior to human-mediated ecological changes as a result of the climatic and /or geological events and thus suggest geographical isolation of the species (Sezonlin *et al*., 2006).

Like many other local phytophagous species, *B. fusca* is hypothesized to have coevolved with indigenous wild graminaceous plants of Sub-saharan Africa, before the domestication of sorghum and the introduction of maize into Africa (Ristanovic, 2001). However, from field data, it appears that the species has had a drastic divergence in host colonization; shifting from ancestral grasses to important cultivated staple cereal crops of the region over the last centuries (Sezonlin *et al*., 2006). For example, recent field studies on the species host range indicate that *B. fusca* is localized and specialized on a narrow range of host plant species especially cultivated maize and sorghum (Le Rü *et al*., 2006a, 2006b; Ong'amo *et al*., 2006). Moreover, marginal utilisation of wild poaceous plants has also been documented for the species in some regions. In the wild, a significant number of young larvae have been recovered from wild sorghum, *Sorghum arundinaceum* (Desv.) Stapf, and *Arundo donax* L. in a number of localities in Eritrea, Ethiopia and South Africa. (Le Rü *et al.,* 2006). Anecdotal recoveries have also been made on *Pennisetum purpureum* (Schumach.) and *Cymbopogon nardus* (L.) Rendle, in eastern Africa,

particularly in Kenya and Tanzania as well as on *Setaria megaphylla* (Steud.) T. Duran & Schinz, in Congo in central Africa (Le Rü *et al.* 2006b). These surveys clearly points to the oligophagic feeding habits of *B. fusca* larvae. Moreover, patchy utilisation of different wild grasses may also point to the possible existence of host plant specific strains within *B. fusca* populations (Le Rü *et al*., 2006a, Sezonlin *et al.* 2006), although little information is available regarding the performance traits of the wild populations on the associated wild grasses to corroborate this hypothesis.

The mechanisms of host plant selection and recognition for oviposition sites have been studied for the adults of *B. fusca* (Juma, 2005), but similar information on larval feeding behaviour has largely been unexplored. For *B. fusca* larvae, the recognition and subsequent choice of host plant for feeding can be hypothesised to partially depend on host plant chemical composition and / or physical characteristics. Nevertheless, morphological differentiation among insect's populations may also be a consequence of different diet breadths. Host correlated morphological differentiation in relation to host choice and use has been described among several insects' species (Langor and Spence, 1991; Bernays, 1991; Bernays 2001). For example, in sap-sucking insects, variations in the lengths of mouthparts, leg segments and the number of sensilla have been associated with different host plant use (Carroll and Boyd, 1992; Bernays *et al.*, 2000; Margaritopoulos *et al.*, 2000). These findings support the theory of adaptability of insect morphology to differential plant use, suggesting that wider diet breadth should be correlated with higher morphological variation in structures involved in host selection and feeding. However, given the importance of host plant selection process prior to larval adoption and the variable behavioural feeding responses during development, it is

likely that *B. fusca* larvae have special structures of high morphological variation among its developmental stages that ensure efficient detection of wide range of plant stimuli that mediate host recognition and subsequent selection of feeding sites. Elucidating how recognition, selection and constraints that affect the emergence of host choice is therefore important to bring into focus the mechanism by which *B. fusca* switched from its ancestral wild grass to cultivated hosts to become an economically important pest of several cultivated cereal crops. The purpose of this study was therefore to understand the basis of host plant selection and acceptance for feeding by *B. fusca* larvae. This data is important for the development of future control and management strategies of this most destructive pest of cereal crops in the Sub-saharan Africa.

1. 2: Rationale

Wild grasses (for example wild sorghum, *Sorghum arundinaceum* [Desv.] Stapf [Poaceae]) were probably the native host plants of *B. fusca* prior to the domestication of sorghum and the introduction of maize into Africa. *B. fusca* which is currently the most economically important pest of maize is speculated to have shifted from the native wild grasses to closely related exotic plants, including *Zea mays* L. [Poaceae]) ultimately expanding its host range. However, the mechanism(s) underlying this host switch is still unknown. Ecological availability of new crops in the field, insect physiological adjustments (adaptive plasticity), efficient sensory system and/or genetic selection pressures are speculated to have heavily contributed to the switch. The existence of different geographic and possibly host plant specific strains have been postulated by Sezonlin *et al.* (2006). Such candidate host plant

associated strains have been reported to inhabit *Arundo donax* L. (Poaceae) found in Ethiopia, Eritrea and more recently, South Africa (Le Rü, 2006b). These conspecific strains seem to show conservatism in feeding on wild grasses suggesting host plant specificity.

Understanding the evolution of host or habitat specialisation in *B. fusca* has important implications not only for evolutionary ecology, but also for the development of sound control strategies. For example, a novel stimulo-deterrent diversionary or the 'push-pull' strategy (Miller and Cowles, 1990) has been developed in East Africa as an integrated pest management approach against *B. fusca* and other stemborer pests (Khan *et al.*, 2000; Khan and Picket, 2004; Cook *et al.*, 2007). The approach exploits the underlying chemical defense mechanisms of trap crops planted as border rows (pull), and repellent and unsuitable plant species (push) intercropped with the cereal crops. At present it is impossible to determine whether in future *B. fusca* might evolve rapidly in host range and adapt to non target plants currently used as repellent intercrops in the push-pull strategy and therefore adversely affect the sustainability of this promising management strategy. Although some of the plants used in this approach are not closely related to the species normal plant diets, it is speculative that as a result of repeated exposure of the pest to these plants, this pest may undergo evolutionary or elicit adaptive changes that may involve expansion of its diet breadth and include non target plants important in this strategy, thereby negatively affecting its management and control.

The current study therefore, sought to infer the recognition, selection, feeding, development and performance patterns of *B. fusca* larvae on various hosts and candidate host plants. Since the likelihood of rapid evolution of shift to a non-target

plant may be judged to some extent by screening populations of the insect for genetic variations and behavioural responses, the genetic structure of the candidate host associated *B. fusca* populations was also studied to infer the correlation between genetic structure and host use, and to provide a framework for the understanding of the mechanisms involved in novel host-plant adoption by larvae of this species. The following questions were thus addressed.

1. Is *B. fusca* larvae able to recognise suitable host plant for colonisation?
2. Which plant factors influence larval development and performance?
3. Do larvae physiologically adapt to chemotaxonomically variable plant diets encountered during its oligophagic foraging habits.
4. Does host use influence genetic sub-structuring of *B. fusca* populations?

1. 3: General objective

This study was undertaken with a broad objective to asses the effects of host plant's chemical and physical attributes on feeding behaviour and performance and to determine the physiological and genetic basis of host plant preference by *B. fusca* larva.

1.3.1: Specific objectives

The specific objectives of this study were:

1. To determine larval sensory structures important in host plant recognition and acceptance;
2. To verify the extent of feeding and growth of *B. fusca* larvae on some selected Poaceae plant species present in the insect's natural habitat.;

3. To determine plant stimuli that influence feeding and growth of *B. fusca* larva:
4. To study the physiological responses / adaptations in larval digestive enzymes and genetic structure of *B. fusca* population in relation to host plant use.

1. 4: Literature review

1. 4. 1: Host range and shift in phytophagous lepidoptran insects

Lepidoptera host plant affiliation is diverse with majority of larvae living at the expense of seed plants and virtually all orders of gymnosperms, angiosperms, ferns, liverworts and mosses. However, most clades in Lepidoptera insect family are primarily restricted to specific higher taxa of angiosperm (Powell *et al.,* 1998). The diversification of Lepidoptera lineages is postulated to have paralleled or perhaps even coevolved with their host plants (Ehrlich and Raven, 1964; Becerra and Venable, 1999).

Most insect species use limited and specific subset of plant taxa as hosts (Strong *et al.,* 1984; Mitter and Farrel, 1991). Generally, within phytophagous Lepidoptera, there is a trend that most generalists are external feeders while specialists feed internally (Gaston *et al.,* 1992; Frenzel and Brandl, 1998) indicating that endophagy reduces the likelihood of oligophagy, a necessary intermediate stage during a host shift (Drès and Mallet, 2002). Moreover, comparison between insect specialists and generalists clearly indicate that generalists are usually fewer than specialists suggesting that a narrow host range is favourable over evolutionary time and space (Schoonhoven *et al.,* 1998). A number of constraints are reported to promote food specialisation in insects. These include, genetically based trade-offs in performance between different habitats (for instance, habitat-specific

adaptations) (Via, 1991; Scheck and Gould, 1993), competition for resources (Mac-Arthur and Levins, 1964), resistance to predators (Dyer, 1995), high cost of information processing (Bernays and Wcislo, 1994; del Campo and Miles, 2003), mate finding (Colwell, 1986), low costs to searching for suitable habitats (Southwood, 1972) and habitat associated deleterious mutations (Kawecki, 1994). In contrast, generalization is favoured by rare or unpredictable habitats (Strong *et al.*, 1984), difficulties with meeting nutritional requirements on a single host and greater resource availability in terms of food supply (Singer *et al,* 1992; Ballenbeni and Rahier, 2000),

Phytophagous insect species can also switch hosts and therefore expand their host range. However, many factors including among others ecological, genetic, neuro-physiological and/or phylogenetic conservatism potentially constraint the evolution or maintenance of host ranges for most insect species (Jaenike, 1990; Bernays and Wcislo, 1994; del Campo and Miles, 2003). Generally, shifts to alternative plants are rare in monophagous insects that often feed on closely related and chemically distinct plants. In contrast, plants in some taxonomic or ecological assemblage may be closely related but less chemically distinct, limiting the barriers to host transfers. This encourage insect host switch among plant species and consequently elevates the incidence of polyphagy (Schoonhoven *et al*., 1998). For instance, many herbivorous insects in temperate deciduous forests, which are dominated mainly by tanniferous angiosperms, are highly polyphagous due to the toughness and low nutritional content of plant mature foliage (Powell *et al.,* 1998). Similarly insect host diversification rates are also influenced by both resource abundance- which leads to

decreased competition (Fox and Lalonde, 1993; Larsson and Ekbom, 1995) and diversity (larger number of potential niches) (Janz *et al.*, 2006).

Several species of phytophagous insects have switched from their ancestral hosts and rapidly adapted new plants (Singer *et al*., 1993; Radtkey and Singer, 1995; Camara, 1997). However, prior to host shift insects must be able to recognize suitable host plants based on the specific plant cues. The apple maggot fly, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), for example, exclusively used hawthorn fruits until the introduction of apple in USA. However, introduction of apple orchards and the subsequent colonisation by the fly occurred rapidly and resulted in the formation of host races that currently differ in host choice, diapause and alloenzyme frequency (Feder *et al.*, 1990).

For lepidopteran species, host plant recognition is a complex process and like many other phytophagous insects, location and subsequent selection of suitable hosts for oviposition by the adults or feeding by larvae appears to be strongly influenced by specific host plant search images which are based on representative chemical, physical and visual characteristics of their host plants (Renwick and Chew, 1994; Städler, 2002). Many examples show that typical volatile compounds emitted by host plants guide insect herbivores while searching for hosts and therefore play an important role in host plant recognition and selection (Honda, 1995; Bruce *et al*., 2005). Similar studies on *B. fusca* for instance, have indicated that volatile cues from Maize, Sorghum and Napier grass mediate host location and oviposition in females (Khan *et al.*, 2000; Juma, 2005; Birkett *et al.*, 2006).

Host selection by most lepidopteran insect species is primarily a function of adult females, since larvae in their early life stages usually have limited dispersal abilities

(Renwick and Chew, 1994). Therefore during host location, females maximize larval fitness by selecting and ovipositing on plants most suitable for future larval survival and development (Pilson and Rausher, 1988). However, as reported by Larsson and Ekbom (1995), there is widespread ability of lepidopteran larvae to also select and feed on hosts not selected for by gravid females. Therefore although not highly mobile, lepidopteran larvae are not always restricted to the host plants initially selected by their mothers. For example, for the gypsy moth, *Lymantria dispar* (L.), extensive host switching can occur during larval development mainly to improve their growth rates (Stoyenhof *et al.,* 1994). For these larvae, switching occurs in both high- and low-density populations indicating that other factors (for instance host quality or phenology) dictate the dynamics of host switching. This indicates that other than the adults, host recognition and selection behaviour by larvae is also an important determinant of host shift (Nylin *et al.,* 2000) and therefore, evolutionary change in host selection may have important consequences on host range.

1. 4. 2: Influence of plant chemistry in host choice

Phytophagous insects are able to discriminate between plants which are acceptable for feeding and oviposition and those which are not. During host plant selection, various types of stimuli play a role; however, chemical factors appear to be decisive in most insect species examined (Bernays and Chapman, 1994). Host colonisations and shifts in many species of insects have been in most cases reported to be strongly influenced by specific phytochemicals including primary and secondary metabolites since most of the insects studied respond behaviourally and physiologically to them.

For example, herbivorous insects within a defined set of hosts distribute themselves among plants according to the specific plant secondary chemicals (Erhlich and Raven, 1964) and may also induce physiological traits that enable them to exploit host plants but with chemicals which in their natural form are potentially toxic to their cellular processes (Duffey and Stout, 1996). Plant metabolites including primary and secondary chemicals have been reported to mediate many aspects of insect behaviour including host finding, host acceptance, danger avoidance and/or mate location (Bernays and Chapman, 1994; Schoonhoven *et al.,* 2005). Some of these phytochemicals stimulate feeding and therefore act as a host plant recognition cues. For example, larvae of *Manduca sexta* (Linnaeus) (Lepidoptera: Sphingidae), a facultative specialist when feed on solanaceous foliage usually develop preference for indioside D, a steroidal glycoside typical of Solanaceae and hence become habituated to such glycoside containing plants. Additionally, this host-restricted larva is able to feed on non-host diets to which the glycoside has been added (del Campo and Renwick, 2000; del Campo *et al.,* 2001; del Campo and Miles, 2003), indicating that indioside D is arecognition cue used to mediate larval host restriction. Apart from mediating host finding and recognition, secondary plant metabolites can also regulate larval feeding in specialist herbivorous insects and are therefore important determinant of the suitability of a particular host plant for survival and development (Mohamed *et al*., 1992). For example, the isoprenoid ketone 6, 10, 14-trimethylpentadecan-2-one (phytone), isolated and characterised from Bermuda grass, *Cynodon dactylon* (L.) (Poaceae) is phagostimulatory to larvae of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Mohamed *et al*., 1992). Similarly, larvae of *Chilo partellus* (Swinhoe)

(Lepidoptera: Crambidae) are stimulated to feed by soluble sorghum phenolic compounds (4-hydroxybenzoic acid, coumaric and ferulic hydroxycinnamics) present in sorghum ethyl acetate extracts (Torto *et al.,* 1991). However, for majority of oligophagous lepidopteran species, primary metabolites including sugars especially sucrose and fructose are the most effective phagostimulants (Sharma, 1994; Bowdan, 1995; Yazawa, 1997).

Moreover, the presence of high concentration of specific phagostimulant in the host plant tissues may in contrary elicit adverse effects to larval feeding when used over a prolonged period of time. For instance, while soluble hydroxamic phenolic conjugates of maize extracts for instance, 2, 4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOA) usually stimulate feeding of European corn borer (ECB) *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae), long-term feeding of DIMBOA throughout larval development, usually results in reduced fecundity and relative growth rates (prolonged development) of ECB (Bergvinson, 1993) or *Ostrinia furnacalis* (Guenee) (Ortego *et al.*, 1998).

However, the phagostimulatory and inhibitory effects of plant chemicals, including primary and secondary compounds, often counteract each other and determine the outcome of insect's decision making process, to accept or reject the plant as a host. In such cases, if all the sensory information gathered during larval test bite is positively judged, along with any physical cues offered by the plant, acceptance of the host is confirmed and feeding is initiated (Schoonhoven *et al*., 1998). The effect of phytochemicals on insect ability to choose a host therefore clearly indicates that, chemical diversity among plants is an important factor underlying host specificity in phytopagous insects.

1. 4. 3: Phylogenetic constraints to host use

Generally, phytophagous insects utilize diverse food resources over their entire geographical range though localized populations may experience different selection pressures. Populations from a distinct region can adapt and utilize a specific host such that a putatively generalist species may actually consist of specialist local populations. This implies that oligophagy may not necessarily mean oligophagy at the individual level (Thompson, 1994). Many clades of herbivorous insects are remarkably conservative in the plants they attack and in many groups; related insects tend to feed on related plants (Erhlich and Raven, 1964; Janz and Nylin, 1998). Hence, species in a higher insect taxon such as the genus or subfamily, will commonly feed on taxonomically related plants, often of the same family. This conservative feeding habit strongly reflects the existence of constraints that lower the ability of insects to adapt to plants that are distantly related to their normal hosts (Schoonhoven *et al*., 1998).

Insects colonize plants that produce specific chemicals and thus plant chemistry plays an important role in determining the variety of plants that can be exploited by phytophagous insects (Bernays and Chapman, 1994). Therefore, shifts to and the subsequent colonization of alternative plants by related groups of phytophagous insects often arise as a result of tracking of specific and suitable plant phytochemical cues in the novel host (Beccera, 1997; Nylin and Janz, 1999). In most cases, such insects become physiologically pre-adapted to a new host plant's secondary compounds that are often not similar to those of the ancestral host. For example, the association between various insects and plants containing furanocoumarins have been interpreted according to this scenario (Camara, 1997). Similarly, related

species of butterflies and beetles often use plants that are chemically similar, even though these plants may be taxonomically distant (Ehrlich and Raven, 1964; Janz and Nylin, 1998). Similarly, although cladograms of papilionid butterflies and chrysomelid leaf beetles (genus *Ophraella*) and their host plants are not congruent (Jaenike, 1990), their cladistic analyses reveal that host shifts in these group of insects are most likely to occur among chemically similar plants.

A residual capacity by insects to use ancestral hosts not in the present range, long after the colonisation of the novel host plant has been described for some insect species and can be evident in a clade many million years following the colonization of a novel host (Janz, *et al.,* 2001; Ikonen *et al.,* 2003). The fact that these ancestral hosts are often kept in a potential repertoire long after colonisations of novel hosts, and frequently re-colonised at a later date, suggests that the observed insect diversification is not due to release from competition but may be due to greater niche diversity as a consequence of greater plasticity in taxa with wider potential host range (Nylin and Wahlberg, 2008).

On the other hand, phylogenetic constraint to host shift is not universal among insects since similar host plant shifts by insects have been documented among plants that are distantly related but chemically or structurally different from their ancestral host species (Dobbler *et al.,* 1996; Crespi and Sandoval, 2000). Species of the leaf beetle of the genus *Tricholochmaea* specialise on willow (Salicaceae), blueberry (Ericaceae), or Meadowsweet (Rosaceae), which tend to grow in similar habitats but do not otherwise have common physical and chemical traits (Futuyma, 1999). Shifts to taxonomically unrelated plants is however, dependent on the geographical distribution of hosts and thus availability of plants (i.e., ecological opportunity),

insect learning or adaptive plasticity among many other constraints (Gomez-Zurita *et al.,* 2000).

Variations in food choice and utilisation among insect populations may also be caused by insect genetic differentiation as a result of differences in preference induction. Induced preference can initially occur as a result of oviposition mistakes by the adult females (Larsson and Ekbom, 1995; Nylin *et al.*, 2000). If such and similar mistakes occur often enough and persist over several generations and keep inducing the modified phenotype, then genetic changes that improve fitness to this specific host are likely to be selected as a result of genetic accommodation (Janz and Nylin, 2008). Similarly, induced preference can also minimise host fidelity and decrease movement to alternative hosts consequently leading to genetic sub-structuring within species (Via, 1991). If gene flow between such populations is limited, this sometimes results in the formation of host-associated sub-populations or host races (Kim and McPheron, 1993; Berlocher and Feder, 2002). Host races are populations of a species partially and reproductively isolated from the other conspecific populations as a direct consequence of adaptations to a specific host. Similarly, speciation can also occur when a specialist's host is patchily distributed increasing the likelihood of differentiation among populations by restricting gene flow. In many cases therefore, diversification to alternative host plants by many insects species are often phylogenetically constrained to taxonomically, chemically, or structurally similar host species (Futuyma and Moreno, 1988; Janz and Nylin, 1998; Janz *et al.*, 2001).

1. 4. 4: Physiological constraints to host use

The physiological basis of selective feeding in phytophagous insects has been examined in many studies, and in most cases, host plant discrimination depends largely on the chemical content of the food plant. Physiologically, phytophagous insects usually possess traits that enable them to exploit chemically diverse host plants, since most of them must deal with toxic or non-nutritive plant secondary chemicals potentially damaging to their cellular processes (Duffey and Stout, 1996). Berenbaum (1990) postulated that specialist insects possess specific enzyme systems capable enough of degrading potential toxins predictably encountered. The production of these enzymes in many insect species, results from mutation of a gene that codes for enzymes that allow the detoxification of the ingested compounds prior to their sequestration in some target organs. If such mutations are advantageous, high gene-flow occurs rapidly to spread the new allele to the whole population and allow adaptations to a new host plant.

Detoxification of plant allelochemicals by specialised insect enzyme systems is widely believed to be the most important factor contributing to the adaptations of insects to different plant diets, host shift and subsequent diversity in host range. For example, the gut microsomal mixed function oxidases (MFO) have been widely implicated in the detoxification of toxic plant allelochemicals through oxidative reactions in many insect species (Brattsten, 1988; Feyereisen, 1999) and hence play an important role in host range determination.

However, although cases of trade-offs in physiological adaptations to different host plants have been described for some insect species and proposed to underlie speciation or the evolution of speciation in phytophagous insects, other factors

including neural constraints to host recognition (Bernays, 2001) or possibly, physiological trade-offs in adaptation to abiotic conditions are often likely to have a greater effect on host plant choice in most insects.

1. 4. 5: "Learning" as a mechanism of host switch

Host switches in insects can also occur without constraint from lineage or chemistry and in most cases may be mediated by learning or induced preference (Tallamy, 2000). Jermy *et al.* (1968) defined induction as the modification of feeding behaviour by a change in host plant preference due to the previous feeding experience. A shift to taxonomically or chemically unrelated novel hosts is primarily mediated by induced preference or adaptive plasticity by insects due to experience (Huang and Renwick, 1995; Agrawal *et al.,* 2002). For instance, previous experience has been shown to have a substantial effects on the rate at which some butterflies locate particular host plants (Papaj, 1986).

In phytophagous larvae, induced feeding preference is an outcome potentially caused by a number of behavioural and physiological mechanisms including habituation to deterrents, associative learning and sensitisation, 'oviposition mistakes' or host deprivation (Huang and Renwick, 1995). The mechanisms by which sensitivity is induced or suppressed in young larvae are not yet known but possibly after hatching, the peripheral gustatory receptor of neonate larvae may become quite malleable and permanently moulded in ways that affect the acceptability of leaf tissue as food source. Conversely, the presence of one or more deterrents in a novel host can permanently suppress the development of sensitivity to these and other compounds thus enabling neonate larvae to feed without ill

effects. This implies that neonate larvae are far more plastic in their food acceptance criteria than the old larvae. However, this is only possible if there is no prior exposure of the larvae to the food that lacks a particular deterrent (Tallamy, 2000). Induction of host preferences for both larval and adult insects has been examined in different insect orders such as Lepidoptera (Anderson *et al.,* 1995; Huang and Renwick 1997; Akhtar and Isman, 2003), Diptera (Jaenike, 1988) and Coleoptera (Rietdorf and Steidle, 2002). In most of these cases, previous exposure to deterrent compounds significantly reduced aversion at subsequent encounters. Dietary experience can influence the ability of insects to taste plant chemicals that signal the feeding suitability of the host. Therefore, repeated exposure of herbivorous insects to a specific non stimulus plant or diet can lead to an increased preference of the host species (Hopkins, 1917) and possibly lead to host switch.

Although, induction of food preference within the larval stages has been reported for many insect groups, only few investigations indicate that larval experience alters adult preference or acceptance to hosts (Anderson *et al.,* 1995; Akhtar and Isman, 2003). Moreover, prior adult females encounter or oviposition on a given host is also necessary for an increased likelihood of future insect preference or acceptance of the specific host plant (Papaj, 1986; Cunningham *et al*., 1998; Egas and Sabelis, 2001). For most lepidopteran species, it has been demonstrated that early larval experience is normally transmitted through metamorphosis and this may explain conservatism of plant preference observed in majority of Lepidoptera species (Barron, 2001; Blackiston *et al*., 2008). Modification of preference by experience either during adulthood or larval stage is however, not universal (Parmesan *et al*., 1995).

1. 4. 6: Ecological constraints to host choice

Phytophagous insect diversification rates can conceivably be influenced by geographic variations in the relative abundance of resource leading to decreased competition among insects utilising the same food source or due to diversity in plants (larger numbers of potential niches) (Singer *et al*., 1992; Denno *et al.,* 1995). For instance, in areas where top-ranked hosts are abundant and oviposition frequent, thresholds for host acceptance would remain high, precluding use of low ranking hosts. However, where favoured plants are rare or their presence is masked by associated members of the plant community, thresholds for host acceptance decrease, making the use of other plants more likely subsequently leading to host range diversification (Singer *et al.*, 1989). Resource diversity therefore creates opportunities for insects to shift to novel host plants (Nylin and Wallberg, 2008). Depending on the spatial arrangement of plants, the scale of intercrops and the movement of insects, intercropping may lead to repeated encounter of non-host plants, greatly contributing to oviposition on non-host plants partly due to oviposition mistakes and subsequent host switch.

1. 4. 7: Influence of plant physical attributes to host choice

Plant selection and acceptance by phytophagous insects is also determined by plant physical characteristics (Calatayud *et al.,* 2006). Prior to feeding on the plant tissues, insect larvae generally palpate the plant surface as a direct response to plant contact cues. Upon contact, larvae obtain information on plant quality for which mechano-chemosensory stimuli are involved**.** Plant physical factors including the presence of trichomes and wax crystal structures, leaf thickness and toughness,

sclerotization and high silica content on the plant surface may strongly influence the avoidance feeding behaviour of larvae (Shoonhoven *et al*. 1998).

