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Integrated Pest Management of *Hellula undalis* Fabricius on Crucifers in Central Luzon, Philippines, with E,E-11,13-Hexadecadienal as Synthetic Sex Pheromone

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Finally I devote this paragraph to all those I may have left out but who had helped me.

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List of Abbreviations

%	per cent
°C	degree Celsius
µg	microgram
♀	female gender
♂	male gender
€	Euro
4-OH	4-Hydroxyglucobrassicin
ANOVA	Analysis of Variance
aqua dest.	*aqua destillata*
AVRDC	Asian Vegetable Research and Development Center
BA	Beet Armyworm Diet
BMZ	German Federal Ministry of Economic Cooperation and Development
Bt.	*Bacillus thuringiensis*
C	control
C/T/N	catches per trap per night
CLSU	Central Luzon State University
cm	centimetre
CRBD	complete randomized block design
CRD	complete randomized design
DAD	photodiode array detector
DAE	days after emergence of seedlings
do	days old
DT	Delta Trap
e.g.	*exempli gratia* (for instance)
et al.	*et alteri, et alii*
Fig.	Figure
Fischer's LSD	Fischer's Least Square Distance Test
FNRI-DOST	Food and Nutrition Research Institute of the Department of Science and Technology
G BT	Green Bucket Trap
g	gram
GBC	Glucobrassicin
GBN	Glucobrassicanapin
GC/MS	gas chromatography/mass spectrometry
GSL	Glucosinolates
GTZ	Deutsche Gesellschaft für Technische Zusammenarbeit
h	hour(s)
ha	hectare
HP	Hewlett-Packard
HPLC	high performance liquid chromatography
i.e.	id est
IDA	iron deficiency anaemia
IDD	iodine deficiency disorders
IPM	Integrated Pest Management
IS	inside
ITC	isothiocyanates
km	kilometre
l	litre

L:D	light: dark
log	logarithm
m	meter
m^2	square metre
MBP	Mung Bean Powder
min	minute
ml	millilitre
mm	millimetre
MSD	Mass Selective Detector
NAP	Gluconapin
NAS	Gluconasturtiin
NEO	Neoglucobrassicin
ng	nanogram
NPV	nuclear polyhedrosis virus
Ø	diameter
OS	outside
p	p-value
PAG-ASA	Philippine Atmospheric, Geophysical and Astronomical Services Administration
PBT	Plastic Bottle Trap
PEM	malnutrition of protein-energy
PRO	Progoitrin
r^2	coefficient of determination
rH	relative humidity
RLP	Radish Leaf Powder
SDE	simultaneous steam distillation and solvent extraction
SE	standard error
SM BT	Self-Made Bucket Trap
SP	synthetic pheromone
TT	Tube Trap
US$	US Dollars
V	Volt
VAD	vitamin A deficiency
VF	virgin female(s)
viz.	Videlicet
W	week
WBT	Water Bowl Trap
WT	Wing Trap
Y/W BT	Yellow/White Bucket Trap

Abstract

Different aspects of the biology and ecology of *Hellula undalis* F. were studied in the lowlands of Central Luzon, Philippines, in the province of Nueva Ecija. Special emphasis was put on E,E-11,13-Hexadecadienal, the identified sex pheromone of *H. undalis*. *H. undalis* is a major insect pest on cultivated crops of the plant family Brassicaceae (Cruciferae). Field trials were conducted to determine possible practical use of the synthetic pheromone in integrated pest management (IPM) with the overall goal to reduce insecticide overuse. These trials were divided in: (i) handling of the pheromone, i.e. determinations of the best trap height, trap design, intertrap distance, pheromone dosage, active substance longevity in the field with new prepared and freezer stored lures, (ii) biology and ecology of *H. undalis*, with determination of natural host plants with analysis of secondary plant metabolites, comparison between E,E-11,13-Hexadecadienal and the virgin female emitted pheromone, influence of virgin females next to synthetic pheromone sources, female sexual maturity, calling time of virgin females and flight activity of males in the field and in the laboratory, (iii) studies regarding the usefulness of the synthetic pheromone in pest control were conducted by comparing E,E-11,13-Hexadecadienal with added Z-11-Hexadecenal in different concentrations, the possible use of E,E-11,13-Hexadecadienal for monitoring and attempts to confuse male *H. undalis* to find females.
The population fluctuations were recorded in different areas of Nueva Ecija partially from October 2001 to November 2003.

The used compound E,E-11,13-Hexadecadienal was specific and attractive for male *H. undalis*. The trap height is best 0.5m above ground, with wing traps and an intertrap distance more than 15 m, when traps are placed crosswind and between 20-30m apart when placed in lines with the wind direction. The best cost/benefit of the pheromone dosage resulted in the use of 10µg impregnated red rubber septa. Lures with 10µg remain highly attractive for two weeks, although males were caught for six weeks in areas with high *H. undalis* density. Lures attracted equal numbers of males when stored for almost a year in a freezer (-7°C) compared to newer prepared ones.

Cleome species are natural host plants. The species *Cleome viscosa* and *Cleome rutidosperma* reached infestations up to 60%. Both species are widespread and play a possible key role as food plant for the larvae at times Brassicaceae are not or only little cultivated. Glucosinolates

(GSL) are metabolites of both *Cleome* species with glucocapparin (methylglucosinolate) as the predominant GSL compound (>90%). Glucocleomin was found in both species as well as traces of Indolyl-GSL such as 4-OH (4-hydroxyglucobrassicin) and glucobrassicin (GBC).
The total GSL content of the aliphatic compounds was much higher in *C. viscosa* than in *C. rutidosperma*. Glucosinolates are known to be host plant attractants for *H. undalis*.

The sex pheromone emitted by virgin females lured up to 25 times more males than E,E-11,13-Hexadecadienal. It is assumed that the sex pheromone might be a blend of chemicals, not only a single compound, or local differences occur in the biochemistry in pheromone production by females of Japan and the Philippines and that might be true for male perception.
The catch rate is not influenced when virgin female baited traps were placed in direct vicinity to traps with the synthetic pheromone. The ratios of catches between both types of baits were in a constant range, which makes E,E-11,13-Hexadecadienal useful as monitoring tool.
Females are sexual mature right after eclosion, i.e. when they emerge from the pupa. No significant differences in the number of males caught were detected between 1 to 5 day old virgin females. The time females start to call and lure males was determined in the field between four and eight o'clock in the morning with most males recorded at six o'clock. This time span could be verified in the laboratory. Males are active in the same phase of the night and do not react when synthetic pheromone is offered outside this period. Already calling females probably influence other females to start calling.

Results of pheromone research in Japan suggested that added Z-11-Hexadecenal to E,E-11,13-Hexadecadienal enhanced the number of males caught. This was not confirmed for the Philippines. Reasons to explain this discrepancy may be in differences in the composition of the pheromone for Japanese and Philippine *H. undalis* strains.

Despite the lesser attractiveness of the synthetic pheromone compared to the natural pheromone emitted by female *H. undalis* seems monitoring feasible. Good correlations existed between trap catches and subsequent larval infestation by monitoring the presence of adult *H. undalis* and larval counts. It was also found that rainfall has no effect on males caught in traps, whereas a significant negative correlation existed between rainfall and larval infestation.

A first attempt was made for the use of the synthetic pheromone to confuse males to locate their mating partners. Although mating was not possible due to the set up of the trial, it was clear that males were able to locate females in the area covered with lures containing 1 µg of the synthetic pheromone and therefore mating was likely to happen. However, an effect might be possible regarding the great differences of males caught in- and outside the treated area. It seems worthwhile to continue the research with this method.

Monitoring the population fluctuations over periods up to 2 years proved *H. undalis* a major pest in crucifer cropping systems throughout the year in Nueva Ecija. Population peaks were found mainly in the dry season with infestation rates higher than 70%. The older the crop grew the higher were the infestation and the damage.

Zusammenfassung

Unterschiedliche Fragestellungen zur Biologie und Ökologie von *Hellula undalis* wurden im Tiefland von Zentral Luzon auf den Philippinen, in der Provinz Nueva Ecija, untersucht. Das identifizierte Sexualpheromon von *H. undalis*, E,E-11,13-Hexadecadienal spielte hierbei eine herausragende Rolle. *H. undalis* ist ein Hauptschädling an kultiviertem Gemüse aus der Pflanzenfamilie der Brassicaceae. Feldversuche wurden durchgeführt, die helfen sollten zu bestimmen, inwieweit der Gebrauch des synthetischen Pheromons im integrierten Pflanzenschutz möglich sei, mit dem generellen Ziel der Reduzierung von Insektiziden. Die Versuchen wurden wie unterschieden in: (i) Handhabung des Pheromons, d.h. Bestimmung der optimalen Fallenhöhe, Fallendesign, Abstand der Fallen untereinander, Dosierung des Pheromons im Dispenser, Langlebigkeit der Substanz im Feld, mit Neuen und im Gefrierschrank gelagerten Ködern, (ii) Biologie und Ökologie von *H. undalis*, mit Bestimmung natürlicher Wirtspflanzen und Analyse von sekundären Pflanzenstoffen, Vergleich zwischen E,E-11,13-Hexadecadienal und dem von den unbegatteten Weibchen abgesonderten Lockstoff, Einfluss von unbegatteten Weibchen die neben der synthetischen Pheromonquelle platziert waren, der Zeitpunkt der weiblichen Geschlechtsreife, der Zeitraum indem die Weibchen die Männchen anlocken und die Flugaktivität der Männchen im Feld und im Labor, (iii) Studien die die Nutzbarkeit des synthetischen Pheromons in Schädlingskontrolle wurden durchgeführt mit Vergleichen von E,E-11,13-Hexadecadienal mit und ohne Zufügen von Z-11-Hexadecenal in verschiedenen Konzentrationen, der mögliche Gebrauch von E,E-11,13-Hexadecadienal als Mittel zur Überwachung der Population und der Versuch die Männchen zu verwirren, sodaß sie keine Paarungspartner mehr orten können.
Die Fluktuationen der Populationsgröße wurde in verschiedenen Gebieten Nueva Ecijas aufgezeichnet, unter anderem von Oktober 2001 bis Oktober 2003.

Die benutzte Verbindung E,E-11,13-Hexadecadienal war spezifisch und attraktiv für männliche *H. undalis*. Die optimale Fallenhöhe wurde mit 0.5m über dem Boden, mit Flügelfallen und einem Abstand bei Seitenwind, der größer als 15m, und in Windrichtung zwischen 20 und 30m, zwischen den Fallen sein sollte. Das beste Verhältnis von Kosten und Nutzen bei der Dosierung ergab den Gebrauch der 10µg imprägnierten roten Gummi Dispenser. Köder mit 10µg waren für zwei Wochen hochattraktiv, obwohl in Gebieten mit einer hohen Populationsdichte von *H. undalis* Fänge bis zu sechs Wochen nachgewiesen

wurden. Die Köder waren nach einem Jahr im Gefrierschrank (-7°C) genauso attraktiv wie frisch präparierte.

Cleome Arten sind natürliche Wirtspflanzen. Bei den Arten *Cleome viscosa* und *Cleome rutidosperma* fanden sich Befallszahlen bis 60%. Beide Arten sind weit verbreitet and spielen eine mögliche Schlüsselrolle als Nahrungspflanzen für die Larven in Zeiten, wenn keine Brassicaceae angebaut werden. Glucosinolate (GSL) sind Metabolite des sekundären Pflanzenstoffwechsels beider *Cleome* Arten mit Glucocapparin (Methylglucosinolat) als vorherrschende Verbindung (>90%). Glucocleomin wurde ebenso bei beiden gefunden wie Spuren von Indolyl-GSL wie 4-OH (4-Hydroxyglucobrassicin) und Glucobrassicin (GBC).
Der gesamt Gehalt an GSL der aliphatischen Verbindungen war wesentlich höher in *C. viscosa* als in *C. rutidosperma*. Glucosinolate sind als Pflanzenlockstoffe für *H. undalis* bekannt

Das von den Weibchen abgegebene Sexualpheromon köderte bis zu 25 mal mehr Männchen als E,E-11,13-Hexadecadienal. Es wird angenommen, dass das Sexualpheromon eine Mischung verschiedener Substanzen ist, nicht nur aus einer einzigen besteht, oder es lokale Unterschiede der Biochemie bei der Produktion der Pheromone durch die Weibchen, in Japan und auf den Philippinen besteht. Dasselbe könnte für die Perzeption der Männchen gelten.
Die Fangrate wird durch benachbart postierte unbegattete Weibchen neben Fallen mit dem synthetischen Pheromon nicht beeinflusst. Das Verhältnis der Fänge beider Arten von Ködern blieb konstant, warum E,E-11,13-Hexadecadienal zur Überwachung der Population eingesetzt werden kann.
Weibchen erlangen sexuelle Reife direkt nach der Entpuppung, d.h. wenn sie aus der Puppenhülle schlüpfen. Keine signifikanten Unterschiede der Fangzahlen von männlichen Tieren bestanden zwischen 1 und 5 Tage alten Weibchen. Der Zeitpunkt, wenn die Weibchen die Männchen anlocken wurde im Feld zwischen vier und acht Uhr morgens bestimmt, mit den meisten gefangenen Männchen um sechs Uhr. Dieser Zeitraum wurde im Labor bestätigt. Männchen sind zur selben Phase aktiv und reagieren nicht auf das Pheromon wenn es außerhalb dieser Zeit angeboten wird. Lockstoff abgebende Weibchen beeinflussen wahrscheinlich andere Weibchen mit dem Anlocken zu beginnen.

Ergebnisse aus Japan ergaben, dass E,E-11,13-Hexadecadienal versetzt mit Z-11-Hexadecenal die Attraktivität für Männchen steigert und die Fangzahlen damit erhöht. Dieses konnte für die Philippinen nicht bestätigt werden. Gründe dafür liegen vermutlich in der Unterschiedlichkeit der Pheromonkomposition von japanischen und philippinischen *H. undalis* Stämmen.

Trotz der geringeren Attraktivität des synthetischen Pheromons im Vergleich zum natürlichen, von den Weibchen abgegebenen, erscheint die Überwachung einer Population möglich. Gute Korrelationen ergaben sich aus der Anzahl der Fänge in Fallen und folgender Befall mit Larven, die durch Überwachung der Anwesenheit von adulten Männchen und Abzählen der Larven an den Pflanzen ermittelt wurden. Regen hatte keinen Einfluss auf den Fang der Männchen, wohingegen eine signifikant negative Korrelation aus Regen und Befall mit Larven ergab.

Ein Versuch wurde unternommen der zeigen sollte, ob das synthetische Pheromon die Männchen so verwirren kann, dass sie ihre Paarungspartner nicht mehr finden können. Obwohl durch den Versuchsaufbau eine Paarung von vornherein ausgeschlossen war, konnten die Männchen ganz klar die weiblichen Köder lokalisieren, in einem Gebiet, welches mit 1µg Ködern bedeckt war. Es ergaben sich jedoch große Unterschiede in der Anzahl der gefangenen Männchen inner- und außerhalb des behandelten Areals. Es erscheint lohnend die Forschung mit dieser Methode fortzusetzen.

Die Überwachung der Populationsschwankungen über einen Zeitraum von bis zu zwei Jahren zeigte deutlich, daß *H. undalis* über das ganze Jahr zu den Hauptschädlingen in Kruziferen-Anbaugebieten in Nueva Ecija zählt. Der Höchststand fand sich hauptsächlich in der Trockenzeit mit Befallszahlen über 70%. Je älter die angebauten Pflanzen wurden, umso höher war der Befall und Schaden an den Pflanzen.

Chapter 1
Thesis Overview and Introduction

1 Introduction

1.1 General Objectives

This thesis is concerned with the sex pheromone of female *Hellula undalis* (Fabricius). The basis for the handling of the synthesised pheromone and possible usage for monitoring or control of the pest was carried out in field trials in a province of Central Luzon, Nueva Ecija, in the Philippines. The overall goal is the reduction of pesticide use in vegetable growing areas for the benefit of producers and consumers.

The main focus comprised studies dealing with the handling of the sex pheromone, biology and ecology, partially with the synthetic pheromone and virgin females as bait and the possible integration in integrated pest management (IPM) programs or even control with it. Additionally, the studies included population dynamics of *H. undalis* in different areas of Nueva Ecija.

1.2 Background of the Study: Malnutrition, Pesticide Use and Vegetable Production Needs in the Philippines

1.2.1 Malnutrition in the Philippines

Malnutrition in the Philippines and particularly in Metro Manila can be seen as one consequence of poverty. Vegetables as one source of micronutrients and vitamins are too expensive and the poor of Manila rely more on rice and meat or fish (ALI and PORCIUNCULA, 1999) as the predominant food source. If consumption of vegetables would increase in Metro Manila, an increase in production is necessary. ALI and PORCIUNCULA (1999) reported a production area of 167 hectare (ha) in the borders of Metro Manila, which could cover 0.52% of the annual consumption there. Thus, vegetables for Metro Manila must come from the surrounding provinces. The needs become even more urgent since the urban growth rate increased to almost double from 1970 (2.4 %) to 2000 (4.3 %) (WORLD BANK, 2001).

Surveys conducted by the 'Food and Nutrition Research Institute of the Department of Science and Technology' (FNRI-DOST) in 1998 reveals that parts of the population of the Philippines suffer of malnutrition of protein-energy (PEM) (BARBA and FELICIANO, 2002; http://www.fn ri.dost.gov.ph/facts/part1.html, 02.10.04). Emaciation was determined for more than 30 % of children between the ages of 0-10 years old. Emaciation had slightly decreased in the sixth survey of the FNRI-DOST in 2003 (http://www. fnri.dost. gov.ph/nns/6thnns.pdf, 02.10.2004) but is still a problem.

Micronutrient malnutrition, particularly vitamin A deficiency (VAD), iron deficiency anaemia (IDA) and iodine deficiency disorders (IDD) are major problems for health (BARBA and FELICIANO, 2002). More than half the children (56 %) of less then 1-year-old, 50.7 % of pregnant and 47.7 % of lactating women do not have the necessary uptake of iron in their diet in the survey of 1998 (BARBA and FELICIANO, 2002). Beside these vulnerable groups, a prevalence of 30.6 % of anaemia caused by iron deficiency in the whole population exists (http://www.fao.org/es/esn/nutrition/phi-e.stm, 02.10.2004). The prevalence of anaemia has increased in the 2003 survey. FNRI-DOST concludes that the nutrient situation has improved compared to 1998 (http://www.fnri.dost.gov.ph/nns/6thnns.pdf, (02.10.2004). Reasons for the malnutrition can be seen as a relationship of poverty, food, and nutrition insecurity as well as in the food consumption with ‘the typical Philippino diet consisting of rice, fish or meat, some vegetables, and occasionally fruit’ (ANGELES-AGDEPPA, 2002; http://www.fnri.dost.gov.ph/ facts/part1. html**,** 02.10.04). The FNRI-DOST concludes that the average Philippino diet is short of recommended dietary allowances and intake of vitamins and minerals are inadequate **(**http://www.fnri.dost.gov.ph/facts/part1.html, 02.10.04).

1.2.2 Vegetables as Source of Micronutrients

Vegetables are essential for human life and provide the body with minerals and micronutrients. The production of nutrients per unit land area is generally higher in staple crops like rice than for vegetables (AVRDC, 1990). Recommendations vary in different countries regarding the daily consumption but vegetables are seen as healthy and in contrast to meat or carbohydrates low in calories. Permanent supply of vegetables, produced with a minimum quantity of pesticides and other agrochemicals is the basis against micronutrient deficiencies and would have a positive effect on the urban poor, which depend highly on vegetables grown in rural areas.

1.2.3 Manila-Peri-Urban-Vegetable-Project

The ‘German Federal Ministry of Economic Cooperation and Development’ (BMZ) supported the project ‘Development of peri-urban production systems for sustainable year-round vegetable supplies to tropical Asian cities’, started in 1998. The coordination of the project was assigned to the ‘Asian Vegetable Research and Development Centre’ (AVRDC), funded by the ‘Deutsche Gesellschaft für Technische Zusammenarbeit’ (GTZ). Aim of the project was to stabilize the supply of safe and nutritious vegetables to metropolitan areas on the example of Metro Manila. Different aspects were examined like socioeconomy, soil and crop nutrition, chemical analysis of pesticide residues, training of farmers and pest management for different crops and pests in Phase I. from 1998-2001 (AVRDC, 2000; 2001).

Among the expected outputs of the second phase, which started March 2001, the major goal was to introduce techniques to decrease the quantity of pesticides used by vegetable growers (AVRDC, 2002; 2003).

Field monitoring in fields of the municipality of San Leonardo revealed *H. undalis,* a pest of crucifer crops, as one of the major pests in the area. Thus, IPM strategies had to be developed. Biology as well as ecology of this pest species is not well examined. Means for detecting the larvae are restricted to monitor larvae of older stages in the field; adult monitoring does not exist except the use of light traps and traps baited with virgin females. Therefore, examination of population fluctuations and developing strategies to control this pest were the aim for the *H. undalis* studies.

1.2.4 Pesticide Use in the Philippines

Insecticides and other agrochemicals are frequently applied onto crops in the Philippines. Surveys of farmers in Nueva Ecija province revealed that all of them were using chemicals for pest control. Biological hazards caused by pesticides have both a negative impact to food for consumers and unprotected field workers. The hazardous use of pesticides is described for instance by HERDT et al. (1994), ROLA and PINGALI (1993) and ANTLE and PINGALI (1994).

Only 20 % of farmers interviewed had knowledge of the existence of natural enemies of insects on vegetables; also the safe handling of potentially hazardous chemicals is not usually practiced even though farmers were aware of dangers to their health (http://www.ruaf.org/1-3/15-16.pdf, 27.09.2004).

Insecticides are used for the most part in rice fields with almost 50 % of the total sales in 1987; the share of insecticides used for vegetables is the second largest in the Philippines (ROLA and PINGALI, 1993) with 19 % of total sales.

The value of insecticides used in the Philippines was 28 Million US Dollars (US$) in 1988 (WOODBURN, 1990) and increased to US$ 50 Million in 1998 and US$ 80 million by 2002 (http://faostat.fao.org/faostat/servlet/XteServlet3?Areas=171&Items=1357&Elements=62&Years=1998&Format=Table&Xaxis=Years&Yaxis=Countries&Aggregate=&Calculate=&Domain=LUI&ItemTypes=Pesticides&language=EN, 27.10.2004), whereas Dioquino (http://www

.nihs.go.jp/GINC/meeting /7th/7profile/phil-rep.pdf, 28.10.2004) reported of US$ 120 million in 1998 for pesticides.

Studies regarding impacts of pesticides on health were conducted with rice farmers in Luzon (PINGALI et al., 1994; ANTLE and PINGALI, 1994). Indications are given that Philippine farmers do not use pesticides in recommended doses nor do they follow the handling, storage, and application in a proper way. ANTLE and PINGALI (1994) conclude that 'farmers do not use pesticides either efficiently or safely'. Medical examinations in the study relate prolonged pesticide exposure with chronic effects on health. Some farmers know about the risks but rely on pesticides since no other possibilities are available, i.e. costs are too high for advanced technologies (protection gear) or they do not know that they are exposed to a potentially health hazardous substance (ANTLE and CAPALBO, 1994). Most pesticides used for controlling rice pests were classified as extremely hazardous chemicals by the World Health Organization (WHO) (PINGALI et al., 1994).

Health of farmers and farm workers and their families is one aspect; another one is related to the environment. Control of pests by beneficial arthropods is strongly reduced, when pesticides are frequently applied (LIM et al. 1986; STERK, 1993).

Impact on beneficial organisms due to non-selective pesticides is described in numerous articles and books (e.g. SMITH and GRIGARICK, 1990 and references therein; HASKELL and MCEWEN (eds.), 1998). Reaching the target is sometimes obtained by only 1 % of the insecticides applied (GRAHAM-BRYCE, 1977). PIMENTEL (1995) estimated an even lower percentage, often less then 0.1 %, reaches target pests; the rest of 99.9 % remains in the environment. Non-target species are exposed to insecticides too and natural enemies of the pests get killed, and are not available for natural protection against the pest. Problems arise when beneficial organisms, which keep minor pests under control within normal conditions, are removed from an area and secondary outbreaks occur (DENT, 2000).

Economically is the use of pesticides an important factor of expenditure ((BRETHOUR and WEERSINK, 2001).

Reduction of pesticides is advantageous to Filipinos in two different ways:

1) - Reduction of chronic diseases caused by pesticides both for producers and consumers, and

2) - Protection of the environment, i.e. reduction in contaminations of soil, water and air and impact against non-target species.

Integrated pest management was developed among other reasons due to environmental concerns, but as well as for health aspects.

Sex pheromone based monitoring of pest species could be one part in reducing the quantity of insecticides applied onto fields. The nature of highly specific and non-toxic pheromones makes them useful as a part within an integrated pest management programme for early detection of the target species.

1.3 Background of the Study: Chemical Ecology, Sexual Pheromones of Moth and Integrated Pest Management with Pheromones

1.3.1 Semiochemicals

Semiochemicals are generally involved in the communication of organisms. They are subdivided into pheromones (Greek, *phérein*- to transfer; *hormán*- to excite) and allelochemicals. Allelochemicals consist of allomones, kairomones, synomones and antimones, which act between species (HOWSE et al., 1998; BORDEN, 1977). The classification follows the effect caused by the releaser of the pheromone and/or the receiver of the signal, whether it is advantageous or disadvantageous. Pheromones mediate between organisms of the same species and are divided into the function or reaction they cause. Examples in the vast number of different kinds of pheromones are alarm pheromones, aggregation pheromones, trail pheromones and sexual pheromones (HOWSE et al., 1998; JUTSUM and GORDON, 1989; BIRCH, 1974).

Sex pheromones were first identified for the silk moth, *Bombyx mori* (BUTENANDT et al., 1959). Since then hundreds of insect sex pheromones became known (ARN et al., 1986, 1997; WITZGALL et al., 2004), with about 530 from female moths (ANDO et al., 2004), and many of them examined in laboratory and field trials (CARDÉ and MINKS, 1997).

1.3.2 Sex pheromones and moth: Biochemistry, Physiology, and Ecology of Sex Pheromone Behaviour of Moth

The sex pheromone communication system of moths consists of chemical signals, produced in special secretory cells, which are in most cases organized in gland organs (PERCY and WEATHERSTONE, 1974). Glands are mostly localized in the abdomen either in the fifth sternite or in the intersegmental membrane between the eighth and ninth segments. The latter is true for most ditrysian families (LÖFSTEDT and KOZLOV, 1997). The pheromone released by male or more often by the female is transmitted through the air and can be perceived by the conspecific sex via the antennae. Sex pheromone molecules, floating in the air, which come in contact with olfactory sensillae initiate a neural response (transduction) followed by a behavioural reaction, when a certain threshold is exceeded (KAISSLING and PRIESNER, 1970; KAISSLING, 1971; PAYNE, 1974).

The chemical signals, the sex pheromones, of Lepidoptera are straight carbon chains (HOWSE et al, 1998). ANDO et al. (2004) classified lepidopteran sex pheromones in two types and various others (10 %). Type 1 consists of primary alcohols and their derivates (acetates and aldehydes) with a long straight hydrocarbon chain (C10-C18). 75 % of the known sex pheromones are identified with such a structure. Type 2 comprises around 15 % of the known sex pheromones and is described as polyunsaturated hydrocarbons and epoxy derivatives with a longer straight chain (C17-C23) without functional group at the terminal position. The high number of differences between the sex pheromones of different species depends on the length of the carbon chain, the functional group (alcohol, acetate, aldehyde), position, and number of double bonds and their stereochemistry (HOWSE et al, 1998). The majority of Lepidoptera release mixtures or blends of different compounds. Pheromone biosynthesis occurs directly from fatty acids, or by the same biochemical pathway fatty acids are build up (BAKER, 1989; TUMLINSON, 1988; ROELOFS and BJOSTAD, 1984; WOLF et al., 1981).

Physiological factors affect pheromone production, release, and response to the chemical signal (SHOREY, 1974). Many species have evolved a circadian rhythm of pheromone behaviour, i.e. release and response to it occurs at the same time (ROSÉN, 2002; CHOI et al., 1998; LINN et al., 1996; FATZINGER, 1973; SOWER et al., 1970, 1971; SHOREY and GASTON, 1965). Age of the imago can influence the production, release, or reaction to the signal. Many species start calling and mating right after emergence but in others several days are between eclosion and calling/mating. This time is needed before eggs are mature and the sex pheromone is produced (GEMENO and HAYNES, 2000; MCNEIL, 1991; TURGEON and MCNEIL, 1982). Age of females can influence the duration, start and end of calling (HOU and SHENG, 2000; RAINA et al., 1986; LAWRENCE and BARTELL, 1972). Mating status, exposure to pheromone prior the imago stage, population density and nutrition are additional factors, which influence the pheromone behaviour of insects (SHOREY, 1974; HOWSE et al, 1998).

Environmental and abiotic factors, such as day length, wind direction and speed, temperature, humidity, seasonality, and food availability for the offspring can have an effect on the sex pheromone behaviour (SHOREY, 1974 and references therein).

1.3.3 Monitoring and Control of Insect Pests with Sex Pheromones

Identification of sex pheromones and research about this topic and the potential use has increased since the first identification of the sex pheromone component of *Bombyx mori* by BUTENANDT et al. (1959). Different directions of research combine knowledge of neurological, physiological, pheromone perception, behavioural, application/use, and evolutionary aspects. Once seen as the most promising way of controlling insect pests, it became clear that many factors influence the behaviour of pest species. Extensive knowledge of biological and ecological aspects of a certain pest species is necessary for the proper use of sex pheromones (HOWSE et al., 1998; BIRCH et al., 1974). Sex pheromone studies are included in the vast field of chemical ecology research with an outstanding number of articles. Although sex pheromones could be used against vectors of diseases like Malaria, Chagas-disease and others, most research was done in regards to pest monitoring and control.

Components consist of the pheromone source viz. the synthetic sex pheromone (in most cases the identical copy of the natural one), a suitable trap and the knowledge of placing the trap in regards to height, location in the area, distance between traps, how long a lure can be used and other factors. All points mentioned above are of lesser information value, when the biology and ecology of a pest insect is not known.

Sex pheromones are used either to monitor a certain pest population, or to control it. The latter point includes two major applications: mating disruption and mass trapping (e.g.: BIRCH et al., 1974; HOWSE et al., 1998; SANDERS, 1997; MINKS, 1997; ARN and LOUIS, 1997; STATEN et al., 1997; SUCKLING and KARG, 1997; and references therein).

Monitoring with a species-specific sex pheromone provides information about the occurrence of the pest in a given area, as an early warning, a general survey for detection purposes. It can also help with decisions of applying measures, whether spraying insecticides or not (HOWSE et al., 1998; CAMPION, 1984; BIRCH et al., 1974). Knowing the threshold level for a pest might help in timing of pesticide applications, or a risk assessment. Another part is survey of population trends, dispersion of pests and the probable effects of control measures (HOWSE et al., 1998).

Mating disruption has proved to be successful in the control of several pests. Mechanisms are stated in SANDERS (1997) in detail and examples for several pest species of successful mating disruption in CARDÉ and MINKS (1997). Mating disruption is not yet fully understood; habituation and confusion are thought to cause the disruptive effects, i.e.,

a) males exposed permanently to high levels of their sex pheromone cannot respond to it since the nervous system gets habituated

b) males cannot find their mating partners due to a masking of the natural pheromone (CAMPION, 1984; SANDERS, 1997; HOWSE et al., 1998).

Mating disruption is used to control commercially important pest species. The codling moth, *Cydia pomonella* (CHARMILLOT, 1990; JUDD et al., 1997; MINKS, 1997), vineyard pests, *Lobesia botrana* and *Eupoecilia ambiguella* (ARN and LOUIS, 1997), the Oriental fruit moth, *Grapholita molesta* (Busck) (RICE and KIRSCH, 1990), and the pink bollworm, *Pectinophora gossypiella* (Saunders) (BAKER et al., 1990) belong to the species the technique was proved to be successful.

Mass trapping reduces the number of conspecific mating partners in such a manner that the mating event will not happen since high numbers of the population are removed from the population (HOWSE et al., 1998). Control or partial control by mass trapping is evident for only a few lepidopteran species (e.g.: CAMPION and NESBITT, 1981; BEEVOR et al., 1993; MAFRA-NETO and HABIB, 1996). Pheromone selling companies offer their products for mass trapping in the majority for Coleopteran and Dipteran species. Only four of 113 commercially available lures are offered for Lepidopteran species (*Sitotroga cerealella, Spodoptera sunia, Tecia solanivora, Tuta absoluta*) at http://www.pheroshop.com/en/species.htm, 9.11.2004.

1.4 Background of the Study: Biology, Distribution, and Control with the Sex Pheromone of *Hellula undalis* F.

1.4.1 Distribution and Pest Status of *H. undalis*

H. undalis is distributed mainly in tropical and subtropical regions (WATERHOUSE and NORRIS, 1989) but can also be found in countries with moderate climates (first identified by Fabricius 1794 in Italy) (WATERHOUSE and NORRIS, 1989). There are no observations of *H. undalis* in North and South America (WATERHOUSE and NORRIS, 1989). The northernmost distribution with outbreaks of *H. undalis* is described for Japan (TANAKA and TANIMOTO, 1979; SHIRAI et al., 1988; SHIRAI and KAWAMOTO, 1990, 1991).

Outbreaks with yield losses up to 100% are described for Hawaii, India, Malaysia, Philippines, Taiwan, Egypt, Iraq and Japan (BUNTING and MILSUM, 1930; HARAKLY, 1968a; HARAKLY, 1969; SANDHU and BHALLA, 1973; RAO et al., 1976; SIVAPRAGASAM and AZIZ, 1992; AVRDC, 1978; SACHAN and SRIVASTAVA, 1972; YAMADA, 1981; TALEKAR et al., 1981; BHALANI, 1984; AVRDC, 1985; SINGH et al.; 1990; AL-JANABI et al., 1990; SINGH and SINGH, 1993; VEENAKUMARI et al., 1995; REJESUS and JAVIER, 1997; SIVAPRAGASAM and CHUA, 1997b).

WATERHOUSE (1992) describes the occurrence of *H. undalis* throughout the pacific region particularly the pacific islands, where *H undalis* is widespread and can cause important damage to planted crucifers.

Peak infestations are described for different regions in India from February to March (BHALANI, 1984), August to October (SACHAN and SRIVASTAVA, 1972; SANDHU and BHALLA, 1973).

For Taiwan, it is serious for the summer season (AVRDC, 1978; CHUANG, 1994) from June to September. In Egypt infestations occur from the beginning of June to November (HARAKLY, 1968b). ISOKO (1995) stated severe damage of radish and Chinese cabbage in Japan. Adults can be found from May to winter with higher populations from July to September.

SIVAPRAGASAM (1994) listed the status of *H. undalis* as a pest with its major host plants for all countries found.

1.4.2 Biology and Ecology of *H. undalis*

H. undalis belongs to the family Pyralidae and subfamily Glaphyriinae (MUNROE, 1972). The imago is small (length of the body between 6-9 mm), with wingspans of 12-16 mm. The colour is greyish brown. On the forewings are proximal undulating lines and more distally just before the wing tip a curved pale patch. The hindwing is pale. The larvae are whitish yellow after hatching and turn to greyish yellow with a dark head capsule. Five broad brownish lines extend from the second thoracic segment to the terminal abdominal segment. The hatching first instar is around 1 mm in length and grows up to 15-16 mm in the fifth instar. Pupae are light to darker brown with a length ranging between 6-10 mm.

Although *H. undalis* can be seen as a major pest on Brassicaceae in many countries, little is known about its ecology. SIVAPRAGASAM (1994) pointed out that no more than 12 parasites were identified so far for this species (WATERHOUSE and NORRIS, 1989; SIVAPRAGASAM, 1994) and biological control seems to be of no effective use.

The biology of *H. undalis* was described by HARAKLY (1968 a, b), AVRDC (1978), TALEKAR et al. (1981), BHALANI (1984), MEWIS (2001), PETER et al. (1987), SHIRAI and YANO (1994), SINGH et al. (1990), SINGH and SINGH (1992), SIVAPRAGASAM (1994), SIVAPRAGASAM and CHUA (1997a,b), YOUSSEF et al. (1973). Eclosion of the imago occurs predominantly in the night (HARAKLY, 1969; TALEKAR et al., 1981). Mating takes place soon after emergence (HARAKLY, 1968b; TALEKAR et al., 1981; BHALANI, 1984). HARAKLY (1968b) observed mating directly after the wings were dried after eclosion and oviposition occurs in most cases within 24 hours after mating.

Females lay their eggs singly or in very few on the same plant at a time (BHALANI, 1984; SIVAPRAGASAM, 1994) in a random manner (SIVAPRAGASAM, 1996). First instar larvae usually mine into leafs. The first two instars are therefore protected largely against pesticides and chemicals, which are applied on the surface of the crop. Later instars migrate leafs, live solitaire and web a net around themselves with incorporated corpuscles of faeces. These webs are symptomatically for *H. undalis.*

The later instars can move from the leaves to feed the growing point (HARAKLY, 1968b; TALEKAR et al., 1981; SIVAPRAGASAM, 1996, 1997b; MEWIS, 2001) causing the death of the infested plant or prevent the plant from developing properly (CHUO, 1973). Studies regarding the intra-plant movements of larvae showed that 84% of the larvae were found on the shoot (SIVAPRAGASAM, 1994; SIVAPRAGASAM and CHUA, 1997b). This behaviour is seen as a protective measure as the shoot provides a natural protection against foraging predators (SIVAPRAGASAM and CHUA, 1997b). Cannibalism of the larvae was observed by HARAKLY (1968b).

Due to the required high external standard of vegetables, damaged or deformed plants are unmarketable. This behaviour pattern of wandering of larvae to the growing point lowers the economic threshold level for damage enormously since a single larva can destroy its host plant.

The time the larvae grow depends on temperature (HARAKLY, 1969; SACHAN and GANGAWAR, 1980, SIVAPRAGASAM 1994). Development below 20°C is slowed down (AWAI, 1958; SIVAPRAGASAM et al., 1994). Temperatures below 0 °C for more then 10 days prevent overwintering in Japan (SHIRAI and YANO, 1994). Hibernation takes place in the last instar larval or pupal stages and was estimated by SHIRAI and YANO (1994) in some parts of Japan.

Development time from egg to imago varied from 14 to 108 days, when the temperature was 35 and 15 °C, respectively (SIVAPRAGASAM, 1994). Exposed to 10°C and 40°C, the survival rate was 0%. The optimum temperature lies therefore between 25 to 35° C, a temperature range that occurs normally in tropical areas. An optimum of 27°C was assumed by HARAKLY (1968a).

Five larval stages were reported by the majority of authors (e.g.: HARAKLY, 1968b; AVRDC, 1978), whereas BHALANI (1984) and SANDHU and BHALLA (1973) detected only four.

The fifth and last instar wanders around when food uptake has finished and looks for a place to pupate. It spins a cocoon, when it reaches maturity and changes into a pre-pupal stage (HARAKLY, 1968b). It becomes shorter and changes its colour to pale yellow. The duration of the pre-pupal stage depends on temperature and lasts between 0.87 to 4.1 days (HARAKLY, 1969).

Pupae are first white/yellow and change to light brown when the cuticle is sclerotized. HARAKLY (1968b) found the pupae in the field in various sites at plants, inside folded and dried edges of leaves, inside stems and on other places of the plants as well as in the soil, buried up to 5 cm into it.

Temperature is also involved in the longevity of the adults. There is a positive correlation between temperature and longevity of female and male *H. undalis*. The cooler it is, the longer the adults can survive. The longevity of the adults ranges from 12 days to 3 days, when the temperature was 35 °C and 15 °C, respectively (SIVAPRAGASAM, 1994; SIVAPRAGASAM et al., 1994).

The host range comprises not only of cultivated crops from the family Brassicaceae but also from the plant family Capparidaceae, containing specific secondary plant substances, mustard oils or glucosinolates. *Cleome rutidosperma* and *C. viscosa* were identified in several countries as natural host plants of *H. undalis* (SIVAPRAGASAM, 1994; SIVAPRAGASAM and AZIZ, 1992; SIVAPRAGASAM et al., 1994; SIVAPRAGASAM and CHUA, 1997a; REJESUS and JAVIER, 1997). The influence of *Cleome rutidosperma* as natural host plant was examined by SIVAPRAGASAM (1994) and SIVAPRAGASAM and CHUA (2000). It is stated that natural host plants could play an important role as food source for the larvae when no crucifers are grown. *C. viscosa* was found as one of the three most dominant weeds in onion fields in a survey of weeds in San José, Nueva Ecija (BALTAZAR et al., 1998).

SHIRAI and YANO (1994) examined the flight ability of female and male *H. undalis*. They conclude that female *H. undalis* are not capable of long distance flights (based on the relation between flight and reproductive success in the laboratory) and males do not show any tendencies of long distance dispersal in a mark-recapture trial (SHIRAI and KAWAMOTO, 1990). However, the potential flight ability ranges from 50-80 km for some days (SHIRAI and YANO, 1994). Temperature also influences the flight behaviour. The velocity increases with higher temperatures. Temperatures of 30°C reduce continuous flight. A temperature of 20°C was suggested as optimum for flight activity.

Both male and female are active during the night (HARAKLY, 1968b; YOUSSEF et al., 1973). Flight times were not observed so far. YOUSSEF et al. (1973) mentions a flight activity peak around midnight. In Taiwan, it is described that the activity starts at dusk (AVRDC, 1978) and continues into the night (TALEKAR et al. 1981).

It appears that female *H. undalis* are selective in the behaviour of egg depositing when different plants are offered.

Host plants such as cabbage and cauliflower resulted in positive correlations regarding size of larvae and pupae and egg laying capacity (EL-SHERIF et al., 1980).

Examinations of preferences between different host plants were conducted in Taiwan (TALEKAR et al., 1981). Nine species and subspecies of crucifers were used. Chinese cabbage and radish were the preferred species. SIVAPRAGASAM and AZIZ (1992) described the host preference, as Chinese mustard followed by cabbage and radish. A detailed study of host plant localizing and female egg laying behaviour with regards to the glucosinolate contents of different host plants was conducted in the Philippines (MEWIS, 2001, MEWIS et al., 2002). The results demonstrate that female *H. undalis* differentiate among host plants. *Sinapis alba* is therefore the most prefered host plant among three species compared, followed by *Brassica juncea* and *Brassica campestris ssp. chinensis.* Glucosinolates play a crucial role in the host plant localisation and stimulate oviposition for several insect species (FEENY, 1977). Occurrence of glucosinolates was found both in Brassicaceae and Capparidaceae (http://bodd .cf.ac.uk/BotDermFolder/BotDermC/CLEO.html, 08.04.04), the glucosinolate Glucocapparin was detected in the seeds of *Cleome viscosa* (KJAER 1960, HASAPIS *et al.* 1981),) and other related plant families (Tovariaceae, Resedaceae, Tropaeolaceae, Limnanthaceae) (http://www.life.uiuc.edu/plantbio/363/lecture17.html, 09. 05.04).

Natural enemies do not have a great impact on *H. undalis* populations in Malaysia (SIVAPRAGASAM, 1994, SIVAPRAGASAM and AZIZ, 1992) with percentage parasitism less then 17% with the braconid *Bassus sp.* Other parasites were the Hymenoptera *Trathala flavoorbitalis, Chelonus sp.* and *Phanerotoma sp.* (SIVAPRAGASAM, 1994). The general parasitism rate was low. Parasites of eggs and pupae are not yet found.

Parasites were detected in Egypt (YOUSSEF et al., 1973), *Apanteles sp.* (Braconidae) and an Ichneumonid, which emerged from pupae, two more braconids (Bassus sp.) from larvae. Others were described by HARAKLY (1969) namely *Nythobia sp.* and *Habrobracon hebetor.*

PETER et al. (1987) recorded two braconid ectoparasites (*Bracon gelichae*, *Bracon hebetor*) with parasitsm rates up to 24 % in India. They also found a bacterial disease caused by *Serratia marcescens* and infected larvae with an unidentified mermethid nematode.

A complete list of natural enemies is given by WATERHOUSE and NORRIS (1989). All known parasitoids are Hymenoptera. No arthropod parasites of *H. undalis* were identified in the Philippines (MEWIS, 2001).

1.4.3 Control of *H. undalis*

The control of *H. undalis* seemed to be successful with insecticides only. Among the work of scientists from all over the world, only very few efforts were made to control *H. undalis* with alternative control methods. There is no known try for biological control (WATERHOUSE and NORRIS, 1989). SIVAPRAGASAM (1996) compared prophylactic insecticide applications with integrated management treatments in Malaysia. Insecticide use could be lowered with the integrated management, but populations were significantly higher and yield was lower.

Two strains of *Bacillus thuringiensis* (Bt.) (H3, H7) were screened and compared with several insecticides in Taiwan (AVRDC, 1985). The results were discouraging and the chemical insecticides gave much better control. CHANG and PENG (1971) were not able to control *H. undalis* with Bt. applications in Singapore. However, the efficacy of microbial insecticides was shown in a field trial in Malaysia (FAUZIAH and AZIZ, 1992) with 10 different brands of Bt. insecticides. Only one failed to protect the plants (Larvo Bt.).