Among the possible plant physical attributes, silica (Si) is among the common elemental chemicals that can accumulate in plant tissues (Jarvis and Jones, 1987; Sangster *et al.,* 2001) and determine host choice in phytophagous insects. The protective effect of silica to plants against insect herbivores, pathogens or abiotic factors is related to the level of its accumulation and polymerization in the plant tissues with highest levels positively correlating with highest resistance to insect feeding (Meyer and Keeping, 2005; Laing *et al.,* 2006). For lepidopteran species, mitigating effects of silica against borer attack has been observed in barley, rice wheat, sugarcane, maize and sorghum (Schoonhoven *et al.,* 2005; Kvedaras *et al.,* 2007a).

Although mechanisms of silica mediated resistance to insect herbivore is still scanty, (Ma, 2004; Hammerschmidt, 2005), silica in plant epidermal cells is thought to provide a physical barrier against borer probing and feeding or pathogen penetration into plant tissues (Djamin and Pathak, 1967; Peterson *et al.,* 1988; Kvedaras and Keeping, 2007). Silica may also increase leaf abrasion which subsequently increases wearing of insects' mandibles and therefore physically deter larval feeding (Raupp, 1985; Massey *et al.,* 2006). Biochemically, silica has been reported to modulate the accumulation of herbivore defensive allelochemicals including phytoalexins, lignin and phenolics in plant tissues (Cherif *et al.,* 1994; Rodrigues *et al.,* 2004; Remus-Borel *et al.,* 2005) or induce the production of plant defensive enzymes such as peroxidase, polyphenoloxidase and phenylalanine ammonia lyase in response to herbivorous insect attack (Keeping and Meyer, 2002; Gomes *et al.,* 2005). These

defensive plant enzymes take part in a number of plant defense processes such as lignifications and/or production of antiherbivore plant metabolites (Goodman *et al.*, 1986; Felton *et al.*, 1994). Nevertheless, the effects of plant tissue silification as a defense mechanism against insect herbivores seem not universal. For example, high silica levels in turfgrass had no influence on feeding and development of *Herpetogramma phaeopteralis* Guenée (Lepidoptera: Pyralidae), nor on growth, survival, feeding preference or mandibular wear of *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) (Redmond and Potter, 2007).

1. 4. 8: Neural constraints to food choice

Constraints on information processing might help to explain the general tendency of herbivorous insects to specialise on relatively few host plants (Fox and Lalonde, 1993; Bernays and Wcislo 1994; Larsson and Ekbom, 1995). Dusenbery, (1992) predicted that identifying, discriminating and choosing among potential host plants usually present a challenge to most phytophagous insects because insect nervous systems have limited capacity to process multiple sensory inputs. The capabilities of insects to discriminate among plant species and the ways in which they use the available information to make such decisions often have profound effect in diet breadth beyond host quality (Bernays and Chapman, 1994; Bernays 2001) and depend on the sensory information gathered during taste bite. When such information is judged positively by the central nervous system, acceptance, the final decision taken in the host-plant selection process, is confirmed and food intake occurs. From an evolutionary perspective, acceptance can then be

considered as the crucial decision taken during host-plant selection, as it has direct consequences for the acquisition of nutrients (Schoonhoven *et al*., 1998).

Lepidopterous larvae have four sets of external chemosensory organs: the antennae, the maxillary palps, the medial and lateral maxillary sensilla styloconica and the epipharyngeal sensilla (Schoonhoven, 1987) that are used in food plant choice. Stimulation of the antennal olfactory receptors by plant odours usually evoke short range orientation towards a plant, whereas activation of the olfactory cells associated with the maxillary palpi may have a phagostimulatory effect (Ishikawa *et al.,* 1969). Gustatory receptors present in the palpi, the maxillary sensilla styloconica and the preoral and buccal cavity (Ma, 1976), further determine acceptability of the plant. Therefore, host-restricted larvae often choose their food based on input from taste receptor cells located within chemosensory sensilla on their mouthparts. Antennal, maxillary palp and epipharyngeal sensilla for example, have been implicated to respond to chemical cues of host plants in non host restricted *Manduca sexta* larvae but seem to have less significant role in the feeding behaviour of host restricted Solanaceae feeding larvae (Glendinning *et al.,* 1998). The responses of the sensilla styloconica to a variety of chemicals compounds have also been examined in a number of insect species. For *M. sexta*, feeding is mediated entirely by the sensilla styloconica on the galea, as removal of these sensilla completely eliminates food preferences of the host-restricted larvae (Flowers and Yamamoto, 1992; del Campo and Miles, 2003). This means that the sensory input from sensilla is both necessary and sufficient for host recognition by host restricted larvae.

As a result of neural confusion, ovipositing insects may ignore the more suitable food for their larvae possibly because host plants lack required recognition cues, present deterrent but harmless chemicals or conversely, may accept unsuitable host plants because of presence of oviposition stimulants (Fox and Lalonde, 1993). On such occasions insect will ultimately select a host plant not within the host range. Another approach in the search for the chemosensory mechanisms involved in food choices involves studying the neural responses of larval chemosensory organs to deterrent compounds. When such compounds are added to artificial diets they modify taste sensilla responses by habituation, resulting in decreased sensitivity to the available stimulus (van Loon, 1990; Glendinning and Gonzalez, 1995). Knowledge of preference behaviour requires a study of the functions of the chemoreceptors involved and the electrophysiology offers a relatively rapid and precise method to elucidate the chemical factors by which plant is recognized. Comparisons of the electrical responses of the chemoreceptors on stimulation with different substances may reveal which chemicals the insect selects through its sense organs from the complex chemical environment.

For *B. fusca* larvae, the chemosensory basis of food plant selection is still at infancy and a more comprehensive study investigating the role of all the known chemoreceptors and important in the discrimination of host from the non-host plants is needed.

CHAPTER TWO

2.0: DISTRIBUTION OF CHEMO AND MECHANORECEPTORS ON THE ANTENNAE AND MAXILLAE OF *B. FUSCA* LARVAE

2. 1: Introduction

Phytophagous insects lay their eggs on plants based on the preferential selection of their host plants. Host finding and acceptance foraging behaviour for such insects are often constrained to particular host species that are thought to represent the suitability of hosts for future larval survival. Host recognition and acceptance in many phytophagous insect species are usually governed by the adults although host recognition and acceptance by larvae is widespread and equally important (Roessingh *et al.,* 2007).

Like many other Lepidoptera, the success of *B. fusca* to recognize and colonize a limited variety of plants is based on the interactive effects of its sensory system and the physico-chemical characteristics of the immediate environment (Calatayud *et al.,* 2006). After hatching under the leaf sheath, *B. fusca* neonates initially ascend to the leaf whorl, where they either feed on the leaves or disperse to other plants via 'ballooning-off' effect (Kaufmann, 1983). However, upon feeding on the leaf whorls and gaining appropriate size, third instar larvae generally descend and bore into the plant stem or migrate in search for more suitable feeding part of the host plant (Kaufmann, 1983). This indicates striking food selective responses by *B. fusca* larva, which appears to be mediated by neural tuning and influenced by larval age and specific plant tissue.

Selective feeding preferences displayed by lepidopteran larvae are based on small set of chemoreceptors present on the larval antennae and maxilla and are thus important in host plant recognition and selection (Roessingh *et al.,* 2007). It is however, not clear whether the sensory structures and their inherent sensitivity to various plant stimuli change with larval age so as to enable the detection of cues emanating from different parts of the host plants used as food source.

Therefore, as a first step to elucidate the basis of host plant recognition and selection, the external morphology and distribution pattern of sensilla present on the maxillae and antennae of neonates and third instar of *B. fusca* larvae were studied. The olfactory and / or contact chemosensory functions of these sensilla were identified based on direct observation made using selective silver staining and dose-response electrophysiological tests.

2. 2: Materials and Methods

2. 2. 1: Insect rearing

Neonates and third instar *B. fusca* larvae were obtained from the Animal Rearing and Containment Unit (ARCU) of the International Centre of Insect Physiology and Ecology (ICIPE, Nairobi, Kenya). Neonates were directly used for experiments one day after hatching. To obtain third instar larva, some neonates were reared on a meridic diet as described by Onyango and Ochieng'-Odero, (1994). Briefly, boiled and cooled (60°C) distilled water was mixed with preweighed quantity of ingredients as listed (in Table 2.1) to make fraction A of the diet. The mixture was then blended in warring blender for three minutes. Fraction B of the diet was made by mixing cold distilled water with a preweighed agar powder and boiling before

cooling to 60°C. Ingredients of fractions A and B were then mixed and blended together for further 3 minutes. 40% of formaldehyde (fraction C of the diet) was measured and mixed with ingredients of fraction A and B and the mixture once again blended for further 3 minutes. Approximately 20 ml of the resulting diet was dispensed into each heat-sterilised glass vial (7.5 cm long x 2.5 cm diameter) using a ketchup dispenser. The diet containing vials were then covered with a clean white cloth and left overnight on the bench in the laboratory to cool and gel.

Four, one day old neonate larvae were inoculated in each vial containing cooled artificial diet. The vials were then stoppered using a cotton wool and larvae allowed to feed and grow in a temperature controlled room until third stadium (after 21-24 days) before they were used for bioassay experiments.

Table 2.1: Amount of ingredients used to prepare a litre of artificial diet used to rear *Busseola fusca* larvae (Onyango and Ochieng'-Odero, 1994)

Ingredients	**Quantity (g) per litre diet**
Fraction A :	
Distilled water	404 ml
Bean powder	88.4 g
Maize leaf powder	25.4 g
Brewers yeast	22.7 g
Ascorbic acid	2.5 g
Vitamin E	2.1 g
Sorbic acid	1.4 g
Methyl p-hydroxy benzoate	2.0 g
Sucrose	35.4 g
Fraction B :	
Agar Techno. N°3	12.6 g
Distilled water	404 ml
Fraction C:	
Formaldehyde 40%v/v	2 ml

2. 2. 2: Scanning electron microscopy

Ten live neonates and ten decapitated heads of third instar larvae were used for the preparations of specimens for scanning electron microscopy. For fixation, the specimen were allowed to stay overnight in a 2.5 % glutaraldehyde prepared in 0.1 M phosphate buffer (pH 7.4) solution. The specimen were then dehydrated in a graded series of ethanol (70%, 90% and 100%) and finally air-dried. The heads of neonates were then separated from the rest of the bodies. Larval heads for both insect developmental stages were then mounted on stubs with conductive double-side adhesive tape, sputter-coated with gold, and finally examined with a JEOL JSM-T330A scanning electron microscope at 10 kV.

2. 2. 3: Silver nitrate staining

Silver nitrate staining was done to determine the presence of pores in the maxillary and antennal sensilla on the heads of neonates and third instars. Intact neonate larvae were stained according to the method of Nayak and Singh (1983) modified as follows: Larvae were first immersed in 70% ethanol containing 1 M silver nitrate for 1 hour and then dehydrated in two concentrations (90% and 100%) of ethanol. Afterwards, heads of neonates were separated from the rest of insect's body. Heads of both instars were separately cleaned in xylene overnight. The specimens were then mounted in Mountex (Histolab) for light microscope observations. A total of 10 head specimens from each of larval developmental stages were examined for pores and distribution of the sensilla.

2. 2. 4: Electrophysiology

Electrophysiological tip recordings from sensilla on the maxillae and antennae of third instar larvae were carried out to determine the contact chemosensory function of the sensilla using tip recording technique (Hodgson *et al.*, 1955). Recordings were carried out only for third instar larvae because the sensilla present on neonates were too small to make electrical contact possible. Larvae were first severed at the thorax and a glass micropipette (recording electrode-thin walled borosilicate glass capillaries, Harvard apparatus) filled with 10 mM NaCl inserted into the body part that contained the head. The glass micropipette was then slipped over a silver wire that served as the reference electrode. The sensilla were then probed for electrical contact with another micropipette filled with 100 mM KCl that sheathed another silver wire and grounded to act as the indifferent electrode. To avoid crystallization and concentration changes at the tip, the electrode was filled with the test substance just a few seconds before the start of the recording. 10 individual larval parts were probed for electrical contact. The recording electrode containing the test solution was placed over single sensilla hairs for 5s with an interstimulus interval of approximately 10 minutes to avoid adaptation. The action potential generated were amplified using a universal AC/DC UN-06 amplifier (Syntech, The Netherlands), recorded on a computer and analyzed using Autospike software (Syntech version 2.1a). The analyses were based on the waveform and amplitude properties of the action potentials generated. A dose-response experiment using sucrose, a feeding stimulus for *B. fusca* larvae when reared on artificial diets (Onyango and Ochieng'-Odero, 1994), was done for sensilla that showed action potentials to provide evidence for a contact chemosensory function. Sucrose concentrations of 0.01, 0.1,

1, 10, 100 and 500 mM prepared in 10 mM KCl were used. The nervous impulses generated by different neurones in the sensilla were discriminated using Autospike software (Syntech version 2.1a).

2. 2. 5: Data analysis

Statistical tests were performed with Statview software (Abacus Concept, version 5.0, USA). For data on the sensilla lengths, means were separated by Mann-Whitney U-test. The spike counts during the first 150 ms of each recordings were log(x+1) transformed in case of electrophysiological data. A linear-regression of log (number of spikes +1) on log (concentration) was done and tested using ANOVA.

2. 3: Results

2. 3. 1: Sensilla present on the larval antennae

Scanning electron microscopy data indicated that the antennae of both neonates and third instars larva of *B. fusca* comprise of three segments (Figure 2.1 a). Located on these segments are three typical insect sensilla: sensilla chaetica, sensilla styloconica and the basiconic sensilla. On the second antennal segment are a pair and dorsally positioned aporous sensilla chaetica (Figure 2.1b, marked C1& C2) and two cone-shaped basiconic sensilla (Figure 2.1b, marked 1 & 3). A similar cone-shaped basiconic sensillum is located on the third antennal segment (marked as 2 in Figure 2.1b). The three cone-shaped basiconic sensilla on the second and third segments are of equal lengths and were shown to possess argyrophilic properties (Figure 2.1c) since they allowed silver nitrate dye to penetrate through their pores and precipitate in their lumen. Three additional but small basiconic sensilla are also present on the

antennae; one located on the second segment while the other two on the third antennal segment (Figure 2.1b, marked as 1', 2', 3'). The presence of pores on the small basiconic sensilla was not discernable and hence their function could not be clearly determined in this study. One aporous styloconic sensillum is present on the third antennal segment adjacent to the cone- shaped basiconic sensillum. Similar type and number of sensilla but with variable sizes were found on the antennae of both neonates and third instar larvae. The size of these sensilla positively correlated with larval developmental stage. For each instar, sensilla chaetica was the longest, then the large basiconic sensilla and finally sensilla styloconicum (Table 2.2).

The action potential obtained for the cone-shaped basiconic sensillum located on the third antennal segment (marked 2 on Figures 2.1b and c) following electrophysiological tip recordings indicated a gustatory function (Figure 2.2). However, similar sensilla on the second segment and the other small basiconic sensilla present on both second and third antennal segments (1 and 3 on Figures 2.1b and c) did not show any action potential activity during the tip recording tests.

Table 2.2: Mean[a] lengths (µm, ± SE; n=4) of antennal sensilla of *Busseola fusca* neonate and third instar larvae

Larval stage	Sensilla type			
	Long sensillum chaeticum (C1)	Short sensillum chaotic (C2)	Large anionic sensilla (1, 2, 3)	Styloconic sensillum (S)
Neonates	47.4 ± 3.7 a	5.7 ± 0.4 a	8.6 ± 0.9 a	7.6 ± 0.5 a
Third instar	200.7 ± 31.3 b	35.2 ± 2.7 b	25.4 ± 2.9 b	14.8 ± 1.1 b

[a]Means followed by different letters are significantly different at 5% level (Mann-Whitney U test).

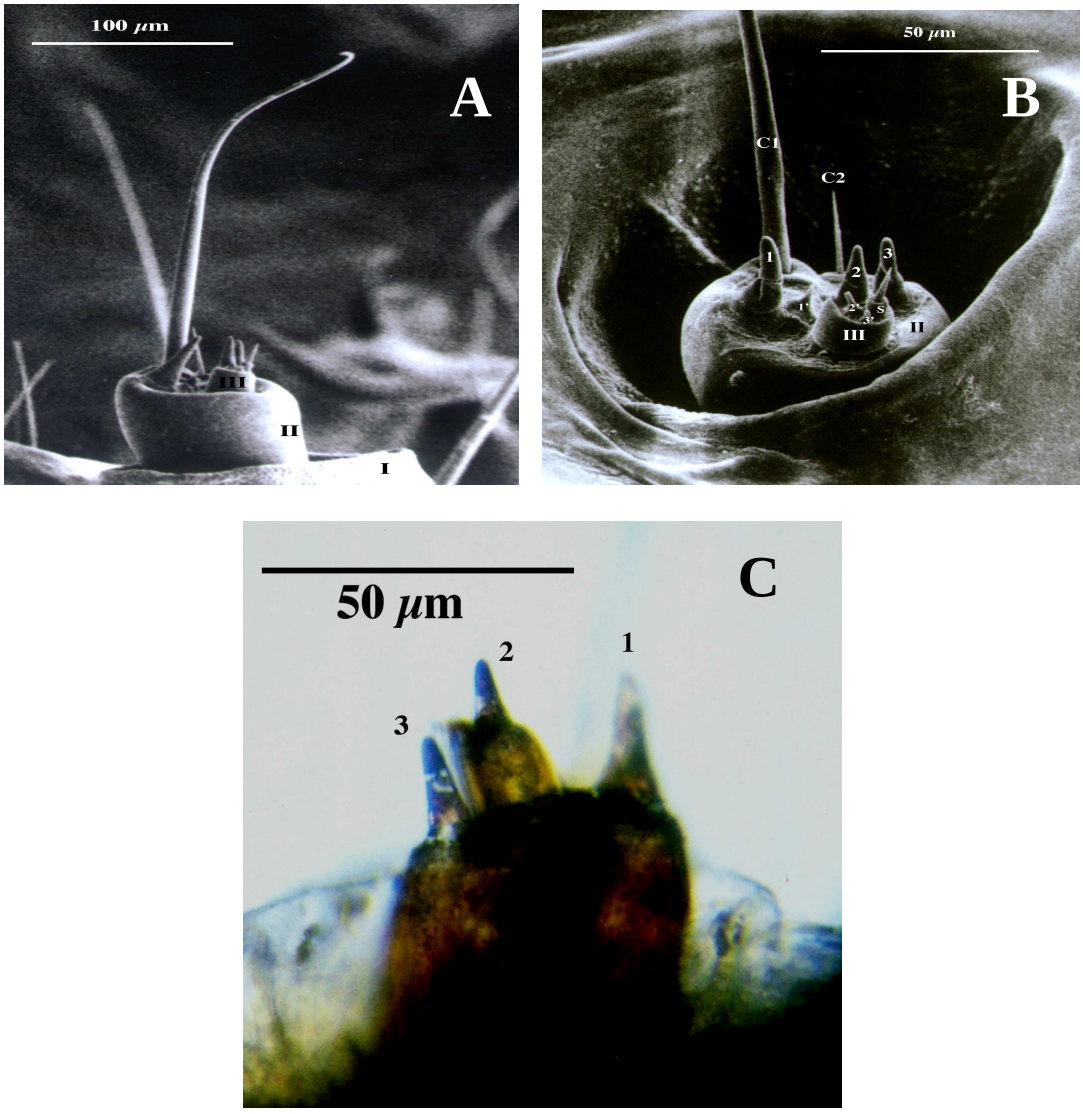

Figure 2.1: Antenna of third instar *Busseola fusca* larva. (A) Tip of right antenna showing the dorsal view of segments I to III. (B) Details of figure A showing two aporous sensilla chaetica (C1, C2), dorsally positioned on the second segment; three cone-shaped basiconic sensilla of similar lengths, two located on the second segment and one on the third segment (1, 2, 3); three small basiconic sensilla (1', 2', 3') and one aporous styloconicum sensillum (S) on the third antennal segment. (C) Dorsal view of the left antenna tip showing 3 large silver stained basiconica sensilla on the 2nd and 3rd segments following staining with silver nitrate.

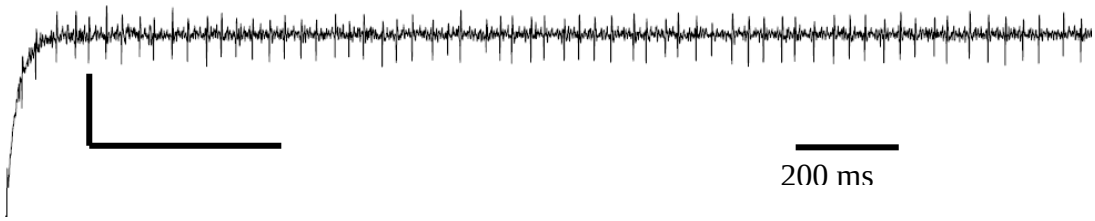

Figure 2.2: Electrophysiological spike activity recordings of the basiconic sensillum on the third antennal segment of a third instar larva in response to 100 mM KCl. Vertical bar: 10 mV, horizontal bar: 200 ms.

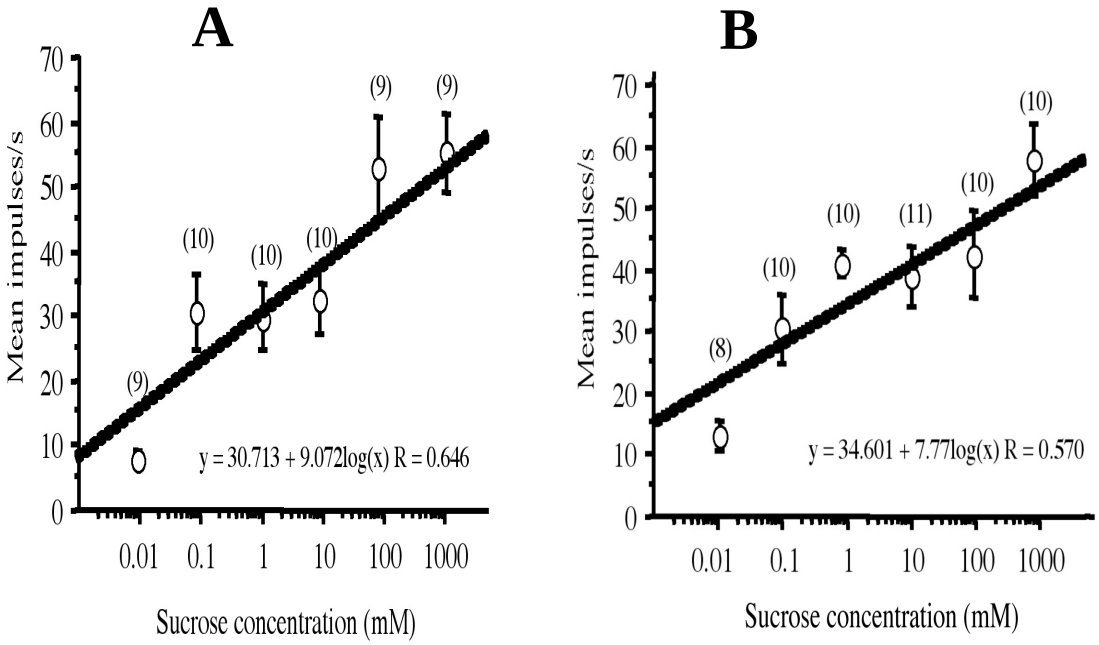

Figure 2.3: Dose-response curves for sucrose obtained after contact with the cone-shaped basiconic sensillum on the third antennal segment (A) and with the maxillary palp (B) of third instar larva. The numbers of replicates are given in parenthesis. Error bars indicate standard error.

2. 3. 2: Sensilla present on the larval maxillae

The sensilla on the maxillary galeae of *B. fusca* larvae were found to comprise of two styloconic sensilla (MS, LS), three basiconic sensilla (B1, B2, B3), and two sensilla chaetica (C1, C2) (Figure 2.4a). The lateral and medial styloconic sensilla stained with silver nitrate and hence were shown to contain a single terminal pore (uniporous) (Figure2.4b). Electrophysiological tip recordings of the neuronal activity obtained from lateral and medial styloconic sensilla (Figures 2.5a and b) indicated their gustatory function. Three aporous basiconic sensilla are located dorsally to the styloconic sensilla (Figure2.4a) while two aporous sensilla chaetica are situated dorsally on the distal part of each galea (Figure 2.4a).

The maxillary palp is two-segmented and contains 8 small basiconic sensilla at the tip (Figure 2.4a) for both neonates and third instar larvae. The number of pores on each small basiconic sensilla on the maxillary palp was not visible but readily stained with silver nitrate indicating their porous property (Figure 2.4c). Similarly, electrophysiological tip recording on each individual basiconic sensilla at the tip of the maxillary palp was impossible because of sensilla's minute size. However, electrical contact and spike strains recorded from this tip (Figure 2.5c) indicated a gustatory function of the palp. As for the antennal sensilla, both larval developmental stages consisted of same types and equal number of sensilla though significantly variable in size. Sensillae chaetica were the longest while styloconic sensilla were the shortest for both larval developmental stages (Table 2.3).

A dose-response electrophysiological tip-recording experiment using sucrose was done on the sensilla present at the tip of the larval maxillary palp and on the cone-shaped basiconic sensillum present on the third antennal segment in order to confirm

their contact chemosensory function. The tip of the maxillary palp was chosen for two reasons: i) it harbours both multiporous and/or uniporous sensilla that have spike trains generated by contact electrophysiological recording (Figure 2.5c) and ii) it is known to respond positively to sucrose in lepidopteran larvae (e.g., Albert, 2003). In this study, significant positive dose-response curves were obtained for both the antennal sensillum ($F_{1, 56}$ = 41.637, P<0.0001; Figure 2.3a) and the maxillary palp ($F_{1, 58}$ = 32.124, P<0.0001; Figure2.3b) when the concentration of sucrose was increased.

Table 2.3: Mean[a] lengths (µm, ± SE; n=4) of maxillae sensilla of neonate and third instar larvae of *Busseola fusca*

Larval stage	Sensilla type			
	Long sensillum chaeticum (C1)	Short sensillum chaeticum (C2)	Styloconic sensilla (LS, MS)	Maxillary palp (P)
Neonates	24.5 ± 0.6 a	12.9 ± 1.3 a	3.7 ± 0.05 a	12.1 ± 0.9 a
Third instar	113.4 ± 0.5 b	43.6 ± 4.4 b	6.1 ± 0.6 b	28.0 ± 3.5 b

[a]Means followed by different letters are significantly different at 5% level (Mann-Whitney U test).