BATTU et al. (1989) detected nuclear polyhedrosis virus (NPV) infections of larvae in Punjab. In a following work (BATTU, 1991) he describes yield levels for occlusion bodies, which could be a potential control agent due to its specificity against *H. undalis*.

SIVAPRAGASAM and AZIZ (1992) examined larvae infected with protozoa, without referring to the taxonomy of the species, in Malaysia.

MEWIS et al. (2003) reported protozoan infections of larvae in the Philippines. The species was not clearly identified, the genus described as *Vairimorpha spec.* Laboratory infections of third instar larvae on an artificial diet caused a mortality rate of 80 %. An average of 16 % of the wild population was infected with *Vairimorpha* with a mortality rate of 75 %.

Biological control with parasitoids is non-existent. Enemies of *H. undalis* were described by several authors' for Egypt, Malaysia, and India (HARAKLY, 1968b; YOUSSEF et al., 1973; PETER et al., 1987; SIVAPRAGASAM and AZIZ, 1992; SIVAPRAGASAM, 1994; SIVAPRAGASAM et al., 1994; SIVAPRAGASAM and CHUA, 1997a).

MUNIAPPAN and MARUTANI (1992) reported the control of *H. undalis* in the field with radish as trap crop in Guam. LUTHER et al. (1996) examined the use of Indian mustard and Tasty cabbage as trap crop for *H. undalis* in Hawaii. Both varieties show potential for effective reduction of larval infestations in the target crops.

BUNTING and MILSUM (1930) have seen the only chance to reduce the pest incidence by destroying the infested plants.

A preliminary resistance trial was conducted in Taiwan with 451 cabbage accessions in 1978 (TALEKAR et al., 1981). Only 13 accessions were tested for there resistance a second time but the level of resistance was not satisfactory.

Another resistance experiment was conducted in Taiwan with 133 cabbage accessions (AVRDC, 1985). Only 19 of those screened cultivars were used for a new trial in 1985. The plants were not treated with insecticides. The percentage of plants infested was high in all but one accession.

BRAR et al. (1989) examined 26 different cauliflower lines towards resistance against *H. undalis*. He found five resistant lines where the percentage of infestation ranged between 10-20%.

LAL et al. (1991) screened sixty-four cultivars of cauliflower with none of them immune or highly resistant against *H. undalis*.

Most attempts to control *H. undalis* were carried out with chemical insecticides (ASSEM and NASR, 1968; HARAKLY, 1969; CHUO, 1973; AVRDC, 1978; TALEKAR et al., 1981; AVRDC, 1985; RAO et al., 1976; CHANG and PENG, 1971; ISOKO, 1995; WATERHOUSE and NORRIS, 1989; ONG and NG, 1988; SUMALATHA et al., 1992; FAUZIAH and AZIZ 1992). As for now, chemicals are the only promising way of reducing this pest, or keep it under control (WATERHOUSE and NORRIS, 1989; FAUZIAH and AZIZ, 1992; SIVAPRAGASAM and AZIZ, 1992). Resistance to several insecticides were found in field trials in Malaysia against methamidophos, deltamethrin, permethrin, ethfenoprox, and insect growth regulators such as diflubenzuron and teflubenuron (Cork, 1997/98, Wisard Project, http://www.wisard.org/wisard/shared/asp/projectsummary.asp?Kennummer=2764, 03.02.2003).

1.4.4 *H. undalis* Sex Pheromone

HARAKLY (1968b) mentioned the possible existence of sex pheromones emitted by female *H. undalis* to attract males. To capture males he placed cages with virgin females beside laboratory windows.

Sex pheromone trials with virgin females were conducted in Taiwan (AVRDC, 1985). Trap design and trap height trials as well as trials with different numbers of virgin females as bait and the age of virgin females were undertaken. Sticky traps could catch most males. Trap placement of 0.5 m above ground attracted a significantly larger number of males than the other heights tested. Four-day-old virgin females could attract most males and the number of 10 virgin females per trap was recommended, proven by the numbers of males caught with this set up.

Monitoring with virgin females was carried out over a cropping season in Japan and positive correlations were found with changes of males caught and number of larvae infesting cabbage (YAMADA, 1981). Males were attracted even without infestation.

Mark-release-recapture-experiments were conducted using virgin females to investigate the flight distance, flight direction, and their relationship with climatic factors (SHIRAI et al., 1988) of male *H. undalis* in Japan. Traps were set in distances from 1.1 to 21.5 kilometres from the release point. The number of recaught males was small (2.4 % and 4.2 %). It is suggested that the temperature has a direct influence on the flight behaviour of male moth.

SIVAPRAGASAM et al. (1996) caught high numbers of male *H. undalis* with virgin female baited traps in Malaysia in absence of cultivated cruciferae. She found two peaks in the flight activity of male *H. undalis* as determined with traps baited with virgin females around 20:30 h after sunset and from 5:30 h to 6:30 h before dawn. There was no correlation between the number of males trapped and larval populations in the field. Rain had no influence in the number of males caught. Virgin females as bait were used in highland areas of Malaysia to monitor incidences of *H. undalis* (SIVAPRAGASAM et al., 1994).

Although identified in the early 1980's (ARAI et al., 1982) the synthetic sex pheromone was not further investigated. The chemical structure of (E, E)-11, 13-Hexadecadienal (Fig. 1) with the aldehyde rest and two conjugated double bonds seemed to be unstable under field conditions in tropical regions.

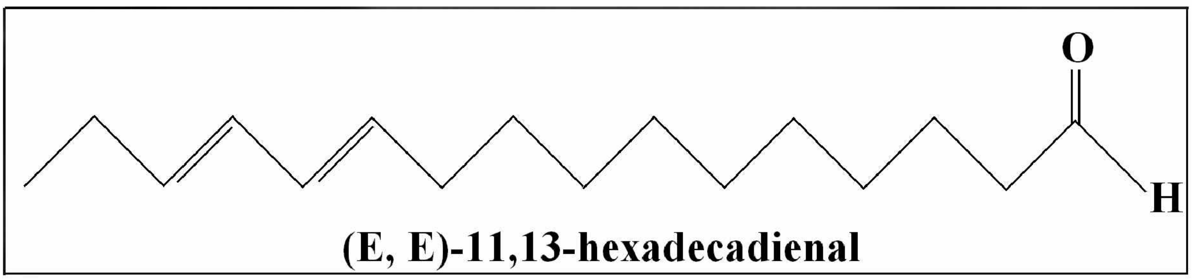

Figure 1: Structural formula of the compound (E, E)-11,13-Hexadecadienal identified as sex pheromone of *H. undalis* (ARAI et al., 1982)

An attempt to develop pheromone traps for monitoring and control *H. undalis* in India, Malaysia, and Taiwan was made 1997/1998 (Wisard Project, http://www.wisard.org/wisard/shared/asp/projectsummary.asp?Kennummer=2764, 03.02.2003). Although putative pheromone components could be identified, synthesised and formulated, field trials could not be undertaken due to degradation of the major component (E, E)-11,13–Hexadecadienal.

SUGIE et al. (2003) conducted field trials with (E, E)-11,13–Hexadecadienal and tested several compounds, which would enhance the attraction activity of (E, E)-11,13–Hexadecadienal to male *H. undalis*. In Japan, it was confirmed to attract males with a mixture of (E, E)-11,13–Hexadecadienal and 15-500 nanograms (ng) of (Z)-11-Hexadecenal in the same rate as virgin females.

1.5 Objectives of the Study

Since no published experiments with synthetic compounds were performed until 2001, basic knowledge of how to use the identified compound was necessary to obtain. The first questions to be answered should therefore clarify whether the sex pheromone is attractive to the male *H. undalis* in the Philippines at all.

The study was realized to examine specific objectives regarding practical use of the pheromone and biological and ecological aspects of *H. undalis* in the Philippines:

- Handling of the sex pheromone, the dosage, placing of traps, trap design, time of persistence and attraction in the field over time and longevity of lures stored for different times in a freezer, trap distance and influence of wind direction
- Ecological aspects, i.e. maturity age of male and female *H. undalis,* calling time and duration of females, flight activity of males and attraction of the synthetic compound in laboratory tests
- Use of the sex pheromone as a monitoring tool, competitiveness of the synthetic compound versus virgin females, influence of calling females in the vicinity of synthetic pheromone baited traps, effects of additional substances as means of enhancers of attraction and confusion of males
- Monitoring of larval population dynamics in different areas of Nueva Ecija, effects of biotic and abiotic factors that influence larval development
- Evaluation of trials conducted in regards to reduce pesticide use on Philippine farms

1.6 Thesis Structure

The thesis is subdivided in five major chapters and a closing general discussion. Chapter 2 describes the location, climates and general working methods; Chapter 3 deals with field trials regarding the necessary components for sex pheromone based monitoring or control. Chapter 4 describes studies of biological and ecological aspects of *H. undalis*, partially examined by applying synthetic and natural pheromones. Chapter 5 describes the possibilities to use the sex pheromone as part of an IPM program. Chapter 6 describes population dynamics of *H. undalis* in two years of monitoring in different areas of Nueva Ecija. Each chapter can be seen as an integral part within this research work and therefore includes with separate discussion of the results. Chapter 7 provides an overall discussion with summary.

Chapter 2
General Materials and Methods

2 Materials and Methods

2.1 Locations and Climates

Trials were conducted from October 2001 to October 2003 in Central Luzon, Philippines. Starting point of all research activities was the Central Luzon State University (CLSU) in Muñoz, Nueva Ecija. The experimental sites were located in five different locations of the Province of Nueva Ecija. The provinces of Pangasinan and Nueva Vizcaya border Nueva Ecija in the north, Pampanga and Bulacan in the south, Aurora in the east and Tarlac in the west.

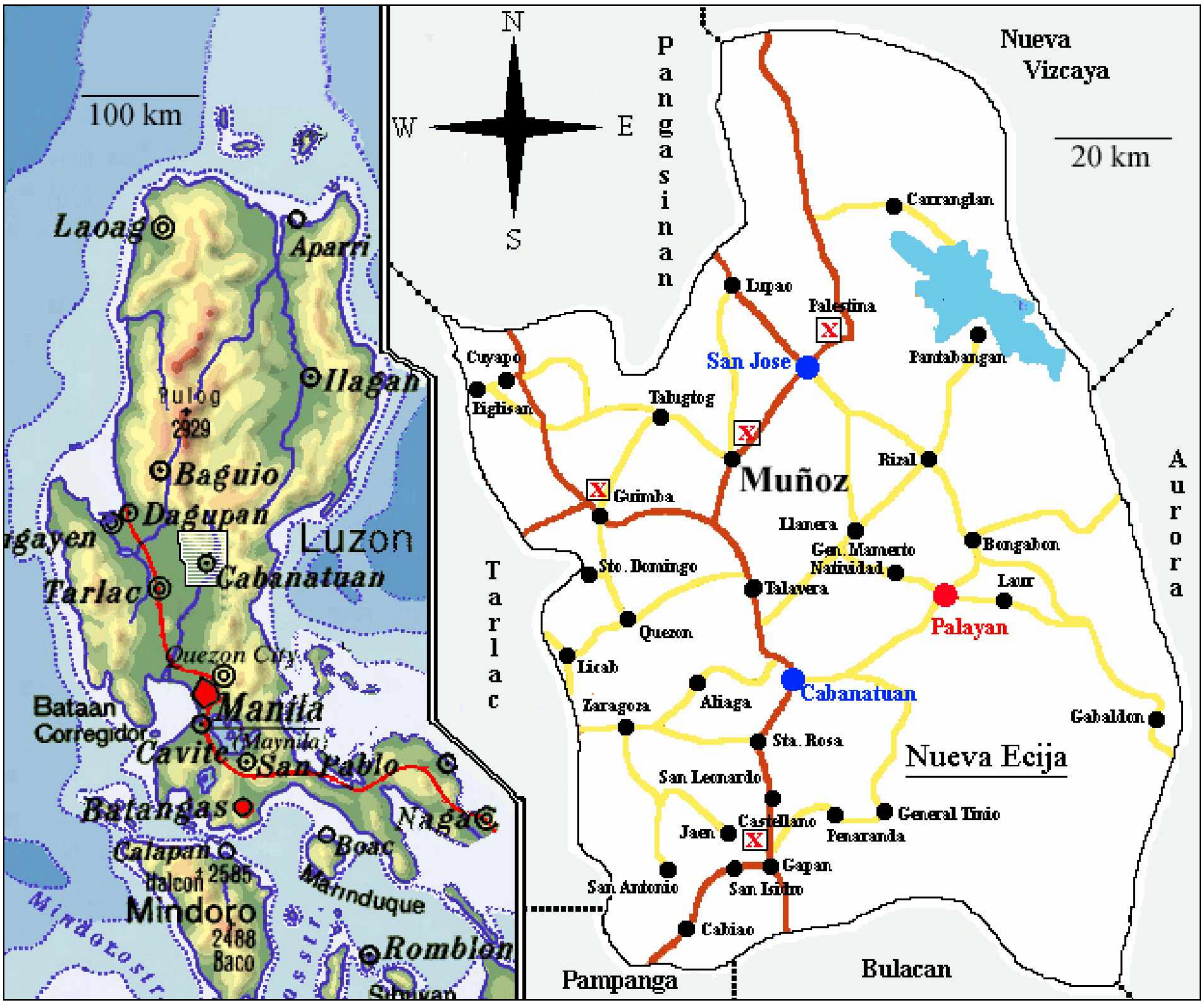

Figure 2: Map of Luzon, Philippines with enlarged province of Nueva Ecija in Region IV. Red x marks represent the sites where sex pheromone trials were conducted and abundance of *H. undalis* was monitored.

Most trials for the examination of the synthetic sex pheromone were located in Castellano, San Leonardo near the provincial border in the south of Nueva Ecija, ca. 70 km from Muñoz. Other sites were Muñoz (experimental area of the CLSU campus and in the Barangay (= village) Matinkis), the Barangay Palestina in San José, ca. 15 km north from Muñoz, and Guimba, ca. 25 km west from Muñoz. No sites where trials were carried out elevate more then 50 m above sea level and fall under the description 'lowlands'.
Nueva Ecija province is referred to as the 'Rice Bowl' of the Philippines (http://www.geo cities.com/lppsec/pp/necija.htm, 23.03.2004). Approximately 75% of the crop production area is used for growing rice (221650 ha). The total field area with crucifer crops is approximately 112 ha with half of the area used for growing *Brassica rapa var. chinensis* (pak choi; 54 ha) (http://rfu3.da.gov/NUEVA_ECIJA.HTM, 12.05.2004).

Geographically the province is situated between 120°36' to 121°21' east longitude and 15°09' to 16°9' north latitudes. The terrain of the province is comprised of low alluvial plains and rolling uplands.
Climatic data was obtained from the 'Philippine Atmospheric, Geophysical and Astronomical Services Administration' (PAG-ASA) weather stations inside the CLSU campus and from the Cabanatuan PAG-ASA station. PAG-ASA is using the 'modified corona classification' for dividing the regions into the different climate types. Therefore, four types are known, based on the distribution of rainfall. Nueva Ecija is classified as Type I, (two pronounced seasons: dry from November to April and wet during the rest of the year). The climatic condition in Central Luzon is characterized by two seasons. The dry season lasts from December to April and the rainy season from May to November, but delays or earlier starting of a season can occur.
Luzon lies within the tropical typhoon belt and typhoons occur mainly from July to October (http://www.pagasa.dost.gov/cab/climate.htm 12.05.2004).
The average maximum temperature is about 31.8°C from April to July. Mean minimum temperature is about 22.0°C during the cool month of November to March.
The mean annual rainfall ranges from 1700-2200 mm from the central plain to the mountainous northern and eastern part of the province, or an average of 1950 mm per year (http://www.nuevaecija.gov/index .php?cat1=2&cat2=1&cat3=1, 12.05.2004). Rainfall data was used either to get the monthly rate or to calculate the rainfall within two monitoring dates. Sometimes the measure gauge for rainfall, both in Muñoz and in Cabanatuan could not seize the true quantity due to low capacity of the device. A 'T' in the weather data sheets stands for

such heavy rainfall. For reasons to include these values it will be calculated with 999.9 mm rainfall on such a day. In the PAG-ASA weather recordings is it not included in the monthly and annual mean values but the possible impact on the insect fauna requires to take such events into account.

2.2 Rearing of *H. undalis*

Rearing was required to use specimen for biological observations such as mating and calling behaviour of males and females. Females are used as bait to attract males in traps and to compare the attraction towards males by the natural and synthetic pheromone. Feeding habits, host range and reproductive success can be determined in the laboratory with the reared species.

2.2.1 Rearing in the Philippines

The rearing of *H. undalis* took place in a windowless room exclusively arranged for rearing purposes in the building the AVRDC-Office was located, inside the CLSU campus. To ensure stable conditions, an air condition unit was installed on the top left side. The total space of the room was 2.7 m x 2.7 m x 2.5 m. The boxes and cages were set on two steel bar shelves with stands in plastic cups filled with a water soap solution to protect larvae and adults against ants. Plastic boxes used were 20 x 10 x 8 cm, the cages made of an aluminium frame and wire screen were 40 x 40 x 40 cm. The number of larvae per box must not exceed 10 larvae. A refrigerator and a working area with a worktable completed the interior of the rearing chamber. The entrance was a double entrance with the first entrance made of a steel bar cage (0.9m x 0.9m x 2m) with the frame covered with black plastic foil. Insects would not enter the dark space between the two doors and therefore not enter the chamber. The air condition unit did not work with proper voltage (~175V instead of 230V), therefore levels for the temperature and humidity were not stable at all times. Frequent power failures complicated the maintenance of constant conditions. The temperature was 30 °C +/- 4°C and relative humidity (rH) was 80 % +/- 5%. The rearing was under a light: dark regimen (L:D) of 12 h : 12 h, regulated with a timer switch.

Food plants (pak choi) for the rearing were obtained mainly from a net house in front of the Research Office. Weekly plantings secured a permanent supply of food for the larvae. Seeds were sown on a seedling tray filled with compost. After 1-2 weeks the plants were transplanted to plastic bags filled with a compost/soil mixture (30/70). The mixture was sterilized in a metal barrel. Fire was lit underneath the barrel that stood on three steel bars welded beneath it. Inside the barrel was a circular wooden plate with holes in a height of 10 cm above the bottom. Water was filled in the space between the bottom and the plate. Above the plate was the compost soil mixture. The mixture was sterilized for at least 1.5 hours.

2.2.1.1 Rearing Procedure with Non-Artificial Diet

First larvae for the rearing were collected in the fields of Castellano, San Leonardo. The larvae were set in plastic boxes in the rearing chamber and fed with leaves from pak choi or radish until pupation. The pupae were sexed (see 2.3) and kept separately and singly in cell culture plates. The emerged adults were placed in mating chambers, for mating and egg lying. These mating chambers were made of big plastic water containers (5 l) with the upperparts removed and covered with fine mesh cloth held in place by a rubber band. The adults were fed with 5-10 % honey solution in a cotton wick, put on a plastic sauce container. The females could lay their eggs either underneath the cloth or at the sidewalls of the water container. One to three pairs were set in one chamber. Dead adults were immediately removed to avoid moulding of the corpses. Eggs were collected or left on the spot where the females had laid them. In the latter case, the hatched first instars were transferred to potted plant or in boxes with plant material using a fine brush. The food was exchanged every two to three days. When the larvae were brought to a whole plant, it was necessary to transfer them again to a box with food after 4-8 days on a plant. The larvae were fed with leaves from pak choi, mustard and radish as well as *Cleome viscosa* and *C. rutidosperma* as non-crucifer species.

Occasional breakdowns of the rearing were determined as the result of protozoan infections. MEWIS et al. (2002) found Microsporidia of the genus *Vairimorpha spec.* infecting *H. undalis* in the Philippines. The rearing chamber was cleaned with Clorox and wiped with Isopropanol 70% when infected larvae were found. The subsequent rearing was started with newly collected larvae from the field.

2.2.1.2 Rearing Procedure with Artificial Diet

Several artificial diet ready mixes were tested for their suitability but only one was proven to be successful. The Beet Armyworm Diet (BA; Bio-serv, # F9219B and F9220B) was the most effective formulation and used for all artificial diet trials. Beside the BA-diet other ingredients were water (distilled), and Agar-agar. Water with Agar-agar was brought to full boil, the ready mix was added and the mixture carefully stirred. The diet was then poured in a liquid state into Petri dishes. The dishes were covered with paper towels until the diet was cooled down to room temperature.

Extra ingredients were mungbeans, pak choi and radish seeds and dried radish and pak choi leaves, all grinded to a very fine powder. These extra ingredients were added in different concentrations and combinations, e.g. for 100 ml of the diet with Beet Armyworm (BA) Dry Mix as Basic Ingredient + Mung Bean Powder (MBP) + Radish Leave Powder (RLP) in a ratio of 85% BA - 10 % MBP - 5% RLP:

Agar Agar	2 g	add to
Aqua dest.	82 ml	bring to full boil
		Add the
BA Dry Mix	15,68 g	
MBP	1,76 g	
RLP	0,88 g	to the hot Agar Agar, stir carefully and

pour the mix immediately into Petri dishes and let it cool down in them.

Larvae of all instars were set next to the food source inside the Petri dishes as well as eggs. The eggs were put on a piece of filter paper and set inside the Petri dish. Since the evaporated humidity could not vanish, it was necessary to place a piece of filter paper on top of the diet to insure the larvae did not drown. This was particularly necessary for the first and second instars. The larvae could develop without any disturbance and were left until pupation.

2.2.2 Rearing in Germany

H. undalis was reared in the Institute of Vegetable Science in Freising, Germany in climatic chambers (Heraeus-Vötsch) with a dark: light regime of 12:12 hours. Humidity and temperature were automatically controlled to 75 % rH and 25°C. Rearing procedure was the same as described in CLSU (see 2.2.1). Due to a microsporidian infection it came nearly to a breakdown of the rearing in the third generation. Since no larvae could be collected from a field, the remaining larvae were reared separately in Petri dishes until pupation and emerging adults were put in mating chambers with only one couple per chamber. Hatching larvae were separated in Petri dishes and plastic boxes and reared in small number of 2-5. The larvae were fed pak choi, radish, mustard leaves and in feeding trials rucola, kohlrabi and three *Cleome* species (*C. viscose, C. rutidosperma, C. spinosa*). Food was exchanged every two days.

2.3 Sex Determination of Pupae and Adults

The sex of pupae and adults was determined by examining the ventral tip of the abdomen (Fig. 3). The pupae were examined for the position of genital and anal openings at the ventral side of the abdominal tip with a dissecting microscope (Nikon). The male openings are closer to each other and lay on two distinct segments, the ninth and tenth. The genital and anal openings of the females are wider and lay on the fused ninth and tenth segments.

Adult males have a slender abdomen, which ends conically. Females have a broad abdomen compared to the males with a very tipped end.

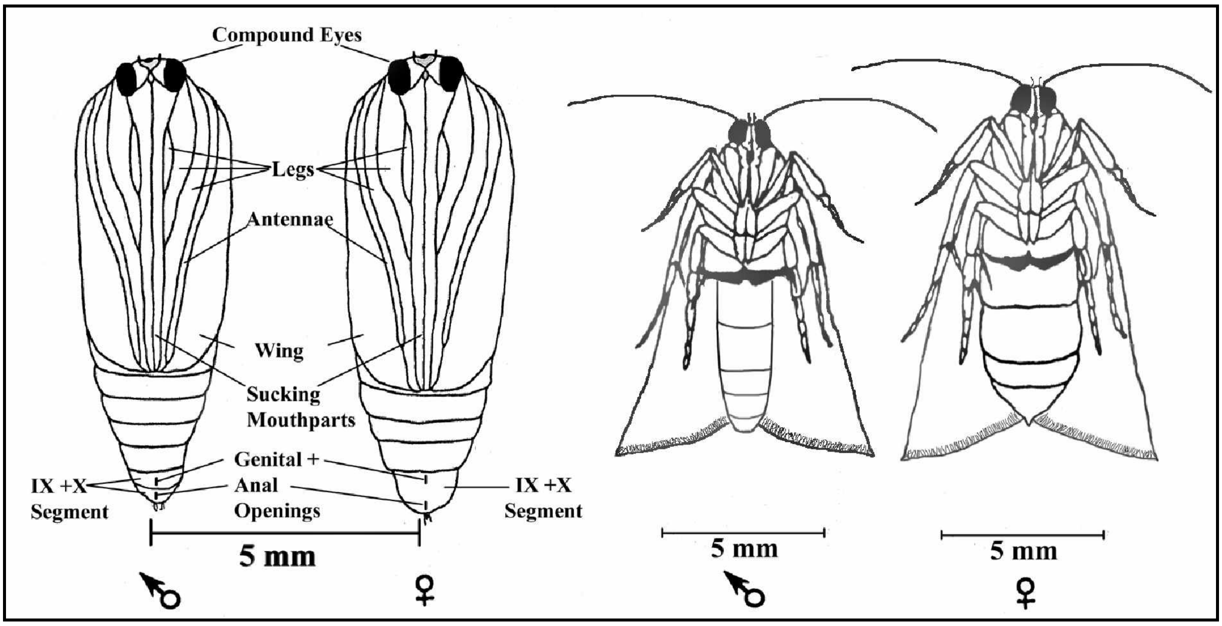

Figure 3: Ventral view of male and female *H. undalis* pupae (left) and imago (right)

2.4 Cultivation of Crucifers in the CLSU experimental area (Area I + II) and College of Agriculture (Area III)

The experimental field area of CLSU is located inside the campus and comprised approximately four ha. The soil is classified as clay-loam. Three water pumps are available for irrigation. A mesh wire fence with four gates, which are closed during the nights and weekends, surrounds the area. Two fields were used in this area. A third field at the College of Agriculture is 1.5 km distant from the other two areas inside the campus. The soil type is the same as inside the campus. The area could be irrigated with a nearby water pump. Strictly, no insecticides were applied in all three areas, only herbicides after the land preparation and again one week prior to sowing.

Weeds were collected and identified up to species level if possible (see 6.1.3.1). Since no herbicides were used after seeds were sown, hand weeding was necessary.

Local scientists performed identification of plant diseases (see 6.1.3.1) on pak choi and radish. The author up to order level performed identification of beneficial arthropods (see 6.1.3.1). Pest insects on fields of Area I-II were identified and numbers recorded (see 6.1.3.1).

2.4.1 Area I (Small Field)

The first area was prepared for the first time in June 2001. The total area comprised 192 m^2 (16 m x 12 m). The beds were raised depending on the season up to 50 cm in height. Due to the small size of the field, no machines could be used and manual preparation was necessary. The size of the beds varied between 0.8m and 2m in width and 15 m in length. Pak choi seeds were sown for the first two-month period (July and August 2001) manually, and from September 2001 with a single row, small seeding machine (K1, Maschinenfabrik Sembdner GmbH, Germering, Germany). Pak choi- 'Black Behi' (*Brassica rapa var. chinensis*) was exclusively planted on this field. The pak choi was line sown and continuously grown from June 2001 to November 2003. Due to typhoons or heavy rains, some crops were destroyed or not planted in succession. Infestation rate of pests was monitored from October 2001 to November 2003 in this area. The planting sequence was managed in such a manner that a permanent food supply (staggered planting) was guaranteed for *H. undalis* larvae. Infestation occurred naturally, only marked males from the rearing were released.

The field of Area 1 served for monitoring of naturally occurring larval populations. Therefore, no chemicals were used against pests and fungi diseases, but an initial herbicide treatment 3-5 days after the preparation of the beds. One side effect of the absence of insecticides and fungicides was the destruction of the crop by pests and fungi before the normal harvest time could be reached.

2.4.2 Area II

The second area in the experimental area was first planted in May 2002. The distance to the first area was 250 m. The field size was 675 m^2 (22.5 m x 30 m). The field was divided in four plots with 9 beds each per plot (1 m x 15 m). Four rows were sown on each bed. The crop of every plot was different, with radish, mustard, pak choi and all three plants mixed on plot, 1, 2, 3 and 4 respectively. The planting was continuous from May 2002 to November 2003 but was also effected, as the plantings from the first area were, by poor weather.

The field of area 2 served for monitoring purposes and the eventual influence of different crucifer crops on the egg laying behaviour of *H. undalis*. Some sex pheromone trials were carried out, with the synthetic pheromone and virgin females as bait and with both at the same time. Flight activity of male *H. undalis* was observed in this area.

2.4.3 Area III

The third area was located outside the campus near the College of Agriculture on an area of 800 m^2 (20 x 40 m). The first planting was in February 11, 2002 and ended in September 2002. The area was monitored for the occurrence of larvae in weekly intervals, beginning 7-12 days after the seedlings emerged from the soil. The first crop planted was pak choi followed by plantings of radish. Irrigation was not available for May 2002 and after October 2002 because of water needs for the surrounding rice paddies. The distance to the next area where crucifers were planted was approximately 2 km. The field was formerly planted to rice, with the first time planted to crucifers.

2.5 Monitoring of Larval Densities of *H. undalis* in Different Areas in Nueva Ecija

The occurrence of *H. undalis* larvae was monitored in different locations in Nueva Ecija. Pest species others then *H. undalis* were also counted and recorded to compare numbers of different pest species.

Beside the areas inside the CLSU campus and next to the College of Agriculture, monitoring took place in the Barangay Matinkis for the duration of the preliminary testing in 2001. Farmers' fields in San Leonardo, San José and Guimba were also examined. The mode of monitoring is described in Chapter 6.

2.6 Identification of Natural Host Plants of *H. undalis*

Plants next to crucifer fields, along field road ditches, beside rice paddies and plants in a ditch near the city centre of Cabanatuan were examined for the occurrence of *H. undalis* larvae. Monitoring was carried out in CLSU campus area, and in Castellano, San Leonardo. Identified host plants found in the experimental area were monitored for the occurrence of *H. undalis* larvae.

2.7 Sex Pheromone Trials

Dr. Frans Griepink, manager from Pherobank (Plant Research International, Wageningen UR) synthesized the compound (E, E)-11,13-Hexadecadienal with a purity of approximately 95 % for the aldehyde and an isomeric purity of 97 %, controlled in the laboratory of Pherobank. The first charge of sex pheromone containing lures was sent to the Philippines in October

2001. The release devices were red rubber septa. The septae were stored in a freezer at ca. –7°C until used. Virgin females from an own rearing at CLSU were also used for trials. Control traps were placed in several trials. All catches were controlled for the presence of accidentally caught females. The number of males caught was recorded in such a case but not included in the analysis. If not mentioned otherwise, all lures used in the different tests contained 10 µg of the active ingredient, and the trap height was usually 0.5 m. Differences in the set up of the trials, particularly the intertrap distances were necessary on account of the size of the fields available at the time the trial was conducted. The largest distance possible was measured to reduce interactions due to trap placement. Location of traps was newly arranged at random, in some trials on a daily basis and whenever possible were set up in line with the crosswind to the prevailing wind direction. Except for the preliminary trials, traps were fastened to bamboo sticks with two threads to prevent trap movement.

Traps used were wing traps if not otherwise mentioned. These traps consist of two parts, a top and lower part with openings in all directions (Fig. 4; 20 a, e, g) and a sticky surface inside the trap (yellow sticky paper was used and attached to the trap with double side tape).

Non-target catches were identified by species or order level and numbers recorded in most cases. In case the sticky surface was covered with specimen, numbers were estimated.

2.8 Statistical Analysis

Numbers of males caught in traps were transformed to log (x+1) and subjected to Analysis of Variance (ANOVA). Means were compared using Fischer's Least Square Distance Test (Fischer's LSD) and the Bonferroni Test.

Correlation tests and linear regression was performed for monitoring data and mean rainfall per month, week, or between two monitoring dates of different areas in Muñoz, CLSU, Castellano and San José. Data were transcribed to log (x+1). The program used was XLSTAT 7.5.

Chapter 3
Handling of Traps and Lures

3 Basic Research of Trap and Lure Handling in Field Trials

Knowledge of various aspects of the handling of the sex pheromone provides the basis for the integration of a specific sex pheromone in an IPM program. Examined were the trap height, different quantities of the active ingredient impregnated in red rubber septa, trap design, placement, intertrap distance and the influence of wind, longevity of the attraction of lures, and level of attraction of lures after different lengths of storage in a freezer. The comparison of male catches is the common basis of all described trials. The set up is for all trials very similar, but changes were necessary depending on the field space available with crucifer plantings. Treatments ranged from three to eight and three replicates were predominantly used. Several trials were repeated to verify already obtained results. Differences among the distances between traps were caused by the available field space. The largest distance possible was chosen to minimize the possible interactions of crossing odour plumes. Since no results with the synthetic pheromone of *H. undalis* was available at the time of the prelimainary test series, intertrap distance was not founded scientifically. Recommendations in the literature was followed by the intertrap distance and the horizontal setting towards the prevailing wind direction, if possible.

3.1 Pheromone Dosage

3.1.1 Introduction

The pheromone dosage is an important factor for the use of a sex pheromone for monitoring. Upper and lower thresholds exist for male Lepidoptera in responding to chemical signals (HOWSE et al., 1998), therefore is it not always possible to attract large numbers of males with the releasing of high rates of the pheromone. Another point is the costs of the pheromone; determinations of the lowest dosage possible meet the requirements for sound economic use of synthetic sex pheromone pest monitoring and control. Since the costs to synthesize a compound not in stock costs 850 € for the first gram and 450 € for following grams (pers. communication F. Griepink, Pherobank, 2001), a major goal was the examination of the right dose for best cost/benefit calculations. Different dosages were tested in three preliminary tests in three different sites in Nueva Ecija. The dosages tested were 10, 20, 50, 100, 200 and 500 µg active ingredient/septum. The set up without replications in two of the preliminary tests (San Leonardo and San José) was choosen to verify effectiveness of the synthezised attractant beside the comparison of different dosages. Another dosage trial with additional doses was carried out at the beginning of November 2002. The aim of this trial was the comparison of dosages already tested in the preliminary trials during October/November 2001 with two smaller doses, 1 µg and 5 µg.

3.1.2 Material and Methods

3.1.2.1 Preliminary Tests with the Synthetic Pheromone

- **Preliminary Test at San Leonardo in October 2001**

Six card paper wing traps (Fig. 4; Fig. 20 e) were baited with lures impregnated with 10, 20, 50, 100, 200 and 500 µg (E,E)-11,13-Hexadecadienal and set on a radish field (ca. 5000 m^2) from October 22 to November 6, 2001. The plants were approximately 4-5 weeks old (canopy height 15-25). Daily monitoring of the traps took place from October 23-28. After October 28 the traps were monitored two more times October 30 and November 6. Arrangement of the traps was newly randomized 8 times.

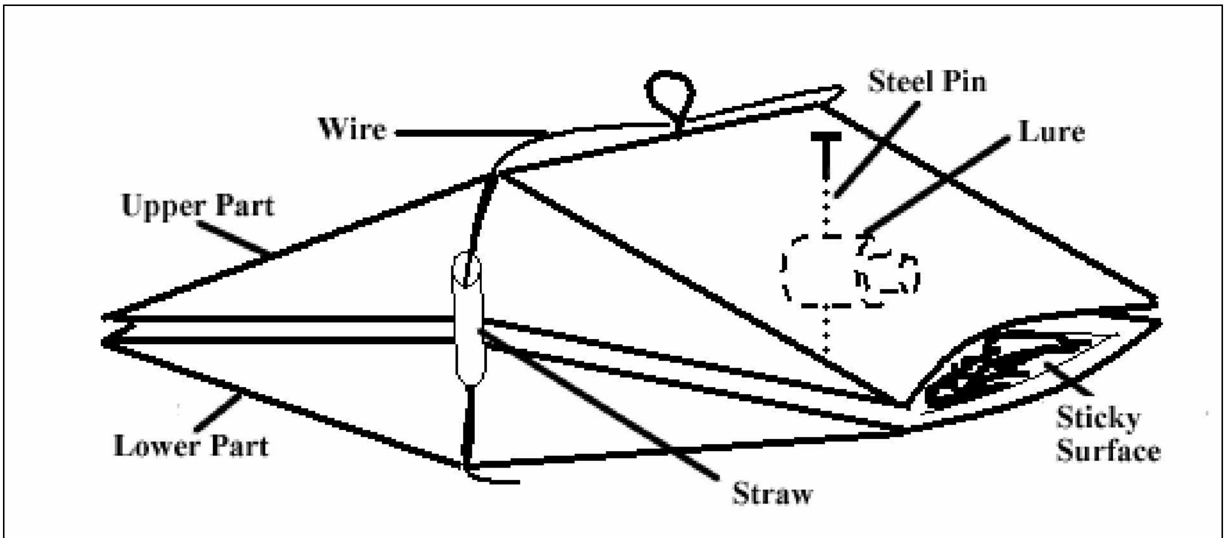

Figure 4: Wing trap design, used for catching male *H. undalis*

The traps were set with a minimum intertrap distance of 30 m. The field was located at Castellano, San Leonardo. At the time of the trial, the surrounding crops were tomato, onion, corn, and pak choi.

Traps were fastened by a wire on the top of the trap to the top of a bamboo stick, which was driven obliquely into ground. Traps were hanging 50 cm above ground. This height was chosen since trap height trials with virgin females as bait yielded in high capture rates (AVRDC, 1985) in Taiwan. The traps were checked every morning around 7:00 – 8:00 h, the

number of males caught was recorded and trapped males removed or the sticky part of the trap replaced with a new one. The lures were put in vials and stored in a cooling box filled with crushed ice during the day. In the afternoon, the lures were again attached with a steel pin pierced from the outside through the upper part of the trap with the lure held inside the trap. The field was monitored one time November 6, 2001.
Weather data were obtained from the PAG-ASA station in Cabanatuan.

- **Preliminary Test at Matinkis in November 2001**

Matinkis, a little Barangay (Community) southwest of Muñoz with small-scale fields, mainly used for growing vegetables, was suitable for an additional sex pheromone dosage trial. Six card paper wing traps, each three baited with 20 µg and 200 µg lures, were set up November 15, 2001 on three sites in Matinkis to check whether *H. undalis* was present in the area. The distance between the sites ranged between 500 m – 2000 m. Two sites of the three were suitable to test all concentrations beginning November 20 until December 3. The traps were placed with a minimum distance of 40 m between two traps. One control trap at a time without lure was set on both sites. The set up was changed during the trial in a randomized manner. Traps were fastened to a bamboo stick that was driven obliquely into the ground in a way that the traps were hanging 0.5 m above the ground. The traps were checked daily at 15:00- 16:00 h, the numbers of males caught were recorded and trapped males removed. The lures stayed in the field during the trial. Pak choi and mustard was planted in both sites, between 2-6 weeks old. Surrounding crops were onion, corn, tomato and rice. The fields were monitored two times (November 19, 23) for the occurrence of *H. undalis* larvae and other pest insects.

- **Preliminary Test Palestina, San José, November 2001**

Six card paper wing traps with lures containing the dose of 10-500 µg of (E, E)-11,13-Hexadecadienal and one control trap without a lure were set up in an area of approximately 2.5 ha with a minimum distance of 50 m between two traps, all of them in a height of 50 cm above ground. One trap with a 2-day-old virgin female was set up in the area for four days. Traps were set November 21; the trial ended December 6. The occurrence of larvae was monitored two times (November 23 and December 6). Pak choi was planted in all fields the traps were set, with an age range of 2-4 weeks after emerging of seedlings.

The virgin female died November 26. One lure, 50 µg, was missing November 27 and was used as a control trap. Data of males caught are presented up until November 26 for the virgin female and November 27 for the 50 µg-baited traps.

3.1.2.2 Pheromone Dosage Trial in San Leonardo

- **Pheromone Dosage Trial I**

Different doses impregnated in red rubber septa were tested in San Leonardo for one night. The tests were carried out in Castellano, San Leonardo on the same fields used for the trap height trial in San Leonardo (Fig. 5). The first trap height trial took place before the dosage trial since the results of the preliminary trials in 2001 indicated the dosage of 10µg as effective as the other dosages tested. For practical reasons are the pheromone dosage trials 1 to 3 described first.

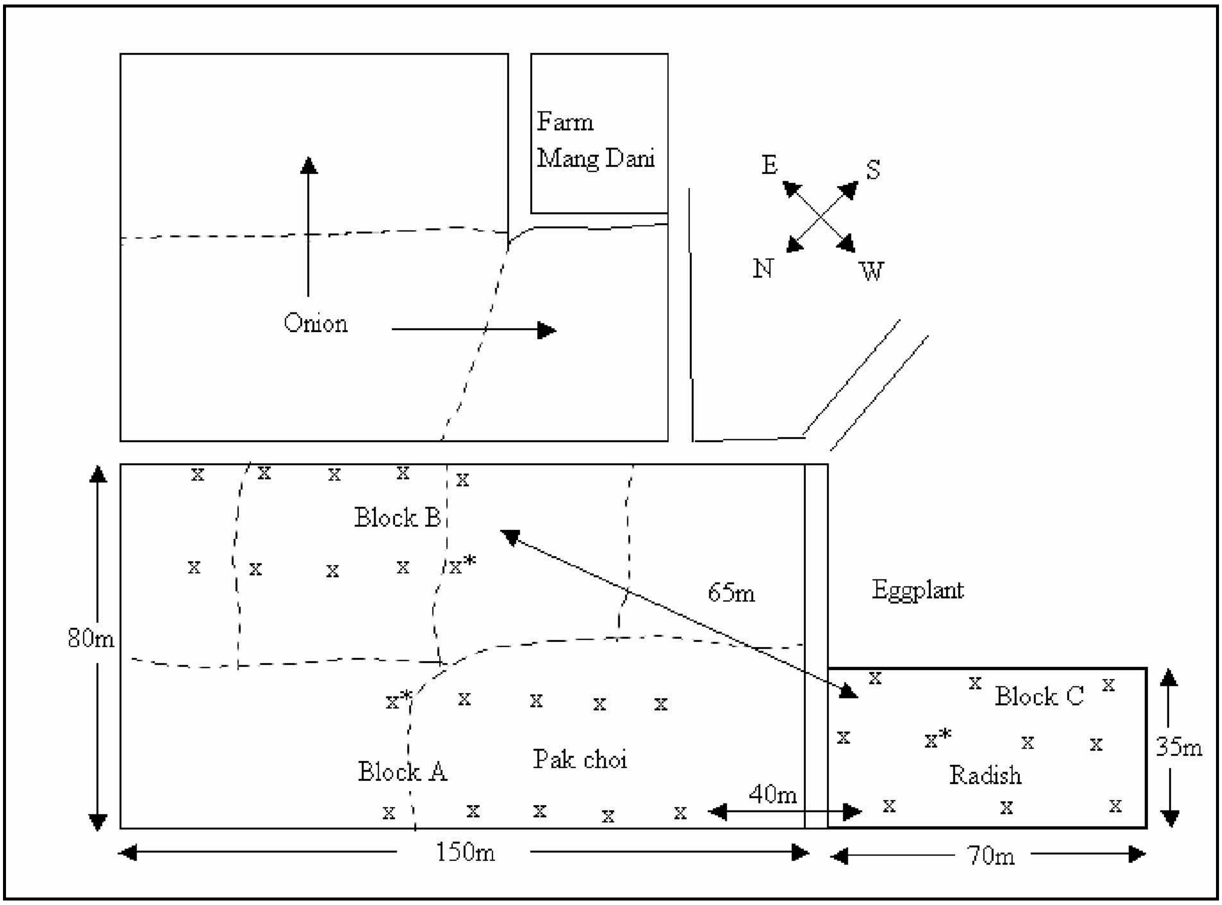

Figure 5: Set up of traps for the dosage trials in Castellano, San Leonardo. Ten traps per block were only set for one night (Pheromone Dosage Trial I), indicated as x*. x: wing trap baited with different dosages of E,E-11,13 Hexadecadienal (1, 5, 10, 20, 50, 100, 200, 500µg). Only blocks A and C were used for 'Pheromone Dosage Trial III'. Prevailing wind direction from east, northeast to west, southwest. (- - - = irrigation ditches)

The red rubber septa were impregnated with 1, 5, 10, 20, 50, 100, 200, 500 µg from October 2001 and 200 µg dosed lures from September 2002 (x* in Fig. 5). Each set with different dosed lures plus one trap without a lure was set up in adjacent field areas seen as different blocks. Traps were set up horizontally towards the prevailing wind direction in two rows in block A and B and three rows in block C. The intertrap distance was at least 15 m and 20 m between rows. The blocks had the same distance as described for the trap height trial (in 3.2.1.2), 40 m between blocks A and B, 40 m between A and C and 65 m between blocks B and C. Rain and wind destroyed wing traps made of card paper in the night of November 3 to 4. Two lures containing 200 µg, sent to the Philippines September 2002 got lost in the field and the remaining lures were not sufficient to set enough replicates for proper analysis. Control traps were set for the first three weeks in every block. The control traps were removed and exchanged with a set of traps with new 10-µg lures from the beginning of the fourth week.