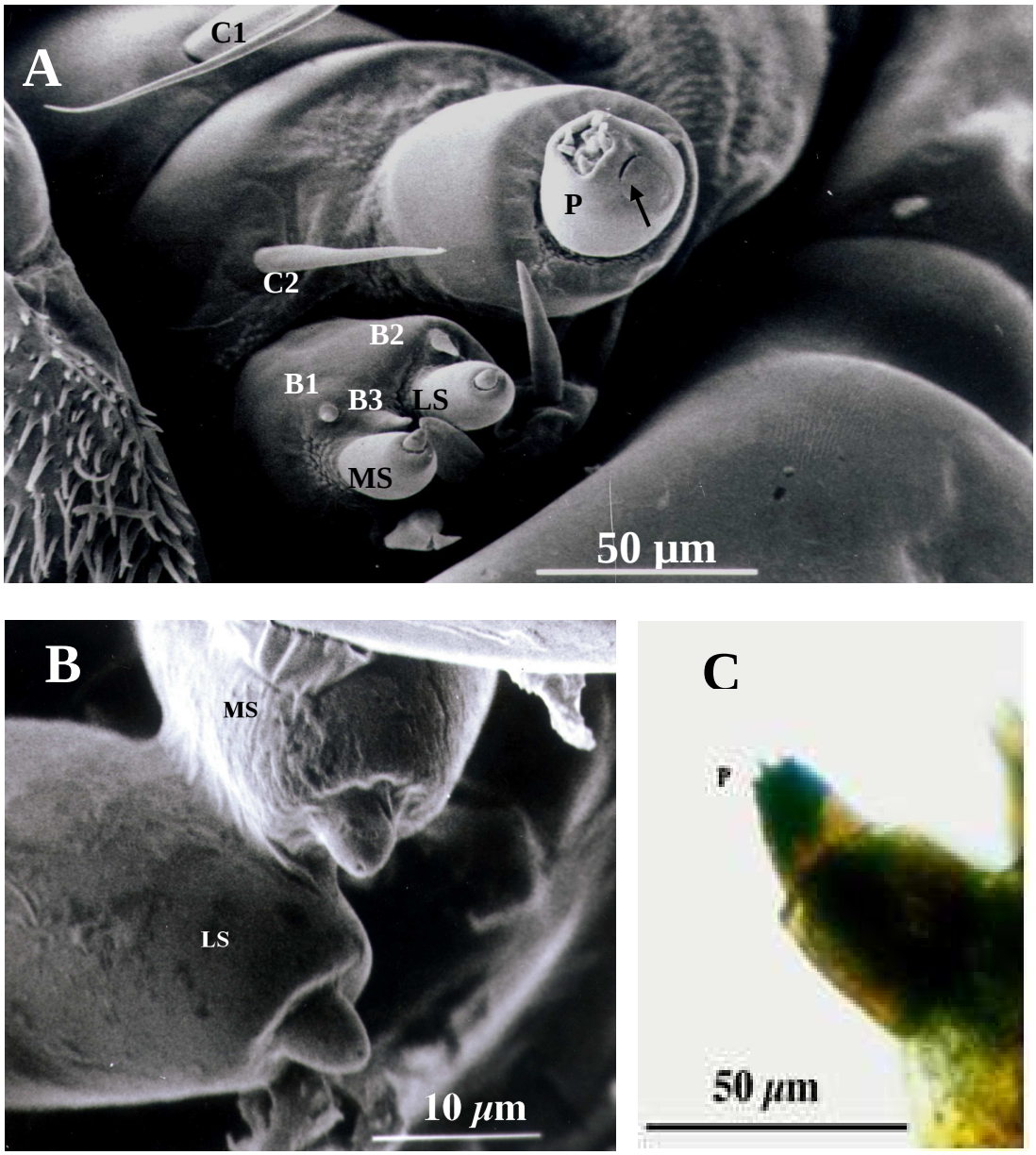

Figure 2.4: Maxilla of third instar *Busseola fusca* larva. (A) Left maxillary galea showing uniporous lateral and medial sensilla styloconica (LS, MS), 3 sensilla basiconica (B1, B2, B3), 2 sensilla chaetica (C1, C2) and the left maxillary palp (P) with 8 sensilla basiconica on the tip and showing laterally a sensillum digit form (see arrow). (B) Close-up of the uniporous lateral and medial sensilla styloconica (LS, MS) with a terminal pore on each. (C) Tip of the right maxillary palp (P), seen dorsally and stained following silver nitrate staining procedure.

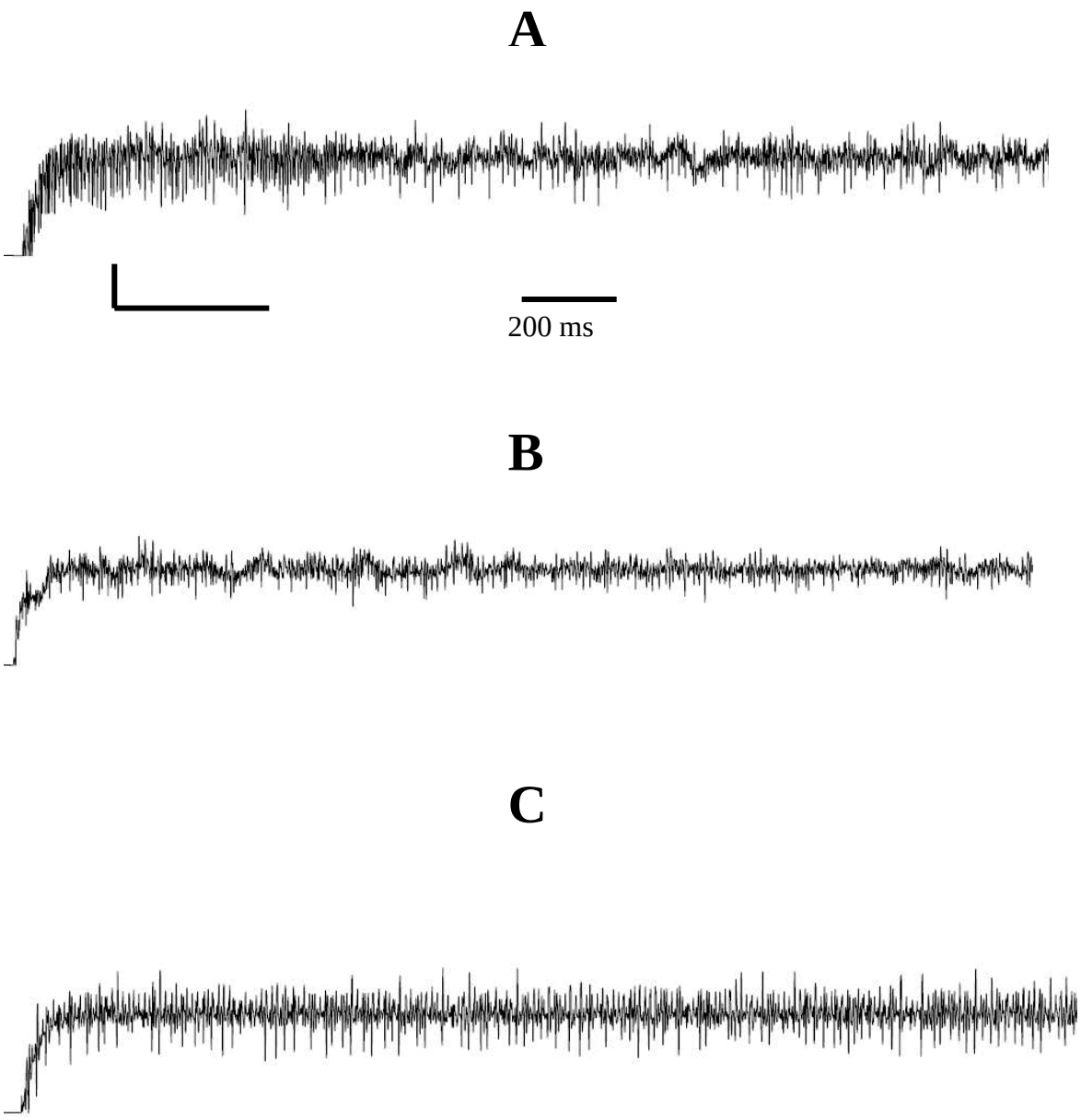

Figure 2.5: Contact electrophysiological recording of spike activity after contact with a lateral sensillum styloconicum on the maxillary galea of a third instar larva (A), with a medial sensillum styloconicum on the maxillary galea of a third instar larva (B) and with a maxillary palp of a third instar larva (C) in response to 100 mM KCl. Vertical bar: 20 mV, horizontal bar: 200 ms.

2. 4: Discussion

The antennae of *B. fusca* larvae are short and simple and comprise of only three segments. The distribution, number and types of sensilla on neonates and third instar larvae are similar and their structure parallels results for other larvae of lepidopteran families, including the Noctuidae, Sphingidae, Arctiidea and Lasiocampidae (Dethier, 1937; Faucheux, 1999). However, this contrasts results obtained for the sensilla present on the heads of the adults of *B. fusca* moths which are long and complex (Calatayud *et al*., 2006). On the second antennal segment, two sensilla chaetica are dorsally positioned. Unlike for adult moths whose sensilla chaetica were porous, similar larval sensilla chaetica were aporous as was indicated by the non-penetration of silver dye in their lumen. As suggested by Dethier (1941) and Faucheux (1999), these aporous sensilla may be adapted for detection of tactile stimuli, thus, informing the insect of obstacles in its immediate environment and of the contours on the feeding substrate.

Three cone-shaped basiconic sensilla of approximately similar lengths are also present on *B. fusca* larval antennae. The walls of basiconic sensilla are generally perforated by numerous pores (Faucheux, 1995; 1999) indicating that the sensilla may present an olfactory function. Olfactory sensilla have receptor neurons that play an important role in discriminating odours that emanate from different plants (Dethier and Schoonhoven, 1969; Dethier, 1980; Faucheux, 1999) and hence are important in the location and selection of suitable hosts. Preliminary results have indicated that both neonates and third instar larvae of *B. fusca* orient significantly towards maize plant volatiles in Y-tube olfactometric tests. Moreover, the electrophysiological tip recordings on one basiconic sensillum located on the third

antennal segment indicated that it has a gustatory function as well. Preliminary observations of the behaviour of second instars showed that prior to feeding; larvae generally tap the plant leaf surface with the tip of the maxillary palp, suggesting evaluation of the plant surface (Calatayud *et al.,* 2006). Furthermore, the cone-shaped basiconic sensilla located on the third antennal segment seem to frequently touch the plant surface during this tapping behaviour, indicating a probable involvement of both sensilla in plant surface evaluation. However, this plant tapping behaviour with antennal sensilla has not been clearly observed and additional studies are necessary for confirmation in order to elucidate the actual role of these sensilla in plant surface evaluation. Although, the presence of pores on the small basiconic sensilla present on the third antennal segment could not be clearly determined in this study, these sensilla are generally multiporous and are probably thought to have olfactory receptor neurons (Faucheux, 1999). In addition, the third antennal segment has one aporous styloconic sensillum. This sensillum may be innervated by cold sensitive receptors (Schoonhoven, 1967; Faucheux, 1999).

Similar to several other Lepidoptera families (Faucheux, 1999), the sensilla on the maxillary galeae of both neonates and third instar *B. fusca* larvae comprise of two uniporous styloconic sensilla (lateral and medial), three aporous basiconic sensilla and two aporous sensilla chaetica. These sensilla showed a marked variation in size between the two larval developmental stages. Electrophysiological tip recordings of the neuronal activity obtained from the uniporous styloconic sensilla on the maxillary galeae confirmed their gustatory function, as has been reported for several other larvae of lepidopteran species (Ishikawa, 1963; Schoonhoven and Dethier, 1966; Dethier and Kuch, 1971; Faucheux, 1999). For all lepidopteran larvae that

have been studied, the styloconic sensilla respond to many different plant compounds and therefore play a major role in discriminating among plant constituents during insect's initial biting process and sustained feeding (Faucheux, 1999). It is therefore proposed that host plant discriminatory ability of *B. fusca* larvae may accrue from the differential responses of the chemosensory neurones in the sensilla stylonica and forms the basis of selective food choices by this oligophagous *B. fusca* larva.

The aporous basiconic sensilla, located dorsally of the styloconic sensilla, may play a role in thermo-hygroreception and in proprioreceptive function by monitoring the position of the styloconic sensilla (Shields, 1994; Faucheux, 1995; 1999). Moreover, the two aporous sensilla chaetica, situated dorsally on the distal part of each galea, probably have proprioreceptors that respond to mandibular movements during feeding as reported in similar studies (Grimes and Neunzig, 1986; Faucheux, 1999).

Three possibilities are reported for other larvae of lepidopteran species on the function of the small basiconic sensilla present at the tip of the maxillary palp. First, all the 8 may be uniporous basiconic sensilla (the most frequent case). Secondly, they may occur as a combination of 7 uniporous basiconic sensilla and one uniporous styloconic sensillum [e.g., in *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae)]. Thirdly, they may consist of 5 uniporous and 3 multiporous basiconic sensilla [e.g., in *Pieris brassicae* (L.) (Lepidoptera: Pieridae) and *Manduca sexta* L. (Lepidoptera: Sphingidae)] (Faucheux, 1999). Although it was not possible to carry out electrophysiological tip recording tests on each individual sensilla, the electrical contact and spike strains recorded from the overall tip of the maxillary palp confirmed the gustatory function of the sensilla on the tip of

the maxillary palp as has been reported for other lepidopteran larvae (Devitt and Smith, 1982; Faucheux, 1999; Albert, 2003).

In conclusion, *B. fusca* larvae have sensory structures that are able to detect volatiles and surface chemical stimuli on their host plants. The olfactory receptors are mainly located on the antennae but they are also present on the maxillary palps. Gustatory chemoreceptors occur mainly on the maxillae and their presence on the antennae of *B. fusca* larvae was demonstrated for the first time and the presence of similar sensilla has never been reported before for any other lepidopteran larvae. This is particularly interesting in view of the antennae and the maxillary palps having receptors that are important in mediating food selection for example, as has been reported in *M. sexta* (De Boer, 1993). The results presented in this study show that the antennae indeed has a chemosensory function, and might be involved in the assessment of host plant suitability. The presence of gustatory sensilla on the antennae offers the larvae with an enhanced ability to quickly and efficiently evaluate appropriate phagostimulatory non-volatile chemical cues present on the plant surface following landing on the plant surface prior to the adoption of plant as a host. This may partly explain the ability of *B. fusca* larvae to easily include a number of host plants with phagostimulatory chemicals on their surface and therefore explain the current diversification and host range scenario of this pest.

CHAPTER THREE

3. 0: EFFECT OF HOST PLANT'S METABOLITES AND SILICA ON SURVIVAL AND GROWTH OF *B. FUSCA* LARVAE

3. 1: Introduction

The evolutionary success of phytophagous insects is dependent on their ability to utilize either many different food plants (generalists) or specialise towards specific plant species (specialists) (Bernays and Chapman, 1994). Majority of phytophagous insects are however specialists (Strong *et al.,* 1984; Thompson, 1994) with a narrow host range but with strong taxonomic conservatism in host plant use (Erhlich and Raven, 1964). Narrow host ranges of herbivorous insects commonly depend on plant chemistry including nutrient composition, primary and secondary metabolites as well as plant physical characteristics (e.g., silica content, spines, and trichomes). These biophysical plant attributes usually inform the foraging larvae about the suitability of a plant for feeding (Bernays and Chapman, 1994; Schoonhoven *et al*., 1998) and hence are important in the acceptance or rejection of host plants.

Plant chemistry significantly influences insects' food choice and subsequent performance (Bernays and Chapman, 1994). Each plant has a unique phytochemical profile that is detected by the insect and forms the basis of food plant selection and discrimination (De Boer and Hanson, 1984). However, most of the allelochemicals present in some plant species are usually active against insects, and renders the plant repellent, toxic or chemically unsuitable for use as food plant (Rosenthal and Berenbaum, 1991). However, larvae of many adapted herbivores will tolerate or use these specific chemical and physical cues to govern the choice of food plant and

determine the suitable feeding host plant (Van Loon, 1996). Recent field studies on the host range indicate that *B. fusca l*arva is highly selective and discriminatory in its food choice and utilisation (Le Rü *et al*., 2006a, b; Ong'amo *et al.*, 2006). As is for the other lepidopteran species, the effects of food on the biology of *B. fusca* is of particular importance in understanding host suitability of infested plant species. It is also important in elucidating the magnitude of injury to the crops attacked which may accordingly help in designing better economic control strategies for this pest.

Moreover, during host selection, young *B. fusca* larvae are capable of selecting their hosts among assemblages of chemotaxonomically dissimilar plants. It has been suggested that during this selection process, *B. fusca* is attracted to its specific host plant within the plant assemblage by blends of volatile and leaf surface metabolites (Juma, 2005). However, the final decision to accept and feed on the particular chosen plant, may involve evaluation of the composition of testants present in or on the plant tissues using larval taste receptors following the first bite. Like for most other lepidopteran species, development of effective strategies for managing *B. fusca* therefore requires a thorough knowledge of the biological interactions of the larvae and its hosts. A very important component of such interactions is that of phytochemical basis of host preference for larval feeding and subsequent host affiliation which is currently lacking for *B. fusca* larvae. In this Chapter, the oligophagic feeding behaviour of *B. fusca* larvae was investigated using some selected Poacea plants found in pest natural habitats. Similarly the larval feeding to crude leaf extracts of the plant species used were evaluated to determine the phytochemical basis of selective feeding and also determine the larval phagostimulatory allelochemicals present in the plant leaf extracts.

Conversely, Poaceous plants often accumulate silica in varying degrees in their leaf tissues. Level of plant leaf silification often varies directly with larval host plant discrimination for a number of lepidopteran species studied (Epstein, 1999). The level of silica in leaf tissues of plants considered were therefore determined to correlate the level of leaf silification with larval growth and survival. Effect of silica on both larval development parameters was confirmed using artificial diet amended with silica. The study focused on both neonates and third instar larvae since neonate stage is critical for the successful host establishment for feeding while the third instar is the critical stage at which boring into the stem and extensive damage to plants occur (Kaufmann, 1983).

3. 2: Materials and Methods

3. 2. 1: Insects

Laboratory-reared *B. fusca* adults have diminished host location and recognition responses compared to their wild conspecifics (Calatayud *et al.,* 2008). However, in a previous experiment, no such relationship was evident between laboratory-reared and larval progeny from adults collected from the wild but to which wild feral individuals had been previously added to rejuvenate the colony. Larvae used in this study were hence sourced from Animal Rearing and Containment Unit (ARCU) of the International Centre of Insect Physiology and Ecology (ICIPE, Nairobi, Kenya). Both first and third instars were used for the experiments. Third instars, were obtained from neonates reared on artificial diet of Onyango and Ochieng'-Odero (1994). For feeding on intact plants, one day old neonates and freshly moulted third instar larvae, initially starved for 24 hours prior to the test were used.

3. 2. 2: Plants

Maize, *Zea mays*, wild sorghum, *Sorghum arundinaceum*, Napier grass, *Pennisetum. purpureum (*Schumach.) *Arundo donax* and *Setaria megaphylla* were used in this study. *Panicum maximum* Jacq. and *Panicum deustum* Thunb the two other Poaceae plant species which are frequently found in the natural habitat of *B. fusca* were also included in the experiments. Maize was grown in 4-litre pots (one plant per pot) from seeds provided by Simlaw, Kenya Seeds Company, Nairobi. All the other plant species were obtained from their natural vegetation in Kenya and propagated from tillers or stem cuttings in 4-litre pots (one plant per pot) in a greenhouse at ICIPE. The environmental conditions for growth were approximately 31/17°C (day/night) with 12:12 hr (L: D) photoperiod. Plants were watered three times weekly and once with a complete nutrient solution. Three weeks old plants were infested with neonates while five weeks old plants were infested with third instar larvae. Because of slow growth compared to other plant species, *S. arundinaceum* plants were infested after the 5^{th} and 7^{th} weeks of growth with neonates or third instars larvae, respectively.

3. 2. 3: Survival and growth of *B. fusca* larvae on different plant species

Potted plants were carefully and randomly assigned in a greenhouse avoiding contact among the neighbouring plants. A thin layer of petroleum jelly was applied on the borderline of each pot to prevent escape, predation or exchange of larvae among the plants tested. Each potted plant was infested with either thirty neonates using a fine camel hair brush or five third instar larvae. Preliminary experiments indicated that any of the seven plant species used could well support an average

number of similar larval population of either the two larval stages used. Both larval instars were weighed prior to plant infestation.

Evaluation of larval performance (growth and survival) was done after, 7, 19 and 31 days following infestation by neonates and 7 and 19 days for third instar larvae. The weight gain and the number of surviving larvae recovered per plant were recorded. The percent larval recoveries per plant (corresponding to the percentage of survival) were also calculated. The relative growth rates (RGR) were calculated by subtracting the initial larval weight from the final weight and dividing the difference by the number of days of infestation (Ojeda-Avila *et al*., 2003). Infestations of plants with larvae were replicated eight and twelve times for third instars and neonates respectively.

3. 2. 4: Preparation and extraction of plant leaf powders

For determination of plant sugars or total polyphenol levels, young leaves of between 4-7 weeks old potted plants were randomly sampled around 10 a.m. for homogeneity. Leaves were cut into small discs and immediately freeze-dried. The weight of leaves prior and after freeze-drying was recorded and the moisture content of each sample evaluated as the difference between the fresh and the freeze-dried weights. Dried leaf discs were then ground into a homogeneous powder in a blender and then stored at -20°C in sealed plastic bags in the dark prior to being used for chemical analyses or bioassays.

Plant leaf powders were extracted first in hexane and then in methanol to remove compounds of different polarities. Two grams portion of leaf powder of each plant species was first extracted for 24 hours in 100 ml pure hexane solvent (Aldrich, 99.8

%). The extract solution was then filtered and the residual material re-extracted using the same solvent and procedure. Both extracts (i.e. filtrate) were pooled and termed as hexane extract. Excess hexane in the residual material was then air-dried off. Thereafter, the same residual material was re-extracted for another 24 hours in 100 ml solution of methanol and water (3:1v/v). After filtration, the residual material was again re-extracted using the same solvent and similar procedure. Both extracts (i.e. filtrate) were again pooled and termed as methanolic extract. The post extraction residual material was finally discarded.

Thereafter, for each plant species, the filtered extracts were each concentrated under reduced pressure in a rotary evaporator (Büchl, Switzerland) at 40°C to obtain a solid crude extract. Based on the freeze-dried weights and the percentage of moisture content of the foliage collected, solid crude extracts were re-dissolved in a similar volume of their respective solvent system to give a final solution of concentration equal to the one found in each plant leaves at the time of collection in the field. Prior to the bioassays, the methanolic extract was divided into 2 equal portions A and B: portion A was used for feeding tests while portion B was used for the analysis of sugars and polyphenol content. All extracts were stored at -20°C until required for bioassays.

3. 2. 5: Feeding preference tests

Each plant extract was applied on cylindrical pieces of Styrofoam carrier matrices (surrogate stems) and tested for feeding activities by means of a laboratory bioassay based on the modified method of Ma and Kubo (1977). Styrofoam carrier matrices

(13 mm diameter, 35 mm length) used, were obtained from a 35 mm thick Styrofoam board by corking with a 10mm diameter cork-borer (Figure3. 1).

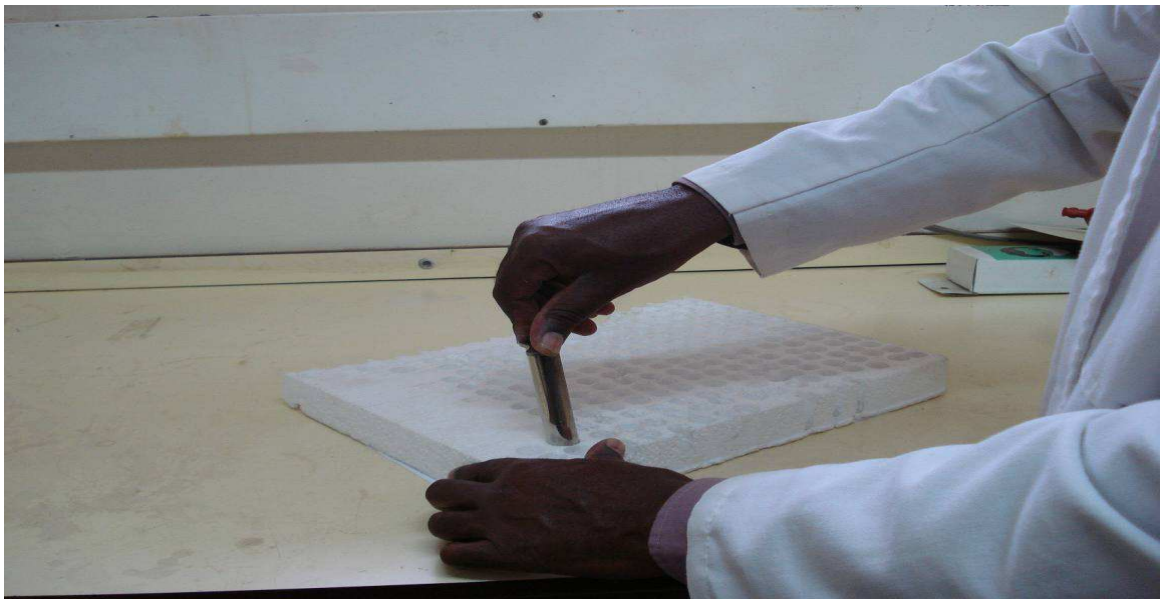

Figure 3.1: An illustration showing the preparation of Styrofoam cylinders (13 mm diameter, 35 mm long) used as feeding matrix for third instar *Busseola fusca* larvae to which plant extracts were applied during larval feeding bioassay.

The bioassay arena consisted of a Petri dish (90 mm diameter) lined with filter paper moistened with distilled water to maintain internal humidity. For all bioassays, an aliquot of 400 µl of each of the plant extracts or solvent (as controls where applicable) were topically applied on the inert Styrofoam carrier matrix. Treated and control bioassay matrices were placed on aluminium foil, air-dried at room temperature prior to randomly arranging them in the afore-prepared bioassay arena (Petri dish). Then, one third instar larva was introduced into each bioassay arena and a Petri dish cover replaced to prevent evaporation of the extract or escape of the larva.

The phagostimulatory activities of third instar larvae to plant‘s extracts were examined both in non-choice and multiple-choice situations. However, similar tests were unsuccessful for neonates because they were unable to bite the Styrofoam matrices onto which the extracts were applied. Choice tests included binary, four and 8-choice experiments. Both hexane (non-polar) and methanol (polar) extracts (i.e. portions A of methanolic extracts) were used for all the bioassay tests except in 8-choice tests in which only methanol extracts were used.

For single choice bioassays (Figure 3.2a), Styrofoam carrier substrates were first impregnated with either hexane or methanolic extracts and tested singly in the bioassay arena. Control treatments run simultaneously, consisted of Styrofoam substrate impregnated with the solvent alone (hexane or methanol) and tested singly in the bioassay arena. In binary choice bioassays (Figure 3.2b), larval preferences for carrier substrates treated with either methanol or hexane extracts were tested together in the same bioassay arena. In 4-choice bioassays (Figure 3.2c), both hexane and methanolic extracts were tested in presence of their corresponding solvent controls in the same bioassay arena. In 8-choice tests (Figure 3.2d), carrier substrates containing each of the seven plant methanolic extracts and a control treated with methanol solvent were simultaneously presented to a single larva in a similar bioassay arena. In all choice tests Styrofoam carrier matrix were differentiated by labelling them with different colour codes prior to submission for bioassay. Each extract impregnated Styrofoam carrier matrix was weighed prior to submission for all bioassay experiments.

Each bioassay experiment lasted for 36 hours in the dark at 25°C and approximately 80% r.h. and a L12:D12 photoperiod. After feeding, the weight of each Styrofoam

carrier matrix was recorded and the feeding response index (FRI) (Cai *et al*., 2002) calculated as follow:

$$FRI\ (\%) = [(X-Y)/X] \times 100$$

Where, X and Y are the weight of the Styrofoam matrix before and after assay, respectively. Each bioassay was replicated 30 times except for 8-choice condition, which was replicated 87 times.

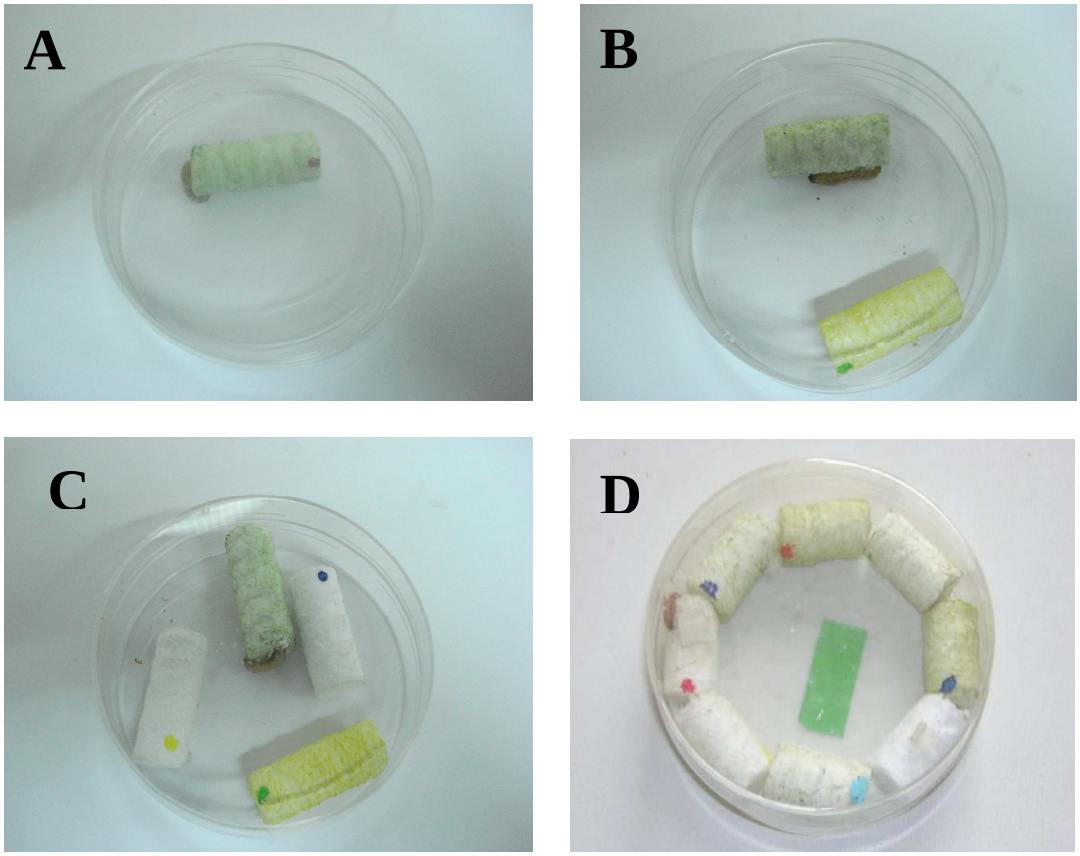

Figure 3.2: Illustrations for bioassay feeding setups for third instar *Busseola fusca* larva: single choice (A) dual choice (B) 4-choice (C) and 8-choice feeding tests (D).

3.2.6: Analysis of simple sugars in plant leaf extracts

Since the hexane plant extracts did not stimulate feeding in either non-choice or choice bioassay tests, only the phagostimulatory methanol extracts of each plant species were subjected to HPLC analysis to determine the stimulatory components present in the extracts. Therefore, twelve (12) millilitres of portion B of the methanolic extracts of each plant species was further concentrated in a rotar vapour at 40°C to remove excess methanol. The resulting aqueous solution was freeze-dried and the solid re-dissolved in distilled water to give a solution of approximately 5 mg/ml. A modified method of Gomez *et al.* (2002) was used to purify crude sugar extracts prior to analysis. Briefly, 0.2 g of polyvinylpyrollidone (PVP) was added to 12 ml of crude extract and thoroughly vortexed. The mixture was then centrifuged at 15000 *g* at 4°C for 30 minutes. The supernatant was recovered using 4 ml syringe and sequentially filtered, first through a C18 cartridge and then through Whatman PTFE membrane filter (0.45 µm). The resulting solution was again freeze-dried and stored at -20°C prior to sugar analyses.

Fifty milligrams of each of the purified and dried plant sugar material was re-dissolved in 1 ml of distilled water and the resulting solution filtered on 0.45 µm PTFE membrane filters prior to loading an aliquot of 2 µl into the HPLC column. All analyses were performed on a Shimadzu auto-sampler chromatograph (Shimadzu Kyoto, Japan) equipped with a quaternary pump and an UV/visible photodiode array detector. Separations were achieved on a supelcosil-NH_2 analytical column (Supelco) (5 µm x 250 mm x 4.6 mm). Isocratic solution with a mobile phase of acetonitrile and water (3:1v/v) was performed at a flow rate of 1.2 ml/min (6.2 mPa) and the eluent monitored at 240 nm at 35°C over 25 minutes. The

identification of the sugars was achieved by comparing their retention time and UV spectrum to those of authentic standards (Sigma–Aldrich Chemie Gmbh, Steinheim, Germany) and confirmed by co-injection. About 90% of the compounds detected by chromatographic analysis were identified. The quantification of the sugars was based on peak areas. All solvents used in the analysis were of HPLC grade (Fisher Scientific, UK).

3.2.7: Analysis of total phenolic compounds in plant leaf extracts

Ten millilitres aliquot of the portion B of the methanolic crude extract of each plant species was centrifuged at 5500 *g* for 20 min at 4°C and the supernatant recovered. A modification of the Folin-Ciocalteau method (Torres *et al*., 1987) was used to determine the total content of phenolic compounds in the methanolic fractions. Briefly, 100 µl supernatant of each plant extract was mixed with 5.9 ml distilled water and 1 ml of 1 N Folin reagent added. Within five minutes following the addition of Folin reagent, 2 ml of 20% sodium bicarbonate solution was added, mixed and the solution incubated for one hour. Optical densities were measured in a 2 ml cell on a Beckman DU 640 spectrophotometer set at 730 nm. The amount of total phenols in the samples were calculated from a calibration curve generated using anhydrous gallic acid standards, and were expressed as the amounts of gallic acid equivalent.

3.2.8: Dose response feeding experiments with sucrose and turanose

The effect of sugars (sucrose and turanose) on the feeding response of third instar larvae were examined using varying concentrations of pure sugar standards (Sigma-Aldrich, Steinheim, Germany). In no-choice feeding conditions, sugar (sucrose or turanose) concentrations used ranged between 0 to 0.1 M, corresponding to the minimum and maximum concentration range obtained among the plant species analysed. Sugar solutions for dose response experiments were prepared in a mixture of methanol and water (3:1 v/v) as the solvent. Moreover, similar concentration (0.1 M) of sucrose is used to prepare the artificial diet used to rear *B. fusca* larvae (Onyango and Ochieng'-Odero, 1994). To determine the synergistic effects of sucrose and turanose on larval feeding, a mixture of solutions of both sugars each in the following proportions were tested: 0 M sucrose/0.1 M turanose, 0.025 M sucrose/0.075 M turanose, 0.05 M sucrose/0.05 M sucrose, 0.075 M sucrose/0.025 M turanose and 0.1 M sucrose/ 0 M turanose. As already described, 400 µl of either sugar solution were topically applied on styrofoam carrier matrix and submitted for feeding bioassay in both single (15 replicates for dose-curve responses and 24 for the synergistic effect tests) and 6-choice situations (24 replicates).

3.2.9: Extraction and analysis of silica in leaves of different plant species

For each plant species used, young fully expanded leaves were thoroughly cleaned with ultra pure water and subsequently harvested. Leaves were sliced into small pieces, put into plastic containers and freeze dried in stoppering tray drier (Labconco-Germany). Dried leaves were milled into a fine powder befoe digestion.

The dilute hydrofluoric acid (HF) extraction and spectrometric molybdenum-yellow method of Saito *et al.* (2005) was used for the analysis of the level of silica in the leave tissues. Briefly, 100 mg of dry weight of each plant powder was digested in 2 ml of HF solution of concentration 1.5 M HF - 0.6 M HCl in 10 ml plastic bottle. Digestion was carried out at room temperature for one hour with occasional stirring (after each 10 min). After digestion, 8 ml of distilled water was added to each sample tube and the resulting mixture homogenised by vortexing before settling down for further 20 minutes. Silica powder was obtained by heating 50 ml of pure sodium silicate (Sigma-Aldrich, 338443) at 950°C for 2 h in a furnace before cooling and grinding the solid into a fine powder. Thereafter, 1mg/ml silica stock solution was prepared by digesting 0.1 g of silica powder in 20 ml of a 0.3 M HF - 0.12 M HCl solution and then topping to 100 ml with distilled water. This stock solution was diluted to prepare standard solutions of concentration ranging between 0 to 1 mg/l.

For spectrophotometric determinations of the amount of silica in the powdered leaf samples, 100 µl aliquot of each plant digest was transferred to a 10 ml plastic tube and 2ml of 0.1M boric acid solution added. Two millilitres of the molybdenum solution (0.025 M Mo - 0.4 M H_2SO_4 - 0.25 M BH_3O_3) was then added, mixed and allowed to stand for five minutes to allow the complete formation of molybdenum yellow complex. Four millilitres of 0.1 M citric acid was then added and thereafter the mixture vortexed. Similar procedure was used to prepare the aforementioned silica standards. Optical densities of both samples and standard solutions were measured in a 5 ml cell on a Beckman DU 640 spectrophotometer at 400 nm between 4-10 minutes following the addition of 0.1m citric acid. The amount of

silica in the samples was calculated from a calibration curve generated using standard solutions. All reagents and solutions for this experiment were prepared in silica free polypropylene plastic ware previously soaked in 0.1% HF solution.

3.2.10: Survival and growth of larvae on silica amended diets

To evaluate *B. fusca* survival and larval growth rates to increasing dietary silica levels, the artificial diet prepared by Onyango and Ochieng–Odero (1994) was amended with varying levels of silica (from the aforementioned silica powder obtained from sodium silicate). The levels of silica in the artificial diet ranged between 0 to 80 mg/ml and paralleled the concentration range found among the plant species analysed in this study. Once prepared, 20mls of each diet fraction (with different silica levels) was dispensed in each heat-sterilised glass vials (7.5 cm long x 2.5 cm diameter), 9 vials per each silica level. Preweighed *B. fusca* neonates were then inoculated into each vial containing the diet fraction (4 neonates per vial) twenty four hours following hatching. For all experiments, vials were tightly fitted with a cotton wool following each inoculation and kept in 80% relative humidity on a 12:12 hr (L/D) photoperiod. Larvae were allowed to feed *ad libitum* during the experimental period and the respective diet fractions replaced as necessary. Thereafter, surviving larvae were counted and weighed after 19 days of development (the minimum period at which significant effect of silica on *B. fusca* performance was discernable).The percent larval recoveries per vial (corresponding to the percentage of survival) were calculated. The relative growth rates (RGR) were calculated by subtracting the initial larval weight from the final weight and dividing the difference by the number of days of development (Ojeda-Avila *et al*., 2003).

3.2.11: Statistical analysis

Data on percent larval recovery per plant were arcsin transformed. Untransformed results are presented in Tables. All means were separated by Fisher's PLSD or Student-Newman-Keuls test following one-way analysis of variance (ANOVA). For data on feeding preference bioassays in single-choice conditions, ranks were generated following the Kruskal-Wallis test, using Proc RANK of SAS 9.1 (SAS Institute, 2003), and means separated using Tukey's Studentized range test (Proc GLM) (SAS Institute, 2003). For multiple choices feeding tests data, means were separated using Wilcoxon signed ranks test (Stat View software, Abacus Concept, USA). Data on plant sugar content and their relative proportions in the extracts were log and arcsin transformed respectively while data on total phenolic content per plant species were also log transformed. Untransformed data are presented in the Tables. All means were separated by Fisher's PLSD test following ANOVA. A linear-regression of feeding response index in relation to the concentration of each sugar (sucrose or turanose) per extract was done and tested using ANOVA.

Data on plant silica content and percent larval recovery per plant were log and arcsin transformed, respectively. Untransformed results are presented in tables. All means were also separated by Fisher's PLSD test following ANOVA. Linear-regressions of silica contents on percentages of larvae recovered and RGR (Relative Growth Rate) were done to evaluate the respective R^2 values and thus the relationships between the silica content and the percentages of survival and RGR. In addition, a linear-regression of percentage of survival (arcsin transformed) and RGR in relation to the concentration of silica in the artificial diet was done and tested using ANOVA. All statistical tests were done using Stat View software (Abacus Concept, USA).

3.4: Results

3.4.1: Effect of host plants on the survival and growth of *B. fusca* larvae

The survival and growth of *B. fusca* neonates on intact plants varied significantly among the plant species tested (Table3.1 ANOVA: $F_{6,77} = 119.1$, $P < 0.0001$; $F_{6,77} = 87.7$, $P < 0.0001$ and $F_{6,77} = 122.9$, $P < 0.0001$ after 7, 19 and 31 days, respectively). Following infestations, maize and wild sorghum supported the greatest larval survival and growth: 33 and 44% larvae survived on maize and wild sorghum after 7 and 19 days respectively. However, after 31 days of infestation, whereas 41% of larvae survived on maize, only 10% survived on wild sorghum. In contrast, the other five plants; *P. purpureum, A. donax, P. maximum, P. deustum* and *S. megaphylla* were poor larval hosts, and a rapid decline in the larval survival on these plants was obtained over the infestation period tested. In addition, no larva survived on *P. desteum* and *S. megaphylla* beyond seven days following infestation.

Similarly, growth of neonates was significantly higher on maize and wild sorghum (Table 3.1, ANOVA: $F_{6,59} = 29.8$, $P < 0.0001$; $F_{4,49} = 34.8$, $P < 0.0001$ and $F_{4,34} = 276.2$, $P < 0.0001$ after 7, 19 and 31 days, respectively). Nevertheless, while larval growth remained significantly higher on maize throughout the infestation period, a significant decrease in growth was observed on wild sorghum after 19 and 31 days. Larval growth was significantly lower on the other five plant species, regardless of the infestation period. A similar trend in both survival and growth was observed when all the plants were similarly infested with third instar larvae (Table 3.2). As expected, highest survival was recorded on maize and wild sorghum (ANOVA: $F_{6,47} = 26.8$, $P < 0.0001$ and $F_{6,47} = 18.9$, $P < 0.0001$ after 7 and 19 days respectively). Except for *A. donax* on which the growth rate of third instar larvae was significantly

lower after 7 days of infestation, growth rates on other plant species did not vary significantly over the infestation period (ANOVA: $F_{4,24} = 4.0$, $P = 0.0119$). No variation in growth rate was obtained after 19 days of infestation on *Z. mays, S. arundinaceum* or *P. maximum*, the only three plant species on which third instar larvae were recovered (ANOVA: $F_{4,12} = 0.6$, $P = 0.5743$), after the experiment

Table 3.1: Percentage of surviving (mean[1] ± SE, n=12) and growth rates (mg d^{-1}, mean[2] ± SE) of *Busseola fusca* larvae recovered after 7, 19 and 31 days of infestation by neonates on different Poaceae plant species

Plant species	After 7 days		After 19 days		After 31 days	
	% larvae recovered	Growth rate	% larvae recovered	Growth rate	% larvae recovered	Growth rate
Z. mays	38.9 ± 1.2 e	0.15 ± 0.012 c	44.5 ± 3.0 c	1.8 ± 0.22 c	41.1 ± 2.9 c	4.51 ± 0.15 c
S. arundinaceum	33.3 ± 1.0 d	0.13 c	42.8 ± 3.7 c	0.4 ± 0.04 b	10.0 ± 1.4 b	1.45 ± 0.12 b
P. purpureum	10.6 ± 1.6 c	0.09 ± 0.013 b	12.2 ± 1.6 b	0.6 ± 0.12 b	2.8 ± 0.9 a	0.07 ± 0.01 a
A. donax	7.8 ± 2.2 bc	0.05 ± 0.013 a	6.1 ± 0.9 a	0.1 ± 0.06 a	2.2 ± 0.7 a	0.21 ± 0.01 a
P. maximum	1.7 ± 0.5 a	0.06 ± 0.002 a	5.0 ± 1.3 a	0.1 ± 0.02 a	0.6 ± 0.4 a	0.04 a
P. deustum	6.7 ± 1.3 bc	0.03 ± 0.004 a	0 a	-	0 a	-
S. megaphylla	3.9 ± 1.2 ab	0.03 ± 0.005 a	0 a	-	0 a	-

Means within a column followed by different letters are significantly different at 5% level ([1]Fisher's PLSD test or [2]Student-Newman-Keuls test following ANOVA).

Table 3.2: Percentages of surviving third instar *Busseola fusca* larvae recovered (mean ± SE, n=8, except for *Z. mays* and *S. arundinaceum* n=7) and growth rates (mg d^{-1}, mean ± SE) after 7 and 19 days of infestation on different plant species

Plant species	After 7 days		After 19 days	
	% larvae recovered	Growth rate	% larvae recovered	Growth rate
Z. mays	85.7 ± 5.7 c	16.4 ± 1.9 b	65.7 ± 9.5 c	12.8 ± 0.4
S. arundinaceum	57.1 ± 9.2 b	10.4 ± 1.5 b	25.7 ± 5.7 b	11.6 ± 1.2
P. purpureum	20.0 ± 7.6 a	10.5 ± 2.3 b	0 a	-
A. donax	12.5 ± 5.3 a	3.5 ± 1.2 a	0 a	-
P. maximum	20.0 ± 6.5 a	10.4 ± 2.9 ab	5.0 ± 3.3 a	11.9 ± 1.8
P. deustum	0 a	-	0 a	-
S. megaphylla	0 a	-	0 a	-

Means within a column followed by different letters are significantly different at 5% level. (Student-Newman-Keuls test following ANOVA).

3.4.2: Influence of plant metabolites on the performance of *B. fusca* larvae

Hexanic and methanolic extracts of the seven Poaceae plant species used in this study were tested for their phagostimulatory activities on third instar *B. fusca* larvae. The results of the feeding tests expressed in terms of feeding response index (FRI) are shown in the Tables 3.3 to 3.6. In all feeding bioassay tests, all plant methanolic used generally induced significant phagostimulatory activities as compared to the hexane extracts. Under no-choice conditions, significant higher RFI values were obtained for larvae fed on methanolic extracts as compared to hexane extracts

regardless of the plant species used (Table 3.3). This result was confirmed when extracts were further tested under 2- or 4-choice conditions (Tables 3.4 and 3.5). However, not all the RFI values obtained for methanolic extracts under no-choice conditions were significantly different when compared. In contrast, under 8-choice conditions, *Z. mays* extract was clearly the most bioactive among the methanolic extracts used as demonstrated by the significantly higher FRI obtained compared to the other plant extracts tested. Extracts of *S. arundinaceum, A. donax* and *P. maximum*, exhibited intermediate but statistically similar feeding response indices. Moreover, extracts of *P. deustum* and *S. megaphylla* were the least bioactive and exhibited significantly less phagostimulatory activity among all the methanolic extracts tested.

Table 3.3: Feeding response indices (%, mean ± SE, n=30) for third instar *Busseola fusca* larvae after 36 hours of feeding on crude leaf extracts of different plant species topically applied on Styrofoam carrier matrices under no-choice conditions

Plant species	Type of extracts	Feeding response index
Control	Methanol alone	0.7 ± 0.2a
	Hexane alone	1.0 ± 0.4a
Z. mays	Methanol extracts	8.2 ± 1.2d
	Hexane extracts	2.2 ± 0.8a
S. arundinaceum	Methanol extracts	4.6 ± 0.8bcd
	Hexane extracts	2.3 ± 0.9abc
P. purpureum	Methanol extracts	3.8 ± 1.2abc
	Hexane extracts	1.9 ± 0.8a
A. donax	Methanol extracts	4.4 ± 0.4cd
	Hexane extracts	1.9 ± 0.4ab
P. maximum	Methanol extracts	6.2 ± 1.4bcd
	Hexane extracts	1.1 ± 0.3a
P. deustum	Methanol extracts	6.6 ± 1.3cd
	Hexane extracts	1.0 ± 0.2a
S. megaphylla	Methanol extracts	5.7 ± 1.2bcd
	Hexane extracts	1.9 ± 0.7a

Means within a column followed by different letters are significantly different at 5% level (Tukey's Studentized Range test).

Table 3.4: Feeding response indices (%, mean ± SE, n=30) for third instar *Busseola fusca* larvae after 36 hours of feeding on crude leaf extracts of different plant species topically applied on Styrofoam carrier matrices under dual choice conditions

Plant species	Type of extracts	Feeding response index
Z. mays	Methanol extracts	6.4 ± 1.0b
	Hexane extracts	1.5 ± 0.5a
S. arundinaceum	Methanol extracts	7.5 ± 1.0b
	Hexane extracts	2.8 ± 0.8a
P. purpureum	Methanol extracts	4.3 ± 0.9a
	Hexane extracts	2.7 ± 0.9a
A. donax	Methanol extracts	9.3 ± 1.1b
	Hexane extracts	1.8 ± 0.6a
P. maximum	Methanol extracts	7.1 ± 1.3b
	Hexane extracts	0.5 ± 0.1a
P. deustum	Methanol extracts	8.6 ± 1.1b
	Hexane extracts	0.9 ± 0.3a
S. megaphylla	Methanol extracts	7.2 ± 1.3b
	Hexane extracts	1.2 ± 0.4a

Means within a column and plant species followed by different letters are significantly different at 5% level (Wilcoxon signed ranks test).

Table 3.5: Feeding response indices (%, mean ± SE, n=30) of third instar *Busseola fusca* larvae after 36 hours of feeding on crude leaf extracts of different plant species topically applied on Styrofoam carrier matrices under 4-choice conditions

Plant species	Type of extracts	Feeding response index
Z. mays	Methanol alone	0.6 ± 0.2a
	Hexane alone	0.6 ± 0.1a
	Methanol extracts	5.9 ± 1.1b
	Hexane extracts	0.9 ± 0.2a
S. arundinaceum	Methanol alone	0.6 ± 0.1a
	Hexane alone	0.9 ± 0.2ab
	Methanol extracts	8.3 ± 1.3c
	Hexane extracts	1.6 ± 0.5b
P. purpureum	Methanol alone	0.6 ± 0.2a
	Hexane alone	0.8 ± 0.4a
	Methanol extracts	3.6 ± 0.8b
	Hexane extracts	0.6 ± 0.4a
A. donax	Methanol alone	0.3 ± 0.1a
	Hexane alone	0.8 ± 0.2b
	Methanol extracts	4.7 ± 0.8c
	Hexane extracts	0.7 ± 0.2ab
P. maximum	Methanol alone	1.2 ± 0.3a
	Hexane alone	1.4 ± 0.4a
	Methanol extracts	8.6 ± 1.5b
	Hexane extracts	1.4 ± 0.4a
P. deustum	Methanol alone	0.4 ± 0.1b
	Hexane alone	0.5 ± 0.2b
	Methanol extracts	5.2 ± 0.7c
	Hexane extracts	0.1 ± 0.05a
S. megaphylla	Methanol alone	1.4 ± 0.6ab
	Hexane alone	0.4 ± 0.1a
	Methanol extracts	2.1 ± 0.8b
	Hexane extracts	1.2 ± 0.6a

Means within a column and plant species followed by different letters are significantly different at 5% level (Wilcoxon signed ranks test).

Table 3.6: Feeding response indices (%, mean ± SE, n=87) of third instar *Busseola fusca* larvae after 36 hours of feeding on crude methanol extracts of different plant species topically applied on styrofoam carrier matrices under 8-choice conditions

Type of extracts	Feeding response index
Methanol alone	0.7 ± 0.1a
Z. mays	4.5 ± 0.6d
S. arundinaceum	2.5 ± 0.4c
P. purpureum	1.8 ± 0.3c
A. donax	2.5 ± 0.4c
P. maximum	2.0 ± 0.3c
P. deustum	1.2 ± 0.2b
S. megaphylla	1.2 ± 0.2b

Means within a column followed by different letters are significantly different at 5% level (Wilcoxon signed ranks test).