- **Pheromone Dosage Trial II**

The same set up as described for the '**Pheromone Dose Trial I'** without 200 µg lures, from the lure charge sent to the Philippines in September 2002, continued the dosage trials. Self-made wing traps made of corrugated plastic were used. The start of the trial was November 4, 2002, and was finished November 28, 2003. Control traps were baited with new 10 µg lures November 21, to examine field-aging effects and compare attraction of used and new lures. Thus, the trial was carried out for two and a half-week with un-baited control traps and another week with new lures impregnated with 10 µg. The area was not visited after November 18 through November 21. All sticky inserts in the traps were covered with dust and catches are not included in the results.

The fields were monitored several times, number of plants (radish and pak choi) and plants infested with *H. undalis* were counted, and the results recorded. Traps from each block were collected and brought to a nearby shed to count the number of male *H. undalis*, to remove all insects caught and/or change the sticky surface. The new set up was re-arranged at random each time after the traps were monitored.

- **Pheromone Dosage Trial III**

The lures from '**Pheromone Dose Trial II**' were used to get an idea of the persistence of the different dosages, changes with different quantity of E,E-11,13-Hexadecadienal in the lures and the effect of degradation of the sex pheromone with different dosages over time. Lures from former Block A and C were used (i.e. they were already in the field for three and a half weeks). Self-made wing traps were set in a horizontal line to the prevailing wind direction in a distance of 15 m. Traps baited November 21 with new 10 µg lures were removed. Traps from block A were moved to a mustard field next to the old site. The traps of block C were placed in a pak choi field next to the old field after it was harvested (November 28, 2002) and stayed until it was harvested. The traps were monitored another five more times (December 6, 9, 12, 18, 26) until the trial was finished December 26, 2002.

3.1.3 Results

3.1.3.1 Preliminary Tests with the Synthetic Pheromone

- **Preliminary Test with the Synthetic Pheromone in Castellano, San Leonardo**

The first test with different dosages of the active ingredient of the synthetic pheromone in Castellano, San Leonardo, resulted in similar numbers of males caught of all tested dosages (Table 1). The highest catch in one night was 75 males in the trap baited with 10 µg lures. Daily settings of the traps at random excluded disadvantages as a result of location over the period the traps were exposed in the field. It is remarkable that some nights with high catch numbers were followed by nights with lower catch numbers.
The infestation rate was examined by monitoring 50 pak choi plants, selected at random, for the occurrence of *H. undalis* larvae and resulted in 24 % plants infested (November 6, 2001).

Dose	10µg	20µg	50µg	100µg	200µg	500µg
Total	200	186	150	120	137	117
% of total	21.9	20.4	16.5	13.2	15.1	12.9
C/T/N	4.4	4.1	3.3	2.6	3	2.6

Table 1: Preliminary sex pheromone dosage trial in San Leonardo, October 22 - November 6, 2001), with six different concentrations in a radish field. Total after 15 days: 910 males; (C/T/N = Catches per trap per night).

- **Preliminary Test with the Synthetic Pheromone in Matinkis**

The test with different dosages in Matinkis, Muñoz confirmed results from Castellano, San Leonardo. No significant differences ($p > 0.05$) between numbers of males caught were found among the different dosages tested for the first two weeks (Fig. 2). In both control traps no catches were found. Infestation of pak choi plants was 14.4 % (19.11.), 7.9 % (21.11.) and 15 % (20.11., 23.11.) with *H. undalis* larvae. Other pests were very small in number and only *Crocidolomia pavonana* (Zeller), *Spodoptera sp.* and *Liriomyza sp.* were identified.
Comparing 20 µg and 200 µg lures in the detection of suitable sites for further tests resulted in non-significant differences of males caught (Table 3).

Dose	10µg	20µg	50µg	100µg	200µg	500µg	C
Total	36	33	17	27	52	43	0
Mean ± SE	18.0 a ± 5.0	16.5 a ± 3.5	8.5 a ± 4.5	13.5 a ± 3.5	26.0 a ± 14.0	21.5 a ± 7.5	0
% of total	17.3	15.9	8.2	13.0	25.0	20.7	0
C/T/N	1.3	1.2	0.6	0.9	1.9	1.5	0

Table 2: Sex pheromone dosage trial in Matinkis, Muñoz (November 20.01-December 3.01). n = 2/x µg; C/T/N: Catches per trap per night; SE: Standard Error; C: Un-baited control traps; Different letters indicate significant differences according to Fischer's LSD Test, at $p<0.05$. Total catch = 208 males

Dose	20 µg	200 µg
Total	33	33
Mean ± SE	11.0 a ± 2.3	11.0 a ± 7.5
% of total	50	50
C/T/N	5.5	5.5

Table 3: Sex pheromone dosage trial in Matinkis, Muñoz (November 18-19.01). n = 3/x µg; C/T/N: Catches per trap per night; SE: Standard Error; Different letters indicate significant differences according to Fischer's LSD Test, at $p<0.05$. Total catch = 66 males

- **Preliminary Test with the Synthetic Pheromone in San José**

The third setting of different concentrations confirms the results of the trials in the two other locations. Numbers of males caught with the synthetic pheromone were smaller compared to the trap baited with virgin females (Table 4). The highest catch (134) in one night was obtained in the trap baited with 10 µg. A virgin female was caught, which attracted males and therefore the number was not added to the number of males caught with 10 µg. The nightly catch rate was between 4 and 10.5 males per night in the first 5 nights and decreased to an average of 3.5 males per night and trap for the synthetic pheromone baited traps. Disregarding the number of males caught by the virgin female baited trap, more then 80 % of the total catch was recorded in the first five nights.

Infestation of pak choi plants was 30.0 % (23.11.) and 47.5 % (6.12.) with *H. undalis* larvae. Multiple infestations with more then 5 larvae per plant were observed at the second monitoring. Other pests were *Spodoptera sp.*, *Crocidolomia pavonana* in small numbers and numerous flea beetles, *Phyllotreta striolata*.

Dose	10µg	20µg	50µg	100µg	200µg	500µg	3-VF
Total	47	51	20**	43	42	46	141*
% of Total	12.1	13.1	5.1	11.0	10.8	11.8	36.2
C/T/N [1]	10.5	8.6	4.0	6.6	6.0	5.8	35.3
C/T/N [2]	3.6	3.9	-	3.3	3.2	3.5	-

Table 4: Preliminary sex pheromone dosage trial in Palestina, San José, (November 22 -December 6, 2001), with six different dosages. Total after (5) 13 nights: (324) 390 males; (C/T/N = Catches per trap per night; [1]: November 21-26, [2]: November 27-December 6). Total catch = 390 males; * in the field November 21-26; ** in the field November 21-27

3.1.3.2 Pheromone Dosage Trial in San Leonardo

- **Pheromone Dosage Trial I**

Male moths were equally attracted by the different doses tested for the first night (Table 5). The number of males caught in the 1µg lures baited traps was lower than in all other traps, but not significantly. No catches were found in the un-baited control traps. The highest catch was recorded in traps baited with 500 µg. The mean value of 500µg baited traps exceeded the means of most other dosages, i.e. 1, 5, 20, 100, 200 and 200*µg, about double.

Dose (µg)	1	5	10	20	50	100	200	500	200*	C
Total	38	60	99	67	91	73	68	141	45	0
Mean ± SE	12.7 ab ± 9.8	20.0 a ±7.6	33.0 a ± 6.0	22.3 a ± 4.6	30.3 a ± 4.9	24.3 a ± 10.0	22.7 a ±3.5	47.0 a ± 22.6	15.0 a ± 1.5	0.0 b ± 0.0
% of total	5.6	8.8	14.5	9.8	13.3	10.7	10.0	20.7	6.6	0.0

Table 5: Catches of male *H. undalis* in traps baited with different dosages and un-baited control traps (November 3, 2002, Castellano, San Leonardo). All lures tested were sent to the Philippines October 2001 except 200* was from September 2002. The mean value corresponds with the nightly catch rate per trap. Means followed by a common letter are not significantly different at the 5% level according to the Bonferroni post hoc test. C: control traps without lures; SE: Standard Error; Total catch = 682 males

- **Pheromone Dosage Trial II**

Male moths were equally attracted by the different doses tested for the first two weeks (Table 6, 7). 7540 males were caught in 24 nights (Table 9), with more than half males caught in the first week. 23 males were caught in the control traps in the first two weeks, equivalent to 0.3 % of all males caught.

No significant increases in the number of catches occurred when traps were baited with new 10µg lures November 22 (Table 8). The nightly catch rate per trap was highest for all dosages in the first week (~25) and decreased in the second (~15) and third week (<5) (Fig. 6).

Infestation with *H. undalis* determined by monitoring the fields, i.e. block A, B and C, differed only slightly on November 12 with 25.2 % in Block A, 18.1 % in Block B and 22.9 % in Block C. The infestation increased November 19 to 33.6 % in Block A, 25.8 % in Block B and 30.8 % in Block C. Plants of a mustard field, two weeks after emergence, were infested by 5.1%. The average of crucifer plants infested in the fields used for the pheromone dosage trial was 22.1 % (November 12) and increased to 30.1 % (November 19).

Dose (µg)	1	5	10	20	50	100	200	500	C
Total	538	507	517	491	495	606	598	612	19
Mean ± SE	179.3 a ± 63.4	169.0 a ± 47.7	172.3 a ± 19.7	163.7 a ± 44.2	165.0 a ± 46.7	202.0 a ± 57.7	199.3 a ± 21.9	204.0 a ± 59.1	6.3 b ± 1.2
% of total	12.3	11.6	11.8	11.2	11.3	13.8	13.6	14	0.4

Table 6: Catches of male *H. undalis* in traps baited with different dosages and un-baited control traps (November 5-11, 2002, Castellano, San Leonardo). Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test. C: control (un-baited traps). SE: Standard Error. Total catch= 4383 males

Dose (µg)	1	5	10	20	50	100	200	500	C
Total	300	214	240	278	276	337	420	432	4
Mean ± SE	100.0 a ± 47.4	71.3 a ± 17.3	80.0 a ± 23.6	92.7 a ± 1.9	92.0 a ± 11.8	112.3 a ± 15.6	140.0 a ± 34.5	144.0 a ± 34.9	1.3 b ± 0.9
% of total	12.0	8.6	9.6	11.1	11.0	13.5	16.8	17.3	0.2

Table 7: Catches of male *H. undalis* in traps baited with different dosages and un-baited control traps (November 12-18, 2002, Castellano, San Leonardo). Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test. C: control (un-baited traps). SE: Standard Error. Total catch = 2501 males

Dose (µg)	1	5	10	20	50	100	200	500	NL
Total	28	40	48	14	82	64	52	38	34
Mean ± SE	9.3 a ± 9.3	13.3 a ± 10.5	16.0 a ± 7.4	4.7 a ± 2.4	27.3 a ± 11.5	21.3 a ± 18.9	17.3 a ± 16.8	12.7 a ± 9.2	11.3 a ± 1.3
% of total	7.0	10.0	12.0	3.5	20.5	16.0	13.0	9.5	8.5

Table 8: Catches of male *H. undalis* in traps baited with different dosages and traps baited with new 10 µg lures (NL) (November 22-28, 2002, Castellano, San Leonardo). Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test. SE: Standard Error. Total catch = 400 males

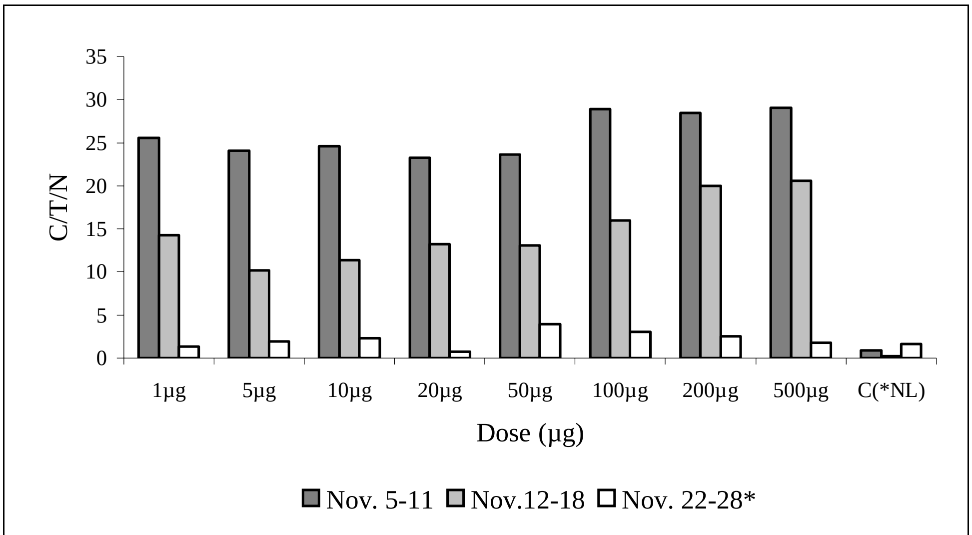

Figure 6: Nightly catch rates per trap (C/T/N) in traps baited with different pheromone dosages in the first three weeks in fields in Castellano, San Leonardo (November 5-28, 2002).Three self-made wing traps were used for each dose tested. C: control; *NL: new lures (in the third week from November 22-28*)

Period	5.11. - 11.11.	12.11. - 18.11.	22.11. - 28.11.
Number Males Caught	4383	2501	400

Table 9: Numbers of males caught in all traps baited with differently dosed lures and un-baited control traps from November 4-28, 2002.

- **Pheromone Dosage Trial III**

Males caught were recorded for all different dosages tested until the end of the trial December 26 (except 1µg for the last week) (Table 10). The nightly catch rate was above 1 male per night until December 18, (Fig 7) except for traps baited with 1µg lures. Catches until December 18 did not fall beneath the estimated value of males caught accidentally (below one catch per night and trap; see 3.1.4), whereas catches from December 19 to 26 in traps baited with lower dosed lures did, or were just above this value (Fig. 7). Numbers of males caught differed between lures containing 1-50µg and 100-500µg at the end of the fifth week and differences increased with the amount of time lures were in the field. 929 males were caught from November 29- December 26 (week 5: 428; week 6; 308; week 7: 171; week 8: 22).

Dose (µg)	1	5	10	20	50	100	200	500
Week 5 (Total)	20	27	20	43	56	59	99	104
Mean ± SE	10.0 b ± 8.0	13.50 ab ± 0.5	10.0 ab ± 3.0	21.5 ab ± 1.5	28.0 ab ± 2.0	29.5 ab ± 22.5	49.5 a ± 23.5	52.0 a ± 5.0
%	4.6	6.3	4.6	10.0	13.0	13.7	23.1	24.2

Dose (µg)	1	5	10	20	50	100	200	500
Week 6 (Total)	10	27	20	28	39	72	51	61
Mean ± SE	5.0 b ± 2.0	13.5 ab ± 6.5	10.0 ab ± 4.0	14.0 ab ± 2.0	19.5 ab ± 2.5	36.0 a ± 28.0	25.5 a ± 2.5	30.5a ± 2.5
%	3.2	8.7	6.4	9.1	12.6	42.1	16.5	35.6

Dose (µg)	1	5	10	20	50	100	200	500
Week 7 (Total)	7	13	8	18	12	48	27	38
Mean ± SE	3.5 c ± 3.5	6.5 abc ± 2.5	4.0 bc ± 3.0	9.0 abc ± 5.0	6.0 abc ± 2.0	24.0 a ± 4.0	13.5 abc ± 6.5	19.0 ab ± 1.0
%	4.1	7.6	4.6	10.5	7.0	28.0	15.7	22.2

Dose (µg)	1	5	10	20	50	100	200	500
Week 8 (Total)	0	1	1	2	1	5	5	7
Mean ± SE	0a ± 0.0	0.5a ± 0.5	0.5a ± 0.5	1.0a ± 1.0	0.5a ± 0.5	2.5a ± 1.5	2.5a ± 2.5	3.5a ± 3.5
%	0.0	4.5	4.5	9.0	4.5	22.7	22.7	31.8

Table 10: Catches of male *H. undalis* in traps baited with different dosages of E,E-11,13-Hexadecadienal 5-8 weeks in crucifer fields at Castellano November/December, 2002. (week 5: 29.11.–6.12.; week 6: 7.12.13.12; week 7: 13.12.-18.12; week 8: 19.12.-26.12.). Totals, means, the nightly catch rate per trap and percentage of total catch are presented. Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test. C/T/N: catches per trap per night

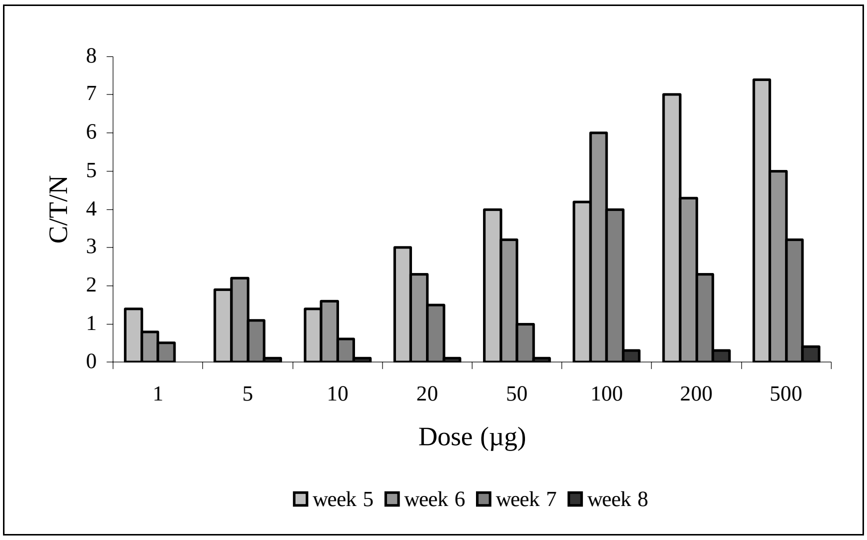

Figure 7: Nightly catch rate per trap with different dosed lures (1-500µg) in Castellano, November 29-December 26. C/T/N: catch per trap per night

3.1.4 Captures of Male *H. undalis* in Un-Baited Control Traps

The results of the concentration and height trials (3.2.1) imply a very low catch rate by chance in San Leonardo. In all set control traps, the catch rate was below one male caught per night per trap. Further catches in such a range will be regarded as an accidental catch. For verification of this result, more control traps were set in other trials, i.e. Trap Design II and Lure Age I in San Leonardo.

Control traps set in CLSU experimental area imply an even lower proportion of males caught by chance. The calculated catch rate is ~0.2 males caught per night per trap.

For both areas, Muñoz and San Leonardo the accidental catches were negligible.

These calculations had practical reasons in setting as few control traps, i.e. traps without lures, as possible. It is assumed that the distance between traps can have an influence on the catch rate. Additional control traps reduce the distance between traps, set in the area to be monitored and therefore reduce the number of replications.

3.1.5 Non-Target Catches

Non-target catches consisted mainly of *P. xylostella* and reached values up to 21 % of the total catch in times of high infestations with this pest (e.g. November 2002) in Castellano. Generally, the numbers were low in times with low infestation rates with *P. xylostella* and increased in times with high infestation rates.

Other insects caught were *Spodoptera sp., C. pavonana* and *Maruca vitrata* of the order Lepidoptera, beetles of the order Chrysomelidae, Scarabeidae and Coccinelidae (*Micraspis*

sp.), Hymenoptera of the family Apidae, Formicidae and unidentified wasps. All specimens mentioned, besides *P. xylostella*, were found in very small numbers and inconsistently.
Spodoptera sp. was found increasingly from October to November 2003 when most fields in the Castellano area were planted with onion.

3.1.6 Discussion

The results presented in this preliminary study show that the examined substance (E,E)-11,13-Hexadecadienal attracted males of *H. undalis*. It was so attractive, that at times males caught saturated most of the sticky area in one night, and made it necessary to change the sticky part. Even at times where the population of *H. undalis* was high in San Leonardo (which in fact was always the case) the numbers of males caught accidentally were very low. In all sites, where the synthetic pheromone baited traps were placed, catches were recorded. Catches up to 190 males could be recorded within one night with the tested synthetic pheromone. The different dosages tested did not influence or enhance the attraction of males. No differences between lures containing 1 µg, the lowest dose tested, and 500 fold the content in the highest dosed lures, were proven statistically different, although numeric values were higher for the higher dosed lures after the first week. The differences between 1 µg and the rest of the doses during the first night might be due to the location the traps were set and pinpoints to the importance of repeating the trial in following nights with a changed (or rearranged) set up.
The level of attraction verifies E,E-11,13- Hexadecadienal as a major component of the sex pheromone of *H. undalis*. Unlike in other species, sex pheromone steered behaviour was not dependent on the dosage for the response. Dose dependent responses are found in many species (TURGEON et al, 1983; ROCCHINI et al. 2003) with higher capture rates in traps baited with higher dosage lures. However, an upper limit exists, where a decrease occurs when dosage is too high (ROELOFS, 1978; KEHAT et al., 1994a). TURGEON et al. (1983) found a dose-dependant response by *Pseudaletia unipuncta*, where the dosage was found to increase the numbers of males caught in higher dosed lures up to 1000 µg followed by a decrease when the dosage was higher then that. High dosages with 10.000 µg containing lures were tested by KEHAT et al. (1992) for the raisin moth, *Cadra figulilella*. Although it was found that differences between such high dosed lures were not significant, lures containing only 100µg attracted significantly less *Cadra figulilella* males.

Dosages used in the conducted trials did not exceed 500 µg per lure and the response even to the smallest dosages tested (1 µg) can be explained with the highly sensitive chemoperception of moth to their specific sex pheromone. The dosage trial and persistence trials (see 3.4) showed that males are attracted above the estimated level they could be caught by chance for more then four weeks. Four weeks with different dosages did not attract significantly different numbers of males. After the fifth week, differences between the lowest and the highest dosage reached a significant level. Until the end of the seventh week in the field, catches were not by chance for most dosages. In the longevity trial conducted with 10 µg lures, results are similar. The number of males caught above the estimated level of males caught until the end of the sixth week was by chance. However, catch performance was best in the first week.

If too high, pheromone dose can attract males from outside the monitoring area. FACCIOLI et al. (1993) found male *Argyrotaenia pulchellana* Haw. (Tortricidae) in italian pear and apple orchards even though no infestations were observed. Males were attracted by the usual dosed lures (1 mg), which was used to time insecticide spraying. By using 1/10 and 1/100 of this quantity, it was possible to attract males from the orchard area alone and could make the monitoring more reliable. MOREWOOD et al. (2000) came to similar conclusions for monitoring the nun moth, L*ymantria monacha* (Linné) in pine forests in Central Europe. Besides attracting males from adjacent forest stands, low dosed sex pheromones reduced the number of males caught, which made the counting of catches over a whole season more comfortable and less insects were attracted by decomposing corpses of the nun moth (ELKINTON, 1987).

SIVAPRAGASAM (1996) supposed that males were attracted from outside the monitored area with virgin females as bait. It can be suggested that such an effect occurred in the Philippines as well, even though the dosages used were relatively low. The effect of nights with high numbers of catches followed by nights with very few, may be connected with the long attraction range of the pheromone. *H. undalis* is widespread all over Nueva Ecija and a reduction of males in a crucifer growing area may lead to an influx of males from outside. Attraction of lures containing less than 1µg probably reduces the number of catches in areas highly populated with *H. undalis*, i.e. San Leonardo and monitoring is restricted to a smaller area. This has to be verified in follow up field trials.

3.2 Trap Placement

Trap placement can have a strong effect on the level of trap catches. The surrounding vegetation can influence the structure and dispersion of pheromone plumes and might affect the capture rates negatively (CARDÉ and ELKINTON, 1984).

Placement of the trap comprises of three points most important to look at. The trap height, the placement of the traps with regards to vegetation, location in- or outside the field, i.e. in the field centre, edges or outside the field and finally the trap density, i.e. the distance between traps (HOWSE et al., 1998; JUTSUM and GORDON, 1989).

3.2.1 Trap Height

3.2.1.1 Introduction

Height as a factor of trap placement can influence the catch level (CARDÉ and ELKINTON, 1984). Wind speed most likely influences the behaviour of males in reaching the source of a chemical signal and therefore the optimum height is important. Inconveniences of placing the trap in heights difficult to reach are minimized by the canopy height of crucifer crops, which is thought to be the place where virgin females call for their mates.

Three trials, two in San Leonardo, one in CLSU, were conducted. The last trial was carried out with virgin females from the own rearing (‘Trap Height Trial II’ in San Leonardo); the others with lures containing 10µg. Trials with the synthetic pheromone were conducted in two sites to compare high and low populated areas.

3.2.1.2 Material and Methods

- **Trap Height Trial I in San Leonardo with Synthetic Lures**

The trial was set up in three adjacent fields in Castellano, San Leonardo (see 3.1.2.2). Four heights were tested, 0.25 m (above canopy), 0.5 m, 1 m and 2 m. The design was a complete randomized block design (CRBD) with three replicates per height. The traps from each block were set up for eight consecutive days. The traps were directed horizontally towards the prevailing wind direction, which was usually from east to west, in one row (block A and B) and two rows (Block C). The distance between rows in Block C was 35 m. The intertrap distance was 15 m. The distance between traps from one block to the closest trap from another block was 80 m from A to B, 40 m from A to C and 45 m from B to C (see Fig. 8). The planted crop was pak choi in block A and B and radish in block C.

Four traps were set at the heights tested in each block with lures and four without (control). The trial took place from October 25 to November 2, 2002. The traps were checked every morning, traps were removed from the bamboo sticks, the number of males caught was recorded, males were either removed or the sticky part exchanged and traps were newly fastened at random.

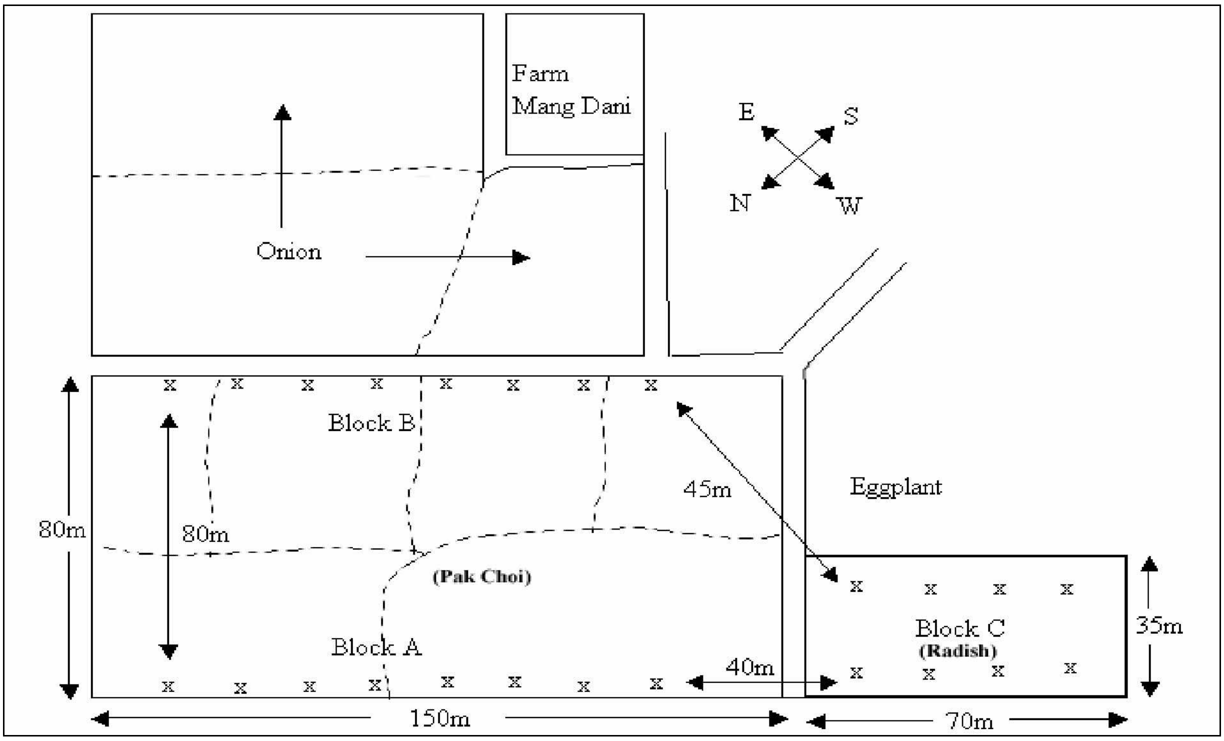

Figure 8: Set up for the trap height trial in Castellano, San Leonardo. Fields of Block A-C were planted with radish and pak choi. x: wing trap baited with 10 µg lure in different heights, 0.1-0.25 m, 0.5 m, 1 m and 2 m, in a complete randomized block design. .Prevailing wind direction from east, northeast, to west, southwest. (- - - = irrigation ditches)

- **Trap Height Trial II in San Leonardo with Virgin Females**

The trial was set up in Castellano from October 25-28, 2003. Five heights were tested, 0.25 m, 0.5 m, 1 m, 2 m and 2.8 m. The set up was completely randomized for all heights tested with five traps in five rows and each height set for each row (see Fig. 9). The rows were located in three adjacent fields with each two rows of five traps in field A and C and one row in field B. Intertrap distance was between 25 m and 30 m; the distance between the rows was between 30 m to 60 m. The traps with different heights were set up at random at the beginning and stayed at their location until the end of the trial. Data from each day was analysed separately. The virgin females used came from the own rearing and were 2-4 days old. Females were put into a prepared film box with both ends removed and sealed with mesh net to guarantee free aeration. Set up of females in traps took place in the late afternoon between 5 to 6 pm. All 25 traps were baited with a virgin female. Females were checked after the first night and replaced when they showed signs of weakness, i.e. reduced mobility. Females, which started to fly around when disturbed, were classified as healthy, remained in their containers and were fed 10% honey solution. Traps were attached to bamboo sticks. Crops planted were radish on all three fields.

No rain was falling in the period of the trial with mean temperatures of 33°C during the day and 22 °C in the night. Catches were counted and recorded in the morning and removed from the sticky surface. The sticky surface was exchanged after every night.

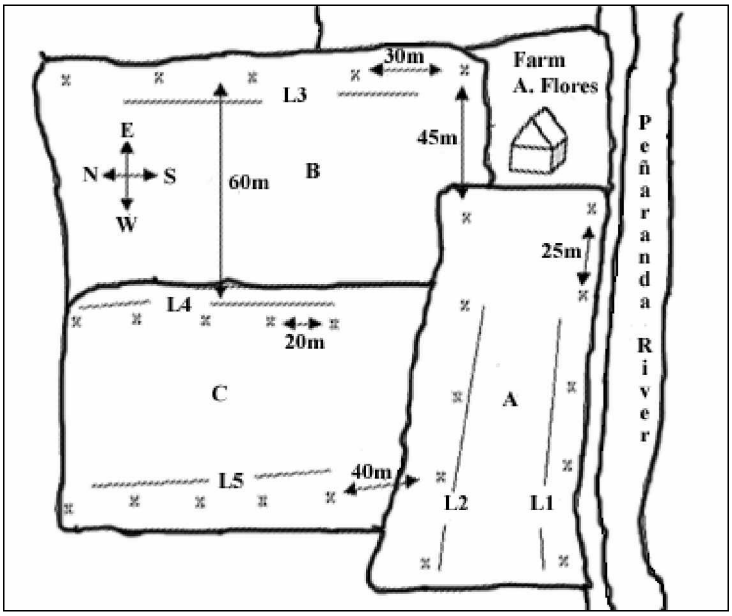

Figure 9: Set up for the trap height trial in Castellano, San Leonardo with virgin females (October 25-28, 2003). Fields were planted with radish (A-C). x: wing trap baited with one virgin female in different heights, 0.25, 0.5, 1, 2 and 2.8 m in a complete randomized block design. Prevailing wind direction from east, northeast, to west, southwest.

- **Trap Height Trial in Muñoz with Synthetic Pheromone**

Nine self made wing traps baited with 10 µg synthetic pheromone in red rubber septa and two un-baited traps were set in the experimental area inside the CLSU campus on Area II. Traps were fastened to bamboo sticks in 0.25m, 0.5m and 1m above ground. Intertrap distance was at least 15 m. Since Area II (675 m^2) was not big enough to place all traps with such a distance between them, it was necessary to set some traps outside the planted area. Treatments were arranged in a complete randomized design. Caught moth were collected and counted daily. Traps were newly arranged at random three times from January 21-29. The control traps were placed in heights of 0.25 m and 0.5 m respectively.

3.2.1.3 Results

- **Trap Height I, San Leonardo**

Significantly more males were caught in traps placed in a height of 0.5 m compared to all other heights tested (Table 11). Traps baited with synthetic pheromone placed in a height of 2 m attracted the smallest number of males. The total of 3102 males caught was recorded in six days. Numbers of males caught within the blocks were significantly lower in block B compared to A and C. An average of 0.3 catches per night per trap was recorded for the un-baited control traps, which is 1.1 % of the total catch. Reasons for the low catch performance in block B is discussed in section 3.2.2. The catch performance differed from day to day, was highest when the trial started October 26 and is fluctuating (Fig. 10).

Males caught	Trap Height							
	Trap with lures				Trap without lures			
	0.25 m	0.5 m	1m	2 m	0.25 m	0.5 m	1m	2 m
Mean ± SE	258.0 ab ± 50.3	327.7 a ± 46.4	231.3 ab ± 24.1	160.0 b ± 28.7	2.0 d ± 1.0	2.7 cd ± 0.3	5.3 c ± 0.9	2.0 d ± 0.6
Total	774	1118	694	480	6	8	16	6
C/T/N	43	62.1	38.5	26.6	0.3	0.4	0.8	0.3
% of total	24.9	36	22.3	15.4	0.2	0.25	1	0.2

Table 11: Catches of male *H. undalis* in wing traps with different heights (Castellano, Nueva Ecija, October 26-November 1, 2002). Traps baited with lures containing 10µg synthetic sex pheromone. Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test. Total catch = 3102 males

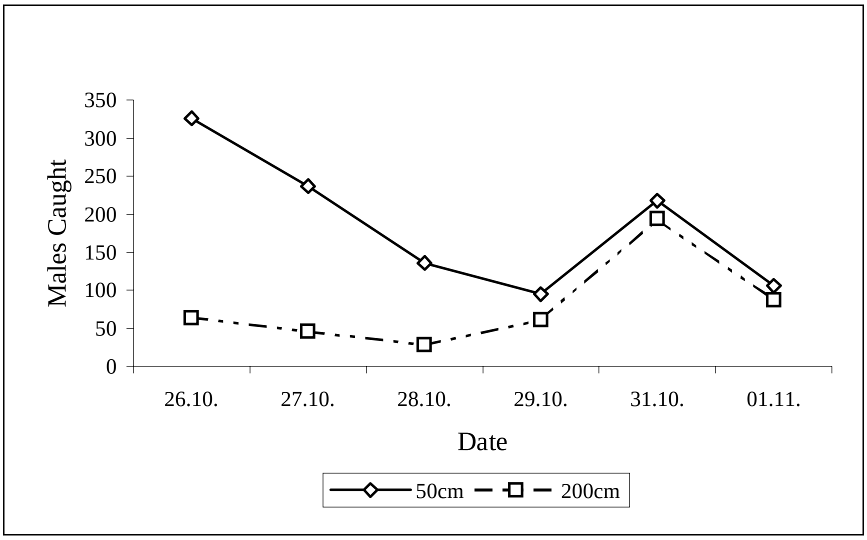

Figure 10: Number of caught male *H. undalis* in traps baited with red rubber septa loaded with 10 µg, 50 cm and 200 cm above ground.

- **Trap Height II, San Leonardo**

Traps placed 2.8m above ground attracted significantly less males than the other heights tested in the first and third night of the trial (Table 12). After the first night, significantly more males were recorded in traps set at lower heights compared to traps at 2.8 m but not 2 m. The number of males caught after the second night reflected significantly more males in traps of 1m, 2m and 2.8m compared to those placed 0,5m and 0.25 m above ground. Traps placed 0.25-2m above ground attracted not a clearly significant different number of males. A high fluctuation regarding the numbers of males caught was detected (Fig. 11) during the respective days. 2683 males were recorded in the first night, 591 in the second and 1852 in the third night. Total number of males caught within three nights was 5126.

Trap Height	26.10. Mean ± SE	27.10. Mean ± SE	28.10. Mean ± SE
0.25 m	150.8 a ± 6.7	4.4 b ± 1.7	94.0 a ± 26.0
0.5 m	136.8 a ± 32.1	8.6 b ± 3.9	91.8 ab ± 36.6
1 m	136.4 a ± 10.9	26.2 ab ± 9.6	86.8 ab ± 27.2
2 m	93.0 a ± 15.9	50.8 a ± 8.6	72.2 ab ± 24.0
2.8 m	19.6 b ± 13.2	28.2 ab ± 16.8	25.6 b ± 20.0

Table 12: Catches of male *H. undalis* in wing traps with different heights (Castellano, Nueva Ecija, October 2003). Traps baited with virgin females (2-4 days old); Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test

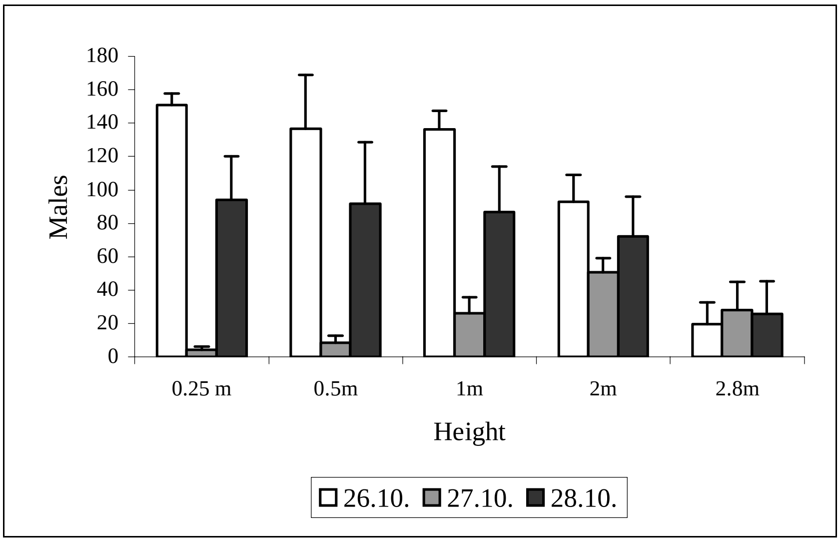

Figure 11: Catches of male *H. undalis* in wing traps baited with virgin females in Castellano, San Leonardo (October 26-28). Five traps were used per height tested (0.25 m, 0.5 m, 1 m, 2 m, 2.8 m). Catches of three days running were analysed separately day-by-day. The fluctuation of catches for every height is presented in the figure.

- **Trap Height in Muñoz**

Significant differences exist between traps placed 1 m above ground and the two other heights tested, 0.25 m and 0.5 m, respectively (Table 13). No catches were recorded in the two control traps.

Height	0.25 m	0.5 m	1m	Control
Males caught (Mean ± SE)	21.7 a ± 6.2	17.3 a ± 3.8	5.7 b ± 2.9	c ± 0.0
Total males caught	65	52	17	0
C/T/N	2.7	2.2	0.7	0
% of total	48.5	38.8	12.7	0

Table 13: Catches of male *H. undalis* in wing traps with different heights (CLSU, Muñoz, January 21-29, 2003). Traps baited with lures containing 10µg synthetic sex pheromone; Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test.

3.2.1.4 Discussion

Trap height is an important factor in the overall setting of traps in the field and it influences the numbers of males caught. The situation of fields in Nueva Ecija is determined by flat topography without elevations except for houses in the cities, villages, farms and trees. These are the only wind breaking structures. Therefore, sex pheromone saturated plumes can disperse over long distances across the fields.

Traps placed 0.5 m above ground in both trials with the synthetic pheromone determined the optimal height. This height was also confirmed using virgin females as bait in Taiwan (AVRDC, 1985), and also by SIVAPRAGASAM (1994), SIVAPRAGASAM et al. (1996) for monitoring male populations in Malaysia. SHIRAI and KAWAMOTO (1990) conducted mark-release-recapture trials in Japan and lured (virgin females) males in traps 2 m above ground. SUGIE et al. (2003) placed traps at a height of 0.4 m during tests with a synthetic pheromone.

Another important pest of crucifers, *P. xylostella*, was monitored with sex pheromone baited traps in Taiwan (CHOW, 1990), placed the same height as the cabbage heads, as well as in Japan (OHNO et al., 1990) 30-40 cm above ground.

Except for SHIRAI and KAWAMOTO (1990), aiming to attract males from long distances, traps were set by all scientists working with *P. xylostella* and *H. undalis* not higher than 0.5m above ground. It seems reasonable to think that females would most likely call from within the field or when sitting on nearby vegetation. TETTE (1974) pointed out that to obtain maximal effectiveness, traps should simulate the natural attractive source as closely as possible and height and placement of the insect's preferred environment should also be regarded.

Optimal trap placement just above or below the canopy was frequently observed for other lepidopteran pests (MITCHELL and HEATH, 1986; AHMAD, 1987; VALLES et al., 1991; SARZYNSKI and LIBURD, 2004). Traps on adjustable poles were placed in sweet corn fields for monitoring *Heliothis zea* populations (Drapek et al., 1990), 20 cm below the top of the plant.

Confusing are the results of the trap height trial with virgin females. It seems males were attracted regardless of the height at which the traps were placed. The pheromone plume can disperse more undisturbed from 2 m and 2.8 m above ground compared with the lower heights, and the reach is probably increased. The effective distance of attraction is most likely beyond the fields, which were monitored and infestation was at the time of the trial above 50% in radish fields in San Leonardo. Additionally, most pak choi and radish crops were harvested and only few fields remained planted with radish. Both females and males were forced to migrate to other locations with suitable host plants.

The consequence would mean that males from the field that traps were placed in were caught in large numbers in the first night. In the second night, males were attracted from greater distances and were mainly attracted by the females in traps placed 1 m above ground. Influx of males occurred all the time and numbers increased for the third night since males were both in the field and attracted from adjacent sites.

The statistical evaluation of the number of males caught analysed day by day for the trap height trial with the synthetic pheromone also gave incoherent results. Over the period the trial was conducted, 0.5m height traps clearly attracted significantly more males and the numeric value was also higher than in other heights most of the time but the differences were not exceptional aside from the highest height of 2 m. It is suggested to adjust the trap height dependent on weather conditions. In the dry season, traps were covered with dust whirled up by wind, at times within two days, and replacing was necessary. Good catching results can be obtained when traps are placed between 25 cm and 1 m above ground. Differences in the attractive range and dispersal of a pheromone plume can be examined by releasing marked males from different distances downwind from traps placed at different heights.

3.2.2 Intertrap Distance

3.2.2.1 Introduction

The influence of intertrap distance in regards to the catch rate of male *H. undalis* was tested in San Leonardo. Traps placed at different distances were examined for differences in catches of male *H. undalis*. The results of this study should help in optimizing intertrap distance for mass trapping and monitoring and to narrow down the distance for distribution of synthetic pheromone for mating disruption applications.

3.2.2.2 Material and Methods

Self-made wing traps, fastened to bamboo sticks, baited with 10 µg of the synthetic pheromone, set in 0.5 m above ground were used for all 'Intertrap Distance' trials.

- **Intertrap Distance I**

Traps set up in three different distances towards each other were tested in a radish field of 0.5 ha in Castellano, San Leonardo. The field was divided to have two replicates and four traps per distance were set up in a square arrangement in each half (Fig. 12). The distances between traps were 2 m, 5 m and 10 m. The distance between the squares of traps was at least 20 m. Catches from March 1 to 6, 2003 were recorded.

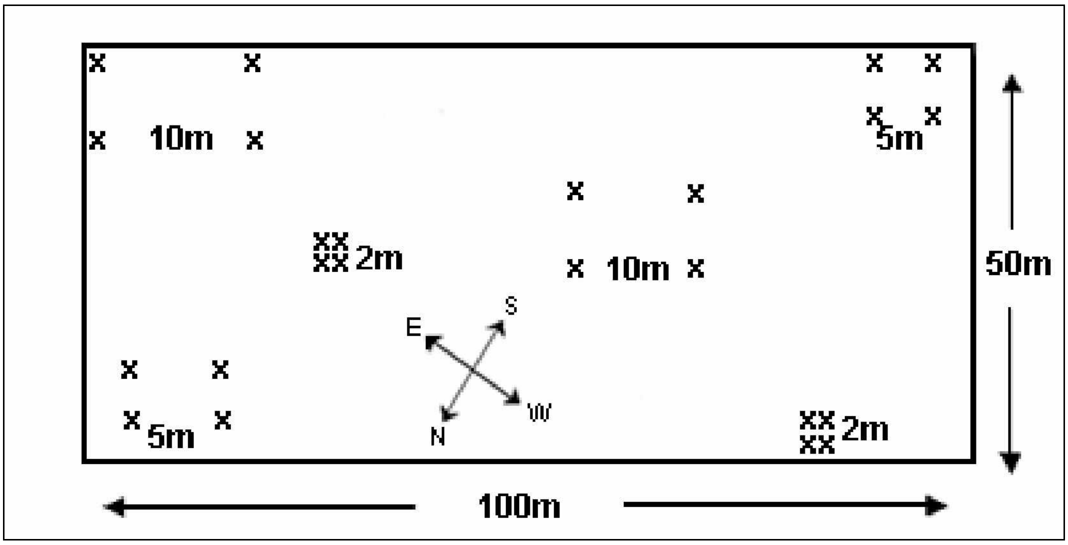

Figure 12: Set up of 'Intertrap Distance Trial I' in Castellano, San Leonardo in a radish field. The field was divided in two halves for two replicates of the different distant placed traps. x = baited wing traps with 10µg lures

- **Intertrap Distance I.1**

The field of the 'Intertrap Distance I `trial was used for a different set up of traps. In total, eight traps per distance were set on the field (Fig. 13). The traps were set in two rows of four traps with a distance of 2 m, 10 m and 20 m. The distance between the different trap distances was at least 20 m. Catches from March 7-22, 2003 were recorded.