Nine naturally occurring sugars were identified in the phagostimulatory fractions of the methanolic plant extracts following HPLC analysis. These included monosaccharide (xylose, fructose, glucose and galactose) and the disaccharides (sucrose, turanose, maltose, lactose and melibiose). Sucrose was the most abundant sugar in each of the plant extracts analysed and varied from 22 to 65µg/mg (corresponding to 0.06 to 0.2 M), of dry leaf weight (Table 3.7). This represented a relative proportion of between 25 to 38% of all the sugar content in all the plant extracts analysed (Table 3.8). The level of sucrose in the extracts however, did not vary significantly among the plant species studied. Glucose and fructose (possibly as

hydrolysis products of sucrose hydrolysis) were also abundant in the extracts. Their relative proportions were similarly constant among most of the plant species tested, except, in S. arundinaceum extracts where their levels were both significantly higher (Table 3.7). Turanose, a disaccharide of glucose and fructose, was the most and significantly variable sugar component of the extracts tested with its levels varying from 5 to 36µg/mg (corresponding to 0.01 to 0.1 M) of dry leaf weight. The relative proportion of this sugar was however lower in *Z. mays, S. arundinaceum* and *P. purpureum* but relatively higher in *A. donax*, *P. maximum*, *P. deustum* and *S. megaphylla*(Tables3.7and3.8).

Table 3.7: Amount of different sugars (µg/mg of leaf dry weight, mean ± SE, n=5) found in leaves of the plant species studied

Plant species	Xylose	Fructose	Glucose	Galactose	Sucrose	Turanose	Maltose	Lactose	Melibiose
Z. mays	2.1 ± 0.4	8.9 ± 2.6a	15.5 ± 4.9a	1.9 ± 0.9	25.7 ± 11.2	4.6 ± 1.6a	5.3 ± 2.1	3.7 ± 0.9	1.7 ± 0.4
S. arundinaceum	1.7 ± 0.6	27.0 ± 7.3b	41.5 ± 11.3b	15.8 ± 10.3	65.1 ± 17.6	10.3 ± 2.7a	5.6 ± 1.6	1.8 ± 0.8	3.9 ± 1.5
P. purpureum	3.3 ± 1.6	15.3 ± 6.6a	16.6 ± 4.4a	2.0 ± 1.7	32.6 ± 16.5	9.1 ± 1.8a	5.1 ± 0.4	3.0 ± 1.0	2.7 ± 0.6
A. donax	2.6 ± 0.7	13.1 ± 3.1a	15.5 ± 3.2a	2.7 ± 0.6	42.0 ± 8.8	33.9 ± 8.1b	16.6 ± 2.9	3.0 ± 0.7	4.4 ± 1.1
P. maximum	1.1 ± 0.4	9.9 ± 3.2a	17.8 ± 5.5a	3.7 ± 1.8	22.4 ± 6.8	18.0 ± 5.5ab	2.8 ± 0.9	2.3 ± 0.6	3.4 ± 1.5
P. deustum	2.1 ± 0.6	7.3 ± 2.4a	17.1 ± 6.0a	4.9 ± 1.2	32.7 ± 12.5	36.0 ± 12.7b	9.4 ± 7.1	7.5 ± 4.8	3.3 ± 1.1
S. megaphylla	1.1 ± 0.4	7.4 ± 0.5a	14.2 ± 5.1a	4.4 ± 1.3	27.9 ± 7.5	28.2 ± 8.8b	2.6 ± 1.1	2.1 ± 0.9	3.8 ± 1.6

Means within a column followed by different letters are significantly different at 5% level (Fisher's PLSD test). No letter was assigned when P>0.05 for ANOVA.

Table 3.8: Relative proportion of sugars (%, mean ± SE, n=5) found in the dry leaves of the plant species studied

Plant species	Xylose	Fructose	Glucose	Galactose	Sucrose	Turanose	Maltose	Lactose	Melibiose
Z. mays	4.3 ± 1.5	13.7 ± 1.6b	23.2 ± 2.4c	2.6 ± 0.7	31.3 ± 4.9	7.0 ± 1.3a	7.7 ± 1.4b	6.2 ± 1.1	3.8 ± 1.3
S. arundinaceum	1.2 ± 0.3	15.8 ± 0.7b	24.0 ± 1.4c	6.5 ± 3.0	38.4 ± 2.2	6.8 ± 1.1a	3.4 ± 0.5a	1.5 ± 0.6	2.4 ± 0.7
P. purpureum	5.3 ± 2.9	16.0 ± 2.1b	18.9 ± 2.1bc	1.6 ± 1.1	32.3 ± 6.6	11.5 ± 3.2a	6.5 ± 1.5b	4.4 ± 2.2	3.4 ± 1.1
A. donax	2.1 ± 0.8	9.7 ± 2.1a	11.4 ± 2.0a	2.1 ± 0.5	31.6 ± 6.0	25.0 ± 4.6b	12.5 ± 1.7b	2.4 ± 0.7	3.2 ± 0.6
P. maximum	3.0 ± 1.5	12.5 ± 1.9ab	19.7 ± 2.8bc	5.0 ± 1.5	26.8 ± 3.6	20.3 ± 2.4b	3.9 ± 1.2a	3.8 ± 1.5	4.9 ± 1.9
P. deustum	3.5 ± 1.6	7.6 ± 1.5a	14.3 ± 2.0a	5.1 ± 1.2	25.7 ± 5.3	26.5 ± 3.8b	7.2 ± 3.0b	6.1 ± 1.9	3.8 ± 1.0
S. megaphylla	1.5 ± 0.9	10.6 ± 2.6a	15.3 ± 2.2ab	4.7 ± 0.7	30.1 ± 2.6	29.0 ± 3.0b	2.7 ± 1.2a	2.5 ± 1.3	3.6 ± 1.1

Means within a column followed by different letters are significantly different at 5% level (Fisher's PLSD test). No letter was assigned when P>0.05 for ANOVA.

The concentrations of total phenolic compounds estimated in plant leaf extracts ranged between 1.6 to 5.1 µg/mg of dry leaf weight (Table 3.9) and were significantly varied among the seven plant species used. *Zea mays* and *P. purpureum* had similar although significantly lowest levels, while *P. desteum*, *P. maximum* and *S. arundinaceum* had the highest levels. However, the concentration of total phenolic compounds in the methanol extracts did not correlate with the feeding response indices of *B. fusca* larva

Table 3.9: Content of total phenolic compounds (in µg gallic acid equivalents/mg of dry leaf weight, mean ± SE, n=10) found in leaves of the plant species studied

Plant species	Total phenolic content
Z. mays	1.8 ± 0.1 a
S. arundinaceum	4.4 ± 0.1 d
P. purpureum	1.6 ± 0.05 a
A. donax	3.6 ± 0.3 c
P. maximum	4.1 ± 0.2 d
P. deustum	5.1 ± 0.2 e
S. megaphylla	2.7 ± 0.1 b

Means within a column followed by different letters are significantly different at 5% level (Fisher's PLSD test).

By increasing sucrose concentration, a significant and positive dose-response curve was obtained for feeding response index (FRI) under no-choice conditions ($F_{1, 73}$ = 45.128, P<0.0001; Figure 3.3a). In contrast, when the concentration of turanose was increased, a significant negative dose-response curve was obtained under no-choice conditions ($F_{1,73}$ = 6.115, P = 0.0157; Figure 3.3b). Similarly, when a homogeneous

mixture of sucrose and turanose were tested for feeding, significantly higher RFI values were obtained as the level of sucrose in the mixture was increased proportionally as compared to turanose levels (Table 3.10). In contrast, FRI decreased significantly with a concomitant increase in the proportion of turanose in the mixture. Similar trends were obtained under 6-choice conditions (Table 3.11).

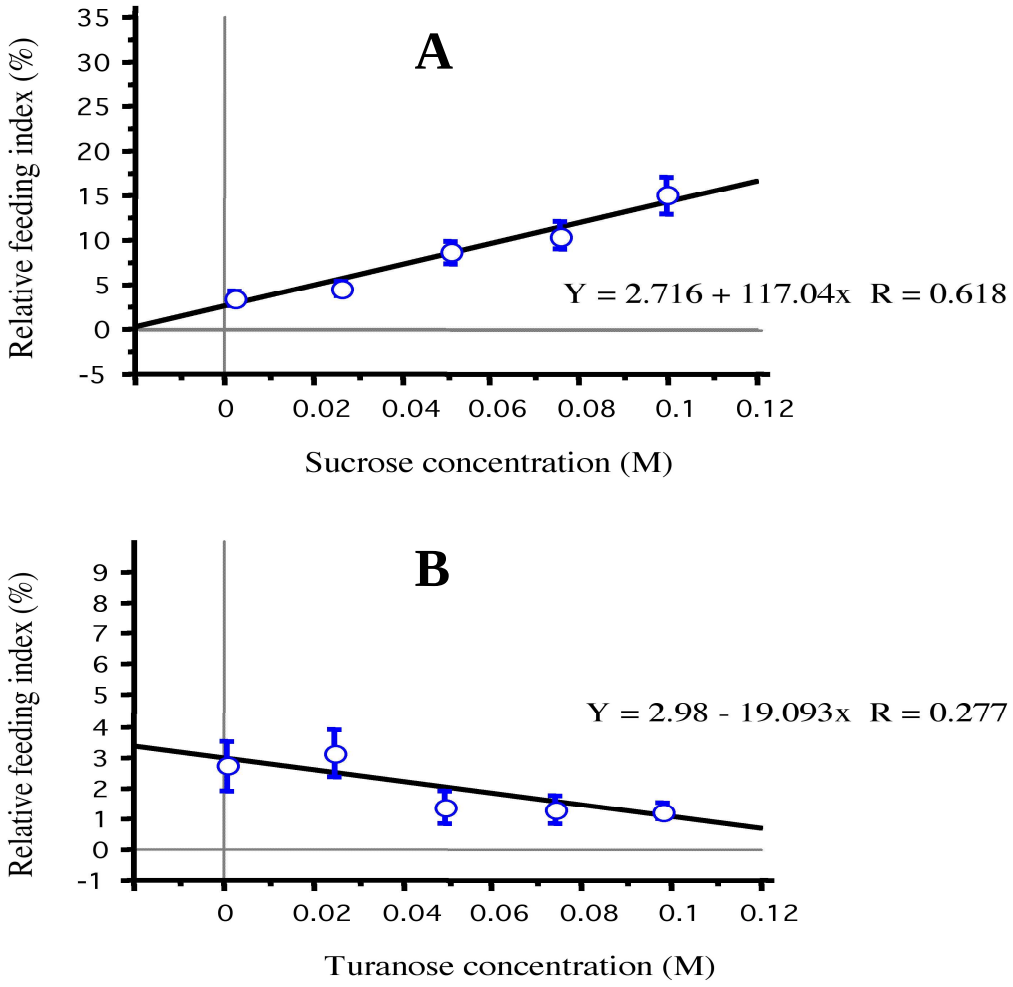

Figure 3.3: Dose-response curves for relative feeding indices (mean ± SE, n=15) obtained by increasing sucrose (A) or turanose (B) levels under no-choice conditions.

Table 3.10: Feeding response indices (%, mean ± SE, n=24) for third instar *Busseola fusca* larvae after 36 hours of feeding on mixture of sucrose and turanose of varying concentration topically applied on styrofoam carrier matrices under no-choice (A) and 6-choice conditions (B).

Sugar mixtures tested	Feeding response index (FRI)	
	A	B
Methanol alone	2.2 ± 0.5a	1.0 ± 0.4a
0.1 M sucrose / 0 M turanose	15.4 ± 1.5c	5.5 ± 0.9b
0.075 M sucrose / 0.025 M turanose	10.3 ± 2.4b	4.7 ± 1.0b
0.05 M sucrose / 0.05 M turanose	3.6 ± 1.2a	5.6 ± 1.1b
0.025 M sucrose / 0.075 M turanose	5.2 ± 1.2ab	1.4 ± 0.4a
0 M sucrose / 0.1 M turanose	1.3 ± 0.2a	0.4 ± 0.1a

(A) [a]Means within a column followed by different letters are significantly different at 5% level (Tukey's Studentized Range test). (B): Means within a column followed by different letters are significantly different at 5% level (Wilcoxon signed ranks test).

3. 4. 3: Influence of plant silica on survival and growth of *B. fusca* larvae

Silica content present in the potted plant leaves varied significantly among the seven plant species analysed (Table 3.11, $F_{7, 248} = 82.8$, $P < 0.0001$). *Zea mays* and *S. arundinaceum* had the lowest silica levels and varied between 20 to 24 µg/mg of dry leaf weight (corresponding to a concentration of 20 to 24 mg/ml of silica as determined in the artificial diet). *P. maximum* and *P. deustum* harboured the highest levels of silica in their leave tissues that varied between 45-55 µg/mg

(corresponding 45 to 55 mg/ml in the artificial diet). *S. megaphylla, P. purpureum* and *A. donax* showed similar but intermediate levels of silica (Table 3.11).

Table 3.11: Amount of silica (mean ± SE, n=34) found in different Poacea plant species used in the study

Plant species	Silica content (µg/mg of dry weight)
Z. mays	19.8 ± 1.0a
S. arundinaceum	24.2 ± 0.3b
P. purpureum	29.3 ± 1.1c
A. donax	29.6 ± 1.2c
P. maximum	45.4 ± 1.1d
P. deustum	55.0 ± 2.3e
S. megaphylla	28.1 ± 0.6c

Means followed by different letters are significantly different at 5% level (Fisher's PLSD test following ANOVA).

Plant silica content negatively correlated with percentages of survival or relative growth rates (RGR) of *B. fusca* larvae when intact plants were infested by neonates (Table 3.1). The highest silica containing plant leaves supported the lowest percentage of larval survival or growth rates. Linear-regressions using the same data (Table 3.1) showed significant negative correlations (with % survival after 7 days of feeding: R=-0.766, $F_{1,82}$ = 43.3, P < 0.0001; with % survival after 19 days of feeding: R= -0.602, $F_{1,82}$ = 46.5, P < 0.0001; with % survival after 31 days of feeding: R= -0.455, $F_{1,82}$ = 21.4, P < 0.0001; with RGR after 7 days of feeding: R= -0.596, $F_{1,62}$ = 34.1, P < 0.0001; with RGR after 19 days of feeding: R= -0.335, $F_{1,54}$ = 6.8, P = 0.0119 and with RGR after 31 days of feeding: R=-0.477, $F_{1,37}$ = 10.9, P =

0.0021). For example, *P. deustum* with the highest silica content was the least preferred plant for larval survival and growth while *Z. mays* with the least silica content was mostly preferred plant for larval survival and growth. Similarly, plant silica content negatively correlated with percentages of survival when all the plants were infested by third instar larvae (Table 3.2), with highest silica containing leaves supporting the lowest percentage of larval survival. Linear regression results using the same data (Table 3.2) showed significant negative correlations with percentage of survival (with % survival after 7 days of feeding: R= -0.432, $F_{1,52}$ = 12.0, P = 0.0011; with % survival after 19 days of feeding: R= -0.337, $F_{1,52}$ = 6.7, P = 0.0126). Nevertheless, no correlation was evident between RGR and the levels of leaf silification when the plants were infested by third instar larvae (Table 3.2).

A significant variation in % survival and growth rates were obtained when larvae were allowed to feed on artificial diets amended with increasing levels of silica (results of ANOVA: $F_{4, 40}$ = 5.1, P = 0.0020 for % survival; $F_{4, 40}$ = 31.9, P < 0.0001 for RGR). A strong interdependence was also obtained between silica content and *B. fusca* larval survival and growth rates after 19 days of development (Figure 3.4). A significant negative correlation existed between the level of silica and the percentages survival and the relative growth rates over the feeding periods tested (R = -0.482, $F_{1, 43}$ = 13.0, P = 0.0008 with % survival; R = -0.855, $F_{1, 43}$ = 117.6, P < 0.0001 with RGR). Highest % survival and growth rates were recorded among larvae fed on low-silica containing diets (0 to 20 mg/ml), while lowest % survival and growth rates were observed among larvae fed on high-silica containing diets (60 to 80 mg/ml).

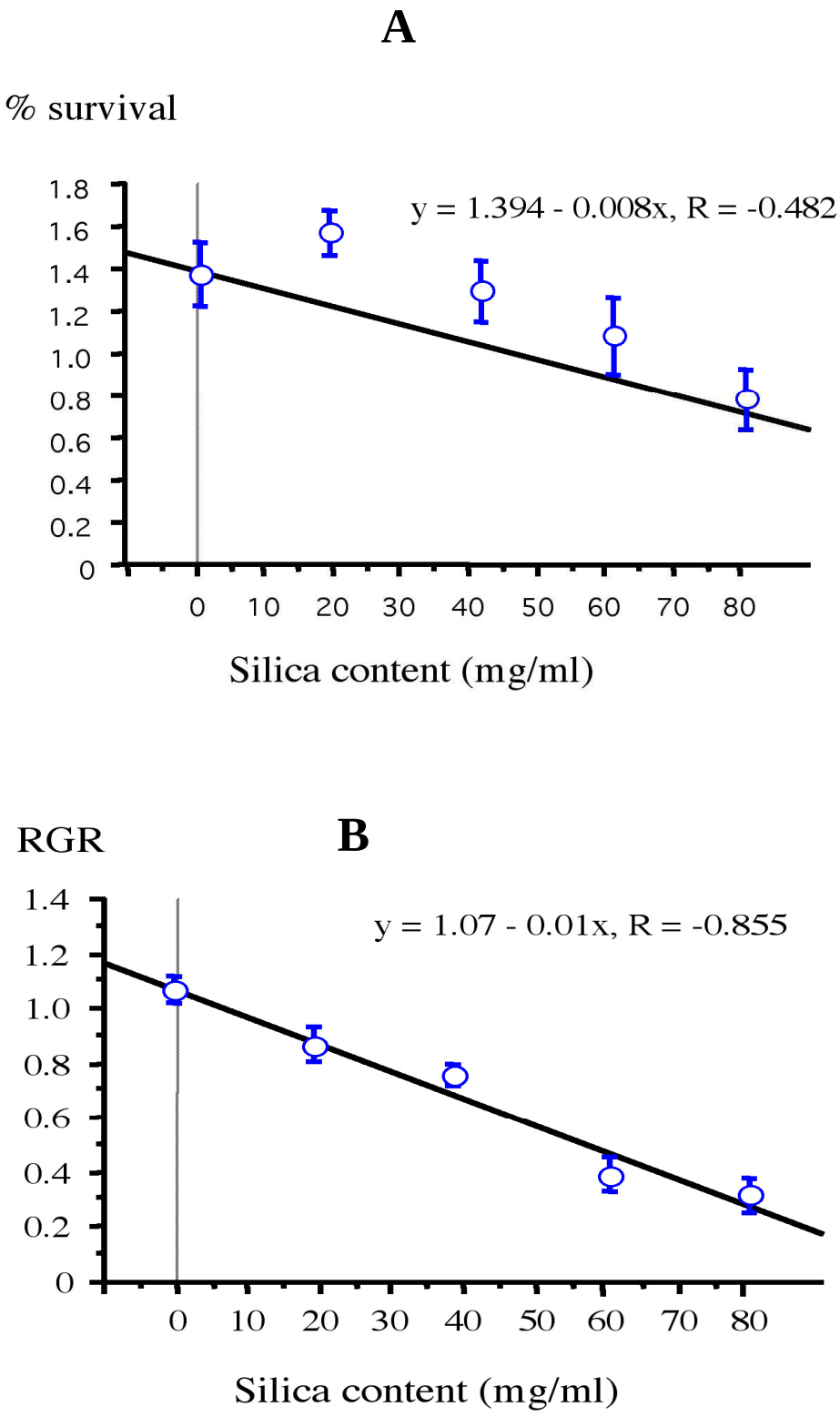

Figure 3.4: Dose-response curves for percentage of surviving larvae recovered (arcsin transformed values, mean ± SE, n=9) (A) and relative growth rates (RGR) (mg.d^{-1}, mean ± SE, n=9) (B) obtained after increasing silica concentration.

3. 5: Discussion

For phytophagous insects, plants can be grouped into three distinct classes: (i) hosts fed upon in nature; (ii) acceptable non-hosts, not fed upon in nature but which can act as food plants in the laboratory; and (iii) unacceptable hosts, neither fed upon in nature nor in the laboratory (Gupta and Thorsteinson, 1960; De Boer and Hanson, 1984). All the seven plant species used in this study influenced significantly the survival and growth rates of both first and third instar *B. fusca* larvae in laboratory conditions. Both larval developmental stages performed better on *Z. mays* and *S. arundinaceum* throughout the infestation period as compared to the rest of other plant species used. The suitability of *Sorghum* sp. and *Z. mays* on the performance of *B. fusca* has been well reported previously (van Rensburg *et al.*, 1989; Haile and Hofsvang, 2002; Le Rü *et al.,* 2006a). However, although neonate survival and growth on the other plant species tested especially *P. purpureum*, *A. donax* and *P. maximum* was inferior to that of *Z.mays* and *S.arundinaceum,* marginal survival recorded on the three plant species suggest the ability of larvae to include not only *S. arundinaceum* but also other related wild plants as food source, probably when the preferred hosts are not available. Marginal utilisations of wild plants by *B. fusca* larvae have also been observed in the field particularly in seasons when maize or cultivated sorghum are absent (Ong'amo *et al*., 2006). Growth rates of Lepidoptera on their host plants are influenced by many factors, but primarily nutritional composition and secondary plant compounds (Herms and Mattson, 1992; Slansky, 1992). Therefore, minimal larval performance on wild plants used in this study may suggest the presence of antibiotic properties possibly due to the presence of plant

secondary metabolites, or similarly these plants lacked nutrients necessary for optimal growth or were physically less suitable for feeding by the larvae.

The acceptance of a particular host plant for feeding by phytophagous insect's larvae is dependent on the phytochemical blends of the plants, which may induce phagostimulant or deterrent bioactivities. Compounds present in both methanol and hexane extracts variably affected feeding responses of third instar larvae. *B. fusca* larvae were stimulated to feed more on methanolic extracts than on hexane extracts indicating that most chemical feeding stimulants were more polar and soluble in methanol. This result is consistent with similar studies in which polar compounds extractable in methanol have been shown to elicit positive feeding responses in *Helicoverpa* species (Lepidoptera: Noctuidae) (Cai *et al*., 2002). In the contrary, hexane extracts elicited aversive or non-remarkable larval feeding responses suggesting that non-polar compounds (e.g. lipids) present in hexane extracts were non-phagostimulatory (as exhibited in no-choice experiments) and less attractive (as exhibited in multiple-choice experiments) to the foraging larvae. Aversive feeding responses therefore suggest either the hexane extracts contained chemicals that deterred feeding or were toxic to the larvae. However, the fact that no larval mortality was observed after feeding on hexane extracts indicated the presence of deterrent rather than toxic allelochemicals in the hexane plant extracts.

The relative contribution of each of the plant's methanolic extract on larval feeding response and preference was however evident in the data obtained for 8-choice bioassay tests. Larvae responsiveness to compounds in the crude extracts varied among the plant species used. Significant feeding response was observed for *Z. mays* extract than for any other plant species used. The hierarchical ranking order of

larval feeding preference on methanolic plant extracts from the most preferred to the least preferred was: *Z. mays* > *S. arundinaceum* = *P. purpureum* = *A. donax* = *P. maximum* > *P. deustum* = *S. megaphylla*. This rank ordering paralleled survival and growth rates on intact plants, where larvae fed on *Z. mays* and *S. arundinaceum*, exhibited the best performance as compared to the residual survival and growth rates on *P. purpureum*, *A. donax* and *P. maximum*. Similar hierarchical ordering of plants according to preference has been observed in the field for *B. fusca* and the species has been reported to use a host lower in the hierarchy especially when the preferred species is not available (LeRü *et al.*, 2006b). The flexibility in host choice presumably enhances the ability of individuals of *B. fusca* to cope up with variation in the environment as has been suggested by Thompson and Pellmyr (1991).

The discriminatory behaviour of *B. fusca* larvae towards some plants used in this study appeared to be influenced by the quantitative and qualitative chemical characteristics of each particular plant species used. The general feeding preferences on methanolic extracts as compared to hexane extracts suggested the presence of soluble phagostimulatory compounds in the methanol extracts. Subsequent isolation and identification of the chemical constituents in the methanolic extracts of each of the plant species used revealed the presence of nine main soluble sugars including xylose, fructose, glucose, galactose, sucrose, turanose, maltose, lactose and melibiose. Most of these sugars identified have been implicated to stimulate feeding in many lepidopteran larvae (Lopez and Lingren, 1994; Bartlet *et al*., 1994). Sucrose represented the major sugar component in all plant methanolic extracts analysed regardless of the plant species. A significant positive dose-response curve for feeding response index as a function of increasing concentration of sucrose,

confirmed the phagostimulatory effect of sucrose towards *B. fusca* larvae. Similar phagostimulatory activity of sucrose to larval feeding has been reported for a number of other lepidopteran species (Albert *et al*., 1982; Bartlet *et al*., 1994; Bowdan, 1995).