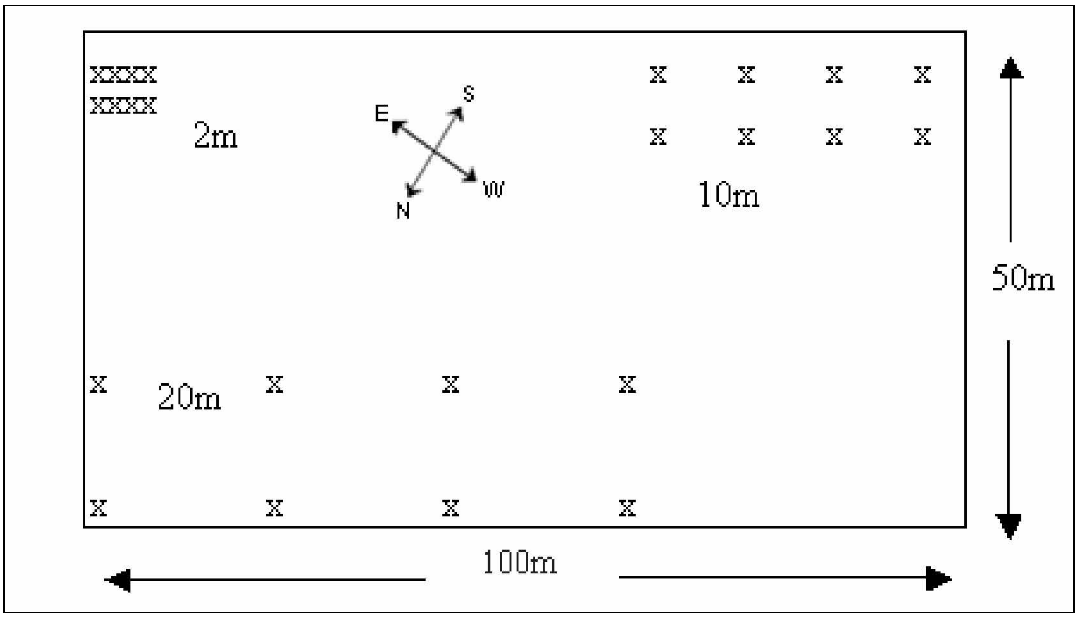

Figure 13: Set up of 'Intertrap Distance Trial I.1' in Castellano, San Leonardo in a radish field without replications. x = baited wing traps with 10µg lures

- **Intertrap Distance II**

Traps set up in three different intertrap distances were tested in two directly adjacent radish fields in Castellano, San Leonardo. The total size of the fields was ~1 ha. The distances between traps were 2 m, 5 m and 10 m (Fig. 14). The distance between grids was 40 m – 50 m and grids were placed in a horizontal line towards the prevailing wind direction (east to west). Catches from March 4-7, 2003 were recorded.

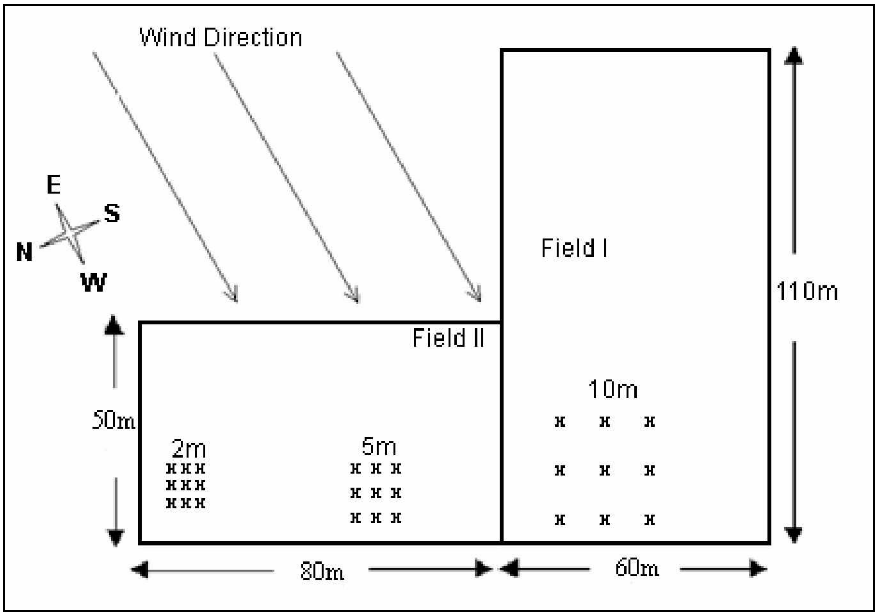

Figure 14: Set up of trap distance trial ‘Intertrap Distance II’ in Castellano, San Leonardo in two adjacent radish fields without replicates. x = baited wing traps with 10µg lures

- **Intertrap Distance III**

Nine traps were set in distances of 5 m, 10 m, and 20 m (Fig. 15). The distance between the grids was 40 m. Catches from March 7-25, 2003 were recorded. The fields were monitored at the beginning of the trial on March 7.

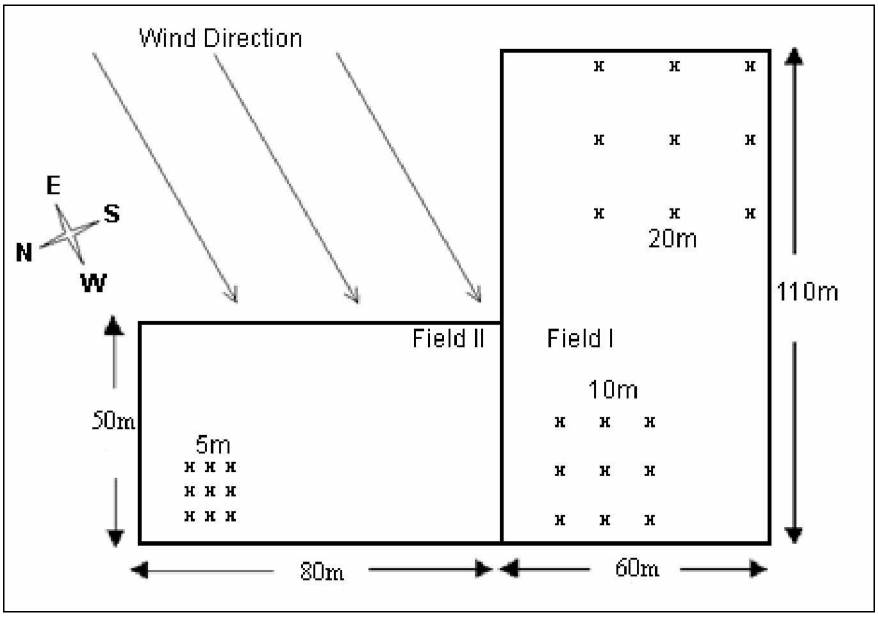

Figure 15: Set up of trap distance trial 'Intertrap Distance III' in Castellano, San Leonardo in two adjacent radish fields without replicates. x = baited wing traps with 10µg lures

3.2.2.3 Results

- **Intertrap Distance I**

The first set up can be analysed statistically. The distance between traps in a grid of ten meters differed significantly in the number of males caught compared to the two other distances (Table 14). Traps set in a distance of 2 m towards each other attracted about half of the males caught in the set up with 10 m between them. Infestations of 37.5 % and 36 % were monitored for the occurrence of *H. undalis* larvae (February 22, 28). Other pests detected were *P. xylostella* with an infestation rate of 27.5 % and 32 %.

Distance	2m	5m	10m
Total	88	145	165
C/T/N	1.8	3.0	3.4
Mean Catch (Mean ± SE)	11.0 b ± 1.5	18.1 b ± 2.1	20.6 a ± 4.2
% of total	22.1	36.4	41.5

Table 14: Catches for four nights of male *H. undalis* in wing traps set in a square of four traps in San Leonardo (March 1-4, 2003). Distance of traps within the square were 2 m, 5 m and 10 m. Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test. Total catch = 398 males

- **Intertrap Distance I.1**

Most catches were recorded in traps placed 20 m apart, followed by traps placed 10 m apart and fewest catches in traps placed 2 m apart. The capture rate is more then double as many in the traps placed 10 m and 20 m apart as in the 2 m distant placed traps (Table 15). Monitoring of the field resulted in an infestation rate of 32 %, 32 % and 50 % for *H. undalis* and 14 %, 52 % and 88 % for *P. xylostella* (March 7, 11, 18). *S. litura* was found March 11 and 18 with infestations of 22 % and 10 %.

Distance	2m	10m	20m
Total	128	320	339
C/T/N	1	2.5	2.6
Mean Catch (Mean ± SE)	16.0 ± 2.8	40.0 ± 3.5	42.4 ± 3.6
% of total	16.3	40.7	43.1

Table 15: Catches of male *H. undalis* in traps set in grids of eight traps in San Leonardo (March 7-22, 2003). Distances between traps within grid were 2 m, 10 m and 20 m. Total catch = 787 males

- **Intertrap Distance II**

In contrast to the first two set ups of trials 5.15.1 and 5.15.2, the distance between adjacent traps of different distances was twice as large (40 m- 50 m) and the set up was performed in a horizontal line towards the prevailing wind direction. The nightly catch rate from traps placed 2 m and 5 m apart is in the same range, but three times less than the 10 m apart placed traps Table 16). An indication is given that placing traps 10 m apart results in an increase of catches compared to traps placed closer together.

Distance	2 m	5 m	10 m
Total	55	58	171
C/T/N	3.1	3.2	9.5
Mean Catch (Mean ± SE)	6.1 ± 0.8	6.4 ± 1.1	19.0 ± 3.2
% of total	19.4	20.4	60.2

Table 16: Catches of male *H. undalis* set in grids of nine traps in San Leonardo (March 4-7, 2003). Distances between traps within grid were 2 m, 5 m and 10 m. Total catch = 284 males

- **Intertrap Distance III**

Taking the results of the first set up, 'Intertrap Distance II', traps were placed even further apart. The total number and the nightly catch rate is almost equal for 5 m and 10 m, but only about half of the results of traps that were placed 20 m apart (Table 17). An indication is given that placing traps 20 m apart from each other results in an increase of catches compared to traps placed closer together.

Monitoring for the occurrence of *H. undalis* larvae resulted in an infestation rate of 25 % and 74.2 % (March 7), 24 % and 44 % (March 11) and 36 % and 32 % (March 18) in field I. and II, respectively. Multiple infestations reached up to nine larvae on a single plant. *P. xylostella* was the only other pest detected (16.1 %, 44 % and 94 %, March 7, 11 and 18) with high infestation rates.

Distance	5m	10m	20m
Total	313	338	662
C/T/N	1.8	2.0	3.9
Mean Catch (Mean ± SE)	34.8 ± 3.4	37.6 ± 5.2	73.6 ± 5.2
% of total	23.8	25.7	50.4

Table 17: Catches of male *H. undalis* in traps set in grids of nine traps in San Leonardo (March 7-25, 2003). Distances between traps within grid were 5 m, 10 m and 20 m. Total catch = 1313 males

3.2.3 Trap Distance and Wind Direction

3.2.3.1 Introduction

The aim of theses trials was the determination of possible interactions with different trap distances placed along the prevailing wind direction. The wind came from east, northeast to west, southwest in Castellano, San Leonardo. There were no windshields in front of the fields, i.e. houses, trees or other elevations, in both sites, which could have an influence on the wind, speed and wind eddies. The direction of placed traps was pin-point by using an electronic compass with an accuracy of ± 2°-3° (Suunto, Vector). Only data for nights with wind direction northeast to southwest were used, which were two nights for the first set up (March 5-6) and seven for the second set up (March 12-18).

3.2.3.2 Material and Methods

- **Wind Direction I**

Nine traps were set up in a 0.25 ha radish field in Castellano. The set up was in lines with the lines horizontally to the prevailing wind direction, which was from east, northeast to west, southwest. Traps were set up in four rows horizontally to this direction. Three traps per row were set in a distance of 15 m between 2 traps in a line and 15 m between the rows (Fig. 16). Traps in one row horizontal towards the wind direction were seen as replicates. The data used were for nights with a northeast to southwest wind direction.

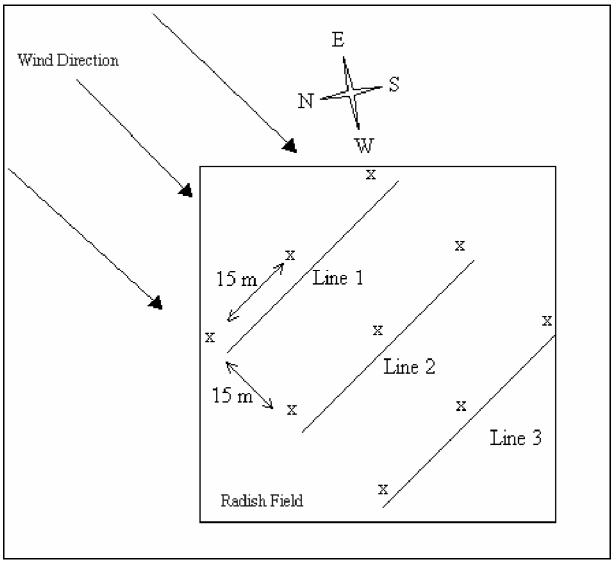

Figure 16: Set up of traps (x) in lines, horizontally to the prevailing wind direction (March 5-6, Castellano, San Leonardo).

- **Wind Direction II**

Twelve traps were set up in two adjacent fields in Castellano, San Leonardo with a size of ~ 1.5 ha (March 12-22). Traps were placed in three rows horizontally to the wind direction (Fig. 17). Four traps per row were set in a distance of 30 m between 2 traps in a line and 30 m between the rows. Traps in one line horizontal towards the wind were seen as replicates. The data used were for nights with northeast to southwest wind direction. Weather data came from the PAG-ASA station Cabanatuan.

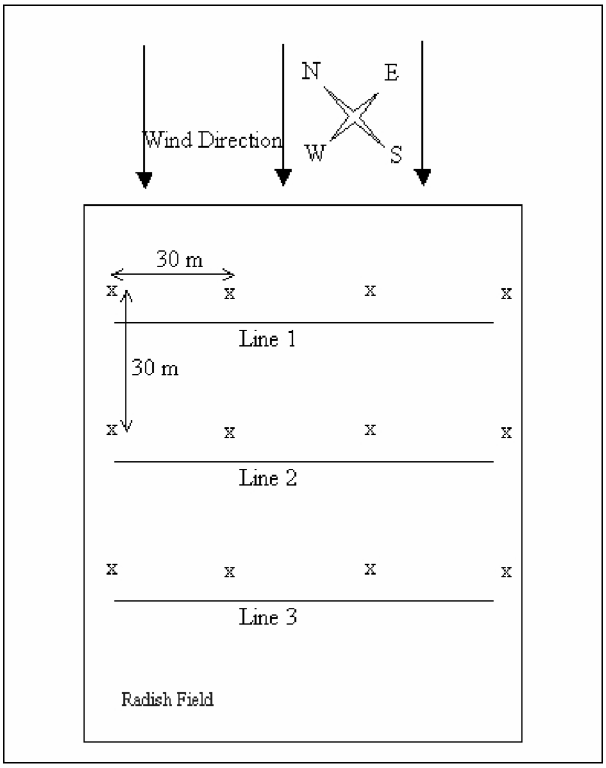

Figure 17: Set up of traps (x) in lines, horizontally to the prevailing wind direction (March 12-22, Castellano, San Leonardo.

3.2.3.3 Results

- **Wind Direction I**

Influence of wind direction and placing of traps one behind the other is significant in the decrease of numbers of males caught in the windward direction of the traps with a distance of 15 m between the traps (Table 18).

← Leeward — Windward

Distance	First (n=3)	Middle (n=3)	Last (n=3)
Total	90	21	16
C/T/N	15	3.5	2.7
Mean Catch (Mean ± SE)	30 a ± 3.5	7.0 b ± 1.5	5.3 b ± 1.2
% of total	59.2	13.8	10.5

Table 18: Influence of wind direction to catch rate with intertrap distance of 15 m in line of wind direction. Only catches were regarded for the analysis when wind direction came from northeast. First means the leeward and Last the windward side. Total catch = 152 males

- **Wind Direction II**

Influence of wind direction and placing of traps one behind the other is not significant in the decrease of number of males caught in the windward direction of the traps with a distance of 30 m between the traps (Table 19).

← Leeward — Windward

Distance	First (n =4)	Middle (n =4)	Last (n =4)
Total	125	123	166
C/T/N	5.2	5.1	6.9
Mean Catch(Mean ± SE)	31.3 a ± 7.8	30.8 a ± 11.5	41.5 a ± 6.1
% of total	30.2	29.7	40.1

Table 19: Influence of wind direction to catch rate with an intertrap distance of 30 m in line of wind direction. Only catches were regarded for the analysis when wind direction came from northeast. Total catch = 414 males

3.2.2.4 + 3.2.3.4 Discussion

Intertrap distance can greatly influence the numbers of males caught. Higher catches in traps placed 10-20 m apart is connected with the area covered by the traps. Traps placed only 2 m apart act as a point source, whereas traps with a distance of 10 m or 20 m cover a wider area and plumes do not mix in the same quality as close placed traps do. The distance between traps is most important for mass trapping and for the set up of comparison trials. For the latter it is important to guarantee the same conditions for all traps to avoid bias due to the location

and traps should not interfere with one another by choosing intertrap distances too short. However, WALL and PERRY (1978) described interactions with an intertrap distance of up to 100 m for the pea moth, *Cydia nigricana*. Traps placed in a line along the wind caught less males when they were placed downwind compared to those upwind (WALL and PERRY, 1980) and the range of attraction was estimated as above 400 m with 100µg containing lures. Interactions and the decrease of catches were found for traps placed in lines along the wind for the centre trap with intertrap distances of 15 m and 50 m (WALL and PERRY, 1981). Subsequent experiments showed that the proportion of moths caught in the downwind traps were sometimes greater than in those placed upwind, but the fewest amounts of moths were always caught in central traps (PERRY and WALL, 1984). ELKINTON and CARDÉ (1988) explain larger catches in downwind traps in that moths enter theses traps first. Dispersion of the plume can be influenced by objects near a field as was assumed in field trials of a tomato field in New Zealand. Significantly, fewer moths were caught at the upwind sites compared with the middle and downwind sites (HERMAN et al., 1994).
Whether more moths are caught in up- or downwind traps depends on effects of vegetation on the plume structure (ELKINTON and CARDÉ, 1988) and surface area, where the pheromone can be adsorbed and re-eluted (WALL et al., 1981). Wind speed is also a factor since dispersion of the pheromone is only possible with an air stream. For the sites in the Philippines there was always a gentle wind blowing with a prevailing direction coming from the northeast. Unfortunately, there is no onsite weather data for the area of Castellano available and the data regarding wind speed and direction used was recorded from a weather station in Cabanatuan, approximately 15 km north of San Leonardo.

It can be shown that catches in traps placed in a vertical line of the wind direction are strongly influenced by the intertrap distance for *H. undalis* in fields of San Leonardo. No effect was observed when traps were separated 30 m, but numbers of males caught in traps with a distance of 15 m were significantly lower in traps of the windward side. This effect was also documented by SAPPINGTON (2002) for boll weevils, *Anthonomus grandis*, when the wind hit lines of traps in an angle more than 22.5° from the perpendicular.

There is an obvious dilemma for a set up on small-scale farms. On one side interactions should be minimized or affect all traps equally (CARDÉ and ELKINTON, 1984), on the other side it is necessary to place traps in the same area or field. Thus, limitation of the number of replicates and treatments exist. In the studies presented, traps were placed in most of the cases in distances exceeding 25 m between lines, when set in more than one line. Horizontally placing towards the prevailing wind direction was performed whenever possible. Absolute diminution of any interference effects cannot be guaranteed, but it was attempted to minimize such effects whenever possible. Re-randomisation was another step of reducing interaction between traps and was carried out for most tests at least twice a week up to a daily re-arrangement of traps at random.

There was no trial conducted to examine the effects of placing the trap outside of a field, centrally or at the edges, but trials at CLSU showed no effect whatsoever between traps placed inside or outside the field. However, ATHANASSIOU et al. (2002) recorded a wider range of catches in peripheral placed traps compared to those in the centre.

The results of the intertrap distance trials and effect of wind to traps placed along the wind direction, emphasize the importance of proper trap placement. Thereafter traps should be placed with the largest distance possible between them. Constraints mentioned above force the experimental conductor to adjust distances for the space available but intertrap distances should be more than 15 m, when traps placed crosswind and at least between 20- 30 m apart when placed in lines with the wind direction.

3.3 Trap Design

3.3.1 Introduction

Traps should be efficient, cheap and practical to monitor. Numerous articles describe the search for the right trap design tailor-made for a certain species. Cost and durability as well as time to handle the traps influence most trap design trials. The trap of choice must be selected dependently of the task it should fulfil. Sensitivity, i.e. detection of the target, is most important for survey. Sticky traps were proved suitable for that. Disadvantageous are the small capture capacities and saturation of the sticky surface, which requires service time for checking and renewing.

Non –sticky traps are mainly used for monitoring populations over longer periods due to their capacity in collecting the target species and for highly populated areas.

Trap design trials compared sticky and non-sticky traps. Since the compound used was not tested in field trials before 2001, sensitivity was evaluated firstly. An attempt was made to find local available materials for construction of cheap traps. The latter was looked at as second priority. Only the Taiwan trap, made of card paper, the yellow/white, and green bucket traps were commercially available. All other traps were hand made to reduce cost. Different trap designs, sticky and non-sticky, were compared for their sensitivity, capacity, and longevity under field conditions in San Leonardo.

3.3.2 Material and Methods

- **Trap Design I**

The set up of different trap designs took place in two adjacent radish fields in Castellano, San Leonardo. The fields had each the same size of 2000 m². The traps were set up in a complete randomized design (CRD). Every design was replicated three times. The distance between the traps was at least 25m. Trap designs tested were wing traps, delta traps, water bowl traps, plastic bottle traps and tube traps (Fig. 20). All traps were hand made, yellow sticky paper was used in wing traps, delta traps, and tube traps. The water bowl and the plastic bottle traps were filled with water and motor oil on the surface and later with a water soap solution due to chemical reactions of the oil, which coagulated and made it difficult to detect the males caught. The tube traps were made of plastic pitchers with the bottom removed, i.e. they were open on two sides. The wing and the delta traps were made of corrugated plastic. The plastic bottle traps had four openings cut in the upper third part of the bottle, bent to the top so far that the cut parts could protect the openings from rain. The water bowl traps were made of plastic bowls with a square piece of corrugated plastic attached to the sides of the bowl, which

covered the surface in a height of 15 cm above the bowl to protect the surface and the lure from direct sunlight. The lures were attached to the cover with a thin wire hanging 3 cm above the surface of the water-oil/ water-soap solution. The same goes for the plastic bowl traps where lures were hanging from the lid about 3 cm above the water level.
The trial was conducted from November 16 to December 6, 2002.

- **Trap Design II**

Plastic yellow/white bucket traps, all green bucket traps and self-made bucket traps as well as self made wing traps were used for the second trap design trial. The bucket traps (Unitrap) were ordered from Gempler's®, or made of modified water dispensers for chicken. Vaportape® strips were put at the bottom of the bucket traps as a killing agent. The active ingredient of Vaportape® is 2,2-Dichlorovinyldimethylphosphate. In total 15 traps were set in a two ha radish field in Castellano in a complete randomized design. The distance between the traps was 25 m. Each design was replicated three times. The trial was conducted from October 4-6, 2003, and was stopped because of small catch rates. The set up was changed the next time the trial was conducted from October 13-16, 2003. The bucket traps were hanging from the centre of tripods made of bamboo; the wing traps were attached to bamboo sticks. Three wing traps without lures were used as control.

3.3.3 Results

- **Trap Design I**

Wing traps attracted more males than all other trap designs. The differences are significant ($p < 0.05$) among the tested designs (Table 20). The wing trap caught 6.7 times more males than the trap with the smallest catch rate, the tube trap. In the other trap designs (delta trap, water bowl trap, bottle trap) 3.3, 4.0 and 2.7 times less males were caught compared to the number of catches in the wing trap design. Almost half of all catches were recorded from wing traps (Fig. 18).

Trap Type	WT	DT	WBT	PBT	TT
Total	652	194	161	236	97
Mean ± SE	217.3 a ± 28.8	64.7 b ± 17.9	53.7 bc ± 3.0	78.7 b ± 6.9	32.3 c ± 6.4
C/T/N	10.3	3.1	2.6	3.7	1.7

Table 20: Catches of males caught with different trap types in San Leonardo (November 16 to December 6, 2002). WT: Wing Trap, DT: Delta Trap, WBT: Water Bowl Trap, PBT: Plastic Bottle Trap, TT: Tube Trap, C/TN: catches per trap per night. In a column, means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test.

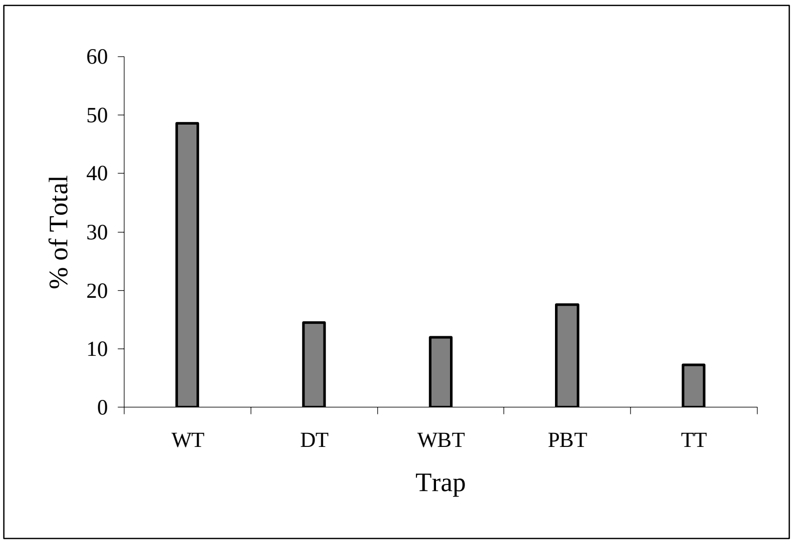

Figure 18: Percentage of caught *H. undalis* in wing traps (WT), delta traps (DT), tube traps (TT) with sticky insert and water bowl traps (WBT) and plastic bottle traps (PBT) with water as catching agent (set up in Castellano, San Leonardo, November 16 – December 6, 2002).

- **Trap Design II**

The first set up had small catches in all trap designs, below 1 male per night and trap (Table 21). Wing traps were the only design with a higher nightly catch rate. The catches could be caught by chance with all designs except the wing traps.

Trap Type	Y/W BT	G BT	SM BT	WT w lure	WT w/o lure
Total	5 a	3 a	6 a	30 a	0
C/T/N	0.5	0.3	0.7	3.3	0

Table 21: Total of males caught in a first set up (4.10. - 6.10.2003, San Leonardo) of four different trap designs and un-baited control traps with the nightly catch rate per trap (C/T/N). Y/W BT: Yellow/White Bucket Trap, G BT: Green Bucket Trap, SM BT: Self-Made Bucket Trap, WT: Wing Trap. Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test.

The total catches in the second set up was higher for all trap designs (Table 22). Most catches were recorded in wing traps followed by yellow/white bucket traps. These two designs differ significantly with all other trap designs, in which the latter is in an intermediate position. In both set ups the green bucket traps were the least attractive in regards to the numbers of males caught. More than half of all catches were recorded in wing traps (Fig. 19).

Differences between the two commercial bucket traps yellow/white and green indicate the influence of colour or brightness of the traps as an additional factor for attracting males. Self-made wing traps were usually made of white, yellow, light green, orange, or red corrugated plastic, the self-made bucket traps were white and red.

Trap Type	WT	Y/W BT	G BT	SM BT
Total	217	84	20	70
C/T/N	18.1	7.0	1.6	5.8
Mean ± SE	72.3 a ± 22.7	28.0 ab ± 7.1	6.7 c ± 1.9	23.3 b ± 7.3

Table 22: Total of males caught in a second set up (13.10. -16.10.2003, San Leonardo) of four different trap designs and un-baited control traps with the nightly catch rate per trap (C/T/N) and mean values. Y/W BT: Yellow/White Bucket Trap, G BT: Green Bucket Trap, SM BT Self-Made Bucket Trap, WT: Wing Trap. Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test ($p<0.05$).

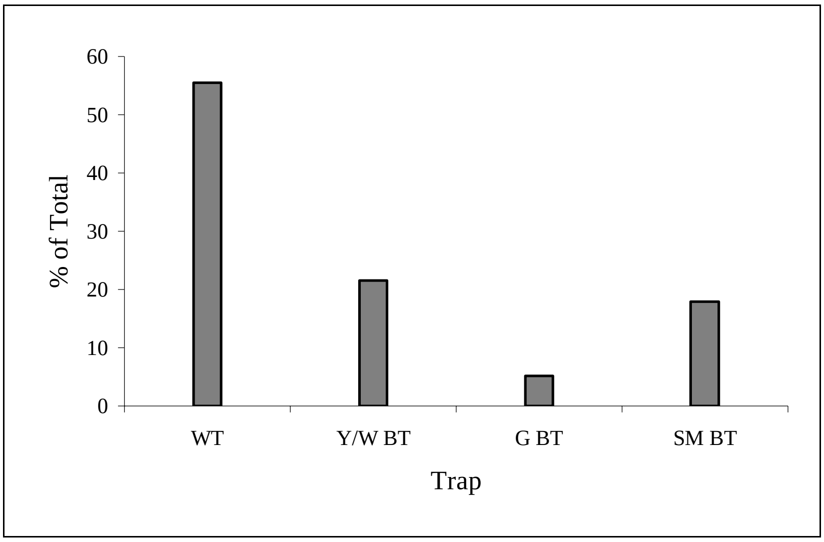

Figure 19: Percentage of total captures of male *H. undalis* in yellow/white (YW BT), green (G BT)and self-made (SM BT) bucket traps and wing traps (WT) (2nd set up in San Leonardo, October 13-16, 2003).

3.3.4 Discussion

Trap design is an important factor in sex pheromone monitoring systems and differences among capture rates are described for many species and trap designs (e.g.: SUBCHEV et al., 2004; DOWNHAM et al., 2003; ATHANASSIOU et al., 2002; AMELINE and FRÉROT, 2001; BARTELS and HUTCHINSON, 1998; LOPEZ, 1998; LOPEZ et al., 1994; KEHAT et al., 1994 a,b; ANSHELEVICH et al., 1993, 1994; GRAY et al., 1991; KEHAT et al., 1991; VALLES et al., 1991; ADAMS et al., 1989; MITCHELL et al., 1985; STRUBLE, 1983).

The most convenient solution would be a trapping system that is easy to maintain, with high capacity and sensitive capture properties. Six different types were tested in this study. Wing traps with a sticky insert caught the most males in all trials. The problem with sticky traps is the need for intensive care, by changing the sticky insert, sometimes on a daily basis when pest population is high, due to a very limited capacity (CARDÉ and ELKINTON, 1984). Dust and water can reduce the sticky effect. Working with the inserts is messy and males caught do not die instantly or within a short time. Depending on temperature, it could take a couple of days. The time necessary for maintaining such traps is particularly high. In the trials conducted in Castellano, sticky inserts were changed, depending on trial, for more then 40 traps a day. Bottom parts were prepared in the laboratory but the old ones had to be removed and new ones had to be put together with the upper part of the trap, which could take half a day with time for counting and resetting. Nonetheless, wing traps with sticky insert were the most sensitive design and detection of males in low populated areas in Palestina was possible even with the synthetic pheromone. Saturation caused immense efforts in changing parts or removing corpses but sensitivity was more important for the studies conducted and complete saturation was found although occasionally only a couple of times in peak population density in Castellano, partial caused by accidentally caught *P. xylostella* virgin females. Saturation effects are widely recognized as a limitation factor for the usage of sticky traps (e.g.: MOREWOOD et al, 2000; CARDÉ and ELKINTON, 1984; RAMASWAMY and CARDÉ, 1982). Wing traps are impractical as trapping systems for longer periods since the time expenditure for checking sticky inserts for saturation or replacing them is far too high.

The initial traps used in this study were commercially available traps made of card paper from Taiwan. Although effective and easy to handle, it was obvious after rainy nights that theses traps were not usable for longer than one night. Morning humidity soaked the card paper and traps fell apart when the wire was loosening, which held the bottom and upper part together. The price for such a trap was approximately 2.50 US$, too high a cost for farmers to probably invest in the Philippines. The self-made traps of corrugated plastic on the other hand lasted for more than half a year and the price for a trap was calculated between 0.40 – 0.60 $ (US) per trap. Preparation is easy and the sturdy materials make it comfortable to work with. Advantageous is the fact that materials are easy to get in bookstores and only a ballpoint, a punch and wire is necessary for assembling. Unitraps have a better storage capacity for dead corpses but prices are much higher (12.50 US$ per trap + 2.50 US$ for the Vaportape®). SANDERS (1986a) examined the effect of killing agents and the influence of dead insects inside the traps. The killing agents he examined, dichlorovos (i.e. the substance used in Vaportape) and a mixture of ethanol and ethylene glycol had a repellent effect in non-sticky traps. Catches were also reduced when dead moths were present inside the traps. High capacity traps like funnel or bucket traps are advantageous when populations are monitored for longer periods; sticky traps are not suitable for such purposes (SANDERS, 1986b). Nonetheless, the efficiency of capturing moth, here the spruce budworm *Choristoneura fumiferana*, was higher in sticky traps than in non-sticky traps (SANDERS, 1986b). No differences between numbers of males caught in wing and bucket traps were found for another tree pest, the Douglas fir pitch moth, *Synanthedon novaroensis*, (ROCCHINI et al. 2003).

What makes wing traps the traps of choice is the current way the pheromone can be used. Adult emergence is the required information to get. Long-term observations would require changing of lures at least twice a month to secure capture rates not influenced by the release quantity of the lure. Additionally, the traps must be monitored daily to detect upcoming migration into an area, which is possible with only a few traps, but they should provide an optimum of sensitivity.

Results of the second trap design trial in 2003 indicate the influence of trap colour to the number of males caught. Colours used in wing traps were usually bright, mainly white, yellow, and orange, less were red or light green. Colours in bucket traps were green for the first-, yellow/white for the second type and transparent/red for the self-made bucket trap. Significantly, more males were caught in the yellow/white design, which is a strong indication for a colour dependent influence of attraction towards the male response to search for a mate. Colour of traps can influence attraction towards the trap both in day- and night-flying species of Lepidoptera, Diptera and Coleoptera (SUBCHEV et al., 2004; KNIGHT and MILICZKY, 2003; SASAKI, 2001; LIBURD et al., 2001; FALACH and SHANI, 2000; MITCHELL et al., 1989; TIMMONS and POTTER, 1981; MCLAUGHLIN et al., 1975) with more night-flying species examined. HERMAN et al. (http://www.hortnet.co.nz/publications/nzpps/proceedings/ 94/94 _154.htm, 5.10.2004) used different coloured traps, white, yellow, and green, to determine catch rates of tomato fruitworm moths, *Helicoverpa armigera*, and related the numbers of non-target species; particularly bumblebees, with the colour of traps. Accidentally caught bumblebees were caught 5 times higher in yellow or white traps compared to green traps. Catches of the target species in green traps were reduced about 58 % compared to yellow or white coloured traps. Influence of trap colours is described for several moth species (MITCHELL et al., 1989; SUBCHEV et al., 2004), where green coloured traps attract lesser amounts of males then coloured ones. For the maize stalkborer, *Busseola fusca* it was found that green coloured traps attracted significantly more males then in yellow/white traps (CRITCHLEY et al., 1997).

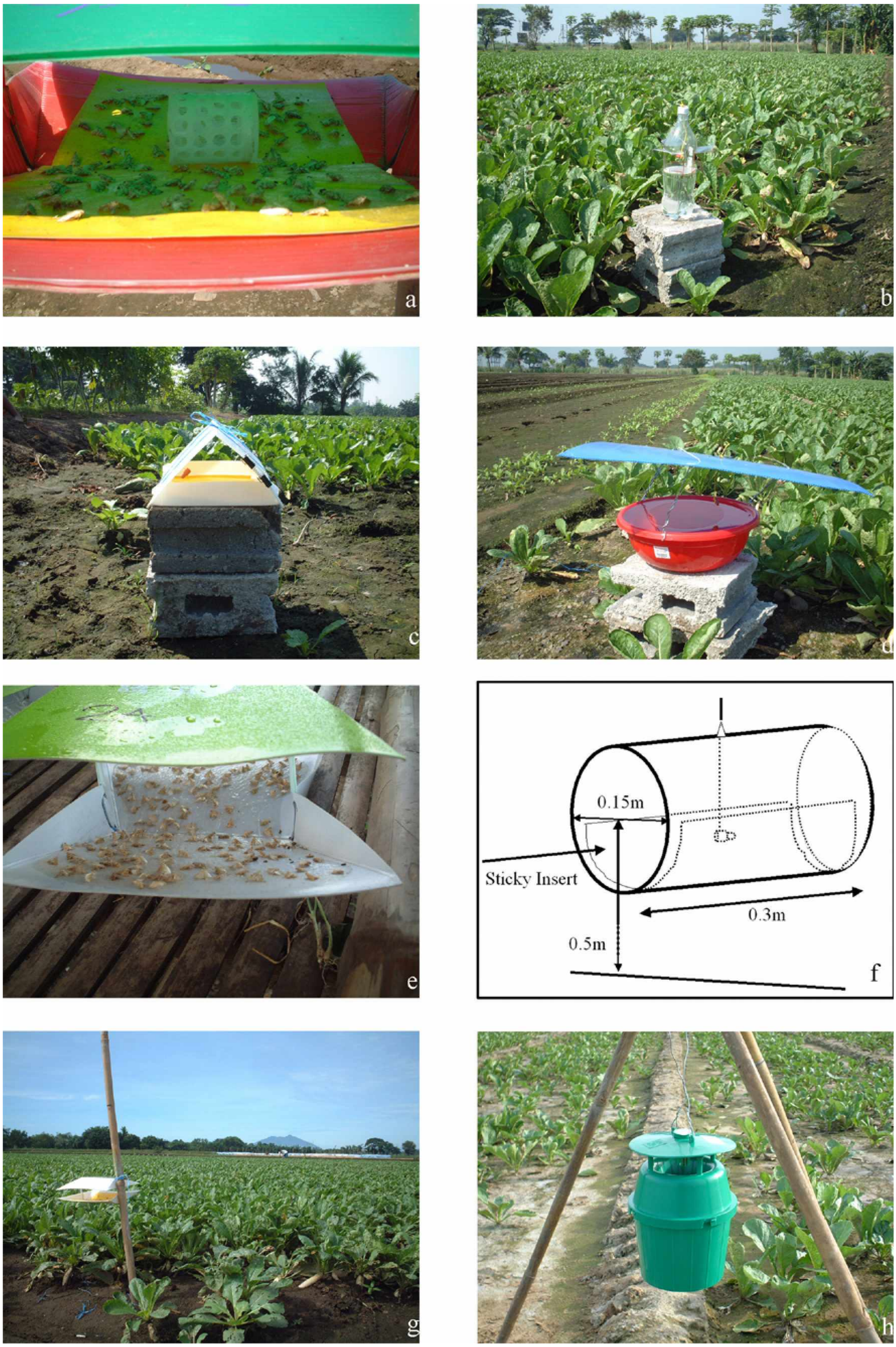

Figure 20: Trap designs. Self made wing trap (SM WT) baited with a virgin female in a film box (a), plastic bottle trap (PBT) (b), delta trap (DT) (c), water bowl trap (d), wing trap (WT) from Taiwan, made of card paper (e), tube trap (TT) (f), self made wing trap set in a radish field in San Leonardo (g) and green bucket trap (GB T) (h).

3.4 Longevity of Attraction of Red Rubber Septa containing 10 µg E,E-11,13-Hexadecadienal

3.4.1 Introduction

Persistence of red rubber septa was tested in two sites in Nueva Ecija in Castellano and Guimba. The aim was to determine the longevity of attraction in heavily (Castellano) and moderately infested areas (Guimba).

3.4.2 Material and Methods

- **Longevity of rubber septa containing 10 µg E,E-11,13-Hexadecadienal in San Leonardo**

In total 12 Traps were set up in a radish field of 4000 m^2 (CRD). Lures used were sent August 2002 from Dr. Griepink; 6 traps at a time baited with septa impregnated with 10µg of a pure and stock solution were used for the trial. The traps were placed with a minimum distance of 24 m between adjacent traps in three lines in the field. The trial started November 8 and finished December 18, 2002. Rearrangement of traps took place two times per week at random to avoid bias due to the placing. Traps were moved from one field to another on November 27 since the first field was harvested and newly prepared. The second area, a radish field nearby, was harvested December 15-18. The traps were controlled every 1-3 day in the first month and then every 3-7 day until the end of the trial. The sticky paper was exchanged whenever necessary.

The field was monitored for the occurrence of *H. undalis* larvae November 12 and 19, 2002.

- **Longevity of rubber septa containing 10 µg E,E-11,13-Hexadecadienal in Guimba**

The set up was identical with the one in San Leonardo (3.4.2). The trial started January 4 and finished February 4, 2003.

3.4.3 Results

- **Longevity of rubber septa containing 10 µg E,E-11,13-Hexadecadienal in San Leonardo**

All traps attracted male *H. undalis* over the period of six weeks in quantities, which were above the catch rate by chance (Table 23). The highest numbers, 1511 and 1553, were caught in the first week from November 8-15 with one third in the first night, November 8-9. The catch rate per trap in the first night was above 80 males per trap.

There is no significant difference between the two solutions, pure and stock, impregnated into rubber septa (Fig. 21). Both attracted equal numbers of male *H. undalis*. Half of the total catches occurred in the first week. The numbers decreased in the second week but increased in the third and fourth week to a quarter of the amount caught in the first week. The mean catch per night and trap lays above the level for accidental catches in all six weeks. Male *H. undalis* were attracted by the synthetic pheromone for six weeks. 5949 male *H. undalis* were caught in a period of six weeks with all 12 traps.

Monitoring of the field November 12 resulted in an infestation rate of 29.2 % with *H. undalis* larvae, 52.5 % with *Plutella xylostella* larvae and pupae, and 22.3 % with *Crocidolomia pavonana*. One week later, November 19, the infestation rate increased to 100 % from 89 examined plants with 160 larvae in total. *P. xylostella* were found on every plant. The crop was older then two months at the second monitoring.

Period	Total Pure (%)	Total Stock (%)	C/T/N-Pure	C/T/N-Stock
First Night	485 (16.2)	508 (17.2)	80.8	84.7
Week 1	1511 (50.3)	1553 (52.7)	35.9	36.9
Week 2	202 (6.7)	256 (8.7)	4.8	6.0
Week 3	449 (14.9)	402 (13.6)	10.7	9.6
Week 4	469 (15.6)	366 (12.4)	11.2	8.7
Week 5	238 (7.9)	211 (7.2)	6.6	5.9
Week 6	133 (4.4)	159 (5.4)	3.7	4.4
Week 1-6	3002 (100)	2947 (100)	12.8	12.6

Table 23: Catches of male *H. undalis* with lures containing 10 µg E,E)-11,13-Hexadecadienal from a pure and stock solution in a six week period (November 9- December 18, Castellano, San Leonardo). C/T/N = Catch per trap and night. (%): Percentage of catches of the total catch.