Apart from sucrose, turanose was the most variable sugar component in most of the plant extracts analysed. High levels of this sugar were present in plant methanol extracts of *P. deustem*, *A. donax* and *S. megaphylla*. Extracts from the three plant species mediated minimal larval survival and growth rates and therefore levels of this sugar correlated negatively with *B. fusca* larval feeding responses. A similar and significant negative dose-response curve of RFI expressed as a function of increasing levels of pure turanose standards, corroborated the phagodeterrent effect of turanose towards feeding by larvae. This was contrary to a similar dose response curve of RFI as a function of increasing sucrose concentration that exhibited positive correlations with larval feeding. Turanose which is structurally analogous to sucrose is a reducing disaccharide with α-(1, 3) glycoside bond between glucose and fructose. It is therefore possible that the opposing effect of turanose and sucrose to larval feeding lies in the small but subtle structural differences inherent in the two sugars that are likely to give rise to variation in molecular fit in the larval taste receptors inducing the opposing behavioural responses. Though the role of turanose in feeding of lepidopteran larvae is limited in the literature, the same sugar has been demonstrated to possess a weak phagostimulatory potential for corn earworms, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Lopez and Lingren, 1994). Turanose has similarly been demonstrated to act as a strong phagostimulant for the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (Vander

Meer *et al*., 1995). Moreover, both sugars appeared to have a synergistic effect on *B. fusca* larval feeding responses when presented together in a solution mixture applied on Styrofoam carrier matrices especially when turanose was weakly concentrated. This implies that the host discriminatory ability and the selective feeding behaviour of *B. fusca* larvae may partly depend on the relative concentration of each of the two sugars in the plant tissue and this could sufficiently explain the oligophagous feeding habits of *B. fusca* larvae.

Plant total phenolic compounds have been reported to correlate negatively with performance of most important phytophagous species (Haukioja *et al*., 1985; Bergvinson *et al*., 1994; Santiago *et al*., 2005). Despite, their possible defensive roles against herbivores, some soluble phenolic compounds have been reported to possess strong phagostimulatory properties to lepidopteran larvae (Torto *et al*., 1991). The amount of total phenolic compounds in leaf extracts studied varied significantly among the plant species tested. *P. deustum*, *P. maximum* and *S. arundinaceum* extracts had the highest concentrations of total phenolic compounds as compared to the other four Poaceae plant species analysed. However, in contrast to sucrose or turanose, the levels of total phenolic compounds in the methanolic extracts did no correlate with the feeding response indices or the growth and survival of *B. fusca* larvae. For example, while both *S. arundinaceum and P. maximum* had similar and high levels of total phenolic compounds, larvae fed more on the methanolic extracts of the former than of the later. It is therefore possible that the determination of total phenolic compounds (i.e. pooled compounds) does not allow correlation of the importance of a particular phenolic compound present in the entire plants with the insect survival and performance parameters.

Silica in plants can constitute between 0.1 to 10% weight of the dry matter of most plant species (Ma and Takahashi, 2002). Results obtained from this study, indicate that the levels of amorphous silica varied considerably among the plant species analyzed. The silica content was about three times greater between the highest (*P. deustum,* 55.0 ± 2.3 µg/mg) and the least (*Z. mays,* 19.8 ± 1.0 µg/mg of dry leaf weight) following the analysis of silica from samples digested in dilute HF Nevertheless, levels of silica present in all plant leaf tissues sampled appeared to be within the concentration ranges as has been previously reported in similar studies (Ma and Takahashi, 2002).

Differences in silica content among plants are frequently associated with plant resistance to insect attacks (Keeping and Meyer, 2002; Kvedaras *et al.*, 2005; Keeping and Meyer, 2006). Therefore, during host selection process herbivorous insects may discriminate between high- and low-silica plants, and feed preferentially on the later. Growth rate of *B. fusca* larvae on the tested plant species correlated negatively with the degree of plant leaf silification. Larvae preferentially fed on *Z. mays* and S. *arundinaceum* foliage as exhibited by increased in growth rates and high larval survival percentages on both plants over the whole infestation period. Similar performance indices were however poor on other wild grasses especially *P. deustum* and *P. maximum,* that had the highest level of endogenous silica in their leaf tissues. This clearly indicated that plants analyzed in this study had different capacities to accumulate amorphous silica in their leaf tissues and high levels of silica in the leaves correlated negatively with the feeding and developmental performance of *B. fusca* larvae. This was further confirmed by using artificial diets amended with increasing levels of silica. It was evident that the detrimental effect of

silica on *B. fusca* larval performance is dose dependant and as little as 20 mg/ml of silica in the artificial diet was sufficient enough to disturb feeding and development of *B. fusca* larvae. For example, larval RGR decreased significantly from 1.06 ± 0.05 to 0.86 ± 0.06 mg d^{-1} following incorporation of 4grammes of silica into 200mls of artificial diet. This further corroborated the negative role of silica on the feeding and development of *B. fusca* larvae and parallels results of similar studies that previously sought to correlate the effect of silica on the feeding behaviour of several lepidopteran stemborer species (Peterson *et al.,* 1988; Kvedaras *et al.,* 2005; Keeping and Meyer, 2006). For *B. fusca*, such correlation can form the basis of host plant/artificial diet discrimination and may indicate that silica content in plants is a reliable predictor of food choice by *B. fusca* larvae.

The deterrent effect of silica that resulted in the reduction in the numbers and growth rates of larva on high silica containing plants may partly be attributed to the effect of silica at both nutritional and physical levels. As reported by Panda and Kush (1995) elevated levels of silica in plant cell wall might increase the bulk density of the diet and discourage larvae from ingesting sufficient quantities of nutrients and water leading to their poor performance. While compensatory feeding behaviour on nutritionally poor diets have been reported for some insects, feeding of *B. fusca* larvae on similar artificial diets amended with silica did not alter the rate of diet consumption as was reflected by the concentration dependent diminutive larval mass gain over the entire feeding period. Similar results have been reported by Keeping and Meyer (2006), Kvedaras and Keeping (2007) and Kvedaras *et al.* (2007b). Moreover, other studies have indicated that most specialist herbivores have limited ability to increase their diet consumption to compensate for feeding on poor

quality plants (Lee *et al.,* 2003). *B. fusca* larvae can therefore be considered more specialist than generalist (Le Rü *et al*., 2006a, b; Ong'amo *et al.,* 2006) and this could have given rise to the greater impact of silica on larval performance.

Despite of the deduced evidence on the importance of silica in deterring host plant choice by *B. fusca* larvae, the underlying mechanism of silica mediated resistance is still speculative and may only support the hypothesis that silica impedes larval penetration of host plants. It was observed that most larvae fed on the relatively high silica amended diets could not easily bore into the diet material but only fed superficially, nor bore into the stems of highly siliconized plants. This provides initial insight into the possible mechanisms of silica mediated resistance against *B. fusca* larvae and provides a correlative support for a physical role of plant silica in impeding feeding of larvae. However, this observation does not preclude the probable induction of biochemical defence pathways facilitated by the presence of soluble silica following wounding of tissues by *B. fusca* larvae.

From an applied point of view the fact that silica augments the resistance of various plants to pest insects (Keeping and Kvedaras, 2008) and that plants have a capacity to accumulate silica (Ma and Yamuji, 2006), is particularly relevant for silica deficient soils of cereal growing regions of sub-Saharan Africa. Silica amendments in plant tissues may provide improved resistance to *B. fusca* in these regions. This has been demonstrated for *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae) following *Z. mays* tissue silification (Sétamou *et al*., 1993) as a result of silica addition to the soils. However, for *B. fusca,* field trials are required to confirm this hypothesis and also confirm the quality of the modified crops for human consumption following silification of their tissues.

CHAPTER FOUR

4. 0: PHYSIOLOGICAL ADAPTATIONS AND GENETIC DIVERSITY OF *BUSSEOLA FUSCA* LARVAE IN RELATION TO HOST PLANT USE

4. 1: Introduction

The diversity of phytophagous insects is thought to be the result of the evolutionary process of host specialisation (Jaenike, 1990; Bernays and Chapman, 1994). Studies on host range and utilisation by *B. fusca* appears to be restricted and specific (Le Rü *et al.*, 2006a). The principal host plants for this species are cultivated cereal crops especially sorghum and maize (Le Rü *et al*., 2006a, 2006b; Ong'amo *et al*., 2006). Although, the species is well adapted to the two important staple crops, it is also reported to marginally attack other Poaceous non-crop plant species, including *Sorghum arundinaceum, Pennisetum purpureum, Arundo donax* and *Cymbopogon nardus. Sorghum arundinaceum, A. donax* and *Cymbopogon nardus* appear the most preferred non-crop host plants for *B. fusca* among the currently known candidate wild plant species. For example, of the 346 *B. fusca* larvae recovered on wild plants in Ethiopia, 328 (95%) were found on *A. donax* (Le Rü *et al*., 2006a), suggesting a strong association of *B. fusca* with the particular plant species.

To exploit a number of food plants but with diverse allelochemicals predictably encountered for growth and which are not in normal repertoire of larva's diet, young lepidopterous larva often depend on their digestive performance especially in enzyme assortment in the gut system (Brattsten, 1991; Peric-Mataruga *et al.*, 1997). For example, food composition has been reported to modify and alter the activity of

insect digestive enzymes (Hinks and Erlandson, 1994; Lazarevic and Peric-Mataruga, 2003). Therefore, the complement of digestive enzymes induced after larval foraging on chemotaxonomically diverse plants should reflect the allelochemical composition of the particular plant species. Such plastic responses to nutritive stresses are important for predicting insect's outbreaks and understanding mechanisms of host plant specialisation.

It was hypothesized by Sezonlin *et al.* (2006) that *B. fusca* abandoned its ancestral Poaceous wild hosts, switched and colonised the current and possibly nutritionally superior cultivated hosts, following their domestication (*S. bicolor*) or introduction (*Z. mays*) into Africa. Like many other phytophagous insects, adaptation of *B. fusca* to these new cultivated host plants following switch from their native wild hosts may have been accompanied by larva's physiological and/or behavioural adjustments due to the process of natural selection (Futuyma and Moreno 1988). Since host choice and oviposition behaviour among phytophagous insects are genetically determined (Jaenike, 1990), natural selection is likely to have favoured oviposition by *B. fusca* to hosts that currently support better growth and survival of the offspring as suggested by Gassmann *et al.* (2006).

Phenological, phytochemical and morphological differences among host plants may also promote utilization of specific hosts following host shifts and may also explain the current trend of host choice and affiliation by *B. fusca.* Sustained and prolonged colonization of adapted plants may induce the selection of adaptive traits and genetic differentiation within insect populations utilising specific hosts (Rice, 1987; Diehl and Bush, 1989; Jaenike, 1990) and may subsequently evolve into host races (Bush, 1994; Prowell *et al*., 2004). In phytophagous insects, species with narrow

host range are more prone to genetic differentiation than insect species with a wide host range (Futuyma and Moreno, 1988, Peterson and Denno, 1998). Host associated genetic differentiation has been documented in a number of moth families including Noctuidae (Pashley, 1986; Leniaud *et al*., 2006; Ong'amo *et al.*, 2008), Tortricidae (Emelianov *et al.,* 1995), Prodoxidae (Groman and Pellmyr, 2000) and Crambidae (Martel *et al.*, 2003; Leniaud *et al*., 2006).

Although geographical differentiation of *B. fusca* populations have been reported (Sezonlin *et al.*, 2006), little is known about the possible genetic isolation of the species by host plant. As Fox (1993) and Forister (2005) pointed out, differences between populations or species of insects in preference for and performance on a plant are usually genetically controlled, there could be a possible existence of genetic differentiation between *B. fusca* larva feeding on cultivated crops and those found to feed on wild plants such as *A. donax*.

The aim of this study was therefore to determine the larval physiological response in terms of digestive enzyme assortment induced in larvae fed on leaves of different plants and also to determine the possible existence of genetic differentiation between two *B. fusca* populations collected from cultivated crops (*Z. mays*) and *A. donax* in Kenya and Ethiopia respectively using cytochrome *b* gene markers. The mitochondrial cytochrome *b* gene was studied because it is informative at the intrageneric level in Lepidoptera (Simmons and Weller, 2001). It was envisaged that the two candidate plant-associated strains represent populations which differ genetically as a result of host specialisation.

4. 2: Materials and Methods

4. 2. 1: Insects

Larvae used in the study were sourced from laboratory reared individuals from Animal Rearing and Containment Unit (ARCU) of the International Centre of Insect Physiology and Ecology (ICIPE, Nairobi, Kenya). Neonates for experiments were directly used after one day following hatching. For molecular studies, *B. fusca* larvae were collected directly from *Z. mays* and *A. donax* plants in Kenya and Ethiopia respectively. Sampling was simultaneously done in both countries because neither country could provide both host plants infested with *B. fusca* larvae at the time of sampling. The Kenyan population were sampled from maize plants in Machakos and Mt. Kenya regions while the Ethiopian larvae were sampled from *A. donax* plants in Abay Mado and Baimraar regions. Five sampling points (localities) were considered for each region sampled. Plants with symptoms of stem borer infestation were dissected *in situ* for larval recovery. Plants from which larvae were recovered were first identified and the recovered larvae kept separately with respect to host plant species and site of collection. Larvae were then reared on artificial diet of Onyango and Ochieng'- Odero (1994) to adult moths. However most larvae sampled from *A. donax* did not develop to adulthood (only two fully developed to adulthood) and were therefore only used after attaining the fifth larval stage and prior to slipping into diapauses. Larvae and adult moths obtained after rearing were thereafter placed individually into 30 ml plastic vials and preserved in absolute ethanol (> 99%) at -20°C prior to DNA extraction.

4. 2. 2: Plants

Potted Maize, *Zea mays*, wild sorghum, *Sorghum arundinaceum*, Napier grass, *Pennisetum purpureum (*Schumach.) *Arundo donax, Setaria megaphylla, Panicum maximum and Panicum deustum* were used in this study. These plants were infested with neonate larva at the age of between 3-7 weeks of growth.

4. 2. 3: Determination of activities of digestive enzymes in larval homogenates of larvae fed on leaves of different plant species.

Dietary exposure to plant allelochemicals can induce physiological responses in neonate larvae that may consequently determine the feeding behaviour of late instars. A fast semi-quantitative analysis of enzymatic activities present in the larvae previously fed on leaves of different plant species was performed using the API-ZYM system (BioMérieux, Marcy l'Etoile, FRA) as described by Rahbé *et al*. (1995). Approximately 100 neonates were used to infest each of the seven plant species used. Surviving larvae were recovered from each of the infested plants 4 days following infestation. Preliminary experiments indicated that four days of growth was the optimum period in which a good number of surviving larvae could be recovered from all the seven plants species tested. Due to the small size of the larvae, it was not possible to isolate the midgut from the rest of the body tissues for gut enzyme analysis. Therefore, a total of forty larvae with decapitated heads (to avoid contamination with the salivary enzymes) collected from each plant species were separately homogenized in 1,300 µl distilled water and centrifuged (18,000 *g*, 10 min., + 4°C). A volume of 65 µl of the resultant supernatant was pipetted to each of the twenty porous plastic micro-cup of the API system (i.e., 19 micro-cups

dispersed with the substrate and one without the substrate (as the control) and the plate incubated (37°C for 4 h). A similar procedure was used for neonates samples previously fed only on water over the 4 days growth period which were used as the control. Enzyme activities were detected by cleavage of a chromogenic substrate (naphtyl derivatives) dispersed dry on a porous plastic micro-cup (~100 µl). Enzymatic reactions were enhanced by an SDS-based acid Tris buffer (Zym A) applied as a drop in each well after incubation of the plate. The reactions were visualized after addition of Fast Blue BB solution (Zym B) to each well. The level of activity was determined by visually assigning a rank value (0-5) corresponding to the intensity of colour produced by each well after comparison with the colour scale provided with the kit (0-5, from ≤ 5 to ≥ 40 nmoles of substrate released) (Figure 3.1). Rank values reflecting enzyme activity from naïve water-fed neonate larvae served as baseline; such that the rank values of this group were subtracted from the rank values obtained for the experimental groups to yield the feeding score value. Score values greater than zero indicated induction of appreciable enzyme activities in the larval homogenate.

The following nineteen enzyme activities commonly found in the guts of lepidopteran larvae were analysed for their presence using the API-ZYM kit system as described above : alkaline phosphatase (pH 8.5), esterase (C4), esterase lipase (C8), lipase (C14), leucine aminopeptidase, valine aminopeptidase, cystine aminopeptidase, trypsin (*N*-benzoyl-DL-arginine-2-naphtylamidase), α-chymotrypsin (*N*-glutaryl-phenylalanine-2-naphtylamidase), acid phosphatase (pH 5.4), naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-

glucuronidase, α-glucosidase, α-glucosidase, *N*-acetyl-beta-glucosaminidase, α-mannosidase and α-fucosidase.

Ranks for enzyme activities were generated following the Kruskal-Wallis test, using Proc RANK of SAS 9.1 (SAS Institute, 2003), and their means separated using Tukey-Kramer test (Proc GLM) (SAS Institute, 2003). Linear regression analysis was performed to determine the relationships between enzyme activities and neonate performance (percent survival or larval growth rates) on the plant species studied over the infestation period.

4. 2. 4: DNA isolation and purification

For DNA extraction, a total of 23 individual larvae (14 from *Z. mays* and 9 from *A. donax*) were randomly selected from 41 *B. fusca* specimens sampled. Total genomic DNA was extracted from each of the individual thoracic muscles using commercial kit (DNeasy^{TM} Tissue Kit, Qiagen Cat # 69506 GmbH, Germany) protocol with Proteinase K digestion as recommended for animal tissues. Briefly, each moth was taken out of the ethanol dried well and the segment containing the thorax removed, cut into smaller pieces and put into 1.5 ml microfuge tubes. Samples were then crushed under ice using electric mortar and pestle. To the crushed samples, 180 µl of lysis buffer (ATL) and 20 µL of proteinase K were added and mixed thoroughly by vortexing. The DNA samples were then incubated for three hours at 55°C in a shaking water bath, after which, they were removed and vortexed for another 15s. To this sample, 200 µL of AL buffer was added and the solution thoroughly, mixed by vortexing prior to incubating for another 10 min at 70°C. A similar amount of absolute ethanol (98 %) was added following incubation and the samples were again

thoroughly mixed prior to pipetting into a DNeasy Mini Spin column placed in a 2 ml collection tube before spinning for a minute at 6000 x *g*. The precipitate was replaced into another DNeasy spin column, washed with 500 µl wash buffer1 (AW1) by spinning for a minute at 6000x g. The DNA precipitate was then eluted by pipetting 100 µl of elution buffer directly onto the DNeasy membrane in the spin column, previously washed with 500 µl wash buffer 2 (AW2) for 3 minutes at 20000 x *g*. The DNA samples were collected in clean 1.5 ml microfuge tube and then incubated at room temperature for 1 minute and then span for another 1 minute at 6000 x *g*. The purified DNA was stored at -20°C until required for amplification.

4. 2. 5: Cytochrome *b* gene amplification and digestion

Polymerase chain reaction (PCR) was used to amplify the 1000 bp Cyt *b* mitochondrial fragment using two primers: CP1 (5′-GATGATGAAATTTTGGATC-3′) (modified from Harry *et al*., 1998) and Tser (5′-TATTTCTTTATTATGTTTTCAAAAC-3′) (Simon *et al*., 1994). The PCR was performed on a Biometra GeneAmp PCR System in a 25 µl reaction mixture containing 1 µl of the genomic DNA, 5X Green GoTaq® Flexi Buffer, 0.24 mM dNTPs, 3 mM $MgCl_2$, 0.4 µM of each primer and 1 unit of Taq polymerase (GoTaq, Promega). After initial denaturation at 94^{o}C for 5 min, PCR condition was 40 cycles at 94^{o}C for 1 min of denaturation, 46^{o}C for 1 min 30 s of annealing, 72^{o}C for 1 min 30 s of extension and a final extension period of 10 min at 72^{o}C. Amplified products were then digested by an enzyme restriction as described by Calatayud *et al.* (2008) as follows. The 25µl reaction mixture containing 100µg/ml bovine serum albumin (BSA), 0.5U Promega *Xho*II restriction enzyme and the amplified DNA incubated for 4 h at 37^{o}C. The digested fragments

were visualised by means of electrophoresis on 1.5% agarose gel previously stained with ethidium bromide. Amplified DNA products were further purified with the Promega Wizard SV Gel purification kit following the manufacturer's protocol prior to sequencing reaction.

4. 2. 6: Cytochrome *b* gene sequencing and analysis

DNA sequencing reactions and the analysis for the determination of the extent of genetic differentiation between the two populations were performed at the laboratoire Evolution, Génomes et Spéciation- CNRS (France). Reactions were performed using the ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems), cleaned using ethanol/EDTA precipitation. Sequences were visualized on an ABI 3130 automated sequencer using Big-Dye fluorescent terminators. The consensus sequences obtained were aligned manually using Mac Clade 4.05 (Maddison and Maddison, 2001).

Basic sequence statistics and number of haplotypes were determined using DnaSP (Rozas *et al*., 2003). The following parameters were used to estimate genetic variability among populations between the two host plants (*Z. mays* and *A. donax*): number of haplotypes (*h*), number of polymorphic sites (*S*), haplotype diversity (*d*) (Nei, 1987), nucleotide diversity (π) (Lynch and Crease, 1990) using the Jukes and Cantor correction (Jukes and Cantor, 1969). The extent of genetic differentiation between the populations (F_{ST}) (Hudson *et al.,* 1992) was performed with the Arlequin 2.000 software (Schneider *et al*., 2000).

4. 3: Results

4. 3. 1: Activities of digestive enzymes in larval homogenates

Results of the semi-quantitative assay of activities of larval digestive enzymes induced following neonates consumption of plant foliage over 4 days period of infestation are given in Table 4.1. Most of the aminopeptidase activities relative to the activities in the control homogenates (homogenates of naive unfed larvae) did not increase significantly regardless of the plant leaves ingested by the larvae. However, activities of most of the sugar degrading enzymes, including *β*-galactosidase, *β*-glucuronidase, *α*-glucosidase and *β*-glucosidase increased appreciably. Highest activities of sugar degrading enzymes were exhibited by larvae exposed on leaves of *Z. mays, S. arundinaceum* and *P. purpureum*. However, the activity of *β*-glucosidase was significantly higher in homogenates of larvae fed only on *Z. mays* and *S. arundinaceum*. Conversely, C4 esterase was significantly higher in neonate larva that consumed leaves of *P. maximum, P. deustum* and *S. megaphylla*. Highest positive correlations between enzyme activities and percentage survival or relative growth rates of neonates were obtained with *α*-glucosidases, *β*-glucosidases and *β*-galactosidase over the infestation period. In contrast, negative correlations between same larval performance parameters and enzyme activities were obtained for esterase (C4) and esterase lipase (C8) (Figure 4.1).

Figure 4.1: A representative plate showing differential staining of the API ZYM plate with ZYM A and ZYM B reagents after 4 hours of incubation of homogenates of larvae fed on plant leaves with chromogenic substrates dispersed on the porous plate. Each coloured well was assigned a rank score (0-5) based on the intensity of colour formed. Differences in rank scores (colour intensities) between the plant leaf-fed and naïve water-fed neonate larva were used to determine the feeding score values for each enzyme activity analysed and used for statistical analysis. Negative control (1), alkaline phosphatase (2), C4 esterase (3), C8 esterase (4), C14-lipase (5), aminopepetidases (6-11) and glycosidases (12-20).

Table 4.1: Semi-quantitative analysis of enzyme activities in the homogenate of entire neonate larvae (with decapitated heads) reared for 4 days on different plants species. Enzyme activities (means ± SE, n=5) correspond to the activities found in the crushed larval homogenate (40 larvae per replicate). Activities correspond to the release of 5, 10 and 20 nmoles of substrate per 4 hours of incubation at 37°C (rate 0: no change in activity)

Enzymes	*Z. mays*	*S. arundinaceum*	*P. purpureum*	*A. donax*	*P. maximum*	*P. deustum*	*S. megaphylla*
Alkaline phosphatase	0	0	0	0	0	0	0
Esterase (C4)	0	0	0	0	0.2 ± 0.2a	1.0a	0.2 ± 0.2a
Esterase lipase (C8)	0	0	0	0	0.2 ± 0.2	0	0
Lipase (C14)	0	0	0	0	0	0	0
Leucine aminopeptidase	0	0	0	0	0	0	0
Valine aminopeptidase	0.7 ± 0.2a	0.6 ± 0.2a	1.0a	0.4 ± 0.2a	0	0	0.6 ± 0.2a
Cystine aminopeptidase	0	0.2 ± 0.2a	0	0	0.2 ± 0.2a	0.2 ± 0.2a	0
Trypsin	0	0	0	0	0.5 ± 0.3	0	0
α-chymotrypsin	0	0	0.7 ± 0.2a	0	0.5 ± 0.3a	0	0
Acid phosphatase	0	0	0	0	0	0	0
Naphthol-AS-BI-phosphohydrolase	1.0b	0.6 ± 0.2ab	1.0b	1.0b	0.2 ± 0.2a	1.0b	0.8 ± 0.2ab
α -galactosidase	0	1.2 ± 0.5a	0	0	0	0.5 ± 0.3a	0
β-galactosidase	1.2 ± 0.3b	1.2 ± 0.2b	1.7 ± 0.2b	0.8 ± 0.2a	0.5 ± 0.3a	0	0.2 ± 0.2a
β -glucuronidase	1.2 ± 0.2a	2.0 ± 0.5b	2.0b	1.0a	1.0a	0	0.8 ± 0.2a
α -glucosidase	1.8 ± 0.2b	1.6 ± 0.2b	2.0b	1.2 ± 0.2a	0	0.5 ± 0.3a	1.0 ± 0.3a
β -glucosidase	1.8 ± 0.5c	1.4 ± 0.2c	1.0b	0.4 ± 0.2a	0.7 ± 0.2ab	0	0
N-acetyl-beta-glucosaminidase	0	0	0	0	0	0	0
α -mannosidase	0	1.0 ± 0.3a	1.5 ± 0.3a	0	0.7 ± 0.2a	0	0
α -fucosidase	0	0.4 ± 0.2	0	0	0	0	0

Means within a line followed by different letters are significantly different at 5% level (Tukey-Kramer test).