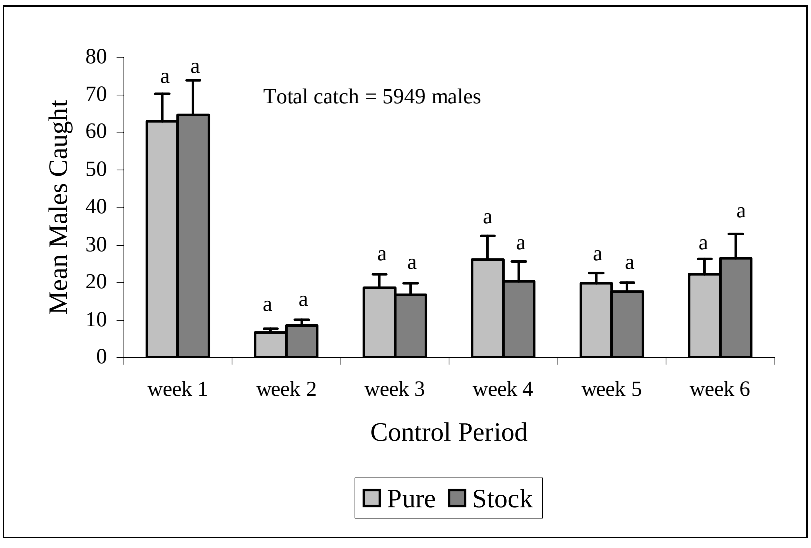

Figure 21: Captures of male *H. undalis* in traps baited with 10 µg of E,E)- 11,13-Hexadecadienal from a pure and stock solution (November 9- December 18). Different letters above bars indicate significant differences according to Fischer's Least Square Distance Test, with $p < 0.05$.

- **Longevity of rubber septa containing 10 µg E,E-11,13-Hexadecadienal in Guimba**

Catches of the first night reached 16 % of the total number of catches during the period the traps were set in the field (Table 24). Lures remained equally attractive for the first two weeks. The attractiveness declines sharply after the fourth week, whereas numbers are steadily decreasing from week 1-4. Differences between lures impregnated with stock and pure solution were not significant; results are listed without referring to the different solutions lures were impregnated with.

	Total (n=12)	C/T/N	% of total
First night	116	9.7	16.3
1 week	268	3.2	37.7
2 weeks	213	2.5	30.0
3 weeks	124	1.5	17.5
4 weeks	85	1.0	12.0
4,5 weeks	20	0.6	2.8

Table 24: Catches of male *H. undalis* Guimba (Janaury 4-February 4, 2003). Total catch = 710 males. Catches of the first night are included in catches of week 1.

3.4.4 Discussion

Aim of the study was testing the compound E,E-11,13-Hexadecadienal for its attraction towards male *H. undalis* over a period of time. Males were attracted 6 weeks in San Leonardo and more than 3 weeks in Guimba. The difference can be explained by the assumed *H. undalis* incidence for both locations, which is higher in San Leonardo. The nightly catch rate per trap indicates that males were not caught by chance. The trial demonstrated that red rubber septa impregnated with 10 µg E,E-11,13-Hexadecadienal do release this compound in such a way that males were attracted. Release rates are perhaps high at the beginning and then continually decline. HOWSE et al. (1998) refer to this effect as to 'first order' release profile, i.e. the release devices tend to release more attractant at the beginning compared to the amount releasing at the end. The ideal would be a release device with a constant release of the same quantity throughout its life (zero order release profile). In both sites, the numbers of males caught was very high with 16 % males caught of the total catch. This initial event is followed with high catches in the first week followed by more or less constant capture rates for the next two-three weeks with another decrease for the rest of the trial. It could indicate constant release rates after an initial phase with a very high release rate. The decline in week 2 in San Leonardo can be explained with the capture of large parts of the local population and duration it takes for other males to immigrate in this area. Numbers for week 3 and 4 are almost identical in San Leonardo.

3.5 Lure Ageing Effects

3.5.1 Introduction

Lures exposed for different durations in a field were tested to determine the time lures are most attractive. The initial concentration was 10 µg /lure. This trial was conducted four times at two different sites in Castellano and once in the CLSU experimental area. New lures versus lures already in use for a certain time were compared in field trials in complete randomized designs and complete randomized block designs. The first trial was running for more than a month whereas the second to fourth trial for a week. The trial in CLSU was running in the Area II for a week from February 27 to March 5, 2003. The used lures stayed in the field for a certain time before they were used for the trials. The age of the lures is described in the respective section.

3.5.2 Material and Methods

Self made wing traps were used for all lure age trials. All traps were fastened to bamboo sticks in a height of 0.5 m.

- **Lure Age CLSU Experimental Area**

Eighteen traps were set in Area II (November 27, 2001). Only new and one week old lures were used. The lures were set at random with a minimum distance of 15 m between two traps. The set up was changed twice during the duration of the trial. The number of males caught was recorded from February 28 to March 5, 2003. Two traps without bait were set in the area as control.

- **Lure Age in Guimba**

Lures in the field for two months were compared with new lures, each containing 10 µg (E,E)-11,13-Hexadecadienal. Five traps each with new and old lures were set in a pak choi field (2 weeks after emerging of seedlings) in Guimba from March 5-11, 2003. The set up was changed three times and rearranged at random. Distance between two traps was at least 24 m. The field was monitored three times (March 1, 8, 11) for the occurrence of *H. undalis* larvae and other pests.

- **Lure Age 1.1 in Castellano**

Traps baited with 10 µg containing lures were set up in a mustard field in Castellano, January 31, 2003. The crop was ~ 6 weeks old. Three traps baited and three control traps were set from January 31 to February 3. Four traps with new lures were set February 3. All traps were placed at random in the field and stayed in their position until the field was completely harvested February 12. Distance between two traps was 25m. The size of the field was approximately 0.25 ha. The lures were further used in trial ‘Lure Age 1.2 in Castellano.

- **Lure Age 1.2 in Castellano**

In total, nine traps plus control traps without lures were set up at random in a radish field (0.5 ha) in Castellano, San Leonardo, approximately 2 km from the area where most other sex pheromone trials were conducted. The start of this long-term observation was 14 March 2003. Lures from trial ‘Lure Age 1.1 in Castellano’ were used. The used lures were in the field since February 31 March 4, so 14 and 11 days in the field. The new lures were taken out of the freezer March 14. At the time the trial was conducted, only one field was planted with a crucifer crop (radish), surrounding fields were planted with onion. The distance between the traps was 20 m; the distance between the lines was 30-45 m. The set up was newly arranged at random in weekly intervals. Traps were controlled three times per week, caught males counted, removed, and sticky paper replaced when necessary.

- **Lure Age 2 in Castellano**

The trial was set in three fields in Castellano next to the farmhouse of the field owners. Every field was seen as a block. Three replicates per block with nine traps were set with 27 traps. The traps were set at random within the blocks. To avoid bias due to the place of a trap, the arrangement was changed at random twice in one week. The distance between the traps from the different blocks closest to each other was at least 40 m, within the block at least 20 m. The ages of the lures were new, one week in the field and two weeks in the field when the trial started. The set up was not changed for the entire week (see Fig. 22). The traps were monitored six times, the catches recorded and removed. The trial was conducted from March 6-14, 2003.

- **Lure Age 3 in Castellano**

The same blocks used in trial 4.17.3 were used (Fig. 22). The lures were the same as in trial 4.17.3 but now one week, two weeks and three weeks in the field and set in a newly randomized pattern. The set up within the blocks was newly set at random two times between March 14 to 22.

- **Lure Age 4 in Castellano**

The blocks used in trial 4.17.3 and 4.17.4 were used again (Fig. 22). New lures, 16 days old and 32 days old lures were set in the three blocks at random. The set up was newly set at random two times, from March 22 to 28.

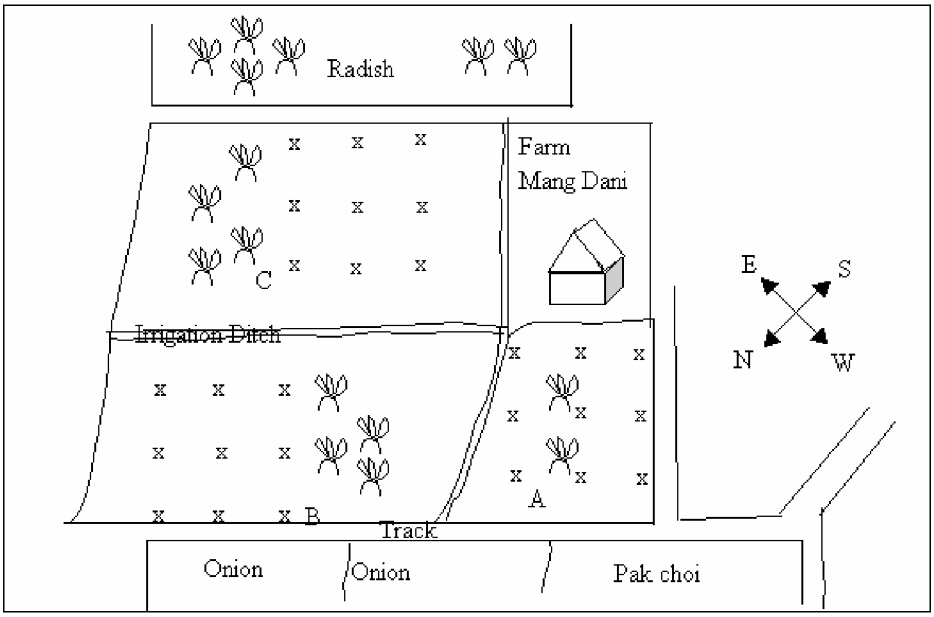

Figure 22: Set up of Lure Age Trial in Castellano. x = synthetic baited wing traps with 10µg lures different times in the field

3.5.3 Results

- **Lure Age CLSU Experimental Area**

The number of males caught was very low; only a total of 53 males were caught within the one-week duration of the trial. The average nightly catch rate per trap was 0.4 males caught. Numbers of males caught does not differ significantly (p= 0.34) between traps baited with new and lures one week old (Table 25). No catches were recorded for the control traps.

Lure Age	1 week old	New
Total	19	34
C/T/N	0.3	0.5
Mean ± SE	2.1 a ± 0.4	3.8 a ± 1.1
% of Total	35.8	64.2

Table 25: Effect of field aging of septa on *H. undalis* males caught in wing traps, baited with red rubber septa loaded with 10 µg (E, E)-11-13-Hexadecadienal. Catches of each nine traps with lures, one week in the field, and new lures were compared (February 27-March 5, 2003). Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test (p<0.05).

- **Lure Age in Guimba**

The total catch and nightly catch rate per trap was very small. Significantly, fewer males were attracted in traps baited with two-month-old lures (Table 26). The nightly catch rate was 5 times lower than with new lures. Infestation rates detected by monitoring plants for the occurrence of larvae resulted in 16 %, 24 %, and 28 %, March 1, 8, and 11, respectively.

Lure Age	2 Month old	New
Total	12	52
C/T/N	0.3	1.5
Mean ± SE	2.4 b ± 0.5	10.4 a ± 2.3
% of Total	19.7	81.3

Table 26: Effect of field aging of septa on *H. undalis* males caught in wing traps, baited with red rubber septa loaded with 10 µg (E, E)-11-13-Hexadecadienal. Catches of each five traps with lures, two month in the field, and new lures were compared (February 5-11). Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test ($p<0.05$).

- **Lure Age 1.1 in Castellano**

Lures in the field for three days and new lures do not attract significantly different numbers of males. Nonetheless, the numeric difference is obvious with a three times higher number of males caught in traps with new lures (Table 27). It is certain that catches in the control traps were caught accidentally. Monitoring of plants for the occurrence of pests resulted in an infestation of 14.5 % (February 6) and 50 % (February 11). *C. pavonana, Spodoptera litura, P. xylostella* and aphids were found in smaller numbers.

Lure Age	3 Days old*	New*	Control
Total	44	129	2
C/T/N	1.6	4.8	< 0.1
Mean Catch/Trap (Mean ± SE)	14.7 a ± 5.4	43.0 a ± 8.1	0.7 b ± 0.7
% of Total	25.1	73.7	1.1

Table 27: Effect of field aging of septa on *H. undalis* males caught in wing traps baited with red rubber septa loaded with 10 µg (E, E)-11-13-Hexadecadienal. Catches of three traps with lures, three days in the field, four traps baited with new lures and three traps without lures were compared (February 3-12). Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test ($p<0.05$). Total catch = 175 males. * Lures in the field, when trial started February 3.

- **Lure Age 1.2 in Castellano**

Significant differences regarding number of males caught were calculated between traps baited with new lures compared to traps baited with lures 14 days in the field for the first two weeks of the trial. Traps baited with lures 11 days in the field are in an intermediate position in that space of time. The newest lures attracted significant more males for the first four weeks (Tables 28-32). The share of total catches for the "new lures" is above 50% for all five weeks the lures were exposed in the field (Fig. 23).

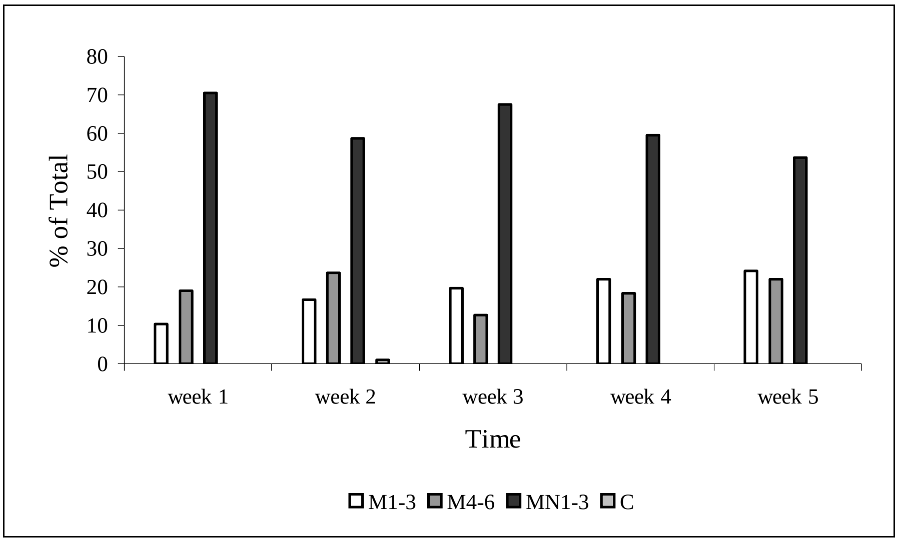

Figure 23: Percentage of captured *H. undalis* in traps baited with new lures, 11 and 14 days in the field, when trial started (February 14). Captures were recorded for a period of five weeks and analysed per week. M1-3: lures in the field for 14 days at the beginning of the trial; M4-6: lures in the field for 11 days at the beginning of the trial; MN1-3: lures in the field for 0 days at the beginning of the trial.

Total numbers of the first week for the different aged lures show a catch rate almost four and seven times higher for traps baited with new lures (Table 28). A similar relation is given for the second week. Numbers of males caught are closer together and total numbers are smaller compared to the first week (Table 29). Differences of males caught in traps baited with lures, two weeks in the field and traps baited with lures three and a half and four weeks in the field at the beginning of the third week, are significant (Table 30). This relation was also calculated for week four (Table 31). In the final week, the numbers of males were caught more or less in a similar range and were not significantly different (Table 32).

Monitoring of plants for occurrence of pests resulted in an infestation of 4 % (February 4), 14.6 % (February 12), 4 % (February 22), 5 % (February 26, 28) and 20 % (March 4, 18). Other pests found were *C. pavonana*, *S. litura*, *Trichoplusia ni and P. striolata* in small numbers and *P. xylostella* in decreasing numbers the older the crop was. The last monitoring resulted in 92 % plants infested with *P. xylostella* with up to 13 larvae on a single plant. Infestation with *P. xylostella*, when seedling just emerged was 6.7 % (February 12).

Lure Age (W1)	Total	C/T/N	Mean ± SE
14-22 do	36	1.7	12.0 b ± 4.9
11-19 do	66	2.1	22.0 ab ± 10.8
1-8 do	245	11.1	81.7 a ± 18.1
Control	0	0	-

Table 28: Effect of field aging of septa on *H. undalis* males caught in wing traps baited with red rubber septa loaded with 10 µg (E, E)-11-13-Hexadecadienal. Each three traps with lures 12 days, 15 days and newly set in the field at the beginning of the trial, and traps without lures were compared (February 15-22, 2003; Castellano, San Leonardo). Total Catch = 347 males. Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test ($p<0.05$). (W: week; do: days old; C/T/N: catch per trap per night; SE: standard error)

Lure Age (W2)	Total	C/T/N	Mean ± SE
23-28 do	50	2.8	16.7 b ± 4.8
20-25 do	71	3.9	23.7 ab ± 6.1
9-14 do	176	9.8	58.7 a ± 14.5
Control	3	0.5	-

Table 29: Effect of field aging of septa on *H. undalis* males caught in wing traps baited with red rubber septa loaded with 10 µg (E,E)-11-13-Hexadecadienal. Each three traps with lures 23 days, 20 days and 9 days in the field at the begin of the second week of the trial, and traps without lures were compared (February 23-28, 2003; Castellano, San Leonardo). Total Catch = 300 males. Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test ($p<0.05$). (W: week; do: days old; C/T/N: catch per trap per night; SE: standard error)

Lure Age (W3)	Total	C/T/N	Mean ± SE
29-35 do	14	0.7	4.7 b ± 2.0
26-32 do	9	0.4	3.0 b ± 1.0
15-21 do	48	2.3	16.0 a ± 1.5
Control	0	0	-

Table 30: Effect of field aging of septa on *H. undalis* males caught in wing traps baited with red rubber septa loaded with 10 µg (E,E)-11-13-Hexadecadienal. Each three traps with lures 29 days, 26 days and 15 days in the field at the begin of the third week of the trial, and traps without lures were compared (March 1-7, 2003; Castellano, San Leonardo). Total Catch = 71 males. Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test ($p<0.05$). (W: week; do: days old; C/T/N: catch per trap per night; SE: standard error)

Lure Age (W4)	Total	C/T/N	Mean ± SE
36-42 do	30	1.3	10.0 b ± 2.5
33-40 do	25	1.0	8.3 b ± 1.5
22-29 do	81	3.4	27.0 a ± 8.9
Control	0	0	-

Table 31: Effect of field aging of septa on *H. undalis* males caught in wing traps baited with red rubber septa loaded with 10 µg (E,E)-11-13-Hexadecadienal. Each three traps with lures 36 days, 33 days and 22 days in the field at the begin of the fourth week of the trial, and traps without lures were compared (March 8-15, 2003; Castellano, San Leonardo). Total Catch = 136 males. Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test ($p<0.05$). (W: week; do: days old; C/T/N: catch per trap per night; SE: standard error)

Lure Age (W5)	Total	C/T/N	Mean ± SE
43-50 do	23	1.1	7.7 a ± 2.0
41-47 do	21	1.0	7.0 a ± 2.6
30-36 do	51	2.4	17.0 a ± 9.8
Control	0	0	-

Table 32: Effect of field aging of septa on *H. undalis* males caught in wing traps baited with red rubber septa loaded with 10 µg (E,E)-11-13-Hexadecadienal. Each three traps with lures 44 days, 41 days and 30 days in the field at the begin of the fifth week of the trial, and traps without lures were compared (March 16-22, 2003; Castellano, San Leonardo). Total Catch = 95 males. Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test ($p<0.05$). (W: week; do: days old; C/T/N: catch per trap per night; SE: standard error)

- **Lure Age 2**

Results indicate a significant relationship between catches of male *H. undalis* and the time dispensers were exposed in the field (Fig. 24). Catches of 2-week-old dispensers were significantly lower than with new septa. Catches in traps baited with one-week-old septa fall in a range of males caught between new and 2 week old lures. The nightly catch rate was above 3 in all traps (Table 33).

Lure Age (do)	Total	C/T/N	% of total
0-7	370	5.9	45.9
7-14	233	3.7	28.9
14-21	202	3.2	25.1

Table 33: Catches of male *H. undalis*, nightly catch rate and percentage of the total catch in wing traps with lures of different age ranges in San Leonardo (March 7-14, 2003). do: days old; C/T/N: catch per trap per night; Total Catch = 805 males

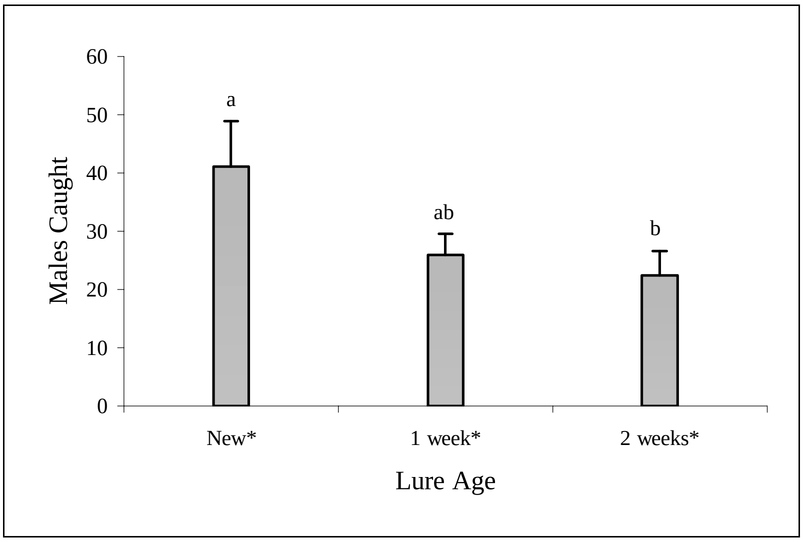

Figure 24: Effect of field aging of septa on *H. undalis* males captured in wing traps baited with red rubber septa loaded with 10 µg (E,E)-11-13-Hexadecadienal (March 7-14, 2003). Nine wing traps were used for each age group (new, 1 week, 2 weeks in the field at the beginning of the trial). Means followed by a common letter are not significantly different at the 5% level by Fischer's Least Square Distance Test ($p<0.05$). *: Lures in the field when the trial started

- **Lure Age 3**

Results indicate a significantly lower catch rate between lures already two and three weeks in the field compared to those only one week in the field (Fig. 25). What is irritating is the catch rate of two-week-old lures, which is lower, than the three-week-old ones. The nightly catch rate is close to one assuming the catches could be by chance (Table 34).

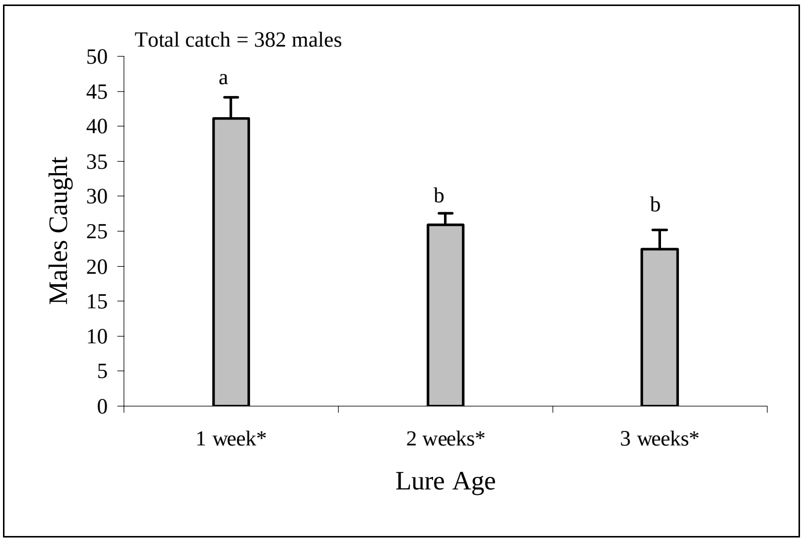

Figure 25: Effect of field aging of septa on *H. undalis* males captured in wing traps baited with red rubber septa loaded with 10 µg (E,E)-11-13-Hexadecadienal (March 15-22, 2003). Nine wing traps were used for each age group (1 week, 2 weeks, 3 weeks in the field at the beginning of the trial).Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test ($p<0.05$). *: Lures in the field when the trial started

Lure Age (do)	Total	C/T/N	% of total
8-16	197	2.7	51.6
15-23	74	1	19.4
22-30	111	1.5	29.1

Table 34: Catches of male *H. undalis*, nightly catch rate and percentage of the total catch in wing traps with lures of different age ranges San Leonardo (March 15 –22, 2003). do: days old; C/T/N: catch per trap per night; Total Catch = 382 males

- **Lure Age 4**

Traps baited with new lures attract and catch significantly more males than those baited with two and four week old lures (Fig. 26). The quantity of males caught between two and four week old lures is also significantly different. The nightly catch rate is above 1 per night and trap for all treatments (Table 35).

Lure Age (do)	Total	C/T/N	% of total
0-6	181	3.4	51.6
16-22	110	2	31.3
32-38	60	1.1	17.1

Table 35: Catches of male *H. undalis*, nightly catch rate and percentage of the total catch in wing traps with lures of different age ranges San Leonardo (March 22 –28, 2003). do: days old; C/T/N: catch per trap per night; Total Catch = 351 males

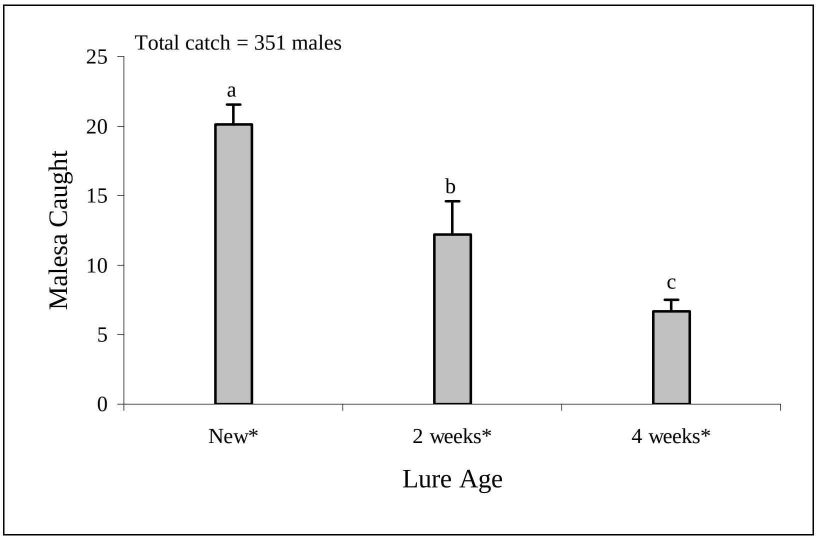

Figure 26: Effect of field aging of septa on *H. undalis* males captured in wing traps baited with red rubber septa loaded with 10 µg (E,E)-11-13-Hexadecadienal (March 22-28, 2003). Nine wing traps were used for each age group (new, 2 weeks, 4 weeks in the field at the beginning of the trial).Means followed by a common letter are not significantly different at the 5% level by Fischer's Least Square Distance Test ($p<0.05$). * Lures in the field when trial started.

3.5.4 Discussion

Attraction of 10 µg containing lures was determined by comparing traps baited with new and lures been used for different durations. The results clearly show the time span of a lure before the attraction of a lure declines significantly. Therefore, the duration of rubber septa loaded with 10 µg of the active ingredient for optimal catch performance was determined to be two weeks.

Longevity of pheromones is highly dependent on the chemical structure. The aldehyde-rest is known to be quite reactive in the air and oxidizes easily to the carboxylic group (HOWSE et al., 1998). Combinations of pheromones consisting of an aldehydic-rest and a conjugated

diene system degrade faster compared to pheromones with an acetate or alcohol rest (HOWSE et al., 1998). One reason that the identified compound was never tested in field trials might be due to the reactive rest. Degradation of E, E-11,13- Hexadecadienal stopped the examination of the synthetic compound in field trials in Malaysia (Cork, Wizard Project, 1997-1998).
Release device in all trials were red rubber septa. Rubber septa are the most used release device due to easy handling and availability in pheromone distributing companies (WEATHERSTONE, 1989) and are considered as industry standard of pheromone lures against which new lures are measured. Fast degradation of aldehydic pheromones was found when rubber septa contained 1,2-dianilinoethane, which reacts with the aldehydes to imidazolidines (STECK et al., 1979). It is proposed to use rubber septa free of this compound (STECK et al., 1979).

Inherent instability of aldehydes under field conditions were assumed by ADATI and TATSUKI (1999) for the pheromone component E,E-10,12 –Hexadecadienal of *Maruca vitrata* and problems of the possible use of this compound in the field. Monitoring *P. xylostella* for a whole summer was reported in trials in Estonia (MÔTTUS et al., 1997) with rubber minidispensers (K-50). The activity of the blend was inhibited when more than 50% of the aldehyde component had decomposed.
It seems that the dosage plays a minor role in attracting males due to the chemical structure of the pheromone, since degradation might be occurring within 2-4 weeks under field conditions. This can be determined by the results of the lure age trials. No differences between new and two week old lures as measured with number of males caught were given and up to three weeks, lures were attractive in the same range as new lures.
The ‘Lure Age Trial 1.2 in Castellano’ shows an adaptation between the catch rates of the different aged lures. Males are equally attracted after five weeks. The nightly catch rate is just above the critical level where males could be caught by chance by the older lures. Accidentally caught males in control traps refer to a very little probability that males were caught by chance (0.3 % of all males caught were found in control traps).
Recommendation for changing lures for monitoring *Ostrinia nubilalis* (European corn borer, a pest of pepper in the USA) is every three to four weeks (http://www.hort.uconn.edu/ipm/veg/htms/pprborer.htm, 10.10.04), whereas for the artichoke plume moth (*Platyptilia carduidactyla*) it is more than two month (http://www.sarep.ucdavis.edu/newsltr/v14n1/sa-4.htm, 10.10.04).

3.6 Level of Attraction of Stored Lures

3.6.1 Introduction

Pherobank, the supplier of the lures used in these trials (http://www.plant.wageningen-ur.nl/default.asp?section=products, 02.10.01), recommends storage of lures in a freezer. Cooling was not possible when packages were sent from the Netherlands to the Philippines by airplane and some problems emerged in the Philippines to get them delivered to CLSU. Lures sent October 2001 stayed in an office in Cabanatuan City over the weekend at ambient high temperatures. Nonetheless, the mean time from sending to receipt was 5 days on average. Lures were protected in screwed up plastic boxes with an extra sealed PE bag. The effect of degradation was examined for lures stored in the freezer up to one year by comparing catch rates.

3.6.2 Material and Methods

Lures sent to the Philippines from Dr. Griepink from October 2001, February 2002, August 2002 (pure and stock solution) all unused and stored in a freezer and lures from August 2002 (pure solution) used for 9 days in the field were set in the CLSU experimental area. The trial took place February 4-10, 2003 in Area II. Intertrap distance was 15-20 m. The set up was changed three times in a completely randomized design. Lures stayed in the field the entire trial. Catches were counted, recorded and removed every morning between 8:00 h-10:00h.

3.6.3 Results

All lures tested show similar catch rates except the ones used for one week in a field (Table 36). The nightly catch rate is above the assumed by chance. Reduced catch rate of traps baited with lures already 9 nights in the field is significant compared to the other lures tested. Lures from February 2002 had a very low catch rate and only about half of catches compared to the other unused lures, but significance is not evident. Lures stored for more than 1 year attracted most male *H. undalis*.

Date Lures prepared	Total	C/T/N	Mean ± SE	% of total
Oct. '01	67	3.2	22.3 a ± 2.6	28.6
Feb. '02	30	1.7	10.0 ab ± 1.5	12.8
Aug. '02-stock	53	2.9	17.7 a ± 6.2	22.6
Aug. '02-pure	60	3.3	20.0 a ± 1.2	25.6
Aug. '02 *	24	1.3	8.0 b ± 3.6	10.2

Table 36: Catches of male *H. undalis* in traps baited with 10µg lures, prepared Oct. 2001, February and August 2002 and used lures prepared August 2002. Total catch = 234 males. Aug. '02*: Lures stayed in the field from 20.1.-29.1.2002 and were then stored in a freezer until used for the trial.

3.6.4 Discussion

Pherobank, the supplier of the used compound, advice to store lures in a refrigerator or freezer (lures are guaranteed for two years when kept by –25°C or one year when kept in a refrigerator, http://www.plant.wageningen-ur.nl/default.asp?section=products&page=/products/pherobank/right.htm). Since (E,E)-11-13-Hexadecadienal was not tested before in field trials, the period it remains unaffected, when stored in a freezer, was uncertain.

Lures stored for more than one year attracted as many, or even more males than lures, that were prepared about one year after the first lures were used in field trials. Concerns that the compound easily degrades cannot be confirmed, at least when lures were kept in a freezer (-7°C) in their original containers. Lures were not affected in their ability to attract males due to the transport. These findings are clearly encouraging, both for the producer but also for the user, that working with this special compound gives reliable results. In another context, it is important to know for the scientist that all lures- new and stored- used, are uniform in their effect and results obtained with lures from different shipments are to assess equally. The comparison with lures already in the field shows the time of optimal attraction but trap placement and possible effects must be considered even though the arrangement of traps was changed. Nonetheless, catches were decreasing significantly within less than ten days when lures were exposed to heat and sunlight.

Chapter 4

Biological and Ecological Aspects

4 Biological and Ecological Examinations of *H. undalis* in the Philippines

4.1 Identification of Natural Host Plants of *H. undalis*

4.1.1 Introduction

H. undalis host range comprises mainly plants of the family Brassicaceae but also plants from closely related families. Although *H. undalis* larvae were found on *Hygrophilia salicifolia* (Acanthaceae) in Malaysia (YUNUS and HO, 1980 in SIVAPRAGASAM, 1994) it remains uncertain whether larvae were actually feeding on it (SIVAPRAGASAM, 1994). Members of the family Capparidaceae were reported as host plants (SIVAPRAGASAM and AZIZ, 1992; SIVAPRAGASAM et al., 1994; SIVAPRAGASAM and CHUA, 1997a; REJESUS and JAVIER, 1997) of *H. undalis* in Malaysia and the Philippines. Larvae feeding on *Cleome sp.* were observed in various countries, both *H. undalis* and another species of the genera *Hellula*, i.e. *H. phidilealis* (ALAM, 1989). *C. viscosa* is one of the three most dominant weeds in onion fields in a survey of weeds in San José, Nueva Ecija (BALTAZAR et al., 1998). Thus, examination of the occurrence of *C. viscosa* and *C. rutidosperma* in different regions of Nueva Ecija and infestation with *H. undalis* larvae was conducted to determine infestation of *Cleome sp.* with *H. undalis* and other pests.

4.1.2 Material and Methods

Plants next to crucifer fields, along field road ditches, beside rice paddies and plants in a ditch near the city centre of Cabanatuan were examined for the occurrence of *H. undalis* larvae.. Monitoring was carried out in the CLSU campus area, and in Castellano, San Leonardo. Monitoring took place in the dry seasons 2002 and 2003. Identified host plants found in the experimental area were monitored for the occurrence of *H. undalis* larvae.

4.1.3 Results

Cleome rutidosperma and *C. viscosa* were identified as natural host plants of *H. undalis*. Monitoring of plants in road ditches and beside fields amounted mostly in very low infestation rates below 10 % of examined plants. Monitoring in the Castellano area and inside the CLSU campus compared the infestation between *C. rutidosperma* and *C. viscosa* with 10 plants of each species examined for larvae of *H. undalis*. In Castellano there were 11 larvae detected on six plants on *C. viscosa*, whereas on *C. rutidosperma* there were only 2 larvae on 10 plants. No other insects or signs of feeding were found on *C. viscosa* beside of *H. undalis*. *Crocidolomia pavonana*, *Pieris sp.*, *P. striolata*, P. *cruciferae* and leafminer signs were found on *C. rutidosperma*. Multiple infestations were observed.

Inside the CLSU campus in an area designated for growing rice *C. viscosa* and C. *rutidosperma* were found in high numbers next to the fields along a path. Infestation with *H. undalis* on *C. viscosa* was higher with six of ten plants infested compared to *C. rutidosperma* with one plant out of ten. Other insects were found on both species, namely two species of fleabeetles *Phyllotreta striolata*, *P. cruciferae* and *Spodoptera sp*. The larvae were found in the blossoms, which were held together with silken threads (Fig. 27). Other parts of the plants with larvae included the shoots and leaves.

Monitoring in the experimental area was carried out three times in February 2003. Infestation of plants adjacent to Area II reached 56 %, 52 %, 36 %, and 52 % on *C. viscosa* and 28 %, 32 %, 24 % and 32 % on *C. rutidosperma*, January 31 and February 7, 14 and 21, respectively. Infestation on planted crucifers in the experimental area was above 50 % both in Area I and in II in January/February and the plants were all in bad condition.

C. rutidosperma was found in many locations in Nueva Ecija blooming throughout the year, whereas *C. viscosa* blooms mainly in the dry season.


Figure 27: *H. undalis* larvae on *C. rutidosperma* (left) and *C. viscosa* (right). Webbing was slightly opened to make 3rd instar larvae visible.

4.1.4 Discussion

Cleome viscosa and *C. rutidosperma* were identified as natural host plants of *H. undalis* and other pests (*P. striolata, P. Cruciferae, Spodopetar sp, C. pavonana*) in the Philippines. Monitoring of plants adjacent to cultivated Brassicaceae showed infestation rates of, particularly *C. viscosa*, similar to those in the planted crops. Larvae of *H. undalis* were found in all regions in which the two *Cleome* species were examined, even inside an urban centre (Cabanatuan), in a road ditch. REJESUS and JAVIER (1997) reported infestations of *H. undalis* larvae on *C. viscosa* in the Philippines and it was found in other countries for *H. undalis* and the related species *H. phidilealis* (ALAM, 1989: cited in SIVAPRAGASAM and CHUA, 2000; SIVAPRAGASAM, 1994; SIVAPRAGASAM and CHUA, 2000). SIVAPRAGASAM (1994) emphasized the potential importance of *C. rutidosperma* as host plant when male *H. undalis w*ere caught in the absence of cultivated Brassicaceae. Infestation rates are rather low with a maximum of 3.5% and are explained by low survivorship of larvae and the assumption that only one larva is usually able to feed on one plant. The high infestation of *C. viscosa* (up to 50 %) found in the CLSU experimental area, next to planted crucifer fields, could be explained with the absence of pesticide use and the general high infestation at this time of the year, but results of San Leonardo with infestations of up 60 % indicate the wide acceptance of *C. viscosa* as host plant. A survey of weeds in cotton fields by PALLER and LIJAUCO (1984) in Nueva Ecija and adjacent provinces showed that *C. rutidosperma* lowered the yield significantly when the control was not successful. Another survey of weeds in rice and onion fields conducted by BALTAZAR et al. (1998) showed *C. viscosa* as one of three most dominant broad leave weeds in onion fields in two locations in Nueva Ecija. *C. rutidosperma* is described as a dominant species of weeds in vegetable growing areas in this survey.

Thus, the role of *Cleome sp*. cannot be underestimated and is probably a key factor in the expansion and survival of *H. undalis* at times cruciferous vegetables are not cultivated in large numbers.

4.2 Identification of Secondary Plant Metabolites in Host Plants of *H. undalis*

4.2.1 Introduction

As mentioned in Chapter 4.1 members of the family Capparaceae such as *C. rutidosperma* and *C. viscosa* were identified as host plants of *H. undalis*. Moreover, members of Brassicaceae such as pak choi, which is cultivated as an important vegetable of the Philippines are also attractive host plants. These plant families belong to the order Capparales whose members commonly possess Glucosinolates (GSL) as characteristic secondary plant metabolites (BENNETT et al., 2004). GSL are sulfur-containing glucosides which are derived from amino acids. More than 100 GSL are known which only differ from each other in the structure of their aglycon moieties; these are generally classified as aliphatic, aromatic, or indole. Each plant species has its own individual GSL pattern which has been suggested as an important chemo-taxonomic criterion for classification of the plant species within the different families (FENWICK et al., 1983). Disruption of the plant tissue initiates a rapid hydrolysis of GSL to yield mainly isothiocyanates (ITC) which besides intact GSL act as host plant attractants of *H. undalis*. Moreover, oxidative degradation products of plant lipids which are produced when enzymes are liberated after plant tissue damage such as 3-hexenal and 3-hexenol also play an important role in plant-insect interactions (REDDY and GUERRERO 2000). In order to provide evidence about the attractivity of the different bioactive substances to *H. undalis*, the GSL in the different host plants and additionally, the essential oil of *C. viscosa* was isolated and identified.

4.2.2 Materials and Methods

4.2.2.1 Analysis of Intact Glucosinolates

Analysis were carried out with both freeze dried leaves and seeds of *C. viscosa*, *C. rutidosperma* and the pak choi cultivar "Black Behi" (*Brassica campestris*) a common cultivated cruciferous vegetable on the Philippines. The freeze dried material was ground with a Culatti MFC grinder equipped with a 1-mm sieve. For glucosinolate extraction 1 g of freeze dried leaves and 0.2 g grinded seeds were extracted twice with 70 % (v/v) of boiling methanol at 75 °C for 10 min by ultrasonic treatment, respectively. The filtrates were combined and concentrated to about 4 ml using a rotary evaporator under reduced pressure. For removal of present protein 1 ml of a solution of 0.4 mol barium acetate was added. The extracts were transferred to 10 ml flasks and filled up with distilled water. For high performance liquid chromatography (HPLC) analysis an aliquot of the diluted extracts were centrifuged by 10 000 rpm for 3 min and afterwards filtered through a 0.2 µm membrane filter.

HPLC analysis was conducted using a Dionex HPLC model P580A equipped with a photodiode array detector (DAD). For separation of the GSL a LiChrospher 100RP-18 column (250 × 4.6 mm i.d., 5 µm) was used. The absorbance of the effluent was monitored at 230 nm. Chromatography was performed at 25 °C, at a flow rate of 1.0 ml min^{-1} using the following solvent system: (A) 0.1 mol NH_4CH_3COO and (B) 0.1 mol NH_4CH_3COO in 33 % (v/v) methanol. The initial solvent condition was 100 % A, hold for 6 min. Afterwards a gradient was used to increase solvent B from 0 to 70 % within 15 min following from 70 to 100 % B within 3 min. Finally, this solvent composition was maintained for 16 min. The total time for analysis of one sample was 40 min.

4.2.2.2 Analysis of Volatile Substances

100 g of frozen leaves of *C. viscosa* were given in a 2 l round-bottom flask. Subsequently, 1 l of distilled water was added. The mixture was extracted by simultaneous steam distillation and solvent extraction (SDE) with pentane as solvent for two hours. For qualitative analysis, an aliquot of the diluted essential oil extract was injected directly into the injection port of the gas chromatograph.

Essential oil extracts were analysed by gas chromatogarpy/mass spectrometry (GC/MS) using an Hewlett-Packard (HP) 5890 series II gas chromatograph equipped with a 60 m × 0.25 mm Supelcowax 10 fused silica capillary column (film thickness 0.25 µm) interfaced to a HP 7971 Mass Selective Detector (MSD). Injections were made in splitless mode at 240 °C for 5 min, and the MSD used a scanning range of 40-250 amu. Carrier gas was helium (1 ml min^{-1}). The oven temperature was 50 °C (3 min) and than programmed at 10° min^{-1} to 120 °C (2 min), at 2° min^{-1} to 155 °C, and finally at 8° min^{-1} to 240 °C (30 min). Compounds were identified by comparisons of the mass spectral data and retention time matches with standard compounds.

4.2.3 Results

4.2.3.1 Glucosinolate Pattern in *Cleome* species

The investigated *Cleome* species had very simple glucosinolate pattern with only three (Fig. 28) and four (Fig. 29) detected GSL, respectively, detected by HPLC.