Table 4.2: Correlations between enzyme activities and percentages of surviving larva of *Busseola fusca* recovered or relative growth rates (RGR) after 7, 19 or 31 days, following infestation by neonates. R-values are given

.	Dependent variables					
Independent variables	% survival after 7 days	% survival after 19 days	% survival after 31 days	RGR after 7 days	RGR after 19 days	RGR after 31 days
Alkaline phosphatase	-	-	-	-	-	-
Esterase (C4)	-0.3	-0.4	-0.3	-0.4	-0.2	-
Esterase lipase (C8)	-0.1	-0.1	-0.1	-0.1	-0.1	-
Lipase (C14)	-	-	-	-	-	-
Leucine aminopeptidase	-	-	-	-	-	-
Valine aminopeptidase	+0.2	+0.3	+0.2	+0.3	+0.03	+0.09
Cystine aminopeptidase	-0.05	-0.2	-0.1	-0.1	-0.1	+0.05
Trypsin	-0.2	-0.1	-0.1	-0.1	-0.2	-0.2
α-chymotrypsin	-0.3	-0.2	-0.2	+0.2	-	-0.4
Acid phosphatase	-	-	-	-	-	-
Naphthol-AS-BI-phosphohydrolase	+0.06	-	+0.2	-	+0.3	+0.2
α-galactosidase	+0.2	+0.1	-0.06	+0.2	-0.08	-
β-galactosidase	+0.4	+0.5	+0.3	+0.6	+0.2	-
β-glucuronidase	+0.3	+0.3	+0.1	+0.5	+0.03	-0.09
α-glucoidase	+0.5	+0.6	+0.4	+0.5	+0.4	+0.3
β-glucosidase	+0.7	+0.6	+0.6	+0.6	+0.3	+0.5
N-acetyl-beta-glucosaminidase	-	-	-	-	-	-
α-mannosidase	-	+0.1	-0.2	+0.3	-0.1	-0.3
α-fucosidase	+0.2	+0.2	+0.03	+0.3	-	+0.07

4. 3. 2: Population distribution

For the purpose of the analysis of the genetic structure of *B. fusca*, *Z. mays* and *A. donax* were considered as host plants without quantifying their relative contribution to population dynamics of the pest in the field. Cytochrome *b* gene analysis assigned individuals found on both plant species in two distinguishable clades, that is, clades *KI* and *KII* (F_{ST} = 0.45, P < 0.001). Clade *KI* was characterised by three DNA fragments (approximately 600, 300 and 100bp) while *KII* was characterised by two DNA fragments (880 and 100bp) (Figure 4.2). Individuals from *KII* clade were more abundant as compared to the *KI* clade (Figure 4.2).

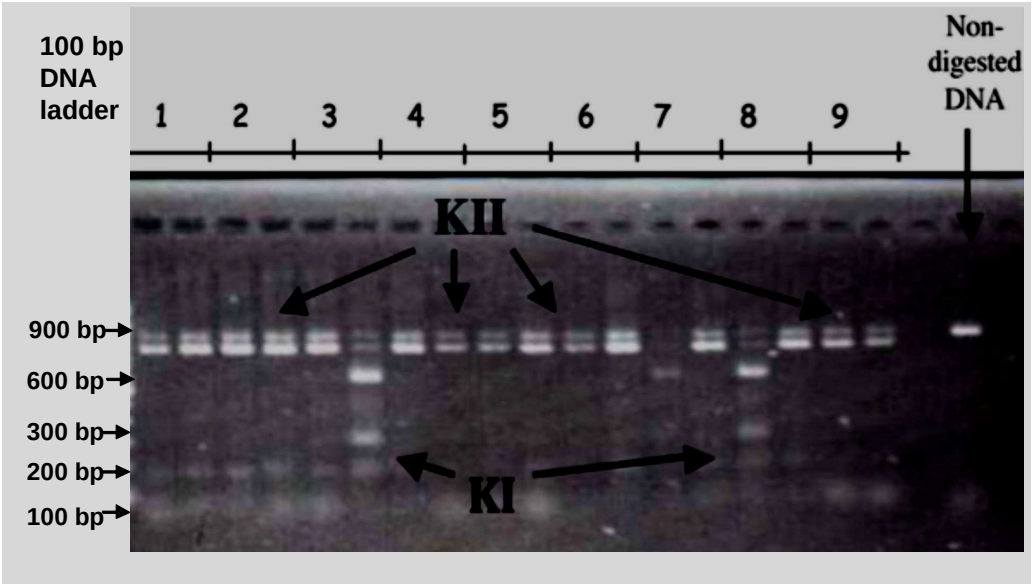

Figure 4.2: A representative agarose gel electrophoresis plate of DNA products of cytochrome *b* DNA fragment of several *Busseola fusca* larvae randomly sampled from *Z. mays* and *A. donax* noted from 1 to 9 following PCR amplification and *Xho* restriction digestion. Distinct bands were identified using 100bp ladder and results showed the presence of two mitochondrial clades labelled *KI* and *KII.*

The spatial distribution of the two clades varied among regions sampled. The Kenyan populations collected from the two localities, Mt. Kenya and Machakos, belonged exclusively to clade *KII* (Figure. 4.2), while individuals from the Ethiopian population belonged exclusively to clade *KI* (Figure 4.2). Similarly, individuals in the *KII* clade were collected from maize while those in clade *KI* were collected from *A. donax*. Results of these clades are summarized in Table 4.3 below. Though, both clades occurred on different hosts they also occurred in different geographical regions.

Table 4.3: Summary of the distribution of *Busseola fusca* clades with respect to host plant species and locality

Extraction code	Country	Locality	Host plant	Sex	Clade
2965	Kenya	Mt Kenya	*Zea mays*	Female	*KII*
2966	Kenya	Mt Kenya	*Zea mays*	Female	*KII*
2967	Kenya	Mt Kenya	*Zea mays*	Female	*KII*
2968	Kenya	Mt Kenya	*Zea mays*	Male	*KII*
2969	Kenya	Mt Kenya	*Zea mays*	Male	*KII*
2970	Kenya	Machakos	*Zea mays*	Male	*KII*
2971	Kenya	Machakos	*Zea mays*	Male	*KII*
2972	Kenya	Machakos	*Zea mays*	Male	*KII*
2973	Kenya	Machakos	*Zea mays*	Female	*KII*
2974	Kenya	Machakos	*Zea mays*	Female	*KII*
2975	Kenya	Machakos	*Zea mays*	Female	*KII*
2976	Kenya	Machakos	*Zea mays*	Male	*KII*
2977	Kenya	Machakos	*Zea mays*	Male	*KII*
2978	Kenya	Machakos	*Zea mays*	Male	*KII*
2979	Ethiopia	Abay Mado	*Arundo donax*	Female	*KI*
2981	Ethiopia	Abay Mado	*Arundo donax*	Male	*KI*
2982	Ethiopia	Abay Mado	*Arundo donax*	Larva	*KI*
2983	Ethiopia	Abay Mado	*Arundo donax*	Larva	*KI*
2984	Ethiopia	Abay Mado	*Arundo donax*	Larva	*KI*
2985	Ethiopia	Abay Mado	*Arundo donax*	Larva	*KI*
2986	Ethiopia	Abay Mado	*Arundo donax*	Larva	*KI*
2987	Ethiopia	Baimraar	*Arundo donax*	Larva	*KI*
2988	Ethiopia	Abay Mado	*Arundo donax*	Larva	*KI*

Table 4.4: Genetic diversity (mean ± SD) of the cytochrome *b* gene of *Busseola fusca* sampled from *Z. mays* and *A. donax* from Kenya and Ethiopia

Genetic diversity parameters	Kenya (*Z. mays*)	Ethiopia (*A. donax*)
No. sequences (n)	14	9
No. segregating sites (S)	18	16
Number of haplotypes (h)	4	6
Haplotype diversity (d)	0.5118 ± 0.0370	0.6302 ± 0.0426
Nucleotide diversity (%)	0.3783 ± 0.1956	0.2221 ± 0.1343

4. 4: Discussion

According to Lindroth (1989), food plant chemicals strongly affect the activity of insect digestive enzymes. The assessment of enzyme activities in the larval homogenates of larva fed on plant leaf tissue indicated that plant foliage consumed by the larvae significantly influences the activity of some of the larval digestive enzymes. Esterase (C4) was significantly induced in larvae fed on *P. maximum*, *P. deustum* and *S. megaphylla*. In addition, larvae fed on the three plant species had the poorest growth rates and minimal survival. Induction of esterase has been positively correlated with increased resistance to allelochemical toxicity in plants in several Lepidoptera species (Ahmad *et al.*, 1986; Lindroth *et al.*, 1993; Hwang and Lindroth, 1997). For example, increased esterase activities have been implicated in the metabolism of toxic phenolic glycosides in several lepidopteran species (Lindroth and Hemming, 1990; Lindroth and Bloomer, 1991; Lindroth and Weisbrod, 1991). For instance, the consumption of phenolic glycosides by neonates of gypsy moth, induced esterase activity and similarly their survival rates were

negatively correlated with increased enzyme activity (Lindroth and Weisbrod, 1991). Although the high activity of esterase induced in *B. fusca* larvae fed on *P. maximum*, *P. deustum* and *S. megaphylla* in this study cannot be directly correlated with the level of total phenolic compounds in plant's leaf tissue; it could still be a possible indicator of the presence of high levels of a specific phenolic glycoside in the respective plant leaves. It is therefore possible that pronounced activity of esterase particularly, C4 esterase in neonate larva fed on the three plant species was a direct physiological response to toxic allelochemicals present in leaves of the three plant species. In fact, *Panicum* sp. and *Setaria* sp. are known to harbour toxic beta-phenylethylamine alkaloids (such as *N*-methyltyramine) and a phenolic acid, setarin (4-allyloxycoumarin), respectively (Steglich *et al*., 2000). It is therefore likely that toxic allelochemicals were present in the less preferred plants and could have been the possible elicitors of the physiological responses in digestive enzymes following their consumption by neonate larva. Similarly, although *Z. mays* and *S. arundinaceum* were the preferred host plants for larvae in this and other related studies (Hofsvang *et al.,* 2001), and were therefore likely to contain phagostimulatory components in their tissues, both plants are reported to also contain toxic compounds such as DIMBOA or gramine (Niemeyer, 1988) and a cyanogenic glycoside (dhurrin) (Conn, 1980), respectively. These compounds have been documented to inhibit the activity of esterase in a number of insect species. DIMBOA has also been documented to inhibit the activity of serine proteases and exopeptidases (tryspin, chymotrypsin and leucine aminopeptidases in *S. nanogrioides* (Ortego *et al*., 1996). In the current study, the level of esterase and the three protease activities were not elevated in the homogenates of larvae that

consumed leaves of both plant species. This clearly indicated the possible presence of these noxious compounds in the two plant species. However, since *B. fusca* larvae has for a prolonged time fed on both plant species (coevolved with) it is probable that larvae of this species developed other efficient enzymatic detoxification mechanisms or genetically based adaptations to cope up with the noxious allelochemicals encountered in the two plant species. Similar adaptations to toxic plant allelochemicals has been reported for example, in the European corn borer, *Ostrinia nubulalis* (Hübner) (Lepidoptera: Pyralidae) (Campos *et al.*, 1989) and for specialised *Heliconius* caterpillars (Lepidoptera: Nymphalidae) which convert harmful cyanogenic glycosides into soluble and harmless thiols (Engler *et al.*, 2000). This biochemical transformation process prevents the later to release cyanide and allows the caterpillars to use the toxic compounds as a nitrogen source. Therefore, it is possible that *B. fusca* larvae have evolved similar mechanisms to cope up with the noxious chemicals present in both plant species as a result of long and close association with the two plants.

For a number of lepidopteran species, the presence of sugars in insect's diets usually induce the production of sugar-cleaving enzymes such as $\tilde{\alpha}$-glucosidases and β-glucosidases in their guts. The activities of most of the sugar metabolising enzymes (glucosidases) were much more pronounced in larvae fed on plant leaves that supported highest larval growth rates and survival. Glucosidases are digestive enzymes that have a critical role in the final stages of carbohydrate digestion; they hydrolyse the -o-glycosyl bonds of sugars. In addition, these enzymes have an important role in plant-herbivore coevolution as they are involved in the catabolism of plant secondary metabolites (Hemming and Lindroth, 1999; Hemming and

Lindroth, 2000). In the present study, levels of glucosidases studied were variably elevated in larval homogenates of larvae fed on leaves of different plant species considered. This indicated the induction of the particular enzyme activities as a response to and in the proportion at which the respective sugar substrates were present in the plant leaf tissues consumed. Indeed identical sugars were found at various concentrations in the leaf extracts of the same plants analysed in this study. Among the glucosidases, *β*-glucosidase was particularly more pronounced among larvae fed on *Z. mays* and *S. arundinaceum.* The activity of *β*-glucosidase enzyme has been reported in many organisms (Yu, 1989) and specifically functions to catalyze biochemical pathways that involve the cleavage of *β*-linked o-glycosyl linkages of many glycosides (Clausen *et al.,* 1990). In insects, *β*-glucosidases play an important role in the digestion of *β*-(1, 4) linked sugars (Mendiola-Olaye *et. al.,* 2000; Zibaee *et al.,* 2008; 2009). Therefore elevated activities of this enzyme in homogenates of larvae fed especially on *Z. mays* and *S. arundinaceum* leaves could be related to larva's physiological response to the presence of high levels of *β*-(1,4) linked sugars (methyl-β- glycosides and cellobiose) in the plant leaf diets. Since *β*-glucosidase is also active on several other glycosides (Zibaee, *et al.,* 2009), its presence could be important in the biotransformation of DIMBOA in *Z. mays* among other harmful glycosides (including cynogenic glycosides, phenols) in sorghum or other plant species to their harmless aglycones via detoxification reactions. This may explain the excellent performance exhibited by larvae on both plant species despite their highest probability of possessing the two harmful allelochemicals.

Moreover, larva fed on *Z. mays, S. arundinaceum* and *P. purpureum* had also appreciable activities of *α*-glucosidase and *β*-galactosidase. *Alpha*-glucosidase,

catalyses the hydrolysis of maltose, sucrose and trehalose, *β*-galactosidase hydrolyse lactose while *β*-fructofuranosides hydrolyse sucrose and raffinose (*Zibaee et al.,* 2008; 2009). But unlike larvae fed on *S. arundinaceum* and *P. purpureum, Z. mays* fed larvae did not exhibit any increased level of *β*-glucuronidase activity. *Alpha*-glucosidases are found in the alimentary canal of almost all insect species and hydrolyze the di- and oligosaccharides. Therefore, elevated activity of this enzyme may be correlated to the presence of phagostimulatory constituents such as sucrose or maltose in the plant leaf tissue. Sucrose has been demonstrated to be a key phagostimulant for many insect species even for *B. fusca* (Onyango and Ochieng'-Odero, 1994). Therefore, the positive correlations between α-glucosidase activities and percentages of surviving larvae recovered or relative growth rates observed throughout the infestation period confirmed that phagostimulation is a key process for *B. fusca* larval survival on a number of plant species.

The activities of larval proteases (aminopeptidases and trypsin) were however lower than the activities of sugar metabolising enzymes in homogenates of larva fed on most of the plant species tested. This indicated that *B. fusca* larva generally and prefferentially relies on simple sugars other than proteins as their food source. This may be possible since the evolutionary advantage for host plant specialisation by herbivorous insects has been hypothesised to involve an increase in energetic efficiency (Zibaee *et al.,* 2008). Therefore, maize and wild sorghum should have a more suitable composition, level or ratio of different carbohydrates required by *B. fusca* larvae for their optimum performance unlike the other plant species considered in this study. Similarly, it is likely that *S. arundinaceum* and other poaceous plants

may serve as alternative hosts for *B. fusca* larvae in the absence of *Z. mays* depending on the relative proportion of sugars present in the plants' leaf tissues.

Large fluctuations in host- plant resources can influence insect populations to the extent that genetic differentiation develops among local populations. However, this often requires substantial genetic drift and strong (long-term) isolation among populations (Berenbaum, 1996; Kawecki, 1997). This has been demonstrated in herbivorous insects that feed on a wide range of host plants: colonization of a new host may induce the selection of adaptive characters and population genetic differentiation (Rice, 1987; Diehl and Bush, 1989). Data on genetic diversity of the DNA samples analysed used in this study indicated a split of *B. fusca* populations into two genetically differentiated groups i.e. clade *KI* and clade *KII*. Clade KI populations were found exclusively on wild *A. donax* while clade KII populations were found on *Z. mays*. Individuals found on *A. donax* (Ethiopian population) were more variable as compared to the *Z. mays* (Kenyan) associated population. This was evidenced by the high number of haplotypes (6) that originated from 9 sequences in *A. donax* associated population in contrast to the lower number (4) haplotypes that originated from 14 sequences of the *Z. mays* associated population (Table 4.4). These data was further corroborated by values of genetic diversity parameters (h, d, π,) obtained following sequence analysis (Table 4.4). The three genetic parameters indicated that Z. *mays* sampled population was indeed more stable but genetically less variable than *A. donax* sampled individuals.

However, according to this data, it is not conclusive whether the observed population differentiation can be attributed to correspond to specific host plant use by larvae since similar genetic demarcation but based on geographical isolation has

already been reported (Senzolin *et. al.,* 2006). According to these authors, genetic sub-structuring of *B. fusca* populations sampled from maize and sorghum across three major biogeographic zones in Africa can be demarcated into three mitochondrial clades; one from Western Africa, and two designated Kenya I and II (KI and KII), from Eastern and Central Africa. These population units display ecological preferences along altitudinal gradients. While the West African population unit preferentially occupy the low altitudes of dry savannah zones (Harris and Nwanze, 1992), the East and central Africa populations predominantly inhabit the highlands characterised by wet and cold seasons (Kfir *et al.,* 2002).

A recent and similar extensive sampling of *B. fusca* larvae on both cultivated maize/sorghum fields and wild plants from the same Ethiopian regions considered in this study revealed the coexistence of both population units. Both units do not display the expected biases in plant host distribution. Additionally, both clades have been reported to coexist on maize or cultivated sorghum plants in some regions in the Kenyan Rift Valley. Furthermore, the Kenyan clade (KII) predominantly found on maize has also been sampled on wild *A. donax* plants in South Africa. Therefore, it is possible that genetic differentiation exhibited by *B. fusca* larvae from larval samples obtained in both countries corresponds to geographical differentiation other than host use. It is hence possible that wild hosts and other crops which are marginally infested by *B. fusca* could only and possibly serve as part of a larger refuge to this species especially in seasons when the preferred hosts (maize and cultivated sorghum) are absent as suggested by Gould, (1998). In the present case, *A. donax* a perennial plant could probably function as sink habitat for *B. fusca* larvae. The plant could only be marginally infested simply for oviposition by

females mainly attracted to from the nearby but dwindling preferred hosts especially during off-seasons, than act as ‘true’ host on which autonomous and large population can develop over several generations. This is well corroborated by the inability of *A. donax* to support survival and growth following its infeststion by larvae in the laboratory as was evidenced in this study. However, this does not rule out firsthand the possibility of the existence of host differentiated species among *B. fusca* populations.

CHAPTER FIVE

5. 0: GENERAL DISCUSSION, CONCLUSION AND RECOMENDATIONS

5. 1: General discussion

Phytophagous insects that damage crops are often polyphagous, feeding on several types of crops and weeds. Recent field studies indicate that *B. fusca* exhibit more oligophagic (Le Rü *et al*., 2006a, 2006b; Ong'amo *et al*., 2006) than polyphagic feeding habits contrary to as previously reported (Polaszek and Khan, 1998; Haile and Hofsvang, 2001). The host plants for the pest are primarily maize and sorghum (Haile and Hofsvang, 2001), although a significant number of populations have also been found on wild Poaceae plants (mostly *S. arundinaceum* and *A. donax*) (Le Rü *et al*., 2006a, 2006b; Ong'amo *et al*., 2006). The purpose of this study was therefore to determine the extent of oligophagy of *B. fusca* by first examining the sensory abilities of the larvae to recognise different plants as hosts and its subsequent performance in terms of survival and growth when fed on the same host plants. Since larval performance on a particular plant of most lepidopteran species is depended on the plant's chemical and physical cues, the study was designed to determine plant stimuli that influence larval host choice and subsequent colonisation. The potential existence of host plant associated populations of the species as was postulated by Senzolin, (2006) was also examined.

The distribution of chemo- and mechanoreceptors on the antennae and maxillae as sensory equipments modulating host choice and acceptance of *B. fusca* larvae were ascertained based on observations made using scanning electron microscopy,

selective staining with silver nitrate, and gustatory electrophysiological recordings. The sensilla present on the larval maxillary galeae are typical of other Lepidoptera family and consist of two uniporous styloconic sensilla that are contact chemoreceptors, three basiconic sensilla, and two aporous sensilla chaetica (Faucheux, 1999). The larval maxillary palp is two-segmented and posses eight small basiconic sensilla at the tip that were found to be gustatory as have been described for other lepidopteran species (Albert, 2003). The antennae of *B. fusca* larvae are short and simple. Similar to other lepidopteran species, the antennal sensilla comprise of two aporous sensilla chaetica, three multiporous cone-shaped basiconic sensilla, three small basiconic sensilla and one aporous styloconic sensillum (Dithier, 1937; Faucheux, 1999). The basiconic sensillum present on the third antennal segment displayed a contact chemoreception response. However, similar sensilla on the first and second antennal segments did not show any action potential activity during tip recording tests and are therefore unlikely to be associated with chemoreception. Nevertheless, the significant and positive dose-response electrophysiological curve obtained with an increasing sucrose concentration for the antennal basiconic sensillum that displayed a contact chemoreception response confirmed for the first time the presence of gustatory chemoreceptors on the antennae of a Lepidoptera larva.

The oligophagic feeding behaviour of *B. fusca* larvae as evaluated based on the performance of larva on the seven plant species used, indicated that their growth rates and survival was better on *Z. mays* and *S. arundinaceum.* Marginal performance was also recorded on *P. purpureum*, *A. donax* and *P. maximum* confirming the oligophagy of the species. Preferential feeding habits of *B. fusca*

larvae on different Poacea plants as exhibited in this study have also been observed in the field (Le Rü *et al*., 2006a, 2006b; Ong'amo *et al*., 2006), further confirming the oligophagous feeding habits of *B. fusca* larva.

Therefore, it appears likely that non-crop plants especially wild sorghum, *S. arundinaceum,* may also serve as alternative hosts for *B. fusca* larvae (Polaszek and Khan, *et al.* 1998; Haile and Hofsvang, 2001), possibly in the seasons when *Z. mays* is unavailable in the field. Marginal survival and growth rates of larvae that was recorded on *P. purpureum*, *A. donax* and *P. maximum*, some of the hypothesised ancestral hosts also suggests that *B. fusca* larva has a residual ability to utilise not only *S. arundinaceum* but also other wild plants present in their microhabitat as food sources in the absence of preferred hosts. Utilisation of several plant species albeit at different degrees, is suggestive that several plant cues from the tested species influence the growth and survival of *B. fusca* larvae.

Plant physical features (trichomes, levels of tissue silification) can easily influence the choice of host plant use by *B. fusca* larvae. Levels of endogenous silica among plants are frequently associated with plant resistance to insect attacks, whereby in most cases resistance is positively correlated with plant silica content (Meyer and Keeping, 2005; Laing *et al.*, 2006). Phytophagous insects will therefore discriminate between high- and low-silica containing plants and preferentially feed on the later during host selection process. In this study, feeding and survival of *B. fusca* larvae on the plant species considered correlated negatively with their silica content. Larvae preferentially fed on *Z. mays* and S. *arundinaceum* foliage with low silica content as compared to the five other plants used and shown to contain high levels of silica. Similar results have been reported for a number of lepidopteran larvae

(Schoonhoven *et al.,* 2005; Kvedaras *et al.,* 2007a). The resistance effect of silica on *B. fusca* larval growth rate and survival was dose dependent as was confirmed by gradual increase of the amount of silica in silica-amended diets. For *B. fusca,* such correlation may form the basis of host plant/artificial diet discrimination and indicates that silica content in plants is a reliable predictor and contributory factor of food choice and discrimination for this species.

Host plant discriminations and preferences by *B. fusca* larvae appear to be partially determined by plant nutritional factors and/or plant primary/secondary metabolites. Polar methanol extractable host plant chemical stimulated feeding of third instar *B. fusca* larvae, compared to deterrent hexane soluble plant compounds. The relative suitability of host plant utilisation by *B. fusca* larva was reflected in the order of preference of host plants which included *Z. mays* > *S. arundinaceum* = *P. purpureum* = *A. donax* = *P. maximum* > *P. deustum* = *S. megaphylla*. This ranking order reflected the effects of allelochemicals present in particular plant leaves and their overall contribution larval choice of the host plant. Similarly, feeding responses of larvae to the host plants' extracts and fractions thereof also paralleled feeding responses on intact plants from which the extracts were obtained implying that that chemical composition are important in controlling food choice behaviour of *B. fusca* larvae. More importantly, the abundance and relative proportion of sucrose and turanose in the host plant leaves appear to determine the basis of plant acceptance or rejection by larvae. Typical to other Lepidoptera larvae (Sharma, 1994; Bowdan, 1995; Yazawa, 1997), sucrose was the most stimulatory sugar to *B. fusca* larval feeding among the sugars identified in the plant extracts. However, turanose, which was highly variable in its content among the plant leaf extracts, appeared

phagodeterrent to larval feeding especially at high concentrations as was confirmed by the negative dose response curves. The overall effects of the two sugars on feeding by larvae was reflected in the relative proportion of the specific sugar in the host plants: plants with high sucrose content stimulated feeding while those with high turanose content deterred larval feeding. Both sugars however, appeared synergistic on the feeding responses by *B. fusca* larvae when presented together in specific concentrations of each sugar, particularly in the ratio of 3:1 sucrose-turanose concentration. Therefore the host discriminatory ability and the selective feeding behaviour of larvae seem to be dependent on the relative concentration of each of the two sugars in the plant tissue and this may sufficiently explain the oligophagous feeding habits of *B. fusca* larvae.

Although the amount of total phenolic compounds in the leaf extracts studied varied significantly among the plant species tested, their concentrations did not correlate with the feeding and survival of *B. fusca* larvae on entire plants. This is contrary to most studies that positively correlated the level of phenolic compounds with insect resistance (Haukioja *et al*., 1985; Bergvinson *et al*., 1994; Santiago *et al*., 2005). The absence of a relationship between the two does not however, rule out first hand the importance of phenolic compounds in host choice and selection by *B. fusca* larvae. This is because the determination of total phenolic compounds (i.e. pooled compounds) as was carried out in this study, does not allow correlating the importance of a specific phenolic compound present in the entire plants with insect performance parameters.