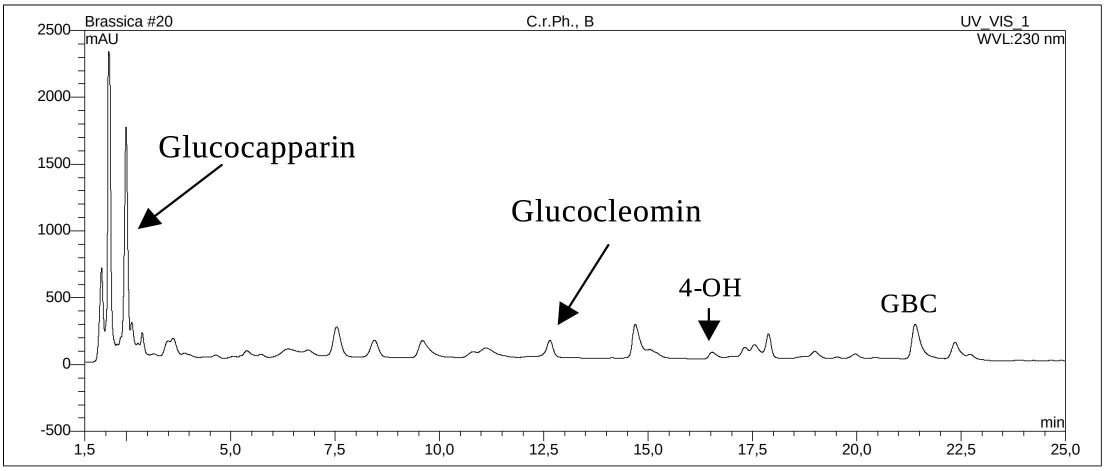

Figure 28: HPLC chromatogram of GSL from leave extract of C. rutidosperma. GBC: glucobrassicin

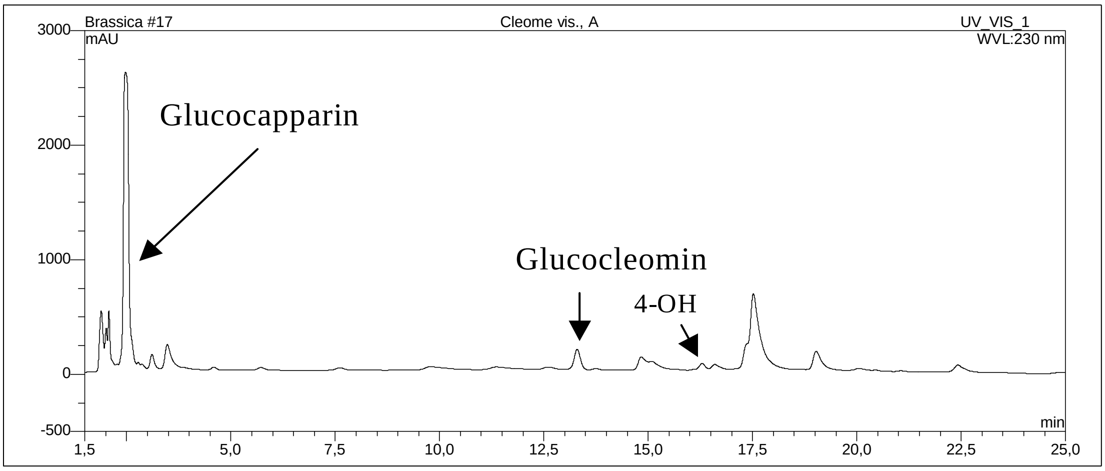

Figure 29: HPLC chromatogram of GSL from leave extract of *C. viscosa.*

Both, *C. rutidosperma* and *C. viscosa* had glucocapparin (methylglucosinolate) as the predominant GSL compound containing more than 90 % of the total. Moreover, glucocleomin (2,2(R,S)-hydroxymethylglucosinolate) was detected in both chromatograms. These GSL is characteristic for Cleome species, therefore its trivial name is deduced of the botanical name. Indolyl-GSL such as 4-OH (4-hydroxyglucobrassicin) and glucobrassicin (GBC) occurred only in traces in the *Cleome* species. The total GSL content of the aliphatic compounds was much higher in *C. viscosa* than in *C. rutidosperma*; the concentration of glucocapparin was about 36 % and glucocleomin at about 30 % higher in *C. viscosa* than in *C. rutidosperma*. On the other hand, the amounts of Indoly GSL were higher in *C. rutidosperma*.

4.2.3.2 Glucosinolate Pattern in Pak Choi

The GSL pattern in leaves of pak choi showed seven individual components (Fig. 30). In opposite to the *Cleome* species, no dominating GSL compound exists in pak choi. Three compounds possess an aliphatic and an indolyl aglucon rest, respectively. Among aromatic GSL only gluconasturtiin was found.

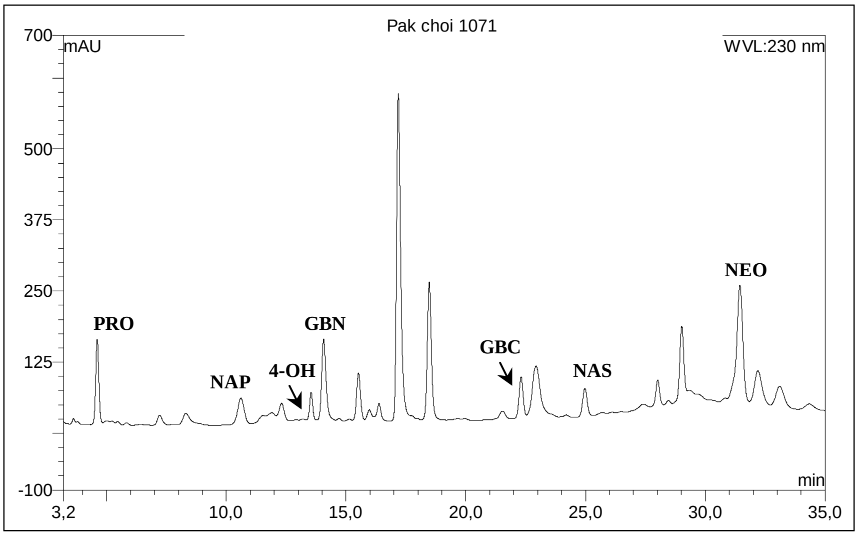

Figure 30: HPLC chromatogram of GSL from leave extract of pak choi. PRO = Progoitrin, NAP = Gluconapin, 4-OH = 4-Hydroxyglucobrassicin, GBN = Glucobrassicanapin, GBC = Glucobrassicin, NAS = Gluconasturtiin, NEO = Neoglucobrassicin

4.2.3.3 Essential Oil of *Cleome viscosa*

It is well known, that green leaf volatiles, such as saturated and nonsaturated short-chain aliphatic alcohols, aldehydes, and acetates among others act as host-plant attractants (REDDY AND GUERRERO, 2000). These compounds are formed by oxidative degradation of surface plant lipids when enzymes are liberated after plant tissue damage.

In order to elucidate the volatile compounds of Cleome leaves the essential oil of *C. viscosa* was extracted and analysed for its composition.

4.2.3.4 Composition of the Essential Oil of *Cleome viscosa*

Most components were mono- and sesquiterpenes which are characteristic volatile compounds of an essential oil existing genuine in the plant (Fig. 31). Moreover, some oxidative degradation products of plant lipids were detected.

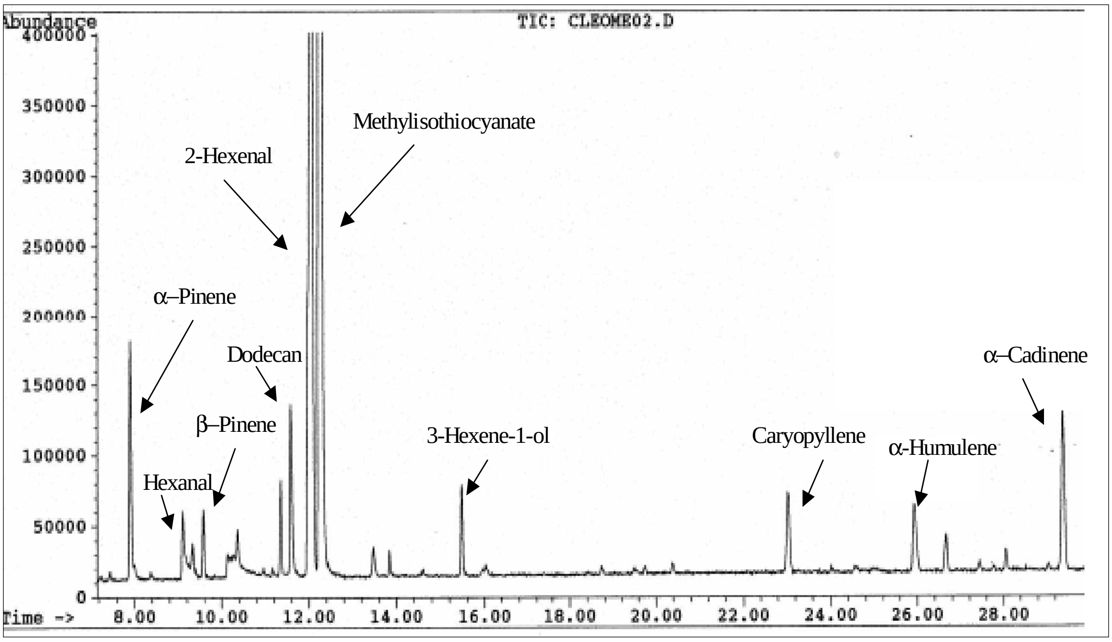

Figure 31: Gas chromatogram of the essential oils of *C. viscosa*.

Major compounds of the essential oil were methyl-ITC and 2-hexenal (Table 37). Methyl-ITC is formed by enzymatic or thermally degradation of capparin (methylglucosinolate), the predominant GSL found in the *Cleome* species. Other volatile breakdown products of GSL were not detected in the essential oil. Main components of the essential oil of *C. viscosa* are listed in Table 37.

No.	Compound	Percent of the total essential oil (%)
1	α–Pinene	1.8
2	Hexanal	0.5
3	β–Pinene	0.4
4	Dodecan	1.0
5	2-Hexenal	21.4
6	Methyl isothiocyanate	69.1
7	3-Hexen-1-ol	0.8
8	Caryopyllene	1.0
9	α–Humulene	0.8
10	α–Cadinene	1.7
11	others	1.5

Table 37: Major volatiles and their percentage in the essential oil of *Cleome viscosa*.

4.2.4 Discussion

It is well known that several hydrolysis products of GSL act as host plant attractants for *H. undalis*. As Cleome are used as natural host plant of them, it seems evident that methylisothiocyanate, the only detected isothiocyanate in *Cleome*, possess high attractivity to *H. undalis*. Moreover, the identified oxidative degradation products of plant lipids, such as 2-

hexenal and 3-hexen-1-ol obviously also act as efficient host-plants attractants, which was the result of investigations with *Plutella xylostella* (REDDY AND GUERRERO, 2000).

4.3 Comparison of the Catch Rate of Virgin Female and Synthetic Pheromone Baited Traps

4.3.1 Introduction

Setting synthetic pheromone baited traps in a field creates competition between the natural and synthetic source of attraction. It was determined that E,E-11,13-Hexadecadienal attracts male *H. undalis*. The quantity of males caught with the synthetic pheromone compared to virgin females as bait gives answers to questions regarding the completeness of the studied compound, whether its competitive or not. Differences in the catch rate could point to the fact that the identified single compound sex pheromone of *H. undalis* comprises of more than a single molecule or geographical differences in the biochemistry of the sex pheromone.

4.3.2 Material and Methods

Virgin female and synthetic pheromone baited traps conducted field trials to determine the catch rate.

Ends of film boxes were removed and covered with mesh net to cage virgin females from the own rearing. Holes were punched with a single hole puncher and openings were covered with mesh net glued on the plastic surface. Steel pins fixed the boxes inside the trap on the bottom.

All trials were carried out in the experimental area at CLSU. Trap distances were between 15 and 20 m. Females used were set in the field from the night following eclosion up to 3 days after eclosion. The age of the females depends upon the night they eclosed, i.e. a female referred to as one day old becomes this age during the night in the course of the trial. Both females and rubber septa were collected in the morning and stored in the office during the day in trials longer than one night. New lures were used for every new set up. Radish, mustard and pak choi were planted on the field; surrounding crops were tomatoes, string beans, pak choi and onion. The number of traps with one or the other bait was not consistently the same (Table 38). Trials 1 and 2 were set in a completely randomized design; trials 3-5 in a complete randomized block design. Due to the size of the field (Area II) some traps had to be placed outside of it. The set up of trials took place around 17:00 h.

	Traps baited with VF				Traps baited with 10 µg SP	
No. of Trial	Date of control	No. of traps	Age of VF (days old)	No. of VF/trap	No. of traps	Lure 1st time in the field
1	31.1. -1.2.	3	1	1	6	30.1.
2	18.2. -19.2.	3 3 3	1 1 1	1(n=3) 2(n=3) 3(n=3)	3	17.2.
3	23.2.	6 5 1	3(n=3) 1(n=5) 5(n=1)	1 1 1	6	22.2
4	24.2. -25.2.	9	3	1	9	23.2
5	26.2	3	3	1	12	25.2.

Table 38: Date, number of traps baited with virgin females (VF) and synthetic pheromone (SP), age of virgin females and numbers of virgin females per trap (Trial 2). The lures used were taken off the freezer directly before the trials were set up.

4.3.3 Results

Trial 1: Traps baited with virgin females attracted significantly more males than those baited with the synthetic pheromone (Table 39). The increase in catches per night is 5.5 times higher in traps with the virgin females (Fig. 32).

Bait	SP (10µg)	VF
Total	21	56
Mean ± SE	3.5 b ± 1.5	18.7 a ± 6.0
% of total	27.3	72.7

Table 39: Comparison of synthetic pheromone (SP; n=6) versus virgin females (VF; n=3) baited traps in a crucifer field (January 31- February 1, 2003; CLSU experimental area). Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test. SP: synthetic pheromone, VF: virgin female, C/T/N: catches per trap per night. Total catch = 77 males

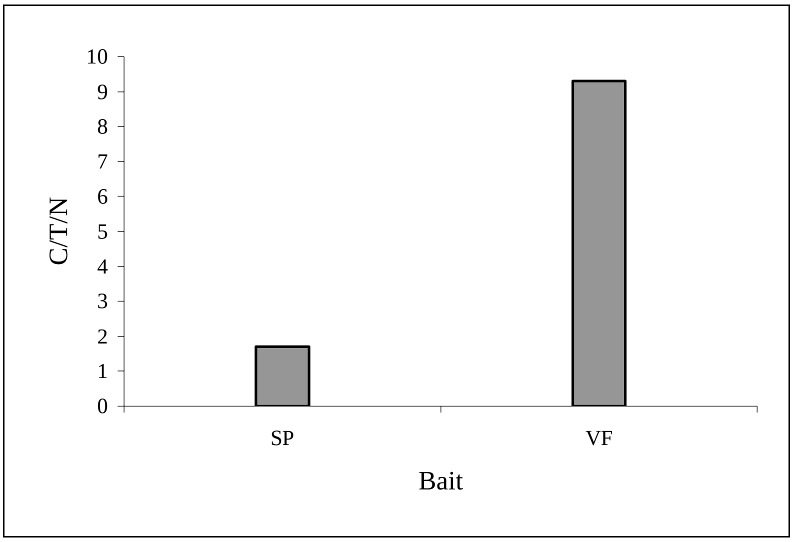

Figure 32: Capture rate of male *H. undalis* per trap per night (C/T/N). Traps were baited with virgin females (VF) and red rubber septa with the synthetic pheromone (SP), (January 31- February 1, 2003; CLSU experimental area). SP: n=6; VF: n=3

Trial 2: Traps baited with different numbers of virgin females attracted significantly more males than those baited with the synthetic pheromone (Table 40). Three virgin females as bait were in an intermediate position. The nightly catch rate is three, four and six times higher with three, one, and two virgin females as bait than traps baited with the synthetic pheromone (Fig. 33).

	Bait			
	1 VF	2 VF	3 VF	10 µg
Total	90	152	78	24
C/T/N	15.0	25.3	13.0	4.0
Mean ± SE	30.0 a ± 12.0	50.7 a ± 17.9	26.0 ab ± 12.5	8.0 b ± 1.5
% of total	26.2	44.2	22.7	6.9

Table 40: Catches of male *H. undalis* in traps baited with 1, 2 or 3 virgin females and 10 µg loaded red rubber septa. Trial was conducted in CLSU experimental area, February 18-19, 2003. Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test. SP: synthetic pheromone, VF: virgin female, C/T/N: catches per trap per night. Total catch = 344 males

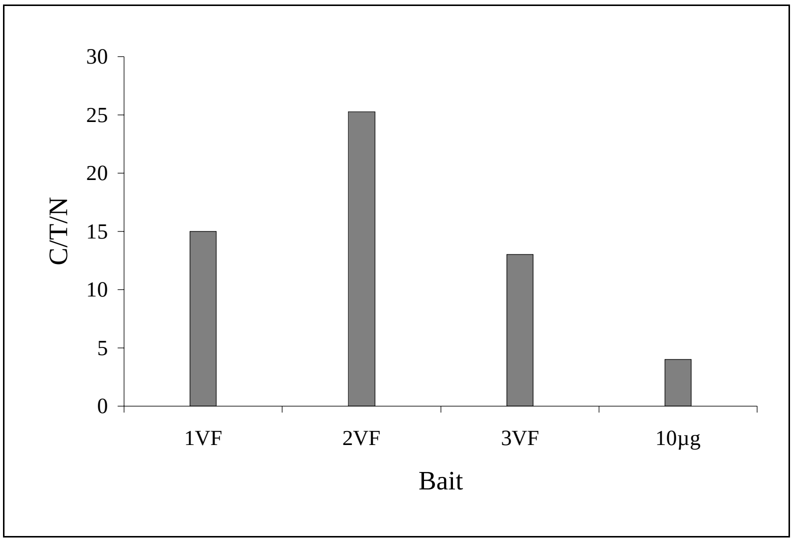

Figure 33: Capture rate of male *H. undalis* per trap and night. Traps were baited with 1,2 or 3 virgin females as bait compared with synthetic pheromone lures. Three traps for each treatment were set in CLSU experimental area (February 18-19).

Trial 3: Traps baited with virgin females with different ages attracted a significantly different number of male *H. undalis* than those baited with the synthetic pheromone (Table 41). The catch rate per night and trap is around 9 times lesser in traps baited with the synthetic pheromone compared to virgin females one or five days old and almost 19 times lesser than the three day old virgin females (Fig. 34).

Bait	SP (n=6)	VF 1*(n=6)	VF 2**(n=6)
Total	9	167	82
Mean ± SE	1.5 b ± 0.8	27.8 a ± 14.1	13.7 a ± 3.5
% of total	3.5	64.7	31.8

Table 41: Comparison of synthetic pheromone (SP; n=6) versus virgin females three days old or with another age after eclosion (VF 1; n=6; VF 2; n=6). Baited traps were set in a field in CLSU experimental area (February 22-23, 2003). Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test. SP: synthetic pheromone, VF: virgin female, C/T/N: catches per trap per night; * females 3 days old; ** five virgin females 1 day old, one female 5 days old. Total catch = 258 males

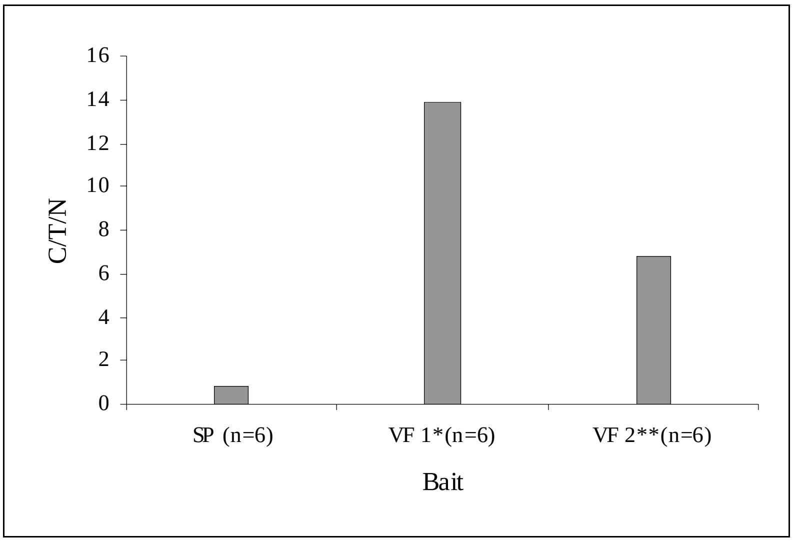

Figure 34: Capture rate of male *H. undalis* per trap and night. Traps were baited with virgin females in different age ranges as bait compared with synthetic pheromone lures in the CLSU experimental area (February 22-23). SP: synthetic pheromone, VF: virgin female, do: days old.

Trial 4: Traps baited with virgin females attracted a significantly different number of male *H. undalis* than those baited with the synthetic pheromone (Table 42). Numbers of males caught in traps baited with the synthetic pheromone were 20 times less compared to virgin female baited traps.

Bait	SP (n=9)	VF (n=9)
Total	9	179
C/T/N	0.5	9.9
Mean Catch (Mean ± SE)	1.0 b ± 0.3	19.9 a ± 7.1
% of total	4.8	95.2

Table 42: Comparison of synthetic pheromone (SP; n=9) versus virgin females (VF; n=9) baited traps in a field in CLSU experimental area (February 24-25). Means followed by a common letter are not significantly different at the 5% level by Fischer’s LSD Test. SP: synthetic pheromone, VF: virgin female, C/T/N: catches per trap per night. Total catch = 188 males

Trial 5: Traps baited with virgin females attracted a significantly different number of male *H. undalis* than those baited with the synthetic pheromone (Table 43). Number of males caught in traps baited with virgin females compared to the synthetic pheromone baited traps was 25 times higher.

Bait	SP (n=9)	VF (n=3)
Total	9	77
C/T/N	1	25.7
Mean Catch (Mean ± SE)	1.0 b ± 0.3	25.7 a ± 15.0
% of total	10.5	89.5

Table 43: Comparison of synthetic pheromone (SP; n=9) versus virgin females (VF; n=3) baited traps in a field in CLSU experimental area (February 26). Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test. SP: synthetic pheromone, VF: virgin female, C/T/N: catches per trap per night. Total catch = 86 males

4.3.4 Discussion

Trap catches per night with virgin females as bait exceeded those with synthetic pheromone up to 25 times.

The identified compound, although a major component, might be incomplete. The only work where the identified and synthesized *H. undalis* sex pheromone and virgin females were compared was conducted in Japan (SUGIE et al. 2003). No significant differences were monitored in the numbers of males caught.

The majority of moth sex pheromones detected are blends of chemicals (ROELOFS, 1977; ARN et al., 1986; JURENKA, 2003) and single compounds acting as attractants, which was found for very few species (MCDONOUGH et al. 1995; QUERO et al., 1997). It remains uncertain if E,E-11,13- Hexadecadienal is a single compound sex pheromone or just the major component of a blend of substances. It is also unclear whether the sex pheromone identified from females of Japanese *H. undalis* represents the sex pheromone for the Philippine population of the species. Differences in the composition of the sex pheromone released by females and male perception in Japanese and Philippine populations of *H. undalis* could be detected by re-identification of the sex pheromone for these two countries.

4.4 Influence of Females in the Catch Performance of the Synthetic Pheromone in the Field

4.4.1 Introduction

As shown in section 4.3 E,E-11,13-Hexadecadienal, although attractive, is by far not as attractive as the natural sex pheromone. Another question concerns the quality of competition between natural and synthetic pheromone, whether virgin females emitting the sex pheromone in direct vicinity to the synthetic pheromone 'poach' can attract males or not. In case the ratios of males attracted by virgin females and released E,E-11,13-Hexadecadienal is constant over a series of tests, the use of E,E-11,13-Hexadecadienal for monitoring seems possible and useful.

The influence of virgin females next to a trap with the synthetic pheromone was carried out in Castellano, with two different set ups. The catch rate between virgin females and synthetic pheromone was also compared.

4.4.2 Material and Methods

Trial 1: Six traps were set in a radish field (6000 m^2) in Castellano. Two traps with virgin females with two traps baited with the synthetic pheromone were placed 2m apart, the same number of traps were placed 5 m apart and two traps with the synthetic pheromone were set alone. The distance between the traps was 50 m and 60 m (Fig. 35). The lures used were sent to the Philippines on October 2003. The trial took place from October 14-16.

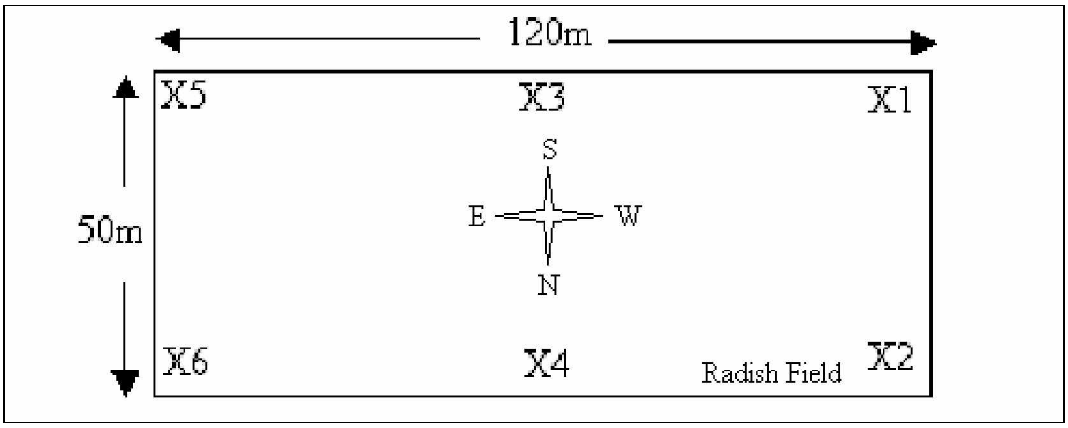

Figure 35: Set up of trial 4.21.1 in San Leonardo, October 14-16. X1 + X6: virgin female baited trap 2 m distant to synthetic pheromone baited trap, X4 + X5: virgin female baited trap 5 m distant to synthetic pheromone baited trap, X2 + X3: synthetic pheromone baited traps

Trial 2: The second trial had 15 trap locations, in total 24 traps were set up in an area of 1.5 ha in Castellano, San Leonardo (Fig. 36). Three traps each with a distance of 1 m, 2 m and 5 m between the trap with a virgin female and a second with the synthetic compound. Each three traps with virgin females and synthetic pheromone were placed singly in the area. The distance between traps was more then 50 m. The set up was completely randomized. First set up was on October 16, 2003. First control was October 17; last control October 20, 2003. There was no new set up after October 16. Within the four days the trial took place, only three virgin females died, two, three and four days after beginning of the trial. Dead moths were exchanged with virgin females from the own rearing. Since the numbers of males caught in the traps with virgin females were so high, the yellow sticky paper was exchanged two times.

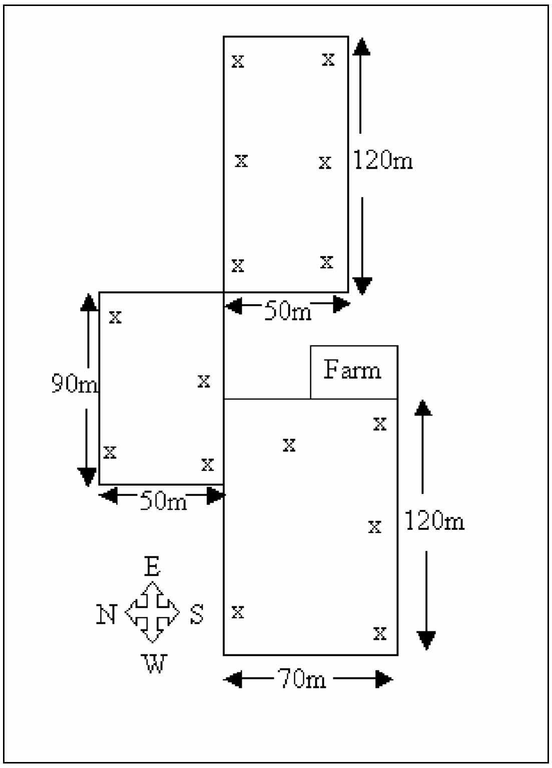

Figure 36: Set up of traps baited with 10 µg and virgin females in three adjacent radish fields in San Leonardo, October 16-20.

4.4.3 Results

Trial 1: Male attractions as determined by numbers of males caught are significantly higher in virgin female baited traps without any exception (Table 44). The distance of placing the synthetic pheromone baited traps near the virgin female baited does not influence the catch rate and is expressed by a non-significant different number of traps next to virgin females. Synthetic pheromone traps placed alone attracted significantly less numbers of males than all other set traps.

	2m		5m		
	SP	VF	SP	VF	SP
Total	57	261	63	194	16
C/T/N	14.3	65.3	15.8	57	4
Mean Catch (Mean ± SE)	28.5 b ± 1.5	130.5 a ± 9.5	31.5 b ± 10.5	97.0 a ± 17.0	8.0 c ± 1.0
% of total	9.6	44.2	10.7	32.8	2.7

Table 44: Influence of virgin female as bait on the catch rate of synthetic pheromone baited traps standing together in a distance of 2 m and 5 m and synthetic pheromone baited traps standing alone. Numbers were calculated from catches of two nights (October 14-16, 2003, Castellano, San Leonardo). Total catch = 511 males

Trial 2: In the second set up, which was conducted for four nights with more replicates (n = 3) than in the first trial, the influence of virgin female baited traps placed next to synthetic pheromone baited is not significant (Fig. 37). Number of males caught in virgin female baited traps is significant higher compared to synthetic pheromone baited traps, but no significant difference in numbers of males caught exists between the different synthetic pheromone baited traps. The ratios of number of males caught with different baited traps were between 0.1 – 0.2 (Table 45).

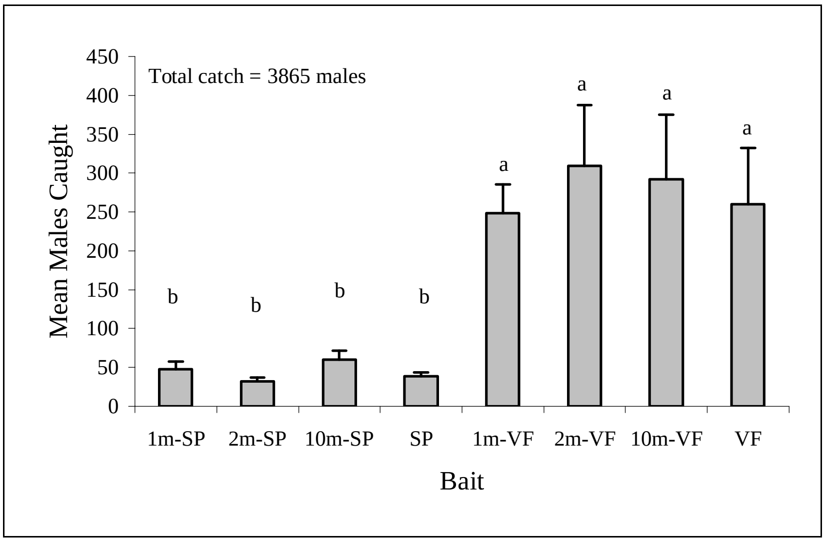

Figure 37: Captures of male *H. undalis* in traps baited with virgin females and synthetic pheromone containing lures. 1 m, 2 m and 10 m referring to the distance between virgin female and synthetic pheromone baited traps; SP: synthetic pheromone, alone; VF: virgin female, alone. Different letters above bars indicate significant differences according to Fischer's Least Square Distance Test, at p< 0.05.

Bait	1 m		2 m		10 m			
	SP	VF	SP	VF	SP	VF	SP	VF
Total	143	745	95	929	180	876	116	781
C/T/N	11.9	62.1	7.9	77.4	15	73	9.7	65.1
SP/VF	0.19		0.10		0.20		0.14	
% of Total	3.7	19.3	2.5	24	4.7	22.7	3.0	20.2

Table 45: Influence of virgin female as bait on the catch rate of synthetic pheromone baited traps standing together in a distance of 1 m, 2 m and 10 m and virgin female and synthetic pheromone baited traps standing alone. Numbers were calculated from catches of four nights (October 16-20, 2003, Castellano, San Leonardo). SP: synthetic pheromone; VF: virgin female; C/T/N: catches per trap per night; SE: standard error; Total catch = 3865 males

4.4.4 Discussion

Results indicate no influence of trap catches with the synthetic pheromone when virgin females were placed in nearby traps with distances of 1 to 10 m. Numbers of males caught did not increase when virgin females were placed nearby traps baited with synthetic pheromone in Trial 2. However, a relevant reduction of males caught was found in traps lured with the synthetic compound placed singly. This reduction is not understood and both weather conditions and placement were not factors, which could have influenced the attraction towards these traps and explain such low catches.

Despite the reduced competitive attraction of synthetic pheromone lures, the ratios of males caught in virgin female and synthetic baited traps were in a constant range between 0.1-0.2. This ratio underlines the possibilities that E,E-11,13-Hexadecadienal could be integrated into monitoring systems, despite the lowered attraction.

4.5 Age of *H. undalis* Female Sexual Maturity

4.5.1 Introduction

Attraction of the conspecific sex in moths depends upon the sexual maturity of female moth (CHAPMAN, 1998). Knowledge of the biology of a pest is the basis for implementation of IPM strategies (JUTSUM and GORDON, 1989) and attraction of males of mature females is one aspect of the sexual behaviour.

HARAKLY (1968b) reported female *H. undalis* mating right after emergence. Time of sexual maturity of female *H. undalis* was determined through setting them as bait in traps and comparing number of catches of different aged virgin females.

4.5.2 Material and Methods

Unmated female *H. undalis*, 1-5 days old (days after emergence from pupae) were used in these trials. Eclosion always took place during the night (with very few exceptions), i.e. the age of 1-5 days was reached in the course of the trial. Three females per trap and age level were set in the CLSU experimental area in a complete randomized design. The distance between the traps was 15-20 m. The traps were set in the late afternoon and checked the next morning after 9:00 h. Emerged females from one night were put in plastic boxes, and fed with honey solution on a cotton wick until used for the trial. The virgin females were put in film boxes where the bottoms and lids were removed and covered with mesh net the afternoon before they were set in the field. The surface was pierced all over with the punch tool of a Swiss army knife.

Females were classified as calling when males were recorded in the trap.

1-3 day old virgin females were set up for three nights, 1-4 day old virgin females for 2 nights and 1-5 day old virgin females for one night. The trials took place from February 2-20, 2003.

Each three females 1-5 days old were used in two consecutive nights February 2-4, 2003 with 1-4 day old females in the first night and 1-5 day old females in the second. Females remained in their marked boxes during the day February 3, to obtain results from the same females.

4.5.3 Results

Catches of male *H. undalis* in traps baited with virgin females did not differ significantly between 1, 2, 3, 4 and 5 day old virgin females (Fig. 38). All females attracted males in the first night after eclosion, referred to as 1 day old. Although the total numbers of males caught fluctuate and differences appear large, is the statistical analysis clear. Percentage of females calling with the age of 1, 2, 3, 4 and 5 days after eclosion with 83 %, 77 %, 66 %, 78 % and 100 %, with three replicates per age level (Table 46).

Numbers of males caught in traps baited with virgin females used for two consecutive nights increased in the second night for 67 % of the females (Fig. 39). The total number of males caught was 417 with 41 % caught in the first (169) and 59 % in the second night (248).

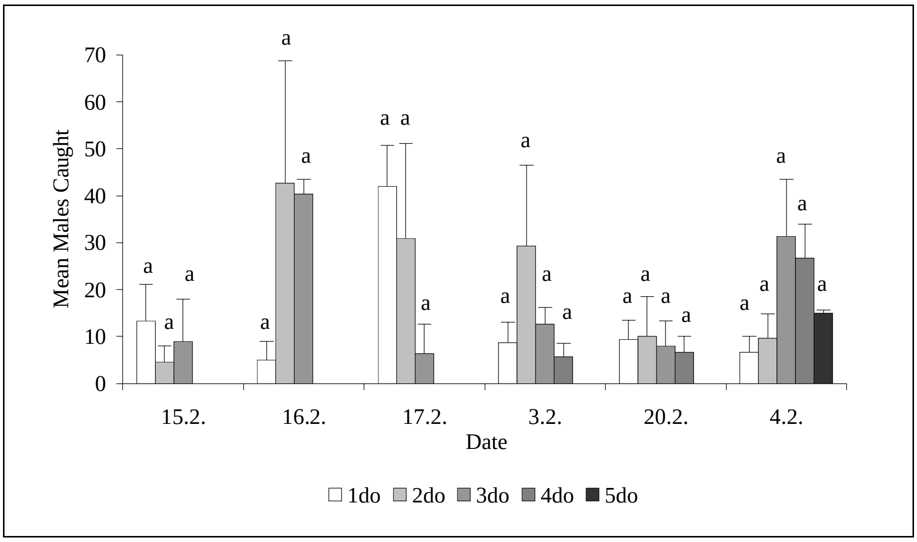

Figure 38: Captures of male *H. undalis* with different aged virgin females (VF). Age of the females ranged from one day to five days after eclosion (1do-5do). Comparisons were conducted in February 2003, CLSU experimental area. Different letters above bars indicate significant differences according to Fischer's Least Square Distance Test, at $p < 0.05$. Total catch 1122 males.

Date	VF 1do (%)	VF 2do (%)	VF 3do (%)	VF 4do (%)	VF 5do (%)
3.2.	100	100	66	100	-
4.2.	66	66	100	100	100
15.2.	66	100	33	-	-
16.2.	66	66	100	-	-
17.2.	100	66	33	-	-
20.2.	100	66	66	33	-
All Nights	83	77	66	78	100

Table 46: Percentage of females calling per age level (n=3) at CLSU experimental area, February 2002. Virgin females 1-5 days after eclosion (do) were used.

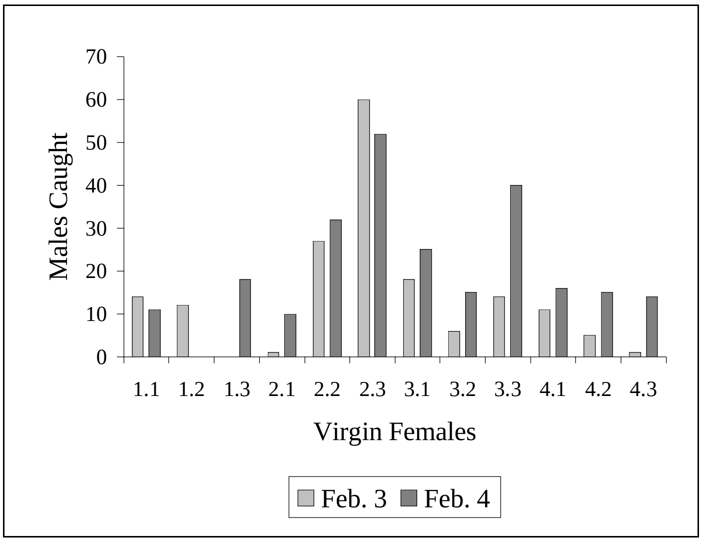

Figure 39: Males caught with virgin females baited wing traps. First Scotophase after eclosion was in the night from Feb. 2-3. Age of the females was 1–4 (February 2) and 2-5 (February 3) days old, respectively. Each age was replicated three times (x.1- x.3; x=1,2,3). Number of males caught was compared for two nights, for the same females.

4.5.4 Discussion

Males were attracted in almost all virgin female baited traps, with females of different ages. Newly emerged females attracted statistically equal numbers of males compared to older females. An average of more than 80 % of the females was calling in the first night after eclosion. This confirms reports from other countries, where *H. undalis* copulated shortly after pupal emergence (HARAKLY, 1968b; BHALANI, 1984; SIVAPRAGASAM, 1994). SIVAPRAGASAM (1996) detected a significant correlation between age of females and numbers of males caught, with older females attracting fewer males than younger ones. Sex pheromone trials in Taiwan come to the result that four-day-old virgin females attracted more males than younger ones (AVRDC, 1985). This cannot be verified for the study in the Philippines. Females lured males regardless of their age. The differences of males caught were not significant. Laboratory tests with virgin females older than 5 days were calling as observed by eight and nine day old females. Effects of reduction in time or quantity of sex pheromone released or produced were not examined; however, field trials with females between 1 and 5 days old indicate no differences in attracting males. This probably reflects the life span of females under field conditions, while in the laboratory females live up to 12 days.

H. undalis was described as non-migratory species (SHIRAI and KAWAMOTO, 1991), however, both sexes are capable of flying longer distances up to 40 and 50 km, determined in laboratory tests (SHIRAI and YANO, 1994). Non-migrant species have a more effective reproduction success when they mate as soon as possible after emergence (MCNEIL et al., 1997) and exploit the resources available in the immediate habitat (McNeil, 1986). A reason for migration of a species is for instance caused by habitat deterioration. Early maturity would be detrimental for reproductive success in such a species (MCNEIL et al., 1997). Female *H. undalis*, although a specialist feeder on Brassicaceae and related families, are unlikely to face problems in finding food plants for the offspring in the Philippines. In Castellano, crucifer crops were planted throughout the year; perfect conditions for *H. undalis* are provided. In peak planting periods where host plants in different age ranges are spread over the whole area, massive applications of insecticides are required to reduce pests like *P. xylostella* and *H. undalis* under a destructive level in which profit can be obtained. In times less crucifer crops are grown in Castellano, adults can move to nearby areas where crucifer vegetables are planted or find the ubiquitous growing *Cleome* species. The availability of cultivated and wild host plants not only in local areas but also in the whole province of Nueva Ecija keeps the population at such a level that outbreaks can occur, with great destructive power and lead to a vicious circle, where farmers are forced to spray more and more poison on the fields.

4.6 Daily Rhythms of Male and Female *H. undalis*

Diel periodicity of female calling and male response (e.g. Cardé, 1974; KOCHANSKY et al., 1977; VAN STEENWYK et al., 1978; RAINA et al., 1986; HAYNES and BIRCH, 1984) and pheromone production (RAINA and KLUN, 1984; RAINA et al., 1986; RAINA, 1997;) is described for many lepidopterous species.

The control of pheromone production of the female moth depends upon age, diel periodicity, mating history, light intensity, temperature, air velocity, and presence and nature of surrounding plants (MCNEIL et al., 1997; RAINA, 1993; MCNEIL, 1991; MCLAUGHLIN et al., 1979; COMEAU et al., 1976; CARDÉ and ROELOFS, 1973) and is regulated by neuropeptides (RAINA, 1993, 1997; RAINA and KLUN, 1984). Existence of a circadian rhythm was found in many insect species (GIEBULTOWICZ, 1999; JACKSON et al., 2000) with a multitude of research concerning pheromone production in moth underlying an intrinsic clock (Rosén, 2002; KAWAZU and TATSUKI, 2002; KAMIMURA and TATSUKI, 1993; ITAGAKI and CONNER, 1988; DELISLE and MCNEIL, 1987). Reasons for calling and responding to it in the same time window could be a potential barrier to cross communication in related species (Cardé et al., 1974; Krasnoff et al., 1983; SCHAEFER et al., 1999) and increases the probability of finding a mate (GEMENO and HAYNES, 2000).

4.6.1 Calling Time of Female *H. undalis*

4.6.1.1 Introduction

Calling females are easy to identify by their behaviour. The tip of the abdomen is bend upwards through the slightly opened wings (Fig. 40). By watching more closely it becomes visible, that pumping of the abdomen occurs and the valves at the end of the tip are moving. Females were therefore observed during the night and the start and end of calling recorded.

Figure 40: *H. undalis* virgin female in calling position.

4.6.1.2 Material and Methods

The observations were done at the Chair for Vegetable Science in Freising, Germany in a climatic chamber (2,2 m x 2,2 m x 2m) with a dark: light cycle of 12h: 12h. The temperature was 25°C, the humidity 75% rH. The light of the chamber automatically switched on at 7 am and off at 7 pm.

In total 27 females were used for the observation. The females were either 3 or 8 days old to see effects of age where calling starts. Each five females, 3 and 8 days old, were set singly in Perspex tubes (height: 30 cm, ø 15 cm). Nine 3- and eight 8-day-old virgin females were set together in each one Perspex tube. The intention of putting more then one female together in a container was to find out possible reactions of non-calling females to ones already calling, i.e. whether the calling females influence the non-calling ones to call by themselves. The tubes were covered with mesh net at the top. The whole development of reared *H. undalis* took place under the dark: light regime of 12:12 h. The females used were reared in the sixth generation in climatic chambers in the Chair of Vegetable Sciences in Freising, Germany. Starting time to observe the females was 18:00 h; the end was at 9:00h.

4.6.1.3 Results

- **First Night (6.8.-7.8.2003):**

All virgin females were calling during the night from August 6-7, 2003 (Fig. 41). The first females started to call at 2:15 h in the container with 9 females set together. Females were eclosed for three nights before observation. More than half of all females, 14 of 26 were calling at 2:45 h, about 8 hours after light off. In the containers with 9 three day old and 8 eight day old females together, ~80% and ~60% were calling, whereas only 1 of five of the three day old and 2 of 5 of the eight day old females had started. All females called when monitored at 4:00 h- 6:30 h. Most of the eight 8-day old females in one container finished calling between 6:30 h and 7.00 h. None of the 8-day old females held singly called after 7.00 am. All other females continued to call until 7:00 h and stopped between 7:00 h and 8:00 h. No females called when monitored at 9:00 h. Calling duration was determined by observing the females placed singly with one female calling from 2:45 h until 8:00 h (3 days old). The average calling duration of the 3- and 8-day-old females was 201 minutes and 228 minutes, respectively. Peak calling time ranged between 4:00 h to 7:00 h. Mean calling duration determined by females held singly resulted in 201 min., 228 min., 216 min. and 208 min. for 3 and 8 day old females August 6 and 4 to 9 day old females for August 7, respectively. No significant differences regarding the calling duration existed between different aged females as well as for the duration of calling for the two consecutive days.

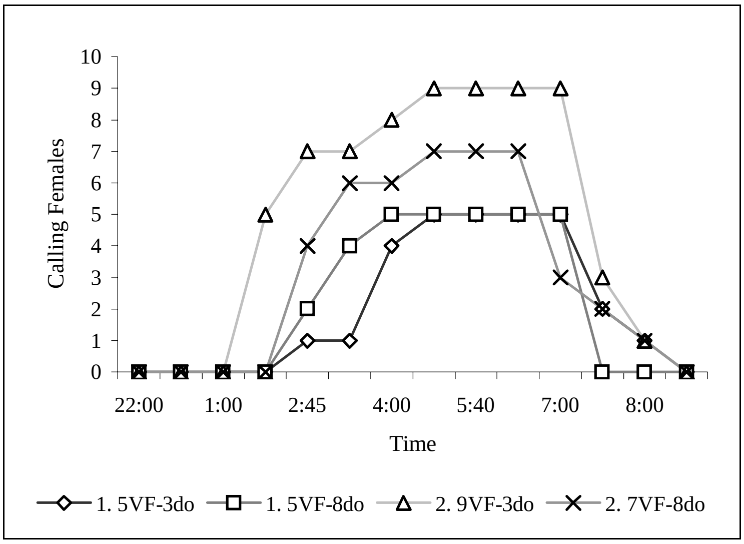

Figure 41: Onset and end of calling of female *H. undalis* observed in the laboratory. Females used comprised different age ranges. Females were set together or singly. VF: virgin females, do: days old (days after eclosion)

- **Second Night (7.8.-8.8.2003):**

The first calling activity ranged between 2:00 and 2:30 hours with 66 % calling for the 9 females, four days old, in one container (Fig. 42). Peak calling time was at 4:30 h, with all females calling. Females singly set in one container were calling until 8:00 in the morning, after the light had already been switched on. Females placed together stopped calling earlier, the last calling females at 7:30 h, half an hour already in photophase. Four-day-old females put singly in the containers started calling later than all other females. Within one hour, all females were calling. Females stopped calling between 8:00 h and 9:00 h.