The assessment of the activities of larval enzymes following feeding on different plants considered in this study using the API-ZYM system (a fast semi-quantitative

analysis of enzymatic activities) indicated that the type of plant foliage consumed by larvae significantly influenced the activity of some of *B. fusca* digestive enzymes. C4 esterase was significantly induced in the guts of larvae fed on *P. maximum*, *P. deustum* and *S. megaphylla*. Moreover, larvae fed on leaves of the three plant species had equally the poorest growth rates and hardly supported larval survival beyond 7days of following plant infestations. Therefore, induction of esterase can be positively correlated with a direct larval physiological response to the toxic allelochemicals present in the specific plant leaves.

Sugar degrading enzyme activities particularly of β-glucosidases were however more pronounced in homogenates of larvae fed on *Z. mays* and *S. arundinaceum* plants that supported highest larval growth rates and survival. Moreover, larva fed on *Z. mays*, *S. arundinaceum* and *P. purpureum* had also a significant increase in the activities of α-glucosidase and β-galactosidase activities. Elevated activities of these enzymes may be attributed to the direct physiological response to the presence of phagostimulatory enzyme substrates present in the respective plant species by the feeding larvae. The positive correlations among α-glucosidase activities and percentages of surviving larvae recovered or relative growth rates registered following 7, 19 or 31 days of infestation by neonates confirmed the importance of phagostimulation for survival and growth of *B. fusca* larvae on host plants. The differential and pronounced levels of glucosidases found in larval homogenates following larval feeding on a variety of plant leaves may be attributed to the balance between sucrose and turanose among the other sugars present in the plant leaf tissues. The activities of proteases (aminopeptidases) were however, lower than the activities of sugar metabolising enzymes in larval homogenates of larvae fed on all

of the plant species studied, indicating that *B. fusca* larvae generally relies on carbohydrates than proteins as food source.

Low dispersal ability, geographical barriers, habitat fragmentation as well as host plant use and availability in the field are considered as key factors responsible for the genetic differentiation in phytophagous insects (Emelianov, *et al.* 1995; Mopper, 1996). Data on genetic diversity obtained using cytochrome *b* genetic marker indicated the split *B. fusca* populations into two genetically differentiated groups. However, genetic substructuring in this species could be attributed to geographical isolation as reported by Senzolin, (2006) rather than influenced by host use.

5. 2: Conclusion and recommendations

The contributions of the olfactory and gustatory organs in food plant discrimination were examined in larvae of *B. fusca*. Larvae of this species have extensive olfactory and gustatory sensilla that allow for the detection of host plant physical and chemical stimuli important in host plant recognition and selection. Both sensilla types are unevenly and unequally distributed on the antennae and the maxillary palp but appear to be important in host plant choice and selection. The gustatory receptors which are mainly located on maxillary palp but sparsely on the antennae are involved in the detection of plant surface chemicals by the help of apical agryrophilic pores. Nevertheless, mapping of *B. fusca* larval sensory structures establishes a basis for the additional electrophysiological and behavioural investigations that may be important for the fully understanding of the relative roles of each of the sensory organ (the antennae, maxillary palpi and the epipharyngeal sensilla) in food plant choice and discrimination for feeding by neonate larvae. Like

has been demonstrated for adult moths (Birkett *et al.,* 2006), further studies are required to determine the ability of young larvae to perceive host plant volatile cues as well as the type and distribution of sensilla involved in such perceptions. Additionally, the nature and function of minute sensilla present on the tip of the maxillary palp of *B. fusca* larvae remains to be fully elucidated and understood.

The patterns of host plant use and the oligophagic feeding behaviour of *B. fusca* larvae as has been reported in a number of field studies (Le Rü *et al*., 2006a, 2006b; Ong'amo *et al.*, 2006; Haile and Hofsvang, 2001) and was corroborated in the current study. Larva of this species are selective in their feeding habits and exhibit a strong feeding preference for *Z.-mays* and *S.arundinaceum* plants, indicating a restricted host plant range. These larva are also able to use other hosts present in their ecological niche albeit minimally, possibly in seasons when the preferred hosts are unavailable. Selective feeding habits of *B. fusca* larvae appear to be partly influenced by host plant physical and chemical cues especially plant silica content and the presence and relative proportion of specific plant sugars.

Similar to other lepidopteran stemborers (Setamou *et al.* 1993; Schoonhoven *et al.,* 2005; Kvedaras *et al.,* 2007a), endogenous plant silica influences *B. fusca* larval performance. High plant silica content negatively correlated with *B. fusca* larval survival and growth. While the identification of the specific mechanisms of silica resistance to *B. fusca* larval discriminatory feeding behaviour may not be crucial for the development of resistant cultivars; such information can facilitate plant breeding or selection programs. Therefore, future studies focused on the mechanism of silica resistance could be useful in understanding host plant resistance to *B. fusca* larvae. Additionally, comparative studies are needed to establish the extent at which various

poaceous plants are able to accumulate silica and subsequently contribute to resistance to feeding by *B. fusca* larvae. Such experiments can be carried out by studying insect performance parameters on plants grown in silica amended soils at various doses. However, from an applied point of view the fact that silica augments the resistance of various plants to pest insects (Keeping and Kvedaras, 2008) and that plants have a capacity to accumulate silica (Ma and Yamuji, 2006), this is particularly relevant for the silica deficient soils in the cereal growing regions of sub-Saharan Africa. Therefore, silica amendments for susceptible cultivars in these regions may provide improved resistance to the insect although field trials are required to confirm the effects and physiological responses of the plants as regard to this amendment. Additionally, the effects of plant tissue silifications on human consumption should be clearly tried and ascertained.

Plant selection and subsequent colonisation by *B. fusca* larvae is similarly influenced by the type and levels of some primary and secondary plant metabolites in the plant leaf tissues. The levels and type of specific metabolites in plant tissues as were established in this study can vary according to plant species and have been shown to mediate food choices in a number of insect species (Mohammed *et al.,* 1992; del Campo and Renwick, 2000; del Campo and Miles, 2003). In the present study sucrose and turanose were the key sugars isolated and appeared to determine *B. fusca* larval food choice. At high concentrations, whereas sucrose was phagostimulatory, turanose was phagodetterent to larval feeding. Moreover the two sugars seem synergistic and can positively modulate larval feeding at specific ratio. The dose response feeding experiments further revealed that *B. fusca* larvae can distinguish by taste the presence of both sugars in their diets and hence both or

either of the sugars may play specific roles in larval food choice especially from plant sources. However, future investigations of the relative proportion of each of the two sugars present in different hosts may reveal a rationale for the selective management of *B. fusca* in agricultural fields. Moreover further laboratory based studies are required to determine the inhibitory mechanisms of turanose to larval feeding. Including turanose in electrophysiological tip recording tests to establish turanose sensitive cells and performing a dose response experiments with turanose amended artificial diets would provide a useful way of determining the role of turanose in host plant recognition and performance by *B. fusca* larva. Although both sugars undoubtedly and greatly contribute to the overall food choice by *B. fusca* larva, the importance of some but yet to be identified metabolites that mediate phagostimulatory/deterrent larval responses from the plants considered can hardly be underestimated and needs to be explored further.

The total phenolic content in the leaf tissues studied varied according to the plant species studied. Although no direct correlation was observed between larval performance and the total polyphenols content in the leaf extracts, this does not explicitly rule out the non-involvement of these compounds in larval host choice and performance. Determination of plant total chemical substance does not allow conclusive information on the importance of single component on a particular insect behavioural trait. And in case of total polyphenols, past phytochemical and experimental studies leave no doubt that individual phenolic compound varies substantially with respect to biological activity (Bergvinson *et al*., 1994; Santiago *et al*., 2005). Therefore, the effect of plant foliage phenolics on *B. fusca* larva may depend on the specific chemical structure of the compound. Ascertaining the exact

role of each and specific phenolic compound on larval feeding responses will require analysis of each phenolic compound and not of pooled contents in the plant leaves.

Study of digestive enzymes in herbivorous insects is not only important for the understanding of digestion biochemistry but also for developing of safe and useful control pest management strategies. Similar to other oligophagous insects, *B. fusca* larvae appear to be dependent on digestive glycosidases (glucosidases and galactosidases) than on protein/amino acid degrading enzymes (proteases) for survival and performance. Therefore by considering the importance of carbohydrate digestion as a target for *B. fusca* control, a study on their digestive enzymes could definitely be crucial in adopting new control procedures. Additionally these enzymes may be important in the degradation of secondary plant metabolites present in *B. fusca* host plants and therefore important in the understanding of insect herbivore coevolution. For example, high levels of C4 esterases induced after larvae fed on high phenolic containing plants (*S. megaphylla, P. deustum* and *P. maximum*) used in this study could be directly correlated with high levels of phenolic compounds in the specific plant tissue. Clearly further biochemical and molecular biological analysis are needed to fully understand the effect of such compounds on the digestive physiology of *B. fusca* larva. Similarly, the mechanisms by which *B. fusca* larvae is able to detoxify harmful allelochemicals or physiologically adapt to host plants containing such toxic chemicals as the larvae and exploits diversifies to variety of host plants needs to be further elucidated.

The refuges constituted by non-crop host plants may be useful in the integrated pest management strategy as has been applied in the Push-pull system (Khan *et al.,* 2000). However the benefits of such refuges may be limited because in some cases

host plant diversity may drive genetic divergence and possibly even host-mediated sympatric speciation (Mopper *et al.,* 1996). However, in this study no host-plant related genetic diversity was visible between populations sampled from *Z. mays* and those sampled from *A. donax* as has been hypothesised (Senzolin, 2006). Moreover the diversity in the genetic structure exhibited between the two populations may be congruent with patterns of geographical differentiation supporting the findings of Senzolin, (2006). However because of the limited samples and the not so efficient methods of sampling used, it is premature to rule out the existence of host associated populations of this species. Better and targeted methods thus need to be developed, such as sampling in the same locality, time frame and on different plant species to conclusively determine the existence of any genetic differentiation in respect to host use among *B. fusca* populations.

In summary, the current study illustrates that an understanding of the specific allelochemicals and plant physical characteristics perceived by insect sensory system in a plant–insect system can give cues as to the type of physiological, biochemical and genetic adaptations that can be exhibited by the insect. Therefore, for sustainable management of *B. fusca* an integrated approach based on the knowledge of important plant cues as well as physiological and genetic adaptations involved in host selection and establishment needs to be adopted to radically influence management initiatives of *B. fusca*.

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7. 0: APPENDICES

Shimadzu CLASS-VP V 6.14 SP1 *External Standard*
Report

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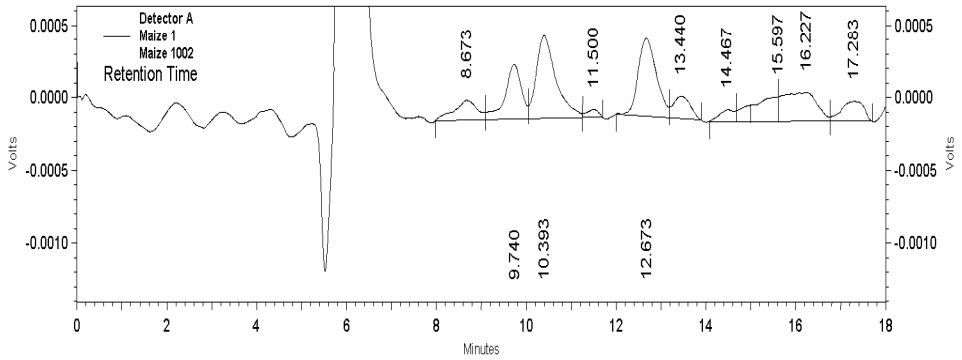

Detector A

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	Xylose	8.673	4707	88.099	MM
2	Fructose	9.740	9512	141.959	MM
3	Glucose	10.393	17488	238.336	MM
4	Galactose	11.500	1013	20.356	MM
5	Sucrose	12.673	15252	248.444	MM
6	Turanose	13.440	4046	62.726	MM
7	Maltose	14.467	1890	34.424	mm
8	Lactose	15.597	5186	107.717	mm
9	Melibiose	16.227	10408	208.751	mm
Totals			69502	1150.811	

A: Representative HPLC Chromagram obtained following injection of 2 µL solution of 10mg/ml dried *Zea mays* methanol extracts reconstituted in water into a supelcosil LC-NH2 column in Shimdzu class-VP autosampler machine.

Shimadzu CLASS-VP V 6.14 SP1 *External Standard*

Report

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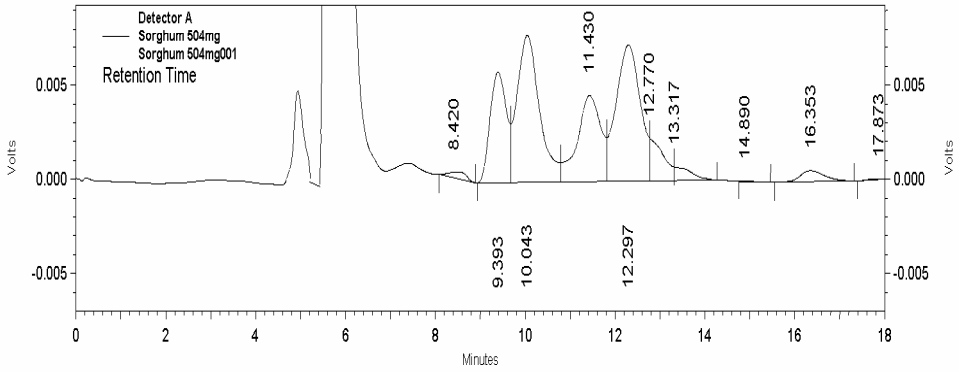

Detector A

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
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2	Fructose	9.393	148369	2214.284	MM
3	Glucose	10.043	271080	3694.424	MM
4	Galactose	11.430	165024	3316.127	MM
5	Sucrose	12.297	271532	4423.061	MM
6	Turanose	12.770	47867	742.098	MM
7	Maltose	13.317	18492	336.807	MM
8	Lactose	14.890	1682	34.936	VV
9	Melibiose	16.353	22313	447.527	VV
Totals			955812	15386.191	

B: Representative HPLC Chromagram obtained following injection of 2 µL solution of 50mg/ml dried *Sorghum arundinaceum* methanol extracts reconstituted in water into a supelcosil LC-NH_2 column in Shimdzu class-VP autosampler machine.

Shimadzu CLASS-VP V 6.14 SP1 *External Standard*

Report

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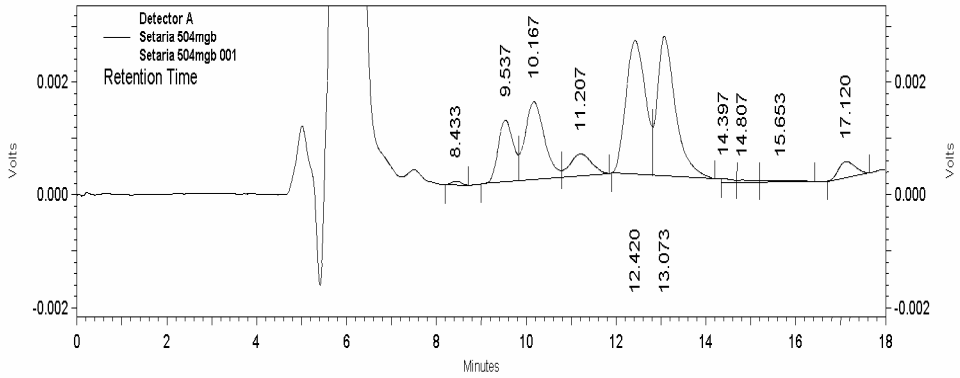

Detector A

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	Xylose	8.433	1018	19.053	BV
2	Fructose	9.537	25656	382.894	MM
3	Glucose	10.167	41653	567.669	MM
4	Galactose	11.207	12897	259.163	MM
5	Sucrose	12.420	71807	1169.684	MM
6	Turanose	13.073	69568	1078.536	MM
7	Maltose	14.397	1029	18.742	VV
8	Lactose	14.807	748	15.536	VV
9	Melibiose	15.653	1329	26.655	VB
Totals			225705	3537.934	

C: Representative HPLC Chromagram obtained following injection of 2 µL solution of 50mg/ml dried *Setaria megaphylla* methanol extracts reconstituted in water into a supelcosil LC-NH_2 column in Shimdzu class-VP autosampler machine.

Shimadzu CLASS-VP V 6.14 SP1 *External Standard*

Report

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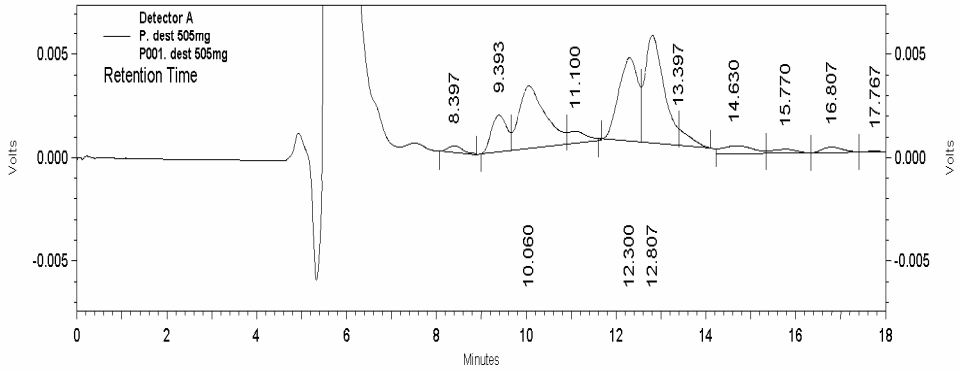

Detector A

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	Xylose	8.397	7634	142.882	BV
2	Fructose	9.393	42220	630.098	MM
3	Glucose	10.060	124395	1695.322	MM
4	Galactose	11.100	16652	334.619	MM
5	Sucrose	12.300	120354	1960.480	MM
6	Turanose	12.807	153406	2378.304	MM
7	Maltose	13.397	14045	255.811	MM
8	Lactose	14.630	17055	354.243	VV
9	Melibiose	15.770	6725	134.882	VV
Totals			502486	7886.642	

D: Representative HPLC Chromagram obtained following injection of 2 µL solution of 50mg/ml dried *Panicum desteum* methanol extracts reconstituted in water into a supelcosil LC-NH_2 column in Shimdzu class-VP autosampler machine.

Shimadzu CLASS-VP V 6.14 SP1 *External Standard*

Report

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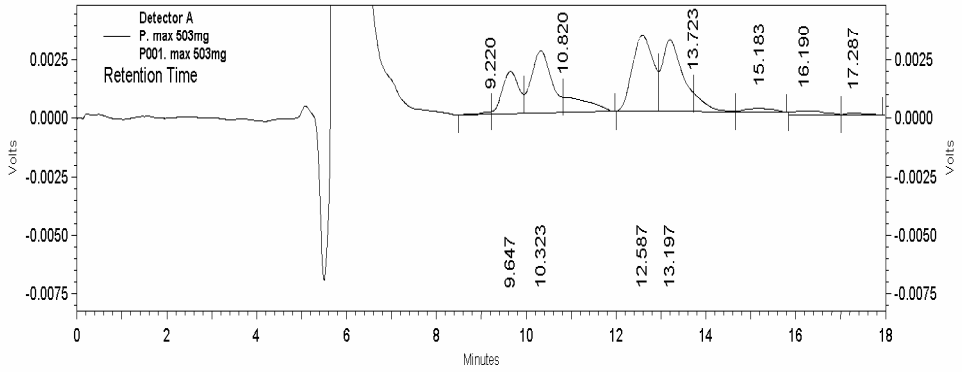

Detector A

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	Xylose	9.220	1789	33.484	MM
2	Fructose	9.647	46160	688.900	MM
3	Glucose	10.323	83453	1137.342	MM
4	Galactose	10.820	26438	531.267	MM
5	Sucrose	12.587	104945	1709.479	MM
6	Turanose	13.197	93701	1452.678	MM
7	Maltose	13.723	13776	250.911	MM
8	Lactose	15.183	7508	155.946	MM
9	Melibiose	16.190	8919	178.886	VV
Totals			386689	6138.893	

E: Representative HPLC Chromagram obtained following injection of 2 µL solution of 50mg/ml dried *Panicum maximum* methanol extracts reconstituted in water into a supelcosil LC-NH_2 column in Shimdzu class-VP autosampler machine.

Shimadzu CLASS-VP V 6.14 SP1 *External Standard*

Report

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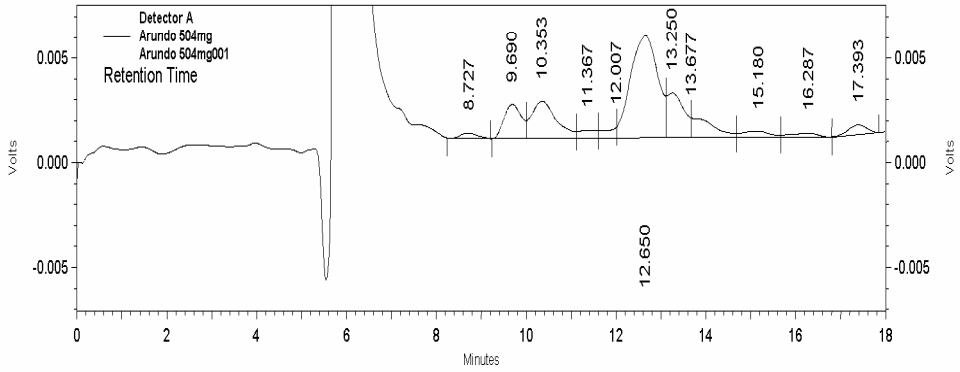

Detector A

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	Xylose	8.727	5219	97.682	MM
2	Fructose	9.690	42014	627.024	MM
3	Glucose	10.353	66141	901.405	MM
4	Galactose	11.367	9642	193.754	MM
6	Sucrose	12.650	204302	3327.932	MM
7	Turanose	13.250	55717	863.799	MM
8	Maltose	13.677	31847	580.050	MM
9	Lactose	15.180	11685	242.705	MM
10	Melibiose	16.287	7182	144.048	MM
Totals			433749	6978.400	

F: Representative HPLC Chromagram obtained following injection of 2 µL solution of 50mg/ml dried *Arundo donax* methanol extracts reconstituted in water into a supelcosil LC-NH_2 column in Shimdzu class-VP autosampler machine.

Shimadzu CLASS-VP V 6.14 SP1 *External Standard*

Report

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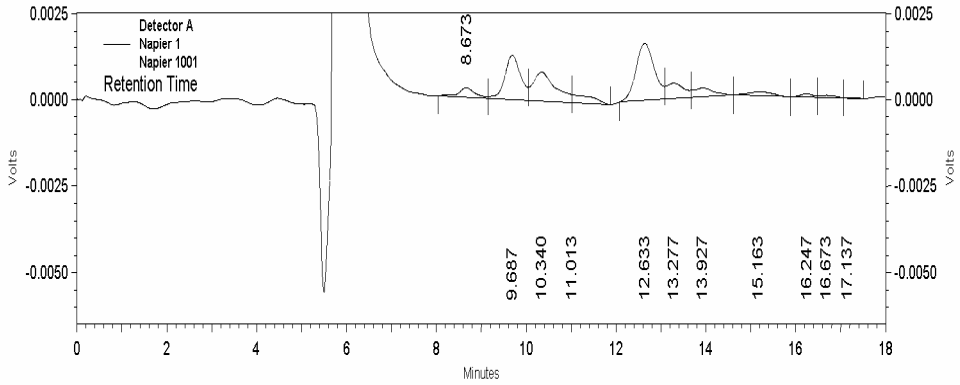

Detector A

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	Xylose	8.673	7199	134.740	BV
2	Fructose	9.687	31465	469.589	VV
3	Glucose	10.340	29181	397.694	Vx
4	Galactose	11.013	7666	154.047	xV
5	Sucrose	12.633	47436	772.698	mm
6	Turanose	13.277	12343	191.358	mm
7	Maltose	13.927	8292	151.028	mm
8	Lactose	15.163	4569	94.901	mm
9	Melibiose	16.247	1858	37.265	mm
Totals			150009	2403.321	

G: Representative HPLC Chromagram obtained following injection of 2 µL solution of 50mg/ml dried *Peniseteum purpureum* methanol extracts reconstituted in water into a supelcosil LC-NH2 column in Shimdzu class-VP autosampler machine.

Shimadzu CLASS-VP V 6.14 SP1 *External Standard*

Report

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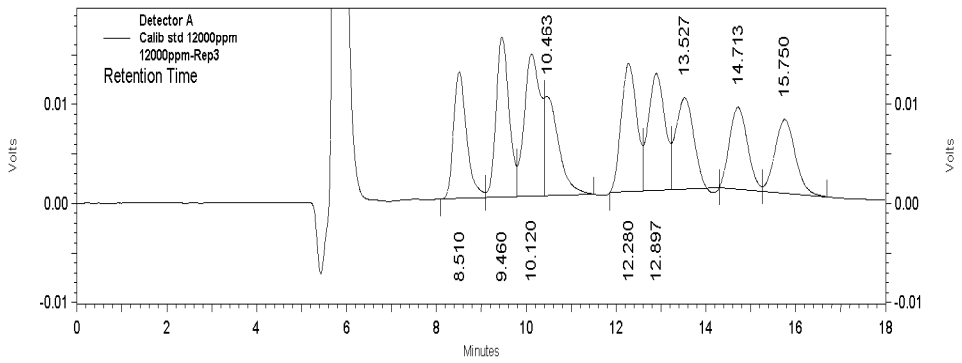

Detector A

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	Xylose	8.510	258551	12000.000 CAL	MM
2	Fructose	9.460	326694	12000.000 CAL	MM
3	Glucose	10.120	361182	12000.000 CAL	MM
4	Galactose	10.463	221407	12000.000 CAL	MM
5	Sucrose	12.280	313930	12000.000 CAL	MM
6	Turanose	12.897	305865	12000.000 CAL	MM
7	Maltose	13.527	252041	12000.000 CAL	MM
8	Lactose	14.713	229990	12000.000 CAL	MM
9	Melibiose	15.750	234013	12000.000 CAL	MM

Totals					
			2503673	108000.000 CAL	

H: Representative HPLC Chromagram obtained following injection of 2 µL solution of 12mg/ml mixture of sugar standard solutions reconstituted in water into a supelcosil LC-NH_2 column in Shimdzu class-VP autosampler machine.