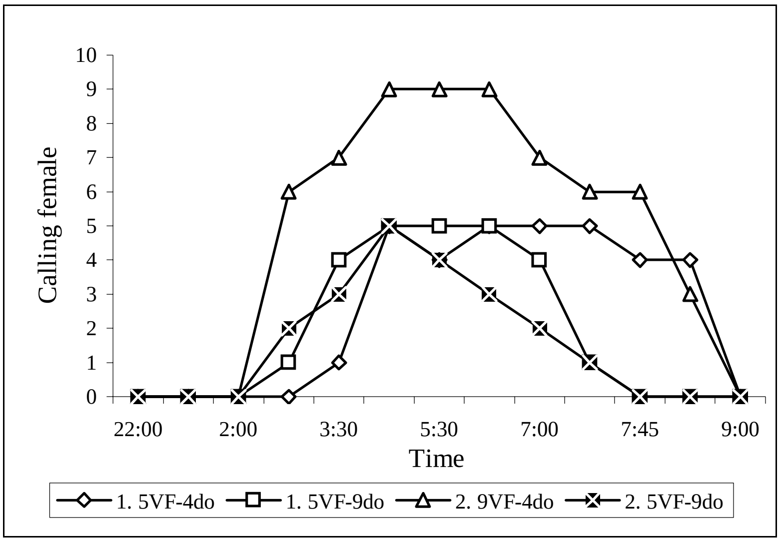

Figure 42: Onset and end of calling of female *H. undalis* observed in the laboratory. Females used comprised different age ranges. Females were put together or set singly in Pespex tubes. VF: virgin females, do: days old (days after eclosion)

4.6.1.4 Discussion

Virgin females call right after emergence, i.e. the first night after eclosion. Field trials with virgin females as bait, 1-5 days after eclosion, attracted males in non-significant numbers between the different ages. The percentage of females calling is high from the first day (> 80%), i.e. 1-day-old females, and remains high for older females. The percentage of females calling decreases to 66% for 3-day old females and increases up to 100% for five day old females but these differences are probably connected with the number of replicates, which were in all settings with three virgin females per age group. Five-day-old virgin females were set into the field for only one night, which makes comparison impossible. Nonetheless, it can be concluded that females attract males in an equal quantity independent of their age.

In the order Lepidoptera, female calling and male response underlies rhythmic periodicity. Initiation of female calling in Lepidoptera is influenced by exogenous and endogenous factors

(McNeil, 1991; Raina, 1993). The age of a virgin female is an aspect of pheromone biology. Some call within the first 24 h of emergence, others vary in the age calling starts. The time females spend calling may increase or decrease on successive nights. Older females may start earlier in the scotophase or extend the calling time later into the scotophase (McNeil, 1991). Pheromone titre in the gland can change with age and time during scotophase (Subchev and Jurenka, 2001; Foster, 2000). Delayed calling behaviour can be related with differences in reproductive maturity (Hou and Sheng, 2000; Swier et al., 1977).

Calling behaviour was observed within 24 hours following eclosion (e.g.: Harakly, 1968b). Females call when they are mature, i.e. eggs are fully developed. This was confirmed in the maturity age trials for females to release pheromone and attract males the night following eclosion. Female *H. undalis* start to call in the first scotophase after eclosion and it is suggested that females are fully mature on emergence. Gemeno and Haynes (2000) examined the calling behaviour of female *Agrotis ipsilon,* the black cutworm moth, and found females calling the first night after eclosion. The percentage of females calling increased from 1- to 3-day-old females and decreased from 3- to 6-day-old females. The results of the maturity age trials of *H. undalis* suggest that the share of females calling and attracting males is not increasing or decreasing within the age ranges tested. The percentage of females calling remained high in all age groups (66 %- 100 %). Duration of calling, as compared with 3-4 and 8-9 day old virgin females show all of them calling in the same level of time (>200 min.).

Personal laboratory observations with virgin females put a) singly and b) several together in containers during night suggest that females could be influenced by already calling females. The time window when females start and end to call was more or less the same but several younger females in one container started to call earlier compared to all other females. More than half of the 4-day-old females called at the same time. Since the observations were made in a climatic chamber, exogenous factors like temperature, humidity, and photoperiod were constant parameters, but field trials in the Philippines with virgin females as bait confirm the findings of the laboratory regarding the time of calling (see 4.3.2).

Temporal changes in the dry season from above 30 °C at daytime are reduced in the night by several °C and could be the cue for females to start calling. The differences in temperature and humidity as well as the time of photoperiod might not be too dramatic, regarding changes between seasons and decreases during scotophase. On site measurements in fields at Castellano in November 2003 from begin of the scotophase at 18:00 h until 4:00 h, revealed a

temperature drop by 3-6 °C. Temperature in the rearing chamber in the Philippines was 30 °C ± 4 °C with the cooling device not always working properly, in Germany it was 25 °C and rearing was possible with both temperatures. However, mating success as rated in numbers of laid eggs was estimated to be higher for the rearing in Germany. GIEBULTOWICZ et al. (1992) examined pheromone gland titers of laboratory reared wild gypsy moths (Lymantriidae). Effects of temperature, age, and photoperiod on pheromone titre were demonstrated. Females held at 33 °C were observed calling even though no pheromone production was detectable by gas chromatography. Exhibiting calling behaviour without releasing pheromone could also be a reason for *H. undalis* females reared under the conditions in the Philippines. Comparing the pheromone gland titre of *H. undalis* kept under different temperatures could probably elucidate any coherence.
The photoperiod varies very little, typically for tropical countries, with photoperiod duration of 10 hours in December and 12 hours in June.

Although virgin females of different ages attracted equal numbers of males, it cannot be excluded that differences in duration of calling and quantity of released pheromone exist. Viewing the numeric values of the trials with the virgin females as bait no tendencies exist of more males caught for a certain age. Most females set for two consecutive days in the field attracted equal numbers of males and the capture rate increased in the second night. Laboratory observations of calling duration with virgin females of different ages for two consecutive days revealed that females of 3, 4 and 8, 9 days old, respectively, were calling for almost the same duration in the same period of the scotophase.

The calling time is important to determine when females possess the most sex pheromone in their glands before it is released, in species it is accumulated and stored until release. By knowing the time of release, it is possible to get the content of the glands before release and obtain a maximum quantity of pheromone per female for analysis. Laboratory examinations of the pheromone titre measured at different times in the photoperiod and scotophase can provide information when females reach the highest titre of pheromone content in the glands. Whether pheromone production takes place just before release (RAINA et al., 1986), i.e. the calling period, or during the whole day and the pheromone is stored in the glands until it is released (DEL MAZO-CANZINO et al., 2004).

4.6.2 Sexual Motivated Flight Activity of Male *H. undalis*

4.6.2.1 Introduction

Many lepidopteran species synchronize the time of their sexual behaviour. Onset of female calling and male response at the same time enhances the probability of successful mating.

Testing of a sex pheromone in the laboratory requires the knowledge of the time span in which males respond to coordinate exposure to the test substance and male sexual activity. Test trials regarding the response of male *H. undalis* to the synthetic pheromone in a wind canal, carried out at noontime in the office at CLSU, resulted in an absence of reactions. Thus, trials had to be carried out during the time males react.

4.6.2.2 Material and Methods

Traps with virgin females and with synthetic sex pheromone as bait were checked during several nights. The controls started at 19:00 h- 20:00 h and were checked repeatedly every 1 or 2 hours until 9:00 h the next morning. Numbers of males caught were recorded at every control time. The traps were placed at the CLSU experimental area. The numbers of traps varied but were never less than nine. The controls were done in January- February. In this period, traps set up in the experimental area were checked six times.

4.6.2.3 Results

- **Flight activity of *H. undalis*, 2001**

Sunset was around at 6:00 h and the expected flight activity was thought from dusk into scotophase. Only one catch was recorded until 22:00 h. After no further catches were found at 23:00 h it was assumed that males were not too active and the traps were monitored early in the morning. Flight activity took place between 23:00 h and 5:30 h in the morning (Table 47).

Time	18:00 h	19:00 h	20:00 h	21:00 h	22:00 h	23:00 h	5:30 h
Males Caught	0	0	0	0	1	0	24

Table 47: Flight activity of male *H. undalis* in San Leonardo, Castellano (October 27-28, 2001), determined with 6 wing traps baited with 10µg, 20µg¸ 50µg, 100µg, 200µg and 500µg E,E-11,13-hexadecadienal.

- **Flight activity of *H. undalis*, 2003**

Male flight activity was predominant after 23:00 h as could be assumed after the first observation of traps after sun set in 2001. Traps baited with virgin females and synthetic pheromone attracted males in a range from 22:00 h until 8:00 h (Table 48). Numbers of males found in traps were very low until 2:00 h compared to the later hours. Peak catch was counted after 2:00 h until 6:00h, with most catches recorded at the 6:00 h monitoring. Expressed in percent, flight activity predominantly took place in the third and fourth quarter of the night (Fig. 43).

Night	Time Males Caught						
	20:00 h	22:00 h	24:00 h	2:00 h	4:00 h	6:00 h	8:00 h
Jan. 27-28.03[a]	0	0	0	2	9	11	0
Jan. 30-31.03[a*]	0	0	0	-	*25*	-	*32*
Jan. 31-Feb. 1.03[a**]	0	0	1	0	-	-	*52*
Feb. 9-10.03[b]	0	0	1	1	5	29	6
Feb. 10-11.03[c]	0	0	1	1	6	8	0
Feb. 11-12.03[d]	0	1	0	-	2	0	14
Feb. 16-17.03[e]	0	0	1	0	52	204	68
Feb. 18-19.03[f]	0	0	0	0	46	102	5
Nov. 19-20.03*	0	2	3	3	1	101	0

Table 48: Catches of male *H. undalis* during nights in January/February 2003.
[a] 12 wing traps baited with 10µg lures; [a*] 10 wing traps baited with 10µg lures, 3 wing traps baited with virgin females 1-4 days old; [a**] 3 wing traps baited with virgin females 1-4 days old; [b] 15 wing traps baited with 10µg lures; [c] 9 wing traps baited with 10µg lures; [d] 13 wing traps baited with 10µg lures; [e] 7 wing traps baited with 10µg lures, 13 wing traps baited with virgin females 1-4 days old; [f] 9 wing traps baited with 10µg lures, 9 wing traps baited with virgin females 1-4 days old; * control of 21 wing traps baited with 10µg lures in Castellano, San Leonardo; Numbers in italic are not included for Fig. 37. Total catch = 795 males

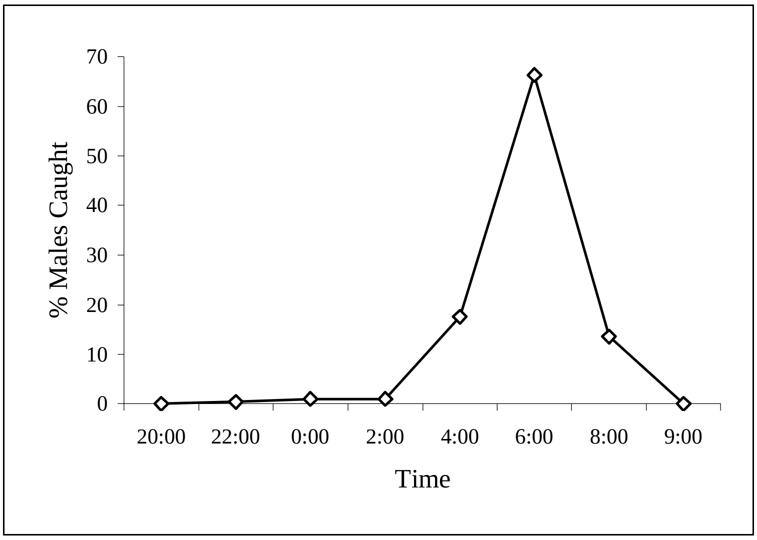

Figure 43: Flight activity of male *H. undalis*. Percentage of males caught between 18:00 h and 9:00 h. No catches were recorded before 22:00 h, therefore 18:00 h is not shown in the figure. Data displayed represent 7 observations in the fields in CLSU, experimental area and Castellano, San Leonardo (Jan., Feb. and Nov. 2003).

Results from 2003 were split into males caught in virgin female and synthetic pheromone baited traps and expressed in percent (Fig. 44). Both baits attracted most males between 4:00 h and 6:00 h (Fig. 44). The quotient of catches per trap per night of synthetic pheromone and virgin female baited traps is 0.3 in three of four nights, indicating a relation of lesser attraction of the synthetic pheromone, however, in equal proportions (Table 49). Males start their flights before any female is calling, but the numbers are very small (Table 50). Females attracted most of the males after 2:00 h until 8:00 h.

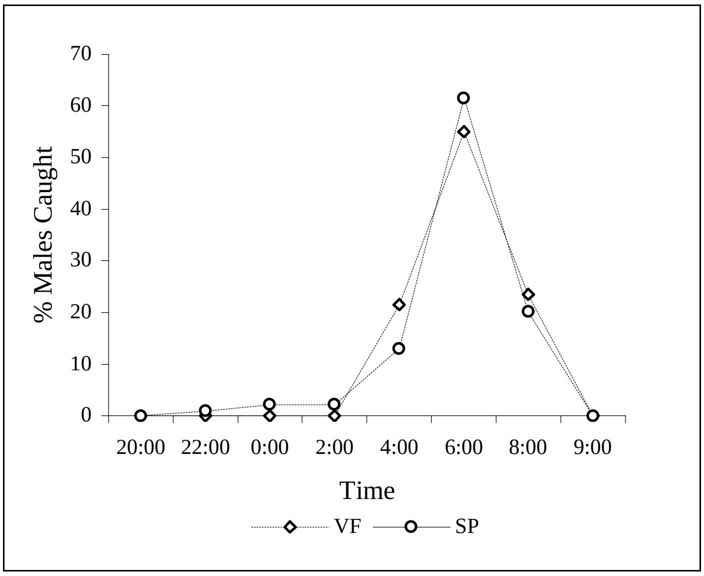

Figure 44: Percentage of males caught during the night with synthetic pheromone (SP) and virgin female (VF) baited traps.

Date	SP- C/T/N	VF- C/T/N	SP/VF
Jan. 30-31	2.9	9.3	0.3
Jan. 31-Feb. 1	2.5	9.3	0.3
Feb. 16-17	6.3	23.1	0.3
Feb. 18-19	1.6	11.8	0.1

Table 49: Nightly capture rate per trap of synthetic pheromone and virgin female baited traps in four nights set together in the field (Jan.-Feb. 2003). SP: synthetic pheromone; VF: virgin female

Bait	Time Males Caught (%)							
	8:00	10:00	12:00	2:00	4:00	6:00	8:00	9:00
VF (n=31)	0	0	0	0	21.49	55.01	23.48	0
SP (n=91)	0	0.93	2.17	2.17	13.04	61.49	20.18	0

Table 50: Percentage males caught in traps baited with synthetic pheromone (SP) and virgin females (VF).

- **Flight activity of male and female *H. undalis* determined by a light trap**

Catches with the light trap were low compared to catches with synthetic pheromone baited traps. Catches from one night were recorded. Female *H. undalis* are therefore more active directly after sunset and do not fly or were not attracted by the black light after 22:00 h (Table 51). Male *H. undalis* were found whenever the bowl was monitored except at the beginning (Table 51).

Date 19.11. -20.11.03	Time								
	18:00 h	19:00 h	21:00 h	22:00 h	23:00 h	24:00 h	2.00 h	3:00 h	4:00 h
Number ♀/♂	1/0	3/2	1/4	0/5	1/3	0/3	0/7	0/5	0/4

Table 51: Male and female *H. undalis* caught in a light trap.

4.6.3 Male Response to the Synthetic Pheromone in the Laboratory

4.6.3.1 Introduction

Behavioural observations on male *H. undalis* exposed to E,E-11,13-Hexadecadienal were carried out in a wind canal to determine the onset of behavioural patterns like antennal movements, wing fanning and moving into the direction of the odour source.

4.6.3.2 Material and Methods

The male response to synthetic pheromone was tested in a wind canal in the library in the Chair of Vegetable Sciences in Freising, Germany. It was built with a Perspex tube of 2 m in length and 0.15 m in diameter. There was a fan fastened on one end of the tube, which generated an air stream of 0.15 m/s. The air stream was measured in the centre of the tube at the mid length with a high sensitive anemometer (Testo 435). The wind speed was infinitely variable.

Males were set in a wind canal at 18:00 h estimated 1.5 m distant to the pheromone source. Males were observed for antennal movements, walking and flying attempts towards the pheromone source. The behaviour was recorded until 4:00 h and 6:00 h in the morning. Ceiling lighting was switched off at 19:00 and only a little lamp was placed in such a way that movements could be just observed and records written down.

Five males were observed July 29-30, ten males August 5-6. The males used were in the first night after eclosion.

4.6.3.3 Results

- **Behavioural observations on five male *H. undalis* exposed to E,E-11,13-Hexadecadienal in a wind canal**

Males did not start to move in the direction of the pheromone source before 23:00 h. Antennal movements were visible before that time, but flying or walking in the direction of the wind stream did not occur. Three of five males in the wind canal got within close distance (5-15 cm) to the pheromone source at 1:10 h. Four males were found at 2:50 h in the same distance range, but all five moved their antennae rapidly, wing fanning combined with walking from one direction to another and flights from one side to the other of the Perspex tube were observed. First contact with the red rubber septum occurred at 4:00 h with one male sitting on the lure. The others were found in close distance to the septum.

- **Behavioural observations on ten male *H. undalis* exposed to E,E-11,13-Hexadecadienal in a wind canal**

None of the males showed any signs of movements. They were not moving their antennae, nor walking or flying around. Males started to move towards the pheromone source at 20:15 h; six did not move / react. Until 21:00 h there were no antennal movements visible. One male was next to the lure at 23:00 h without wing fanning and antennae moving. All other males were still in the last third of the tube. Another male was found near the lure at 0:30 h, where it stayed until 1:00 h.

Around 2:00 h all males were flying and intense antennal movements were visible with five of them close to the lure. All males were active until 5:30 h, flying back and forth in the direction of the lure, sitting next to it and walking in close distance. When checked the last time at 6:00 h some males moved their antennae, others walked and flew, with periods without any movements.

4.6.2.4 + 4.6.3.4 Discussion

SIVAPRAGASAM (1996) reported a bimodal activity pattern of *H. undalis* males with capture peaks at 20:30 h and 5:30 h to 6:30 h in Malaysia. In the Philippines, when traps were monitored at 20:00 h no catches were found at this time. Traps had been in the field for at least 2 hours by then, so the synthetic pheromone could have attracted males. The response level of male *H. undalis* reached a maximum in the last fourth of scotophase with Peak capture time with more than 60 % of all males caught after 4:00 h to 6:00 h. Rainfall had no effect on the flight activity, which confirms results from Malaysia (SIVAPRAGASAM, 1996).

Flight activity was observed in high-populated areas in Castellano but with the means used, i.e. the sex pheromone baited traps it is not detectable. However, catches in the light trap show activity early in the evening. Activity at dusk was reported from Taiwan (AVRDC, 1978) without mentioning the method for obtaining the data.

Flight activity is not necessarily always directly coupled with luring and mating. Females have to find a suitable place from where they start calling and try to attract a male near plants where eggs can be laid. When traps were monitored in the scotophase, it could be observed that males were sitting on the outside of traps, beginning at 21:00 h. Catches were not recorded until 22:00 h with only small numbers of males caught. Males did not enter the inside of the traps, which could mean that they are not too receptive for the pheromone in the early scotophase. It is suggested and results confirm that calling and the response to it are underlying a circadian rhythm. Laboratory trials with male *H. undalis* exposed to the synthetic pheromone caused nearly no reactions before midnight. Males became very active with moving their antennae and fanning their wings during the second half of the night. These behavioural patterns can clearly be addressed as mate searching. Consequently, it can be concluded that male *H. undalis* reach their sexual maturity, like the females, right after eclosion.

Chapter 5

Use of E,E-11,13-Hexadecadienal for Pest Management

5 Use of E,E-11,13-Hexadecadienal in Pest Control

5.1 Comparison of the Sex Attractant (E, E)-11,13-Hexadecadienal with and without added (Z)-11-Hexadecenal

5.1.1 Introduction

In the course of the work, an article regarding sex pheromone components of *H. undalis* was published (SUGIE et al., 2003). Results were promising to enhance trap catches by blending Z-11-Hexadecenal with E,E-11,13-Hexadecadienal. F. Griepink (Pherobank, Wageningen, Netherlands) prepared blends in different concentrations for field-testing in the Philippines.

5.1.2 Material and Methods

All pheromone lures contained 10µg of (E,E)-11,13-Hexadecadienal (control), (E,E)-11,13-Hexadecadienal + 0.1 % Z-11-Hexadecenal, E,E-11,13-Hexadecadienal + 1 % Z-11-Hexadecenal and E,E-11,13-Hexadecadienal + 5 % (Z)-11-Hexadecenal, respectively.

0.1 %, 1 %, and 5 % added (Z)-11-Hexadecenal is equivalent to 10 ng, 100 ng and 500 ng per lure. Traps were set in Castellano, San Leonardo (October 8-12, 2003) with four replicates per treatment. Beside the traps baited with synthetic substances, traps with 2-4- day old virgin females were set. The distance between two traps was 30 m. The trial was conducted on a 1.2 ha area in Castellano, San Leonardo, with radish as crop plant. The location of traps was rearranged at random every morning beginning with October 8. The trial ended October 12, 2003.

5.1.3 Results

All synthetic compounds tested attracted different numbers of males. The tested synthetic compounds differed significantly in the number of males caught compared with virgin females as bait (Table 52). Substances tested attracted about five times less male *H. undalis* than virgin females.

Bait	C	C ± 0.1%	C ± 1%	C ± 5%	VF
Total	274	189	261	231	939
C/T/N	13.7	9.45	13.1	11.6	46.9
Mean Catch (Mean ± SE)	68.5 b ± 9.9	47.3 b ± 17.3	65.3 b ± 7.2	57.8 b ± 12.8	234.8 a ± 24.9
% of total	14.5	10.0	13.8	12.2	49.6

Table 52: Catches of male *H. undalis* in traps baited with (E,E)-11,13-Hexadecadienal (control) with and without added (Z)-11-Hexadecenal in different concentrations and virgin females as bait (October 8-12, 2003; Castellano, San Leonardo; n = 4). Means followed by a common letter are not significantly different at the 5% level by Fischer's LSD Test. SP: synthetic pheromone, VF: virgin female, C/T/N: catches per trap per night, C: control. Total catch = 1894 males

5.1.4 Discussion

SUGIE et al. (2003) found *Z*-11-Hexadecenal in quantities of 50-150 ng added to E,E-11,13-Hexadecadienal to enhance the capture rate significantly. In a first trial, the daily catch rate was 7 times higher with the synthetic blend compared to virgin females. Differences of males caught in their comparative study were small for virgin females and the blend of 5 µg of E,E-11,13-Hexadecadienal and 15–500 ng of *Z*-11-Hexadecenal. The results are difficult to interpret since numbers of males caught were monitored with only one trap per tested substance. The distance between traps was described as more than 7 m and day's traps, which stayed in the field, were seen as replicates.

The results of the study in Japan are not comparable with those obtained in San Leonardo. In the Philippines, virgin female baited traps attracted and caught significantly more males then the control substance *E*,*E*-11,13-Hexadecadienal and blends with different ratios of *Z*-11-Hexadecenal added to the control substance. No significant differences were found between the control, *E*,*E*-11,13-Hexadecadienal, and the blends. Enhancing effects with the added chemical were not detected.

One reason could be differences in the composition and perception of the sex pheromone of Japanese and Philippine populations, as mentioned above in comparison trials with virgin females and the synthetic pheromone baited traps. The findings of SUGIE et al. (2003) indicate differences in the composition of scents for attracting males for *H. undalis* in Japan and the Philippines.

5.2 Monitoring Males with E,E-11,13-Hexadecadienal Baited Traps and Larval Counts on Plants

5.2.1 Introduction

Monitoring larval densities in the field is a time consuming, exhausting work, which requires decent skills to detect and identify different pest species. Monitoring adult populations with the means of a chemical attractant is species specific and mostly sensitive enough to detect pests even in low population densities. Problems concerning monitoring of larvae of *H. undalis* were described; particularly the first and second instar which cannot be detected with normal eyesight in the field. Monitoring of the adult population would provide information on the occurrence and the incidence of this pest in crucifer plantings.

5.2.2 Material and Methods

The usage of the synthetic pheromone as monitoring tool for detection, timing of treatments and combining this method with other sampling methods, as well as estimation of population trends was examined in two locations in Nueva Ecija. The monitoring took place during the rainy season in San Leonardo and the transition from the rainy to the dry season in San José, Palestina.

- **Monitoring in Castellano, San Leonardo**

Monitoring of crucifer fields with synthetic sex pheromone baited traps and plant examination was carried out from February to October 2003 in fields in Castellano. Traps and plants of the fields were monitored once a week. Lures were changed every fortnight; the sticky part of the trap was exchanged weekly from April to August. In the months of February, March, September, and October catches of synthetic pheromone baited traps used in trials or set for monitoring were sampled and means per week were calculated. Only catches of traps baited with lures not older then 2 weeks were used for analysis.

Heavy rains and typhoons prevented field monitoring during the fourth week of July and in the first week of August. Traps were blown away due to a typhoon July 22 and new set up of each four traps in two sites was only possible on July 30.

The age of the planted crops in the different areas monitored was between 2 and 6 weeks. Radish and pak choi was planted in Castellano. Web blight, a fungal infection caused by *Rhizoctonia solani*, affected the plants during the whole period the area was monitored.

Since the population of *H. undalis* was high in Castellano, the field and trap monitoring will give information for the relation of trap catches, i.e. the number of males in the area and the infestation of plants by larvae. Data were transformed to log (x+1) and correlation test and regression analysis performed.

- **Monitoring in Palestina, San José**

Monitoring with four traps baited with 10 µg lures and plant monitoring took place in Palestina, San José from September 22 to October 22. Approximately two-week-old pak choi seedlings were transplanted in a field of 0.15 ha September 22. The four traps baited with 10 µg lures were set on September 22. The distance between traps was 35 m and the set up was horizontally towards the wind direction. Monitoring the same day the traps were set was not possible due to the condition of the plants directly after transplanting and was first practised September 26. Until harvest of the crop, monitoring of 100 plants and checking traps for catches was done every 1-2 day.

The field was monitored eleven times in four weeks, i.e. field monitoring and control of the catches in the traps. The lures were changed after two weeks, the sticky part whenever necessary. Fungicides but no insecticides were applied once a week. Another field directly next to the one used for monitoring was transplanted with pak choi on October 4. Data were transformed to log (x+1) and correlation test and regression analysis performed.

5.2.3 Results

- **Monitoring in Castellano, San Leonardo**

Monitoring in Castellano comprised 13 plantings. The ages of crops ranged between 5-day-old seedlings and up to 4-week-old plants. Mainly pak choi was planted in the period from April to the end of August and most monitoring and setting of traps took place in such fields but also in radish and in a mustard field. Infestations differed from field to field and depended upon the age of plants (Fig. 45). Monitoring with traps resulted in an equal distribution of males caught in the whole area, whereas field monitoring depended on plant age and was affected by pesticide applications. Significant correlation was found between infestations and catches per trap per week ($p = 0.017$). Weekly catches of male *H. undalis* explained 16 % of the variability of the infestation rate ($r^2 = 0.16$). No correlation exists between the number of males caught and rainfall, whereas the relation between infestation and rainfall is positive correlating ($p = 0.004$; $r^2 = 0.237$).

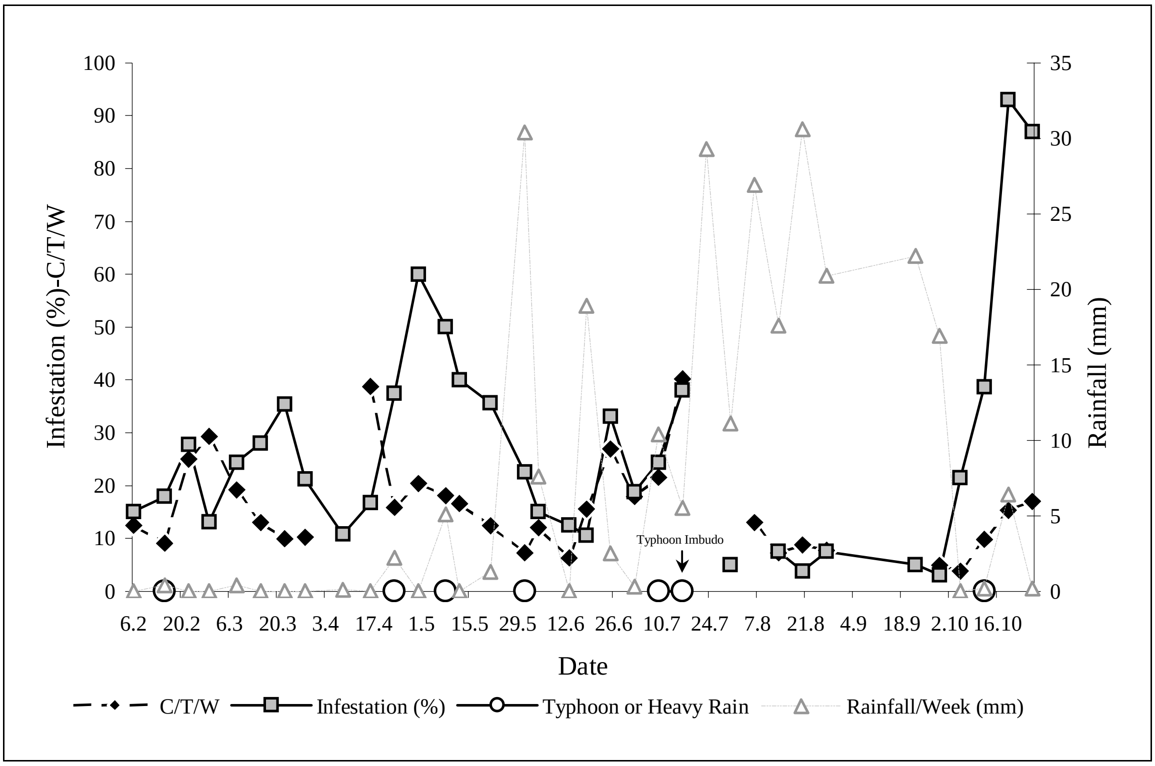

Figure 45: Captures of male *H. undalis* and monitoring for larvae in fields in Castellano, San Leonardo (February-October, 2003). Number of males caught is expressed as males caught per trap per week, infestation as percentage of larvae found on plants in different fields monitoring between 40-100 plants per field); Monitoring was performed at least once a week. Rainfall in mm is the mean rainfall of one week before the monitoring took place or between to monitoring dates. Typhoon or heavy rain occurred seven times in the monitoring period. No data were measured by PAG-ASA on such days. Precipitation was locally higher then in the figure presented. C/T/W: Catches per trap per week

- **Monitoring in Palestina, San José**

Infestation was not detectable when first catches of male *H. undalis* were recorded on September 26. *H. undalis* larvae were found for the first time, about two weeks after transplanting (Fig. 46). Number of larvae on plants and catches of males in traps are positively correlating ($p = 0.003$). Infestation can be explained by 60 % with males caught, i.e. occurrence of adult *H undalis* in the area. Both catches in traps and infestation of plants is increasing with the age of the crop and reaches a peak of 29 % infested plants three weeks after transplantation (October 14) and decreased to 21 % and 6 % prior to harvest. The developmental stage of first found larvae was estimated as third instar. Regarding the time needed for development of larvae from egg to third instar, between four to eight days, eggs must have been deposited between September 26-30; four days after the plants were transplanted. Taking action, i.e. spraying pesticides or protecting the young plants with nets, would have been necessary directly after the plants had been transplanted. It was raining heavily in the period that the first eggs were laid.

No correlations exist between rainfall and infestation rate (p = 0.927) as well as rainfall and number of males caught (p = 0.609).

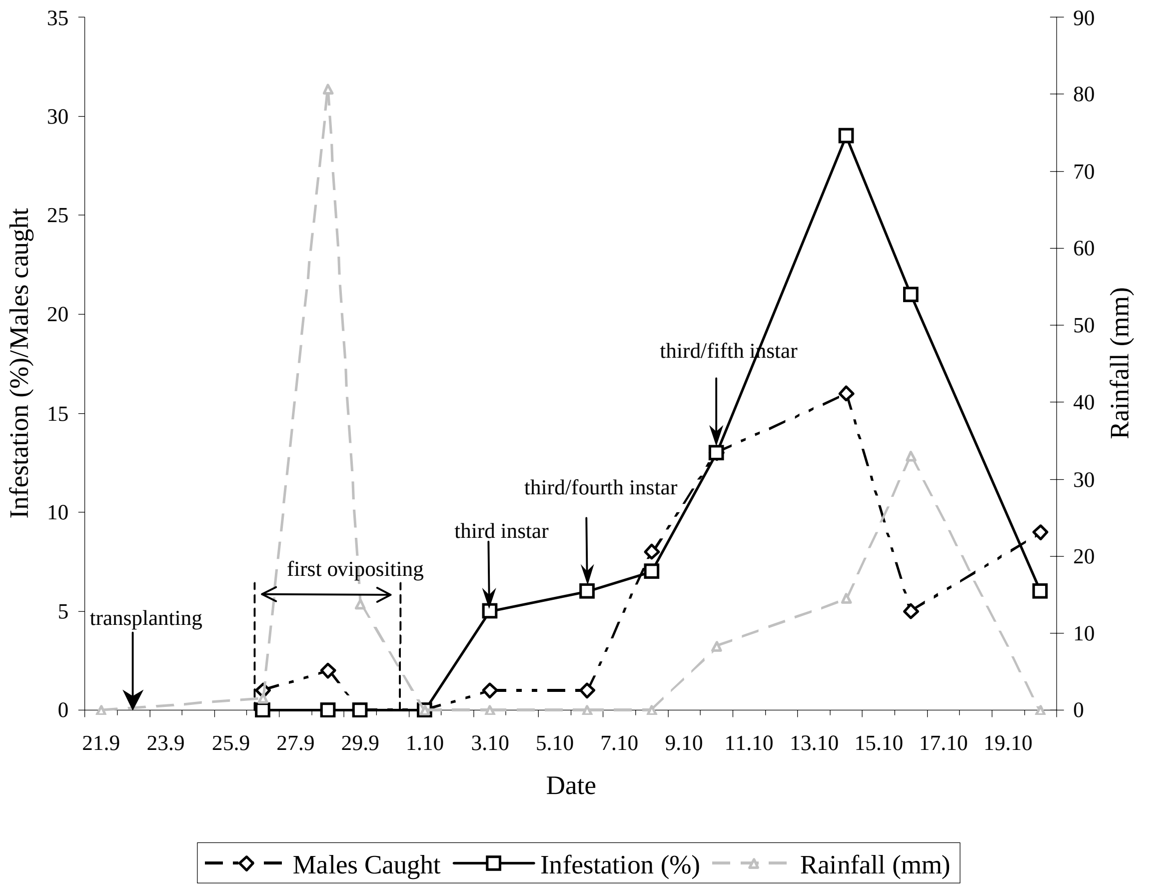

Figure 46: Captures of male *H. undalis* and monitoring for larvae in a pak choi field in Palestina, San José, over a completely cropping period. Seedlings were transplanted September 22. Harvest took place October 20-22.

5.2.4 Discussion

TURGEON et al. (1983) stated that the most effective use of monitoring pest populations is obtained when good correlations exist between trap catches and subsequent larval damage. Results of the study in the Philippines were correlating significantly and in San José, highly significant. TRUMBLE (1997) listed three basic approaches for sex pheromone use in the monitoring of vegetables: flight phenology, economic injury levels, and pesticide resistance.

Studies using virgin females as bait to detect male populations in crucifer fields were conducted by SIVAPRAGASAM (1994) in Malaysia and YAMADA (1981) in Japan in cabbage fields. In the study of SIVAPRAGASAM, the actual larval population exceeded the number of males caught and was already high at an early stage of the planting. She concludes that males must be attracted from further away and therefore the local population has no or only little influence on number of catches. Monitoring with the synthetic pheromone in the presented study indicates a correlation of males caught and larval population on plants though. YAMADA (1981) proposed the use of synthetic pheromone for forecasting seasonal appearance of *H. undalis* since virgin female traps attracted males even though no larval occurrence was detected. Monitoring of pests with sex pheromones and the establishment of relations with the actual larval infestation of a certain crop to determine reliable forecast prognosis proved to be successful for some lepidopteran pests, e.g. the pink bollworm, *Pectinophora gossypiella*, Saunders, a pest of cotton. Correlations were found between trap catches and larval densities in the USA and Pakistan (HENNEBERRY and CLAYTON, 1982; QURESHI et al., 1993). PRASAD and GUERRERO (2001) compared trap catches with larval densities and timing of insecticide applications for controlling of *P. xylostella.* Adult catches in a range of 8-16 males per 12-24 hours, depending on the crop, were followed by insecticide spraying and gave better results than fixed application dates.

The first catches of males in San José were recorded, when the eggs were probably laid. That means that protection must take place directly after the plants have been transplanted, e.g. applying insecticides or protecting the young plants with nets.

Number of males caught exceeded the actual larval population in the monitored field and males were probably attracted from further away. It cannot be determined exactly where the males were coming from, but it suggests that males must have been attracted from areas beyond the monitored area. Interpretations of trap catches are difficult in this case, but also show that the population density is always above a tolerable level for growing crucifer crops in San Leonardo.

Lowering the dosage in a pheromone dispenser is suggested by several authors (TURGEON et al., 1983) to attract males within the radius of the monitored crop. However, the dosage trials showed no differences in attraction for the dosages tested. It could either mean a permanent high number of males in the direct vicinity of the traps or a wide range of attraction of lures loaded with 1-500µg of E,E-11,13-Hexadecadienal. The latter is more probable since high catches were often followed by very low catches the next night and increased catches in the third. Males were most likely caught in the area in a relative high percentage and some time was needed for males to immigrate from outside the monitored area.

The benefits of monitoring and scheduled usage of chemicals adjusted to this information can reduce pesticide application up to 75 % (WALKER et al., 2003).

5.3 Attempt to Confuse Male *H. undalis* for Locating Females

5.3.1 Introduction

Mating disruption is the most successful method for controlling pests beside pesticide use. However, it is successful for only a few species and requires profound knowledge of the biology, ecology and exact determination of the compound or blend most attractive to the receiver of the signal. Conditions favouring this method are described for species with specialized or limited range of host plants. There are further species with restricted flight capabilities. The numbers of generations, synchronous or asynchronous reproduction cycles, and longevity of the pest, population density and possible rise of secondary pests determine the successful application of the mating disruption method.

All points mentioned above seem not to apply for *H. undalis*. The aim of the described trials in section 5.5 was to look for signs of confusion and can only be seen as a first step in a series of experiments regarding the use of E,E-11,13-Hexadecadienal for pest control via mating disruption.

5.3.2 Material and Methods

Field trials were conducted to examine the capability of E,E-11,13-Hexadecadienal for confusing males by means of interruption or the delay of mating.

- **I. Palestina, San José**

In the first trial in Palestina, San Jose, 100 red rubber septa lures were set in a pak choi field in a square of 10 x 10 m. To protect the lures from direct sunlight, they were pinned at the bottom of little plastic beakers that were hanging inside the beaker. Ten of those beakers were then attached to a thread, with a distance of 1m between the beakers and with the opening towards the ground. The threads were attached to bamboo sticks. All lines were placed in a distance of 1m towards the adjacent lines. Inside the square were two sticky wing traps baited with virgin females, 2-4 days old. Outside the square were both two virgin female baited traps in a distance of 40 m in up- and downwind direction. Four traps baited with 10µg red rubber septa were set in a distance of 45 m in the cardinal directions of the square and approximately 20-25 m distant of the outside placed virgin female traps.

The number of males caught was recorded daily until the trial was finished. Males caught were removed, or the sticky part exchanged when necessary. The trial took place from October 24-31, 2003.

- **II. Castellano, San Leonardo**

The second trial was conducted in a field in Castellano, San Leonardo. The field was harvested in the week before the trial started. The surrounding crops were mainly radish from 2 to 5 weeks old. Three hundred ninety-one red rubber septa loaded with 1µg of the active ingredient were set in an area of 900 m^2 (Fig. 47). The distance between the lures was 1.5 m. The lures were pierced through a little piece of corrugated plastic, as protection against direct sun light. The corrugated plastic was attached to bamboo sticks in a height of 0.5m. At the edges of the square were wing traps baited with 10 µg lures and in the centre and 10 m from the outer edges towards the centre were five wing traps baited with 2-4 day old virgin females. Around the area were another nine wing traps baited with 10µg lures and 16 wing traps baited with virgin females 2-4 days old. The distance from the outline of the treated area to virgin female baited traps was 10 m and 40 m, the synthetic pheromone baited between the outer placed virgin female baited traps (see Fig. 47). Catches were counted, recorded and removed daily. The sticky paper was exchanged whenever necessary. New eclosed virgin females, from the own rearing, replaced dead ones.

The trial took place from October 30 to November 11, 2003.

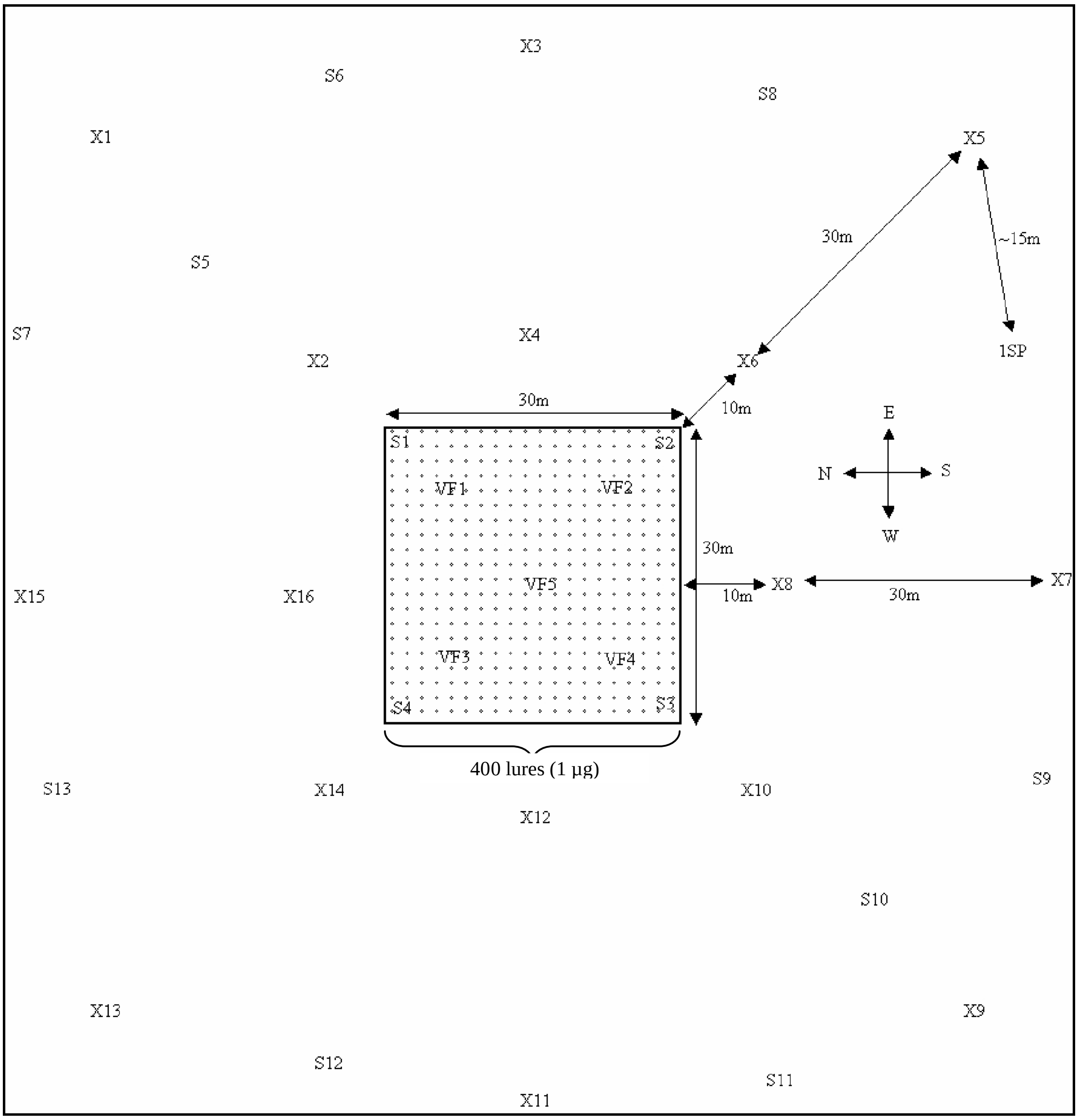

Figure 47: Set up of confusion trial in Castellano, San Leonardo. X1-16: virgin female (VF) baited traps placed 10 m and 40 m distant to the outlines of the square with equidistant placed lures, 1 µg. S1-13, 1SP: synthetic pheromone (SP) baited traps in- and outside the square. VF 1-4: virgin female baited traps inside the square. : : : : traps suspended to bamboo sticks with a distance of 1.5 m between two traps.

5.3.3 Results

- **I. Palestina, San José**

Number of males caught differed significantly between synthetic pheromone and virgin female baited traps, placed outside the square of 100 set 1-µg lures (Table 53). Virgin female baited traps inside the grid are in an intermediate position; fewer males were attracted but it is not significant. It could indicate a possible effect of confusion though.

Bait/Position	SP/OS (n=4)	VF/OS (n=2)	VF/IS (n=2)
Total	57	350	204
C/T/N	2.0	17.5	7.2
Mean ± SE	4.8 b ± 0.8	43.8 a ± 6.9	17.0 ab ± 6.2
% of total	9.3	57.3	33.4

Table 53: Catches of male *H. undalis* in traps baited with synthetic pheromone and virgin female baited traps. From the latter were two traps inside a grid of 100 lures containing 1 µg of (E,E)-11,13- Hexadecadienal, two were placed 40 m outside the grid in a distance of 40 m in up- and downwind direction. Total catch = 611 males

- **II. Castellano, San Leonardo**

The statistical analysis shows clearly significant differences in the number of males caught in- and outside (Fig. 48). The farther from the grid traps they were placed the more males were caught. The same is true for the synthetic pheromone baited traps, with an increase of three times of males caught in the outside traps (Table 54). Remarkable are the differences in the nightly catch per trap: up to five times more males caught in the outside traps. Nonetheless, it is also true that a proportion of 7.3 % of the total catch was attracted by the pheromone sources inside the grid.

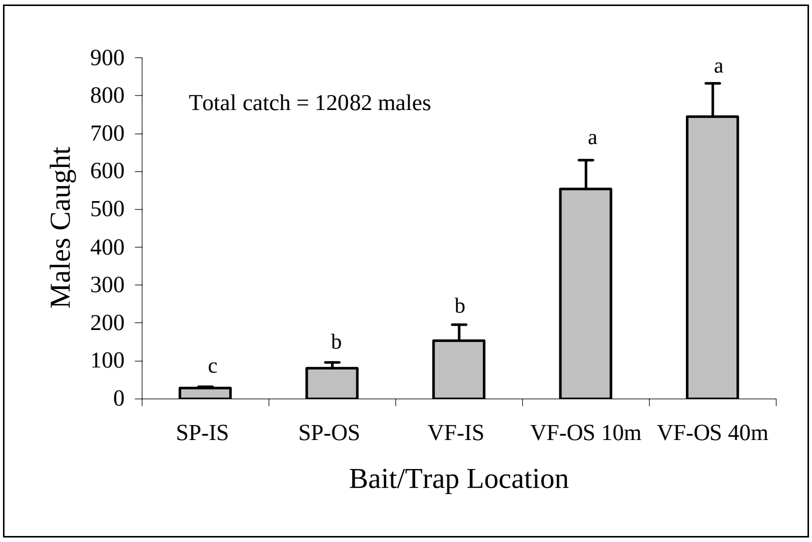

Figure 48: Captures of male *H. undalis* in traps with virgin females and synthetic sex pheromone. Traps were placed in- and outside (IS, OS) a grid with 400 lures containing 1 µg (E, E)-11,13- Hexadecadienal. Virgin female baited traps were placed inside (n = 5) and with distances of 10 m (n = 8) and 40 m (n = 8) outside the grid. Synthetic pheromone baited traps were placed at the outer edges inside (n = 4) and in various distances outside the grid. SP-IS: synthetic pheromone inside; SP-OS: synthetic pheromone outside; VF-IS: virgin female inside; VF-OS 10m: virgin female 10 meter outside; VF-OS 40m: virgin female 40 meter outside; Different letters above bars indicate significant differences according to Fischer's Least Square Distance Test, at p< 0.05.

Bait/Position	SP/IS (n=4)	SP/OS (n=10)	VF/IS (n=5)	VF/OS 10m (n=8)	VF/OS 40m (n=8)
Total	110	813	769	4432	5958
C/T/N	2.3	6.8	12.8	46.2	62.1
% of total	0.9	6.7	6.4	36.7	49.3

Table 54: Catches of male *H. undalis* in a radish field in Castellano (October 30 - November 11, 2003). Traps were placed inside (IS) a square with 400 lures attached to bamboo sticks and were baited with virgin females (VF) and synthetic pheromone (SP). Outside (OS) the square were traps baited with virgin females set in distances of 10 m and 40 m and synthetic pheromone baited traps between the virgin female baited traps. C/T/N: catches per trap per night. Total catch = 12082

5.3.4 Discussion

The aim of the trials conducted in San José, a low populated area of *H. undalis*, and San Leonardo, a high-populated area, was the potential effect of disturbing mate location with the distribution of sex pheromone in a certain area. Mating events could not be observed due to the setting of the females in perforated film boxes, but it is very likely that mating was not inhibited as could be shown with the numbers of males caught in traps baited with virgin females in the area covered with sex pheromone containing lures. However, the numbers of males caught in virgin female baited traps in- and outside the grid of 400 lures, (1 µg) implies a probable effect.

The number was significantly lower inside the treated area in San Leonardo compared to catches outside of it. Regarding the population density of *H. undalis* in San Leonardo, the results of the confusion trials indicate a possible effect of mate location disturbance. The results in San Leonardo, although clear in regards of decreased numbers of males caught inside the treated area, can also be interpreted in such a manner that traps placed outside the treated area caught the majority of males entering the field before they could reach the traps inside the treated area. However, the distribution of males caught in traps placed around the treated area was equivalent, i.e. catches from the windward and leeward side were on a same level.

In San José, the treated area was even smaller than in San Leonardo and males were able to localize the females in the treated area.

Establishing a mating disruption method for *H. undalis* in the Philippines is not likely to be successful for the province of Nueva Ecija. Small fields, for personal consumption or for market sale, are widely distributed; permeating the area only in Nueva Ecija would be an impossible task, due to the size and arising costs. Besides, *H. undalis* is present throughout the year with overlapping generations, which would complicate a mating disruption treatment. JUTSUM and GORDON (1989) describe a univoltine (one generation per year) pest with limited mating period as most susceptible to this kind of control, whereas multivoltine (more generations per year), asynchronous pests would need treatment over the whole season with prohibitive expenses. CAMPION (1984) stressed that it is important to treat as large an area as possible or the area treated should be semi-isolated to reduce influx of migrating pest species.

Mating disruption is successful for control of some important pests of cotton, stone fruits, tomato, grape, apple, black-currant (*Pectinophora gossypiella, Grapholita molesta, Keiferia lycopersicella, Epiphyas postvittana, Eipoecilia ambiguella, Lobesia botrana, Cydia pomonella, Synanthedon tipuliformi)* (CARDÉ and MINKS, 1995; HOWSE et al., 1998; MINKS, 1997; ARN and LOUIS, 1997; STATEN et al., 1997; SUCKLING and KARG, 1997). CARDÉ and MINKS (1995) point out that success is related to the motility of a certain pest, the initial population levels of the pest and release characteristics of the sex pheromone formulation.

Problems with the set up of the confusion trials resulted from distribution of *H. undalis*, the area where crucifers are grown and how these areas are connected, the availability of natural host plants and their distribution all over Nueva Ecija. The treated areas were too small and the dosages of the dispensers probably too low to state an effect in the direction of mating disturbance. Additionally, the time frame the trials were conducted was too narrow and effects are not likely to be seen with the chosen set up.

The threshold of damage of *H. undalis* is high because of the behaviour of larvae to move towards the growing point, with single larvae able to cause maximum damage. Influx of mated females cannot be stopped by the mating disruption method. Also problematic with this pest is the egg laying behaviour, with eggs laid singly or only a few per plant. The consequences result in a high risk caused by only a few mated females immigrating into a crucifer field.

Chapter 6

Population Fluctuations of *Hellula undalis*

6 Population Fluctuations of *H. undalis* in Crucifer Vegetable Fields in Nueva Ecija

6.1.1 Introduction

The occurrence of *H. undalis* larvae was monitored in different locations in Nueva Ecija. The fluctuations of pest incidences were followed from November 2001 to November 2003 in Area I, from February 2002 to November 2003 in Area II, from February 2002 to September 2002 in Area III, and from November 2002 to November 2003 in the area Castellano in San Leonardo. The aim of this long-term observation was more of a descriptive nature than assessment of reasons, and was conducted to study the abundance of *H. undalis* in field's treated and un-treated with pesticides. Seasonal patterns were tracked for infestations in CLSU, Area I and San Leonardo. The number of larvae on a single plant was rated as infested regardless if only one larva or several were found. The observations presented, describe population fluctuations in the state it was found.

6.1.2 Monitoring Procedure

Randomness in selecting the sites for monitoring was secured by dividing the field in a grid with numbers per square and then using cards for each site chosen, both when whole square meters were monitored as well as for 40-50 plants in the entire field. To reduce bias when a number of plants were monitored, a group of 4-6 plants was examined in the selected sites of the grid until the number of 40 or 50 plants was completed. Examination of the plants followed the same routine, with inspecting for larvae of different species of pest insects on the surface and underneath the leaves, the growing point and the petioles. Insect frass (debris from feeding and faeces of the larvae) and webbings were always signs that infestation might have occurred; however, the plant was not counted as infested without the detection of the pest.

Rainfall over longer periods occasionally prevented continuous plantings. The fungus *Rhizoctonia solani* caused the web blight disease and heavy pest incidences destroyed young crops and led to gaps within the 2001 to 2003 monitoring data. The 2003 typhoons destroyed many crops from the beginning of May until mid August and preparation of the field was impossible.

Infestations were calculated with the proportion of plants infested, expressed as percentage of plants infested, no matter whether one or more larvae were found on a single plant. This kind of data interpretation was used since a single larva can cause severe damage or kill the plant. Multiple infestations were recorded but not used for calculations.

The weekly monitoring started seven days after seedlings emerged from the soil. Data regarding abiotic factors like rainfall, humidity and temperature were obtained by the CLSU-PAG-ASA weather station. Influence of rainfall to population fluctuation was subjected to regression analysis. Average amount of rainfall was used for Area I when the area was monitored through the month. The amount of rainfall until monitoring date was used when the area was monitored only one time. One monitoring in one month occurred April 2002 and May 2003. For April 2002, rainfall was very low and no adjustments were necessary. For May, the amount of rainfall was zero the last two weeks before the area was monitored. This value was used for regression analysis. Mean rainfall was 16.2 mm for May.

For Area II and III the amount of rainfall during one week before the monitoring was used as the reference value.

Selected crops grown in different years and from both seasons demonstrate the population fluctuation within one crop.

- **Monitoring in Muñoz**

The staggered planting in Area I provided food for larvae continuously and plants in different age stages and percentage of infestation was calculated with the average of plants infested for every month monitoring took place.

One crop after the other was planted in Area II and III. Infestation is represented for every monitoring date.

Area I

Monitoring in the CLSU-experimental area in Area 1 was carried out from July 2001 to November 2003. The monitoring method in this long-term observation was adjusted to the conditions found in the field regarding weather, development of crop, i.e. age of the plants. This information changed from monitoring several square meters for the whole area to the monitoring of fixed numbers of randomly selected plants. Monitoring of plants inside a randomly selected square of one meter was carried out from July 2001 to March 2003. Data obtained from July to Mid September 2001 is not included since the mode of monitoring had to be practised and an unmistakable determination of the different species was not guaranteed.

Between 4 m^2 and 8 m^2 of the field was selected, all plants counted and species and number of pests recorded. Forty plants in the whole area were monitored beginning in April until September 2003, 50 in October and November 2003. Monitoring was scheduled on a weekly basis. In the period from July 2001 to November 2003, 22 crops were planted and maintained.

Area II

Monitoring in the CLSU-experimental area in Area II was carried out from February 2002 to November 2003. No monitoring took place April 2002, from mid June until mid August 2003 and was possible only one time in September 2003.

Area III

Monitoring in the CLSU-experimental area in Area III was carried out from February 2002 to September 2003. No monitoring took place May and June 2002.

- **Monitoring in San Leonardo**

Larval infestations in San Leonardo were monitored in the Castellano area. Fields from two farm families, which comprised a total area of ca. 8 ha, could be used for monitoring as well as for setting up sex pheromone trials. All fields where pest abundance was monitored and sex pheromone trials were carried out were sprayed intensively with herbicides, fungicides and particularly with insecticides. Different insecticides were mixed together. Spraying occurred frequently, and depending on the farmer’s perception, from one time per week up to daily applications. The fields monitored were subdivided to obtain a grid where randomly selected sites were chosen and square meters were examined as well as single plants.

- **Monitoring in San José**

Weekly monitoring was performed from September to October 2003 with simultaneous monitoring of traps set in fields with transplanted pak choi. The monitoring started the day the plants were transplanted and stopped when the field was harvested. The method of randomization in San Leonardo was practised for monitoring 100 plants per monitoring date. Data are presented in Chapter 5.

6.1.3 Results

- **Monitoring CLSU-experimental area: Area I**

H. undalis was present throughout the year (Fig. 49). No data was available for May to mid August 2003 due to heavy rain and typhoons, which made it impossible to prepare the field. The size was too small to work with machines and manual preparation with the heavy soil was not practical. Infestations above 25 % were monitored mostly in the dry season. The highest infestation rate in May 2003 was achieved due to the crop being old (38 days old). Thus, only one monitoring took place on May 5, when no rain had yet fallen.

Precipitation above 10 mm on average per month resulted in a mean infestation rate of 10-12 % infested plants, whereas average precipitation below 5 mm per month caused infestation rates above 20 %. The correlation is significant ($p = 0.04$). Percentage of infestation is explained by 25 % with amount of rainfall ($r^2 = 0.25$).

P. xylostella had at no time any severe impact on the crops in CLSU. Only *P. striolata* was occasionally found in higher numbers. *C. pavonana* and *S. litura* were found regularly, but without spread over the entire crop, more locally on a few plants with up to 40 larvae on one plant. *H. undalis* and *P. striolata* were the most destructive pests in the CLSU experimental area.

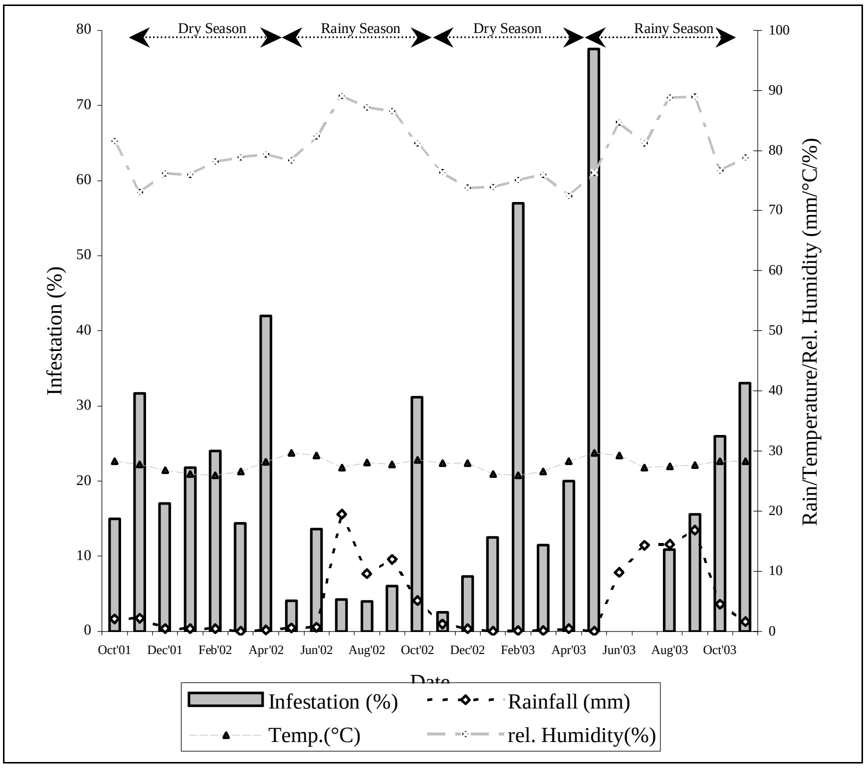

Figure 49: Infestation of a pak choi field with *H. undalis* larvae in CLSU experimental area (Area I, October 2001-November 2003) and climatic data of monthly means.

- **Comparison of infestation rates of crops 2 and 5 weeks after emergence of the seedlings in CLSU experimental area, Area 1**

The majority of crops, planted in Area I, could not be monitored for five weeks since pest insects destroyed most of them before monitoring for the fourth time. Infestation after five weeks was higher than two weeks after emergence of seedlings in all examples (Fig. 50).

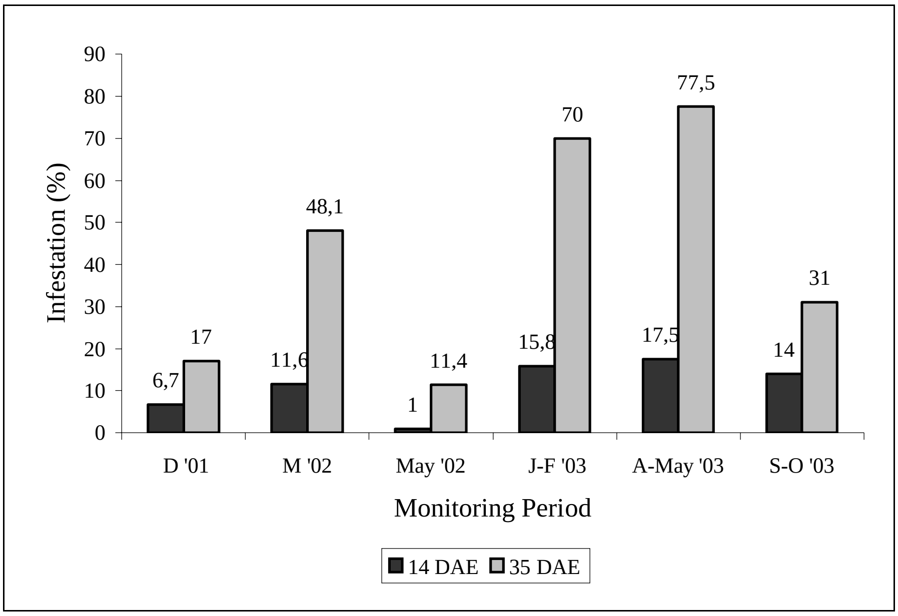

Figure 50: Infestation rates of pak choi crops with *H. undalis* larvae in different month between 2001-2003, with plants two and five weeks after germination. Crops were planted in Area I. DAE: days after emergence of seedlings

- **Monitoring in CLSU-experimental area: Area II**

An increase in infestation is a factor of age of the crop. The relation is significant ($p<0.05$). Infestation is explained by 50 % with the age of the crop ($r^2 = 0.5$). The influence of rainfall is not significant ($p> 0.05$, $r^2 = 0.07$). Since monitoring most of the rainy season 2003 was not carried out, the influence of rainfall cannot be analysed properly.

Infestation with *H. undalis* larvae reaches levels up to 80 % of plants infested (Fig. 51). The infestation rate is very similar to Area I.

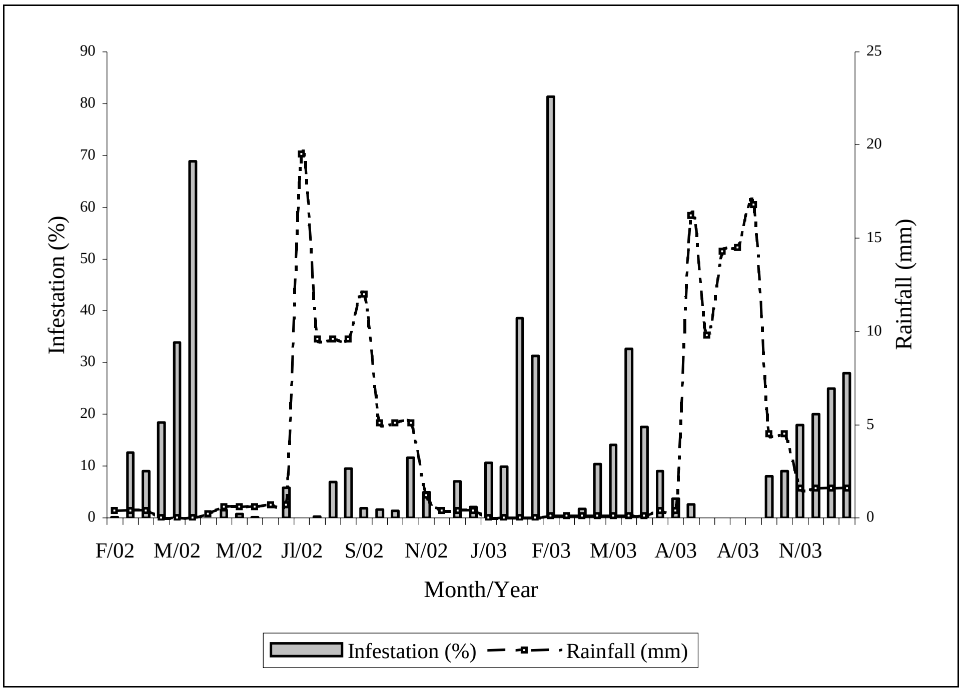

Figure 51: Monitoring of infestation with *H. undalis* larvae in a pak choi field in CLSU experimental area (Area II, February 2002-November 2003).

- **Monitoring in College of Agriculture area: Area III**

Infestation was first monitored twelve days after the seedlings from the first crop emerged. The quantity of plants infested with *H. undalis* larvae increased from 1 % to 36 % within 26 days after seedlings emerged (Fig. 52). Pest insects destroyed this first crop and new preparations were necessary. The following crops used were radish, to extend the monitoring time per crop.

Increase of infestation can be explained with 90 % as function of age for the first and second crop ($r^2 = 0.90$; $r^2 = 0.99$). The relation is significant for the second crop ($p = 0.005$), but not for the first ($p = 0.2$). The third crop, planted in the rainy season, differs in percentage values of infested plants from 1 % to 20.8 %. Age of the crop is significant related to the infestation rate for the first six times monitored ($p = 0.006$, $r^2 = 0.88$). This relation is not valid for the remaining monitoring dates ($p = 0.7$, $r^2 = 0.06$).

Rainfall does not explain the increase or decrease of infestation in all crops with the existing database. The overall infestation rate is much higher in dry season crops than in the rainy season with an average of 30.5 % compared to 6.8 %, but no correlation is given between rainfall and infestation level. Since no crucifer crops were grown in the rainy season from May-August, no regression analysis was carried out. Rice paddies surrounded the field; the next crucifers were grown in the experimental area inside the campus, ~ 2 km distant. Invading adults must have been coming from natural host plants or from areas where crucifers are grown. Invasion and establishment of *H. undalis* occurred in less than one month.

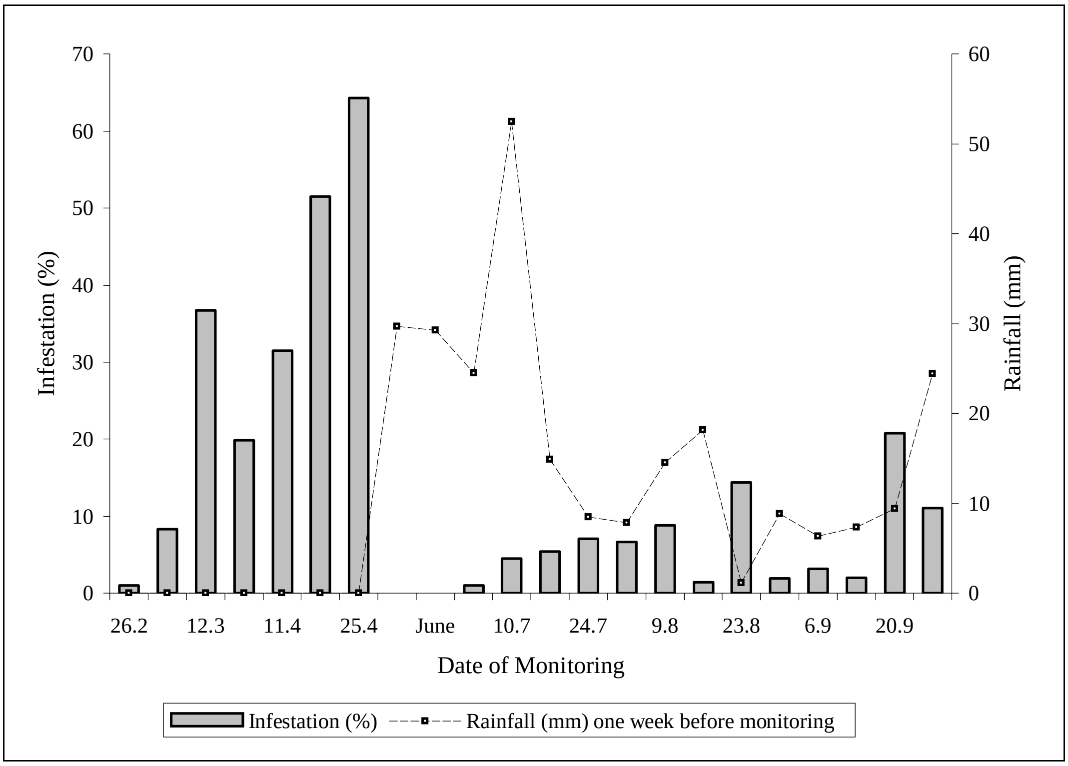

Figure 52: Monitoring of infestation with *H. undalis* larvae in a pak choi field in College of Agriculture area, (Area III, February- September 2002).

- **Monitoring in Castellano, San Leonardo**

H. undalis was present throughout the year in Castellano, San Leonardo. Results in 'Figure 53' are means of different fields, crucifers, and crops in an age range from seedling to plants shortly before harvest (for radish- ~60 days). Locally, infestations close to 100 % existed in older crops. Regression analysis between infestation and rainfall shows no significant relation ($p = 0.4$, $r^2 = 0.06$) with the data used for analysis. In the second half of the dry season, farmers switched to grow onions rather than crucifers due to the pest pressure by *H. undalis* and particularly *P. xylostella*. Abundance of pest species can reach extreme levels with *P. xylostella*, which increased in numbers when the temperatures went down beginning in November.

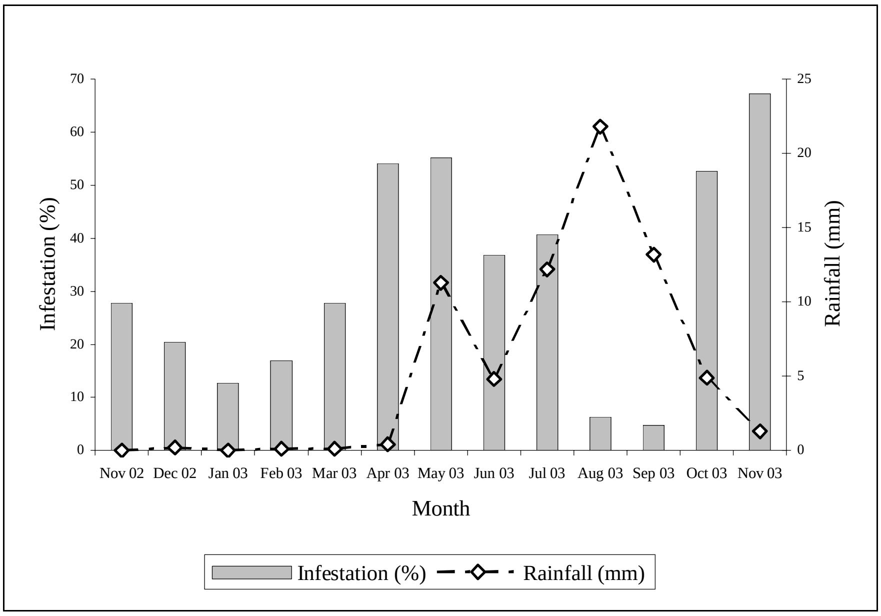

Figure 53: Monitoring of infestation with *H. undalis* larvae in fields in San Leonardo (November 2002-November 2003).

6.1.3.1 Arthropods, Weeds and Plant Diseases in Crucifer Crops monitored 2001-2003

- **Arthropods in crucifer fields, CLSU**

No parasites of *H. undalis* could be identified in the period from March 2001 to November 2003. Spiders were the most abundant predators besides red fire ants *Solenopsis geminata* (F.). Different species of dragonflies (Odonata) were common and it could be observed that they caught disturbed and flying away *H. undalis* occasionally in the air. Species of the family Coccinelidae, adults and larvae, were found both in CLSU and in San Leonardo.

The general occurrence of beneficial arthropods was small in all monitored sites.

Other abundant pests beside *H. undalis* on pak choi, mustard or radish were the striped and black flea beetle, *Phyllotreta striolata* and *P. cruciferae* (Chrysomelidae) of the order Coleoptera, *Crocidolomia pavonana* (Pyralidae), *Spodoptera litura* (Noctuidae) of the order Lepidoptera and Aphids (Homoptera), which were not identified on genus/species level. Found occasionally were *Trichoplusia ni* (Geometridae), *Spodoptera exigua* (Noctuidae) and *Plutella xylostella* (Yponomeutidae) both Lepidoptera, *Nezara viridula* (Pentatomidae) and *Nephottetix virescens* (Cicadellidae), both Homoptera. *Liromyza sp*. (Agromyzidae) of the order Diptera could be found in moderate numbers along with signs of damage on leaves.
Hellula undalis, *Phyllotreta striolata* and *Crocidolomia pavonana* were the major pest species in CLSU, whereas *Plutella xylostella* and *H. undalis* in San Leonardo.

- **Weeds**

A multitude of weeds grew on the fields of the experimental area of CLSU. The dominant species' of Area I + II were the broadleaf *Portulaca oleracea* and the sedge *Cyperus rotundus*. Other broadleaves belong to the genera *Ludwigia sp*., *Cleome viscose, C. rutidosperma* and *Trianthema portulacastrum*. Identified grasses were *Echinocloa sp.* and *Digitaria ciliaris*, sedges of the genus *Cyperus sp.* were found in different species.
Hand weeding was a daily task for the field aids particularly in the seedling stage of the crop plants.
Due to rice hull burning on fields in the Castellano area, weeds were not a major problem. Hand weeding and application of herbicides were done on a regular basis though.

- **Plant Diseases**

Predominantly in the rainy season, web blight, caused by the fungus *Rhizoctonia solani*, affected plants. Infection rates of more then 80 % were recorded in sites monitoring took place September-October 2003, namely Castellano, Muñoz and Palestina. Other diseases were not identified.

6.1.4 Discussion

Populations are regulated and controlled by density-dependent processes. Parasites, predators, and diseases cause these density-dependent controlling factors as well as competition for food, availability of host plants and decrease in fecundity (STRONG et al., 1984). Mortality and natality determine the population size (BEGON et al., 1996).

The monitoring of *H. undalis* in different areas, treated frequently with pesticides (Castellano) and absolutely pesticide free (experimental area CLSU), proves this pest as a major one for crucifer cropping systems in Nueva Ecija and the urgency to develop strategies against it. The relative abundance of *H. undalis* larvae on crucifers, i.e. radish, pak choi and mustard, although variable, reached levels intolerable for farmers in San Leonardo throughout the year. Use of pesticides in San Leonardo seems to be ineffective. The decline of plants infested after July 2003 and the relative low infestation August and September was due to the impact of a typhoon. Most of the fields were flooded for a week after 'Imbudo' hit Central Luzon, July 22. Plantings following this event were instantly re-infested and the population increased within two months to reach levels above 50 % in October and November. The flooding destroyed all crops planted at that time and it can be assumed that larvae would not have survived. Flooding prevented planting in Area I until August; however, infestations were detected as soon as crucifers were planted in both sites. The build up of larval levels above 10 % was directly followed, in Castellano after the flood, in Muñoz after 2 months without planted crucifers.

The tendency of higher infestations in the dry season is given for Castellano and Muñoz. Plants were basically in better conditions in the dry season, when water for irrigation was available. Fungal infections were observed exclusive in the rainy season, affecting large numbers of plants, reducing the quality of the vegetable and influence most likely the development and survival of larvae.

Rainfall is a limitation factor in Muñoz, but not in San Leonardo. Plants are generally in bad conditions in the rainy season and direct impact of rainfall, i.e. washing off of larvae from the plants and increased mortality caused by fungal infections, explain the lower infestation rates.

SIVAPRAGASAM and AZIZ (1992) reported peak populations in drier periods in Malaysia, where rainfall was a mortality factor. Webbing seems to protect older larvae too but might be an important factor during inanition of young larvae. Mortality was not determined for eggs and larvae in 89 % and 75 %, respectively. They also found very low parasitism rates, since parasites were not specific.

The population fluctuation presented here supports the findings of SIVAPRAGASAM and AZIZ (1992) regarding parasitism and rainfall. No macroscopic detectable parasites were found in the period from 2001 to 2003. Earlier studies by REJESUS and JAVIER (1997) and MEWIS (2001) did not prove parasites specialized on *H. undalis* in the Philippines. Rainfall might be a mortality factor depending on the population density. Thus, in low populated areas the impact is higher than in high-populated areas. MEWIS et al. (2003) described infections with a microsporidium (*Vairimorpha sp.*) of 16 % of larvae collected in fields of Nueva Ecija with a mortality of 75 %.

It is most surprising that larval infestation in Area I and Castellano was not completely different in regards to the number of plants infested, since massive pesticide applications were used in Castellano in contrast to Area I. The conclusion of this can be ineffectiveness of the pesticides used and high population density around the area of Castellano. What could be considered critical is the application method and whether they reach the target or not. Resistance developed by *H. undalis* against the used pesticides might be another possibility.

For all crops monitored the age of plants was crucial in the increase of infestation. The increase in infestation of crops two and five weeks old, up to ten times, shows a general problem in field monitoring of small organisms on the one side and the importance to remove old plants and plant material from the field. Larvae can be detected under field conditions from the third instar upward, mining of the larvae and simple size prevents them from being seen before. Thus, monitoring larval infestation is not reliable for detecting early stages of the pest and must be carried out in a laboratory with magnifying glasses or dissecting microscopes (which is not an option for the ordinary farmer).

Broadleaf weeds of the genera *Cleome* are the natural resource of *H. undalis* and other crucifer pests, when farmers switch to onion in large parts of Nueva Ecija. The role of *Cleome* as alternate host plants is discussed by SIVAPRAGASAM and CHUA (2000). They conclude that *Cleome* provides the consistent source of recruitment for newly planted crucifers. This can be assumed for the Philippines too.

Chapter 7

General Discussion

7 General Discussion

7.1 *H. undalis*, a Major Pest in the Philippines

H. undalis was becoming a major pest in crucuferous crops in the Philippines within a very short period, assuming it was introduced. That was also suggested for Malaysia by OOI (1979; cited in SIVAPRAGASAM, 1994). Importation of insect pests and other invertebrates via imported cabbage was positively tested by LIN and SOON (1985). Their results clearly demonstrated that pests could be transferred via their host plants from one country to another.

From the first detection until now only slightly more than 20 years have passed. Excessive use of pesticides is the only way farmers try to control this pest in a not specific way. Insecticide applications are prophylactic and on a regular basis. The majority of farmers do not carry out monitoring of actual insect population density as information for the decision taking process. Reduction of pesticide quantity and sound use of chemicals for controlling *H. undalis* was the cause to study this pest. Examinations of the biology, ecology and chemical ecology of *H. undalis* in field trials will provide knowledge about this pest species and enhance the possibilities to find measures for control or to keep populations in a level tolerable for farmers.

The research done comprised biological, ecological and chemical ecology aspects of the pest *H. undalis* as well as technical basics for the usage of E,E-11,13-Hexadecadienal for monitoring. The knowledge of the above mentioned areas will help to develop IPM strategies.

The employment of the compound in a practical sense concern (a) the positioning of the trap, i.e. intertrap distance, trap height and position relative along the prevailing wind direction, (b) dosage, longevity of the lures used, lure ageing and usability of stored lures and (c) trap design.

Additional aspects of the biology and ecology not yet known or unclear were studied. These included (a) natural host plants (b) maturity of females and (c) activity patterns of males and females.

Based on the results it will be possible to use synthetic pheromone for monitoring and control of *H. undalis.*

Population fluctuations of larval infestations in crucifer crops in the period from 2001-2003 demonstrate the status of *H. undalis* as a major pest and the need to change the current state.

7.2 Impact and Pest Status of *H. undalis* in the Philippines

Observations of the population fluctuations in the locations of Muñoz and Castellano proved that *H. undalis* is the second most important pest on crucifers in Nueva Ecija and probably for all lowland areas of Central Luzon. Outbreaks occurred regularly from 2001 to 2003 with infestations of up to 100% and multiple infestations with more then 10 larvae on a single plant. Depending on the crop, the yield losses are considerable (AVRDC, 2000), most severe in pak choi and mustard compared to other crucifer crops, since cosmetic standards for these leafy vegetables are very important for the consumers (AVRDC, 2002).

Noticeable is the speed of building up high population levels after the typhoon Imbudo hit the Philippines in July 2003. Fields were flooded in most of the San Leonardo area. Fields were planted again in August and September and pest monitoring shortly after was below 10 %. In October increased the ratio of infested plants to just below 50 % and in November already above 60 %. The speed the population growth can only be explained with a general high *H. undalis* population density in the whole area, the ineffectiveness of the pesticides used, probably wrong applications as well as resistance to the applied chemicals. It demonstrates the necessity of a fundamental change in pest control. Growth of insect population at CLSU after Imbudo did not reach the level at San Leonardo and pesticides were not used, but from the first planting of pak choi to infestation rates above 10 % passed only one month. After four months of continuous crop cultivation, it was above 30 %. REJESUS and JAVIER (1997) already recognized *H. undalis* as a next pest. This can absolutely be confirmed. Beside *P. xylostella* is *H. undalis* now the most important factor for yield reduction of planted crucifers with several side effects, like pesticide overuse with hazards to growers and consumers. However, without treatments, it seems impossible to cultivate Brassicaceae commercially for five to six weeks until harvest. The situation in San Leonardo is so difficult, that the only conceivable solution would be the renunciation of growing crucifers for a certain time. Monitoring natural host plants, i.e. *Cleome sp.*, and the use of synthetic pheromone baited traps would provide informations, which can help taking the decision when crucifers can be planted again.

7.3 Synthetic Sex Pheromone Components and Monitoring

Probably the most promising result of the studies was the evidence that E,E-11,13-Hexadecadienal impregnated in red rubber septa attracted males very specific. This made it possible to examine techniques for dosage, trap height, longevity, trap design and field placement, necessary information's to continue studies.

Unlike in trials conducted in Japan and Malaysia (SUGIE et al., 2003; CORK, WIZARD PROJECT 1997/98) it was possible to attract larger numbers of males over several weeks with the compound synthesized by Pherobank. Compounds different from E,E-11,13-Hexadecadienal were identified in the Wizard Project (CORK, WIZARD PROJECT 1997/98), but field tests were not carried out. The results of the comparative studies between virgin females and synthetic pheromone baited traps point to the possibility that the identified compound (ARAI et al., 1982) might be incomplete, at least for the Philippine population of *H. undalis.*

Usage for early detection of this pest with the synthetic pheromone can be carried out in low *H. undalis* densities in areas like San José and Muñoz but not in San Leonardo. For the latter it is very difficult to estimate the possible population size and determine the goal-directed application of insecticides. *H. undalis* was always present in high numbers with adults and larvae on plants.

Monitoring of pests by means of sex pheromones can be used to time insecticide applications. Relying on a fixed schedule to apply insecticides against *P. xylostella* in India, caused significantly higher numbers of eggs, larvae and damage to crucifers than spraying according to threshold levels by sex pheromone baited traps (REDDY and GUERRERO, 2001). JOHNSON (1983) and SANDERS (1988) found correlations to other pest species, *Heliothis virescens* and *Choristoneura fumiferana,* respectively, for trap catches and number of larvae or laid eggs. In San Leonardo with frequent applications of mixed pesticides, it looks very difficult to control pests with whatever means but reduction of chemicals is important not only for the local people and for consumers in Metro Manila, but to improve the environment for beneficial arthropods and vertebrates to control crops more naturally. With lower *H. undalis* densities like in San José, the information that *H. undalis* is present should be considered as date to apply insecticides. The area was monitored for trap catches and larval occurrence but it is not certain that no pesticides were applied, as the farmer said. It will be difficult to say whether the population growth was influenced by the use of chemicals. However, the calculated ovipositing must have been taking place when first catches were recorded.

In such lower populated areas, like San José, it can be of great advantage to scout for the presence of *H. undalis* before planting to determine the insect population in the area on natural host plants. In San Leonardo, though, *H. undalis* is present at all times and scouting for the presence of *H. undalis* might be useless but the actual population density is worthwhile to monitor.

7.4 Control of *H. undalis* with E,E-11,13-Hexadecadienal in the Philippines

Virgin females as bait were used in three different sites in Nueva Ecija and numbers of males caught was in female baited traps up to 25 times higher than in synthetic pheromone baited. Comparison of catch rates between virgin females and the synthetic pheromone used to attract males, clearly showing a significant preference of the males.

Usage of incomplete synthetic pheromone blends was detected for many species (AMELINE and FRÉROT, 2001; LINN and ROELOFS, 1989; TUMLINSON, 1988) but monitoring or control, by mating disruption with such a compound is unlikely to be successful (SANDERS, 1984; Borden, 1993). AMELINE and FRÉROT (2001) stated that monitoring is still possible due to constant relations between catches of synthetic and natural pheromone baited traps for *Sesamia nonagrioides* at different days. Males are attracted even the blend or compound is not the exact copy of the natural sex pheromone and small dosages of the major compound leads to a response of the male (Vickers et al., 1991).

Examples of single compound pheromones exist, for example Disparlure, the sex pheromone of the gypsy moth (BIERL et al., 1970). *Heliothis zea* is not attracted by the major component alone and only the blend causes reactions (KLUN et al., 1980). Two mechanisms, which influence the behaviour of a male exposed to a sex attractant help to explain the role of multi-component pheromones (LINN et al., 1986 a,b; TEAL et al., 1986). The 'component hypothesis' states that males orient first when the major component is sensed and initiates upwind flight, minor components are important in close range. The 'blend hypotheses' states that the pheromone blend acts as unit to attract males. Both mechanisms are probably valid depending on the examined species (CHRISTENSEN, 1997). The number of male *H. undalis* caught in the presented study is an example of attraction to the major component, although restricted compared with the natural one.

Supposing the identified compound of *H. undalis* is not the whole sex pheromone blend, males were even attracted by the lowest dosed lures of 1 µg active ingredient. This points to the fact that, 1) usage as monitoring tool is possible and 2) re-identification is necessary.

Differences can also exist within different strains or races of one species, which should be taken into account for further investigation of the sex pheromone of *H. undalis*. ARAI et al. (1982) identified the compound from Japanese moths. Production of the sex pheromone by females might vary for *H. undalis* in different populations. Examples of geographically separated species using different blends for attracting the conspecific partners are for instance *Agrotis segetum* (TÓTH et al., 1992; HANSSON et al., 1990), *Pectinophora gossipiella* (HAYNES and BAKER, 1988), and *Trichoplusia ni* (HAYNES and HUNT, 1990).

Unlike the beginnings of sex pheromone analysis in the late fifties (identification of the *Bombyx mori* sex pheromone by BUTENANDT et al., 1959) it is nowadays possible by improved analytical techniques to identify the gland content of only a few specimens.

Adoption of pheromones for pest control depends individually upon the social and economic situation of farmers and characteristics of national farming systems. The influence of the government to subsidize control measures others than pesticides or the simultaneous adoption of groups of farmers (HEBBLETHWAITE, 1989) plays an important role for adoption pest control with pheromones. In the case of *H. undalis* in the Philippines is the government probably the only authority able to change the current situation.

7.5 Farmers Practice and IPM

Many farmers in the Philippines do not understand complex ecological connections. Life cycles of lepidopteran pests are not known or incompletely and larvae cannot be related to adults. A case study of insecticide application with untrained Philippine rice farmers concluded that 'farmers were deficient in pest identification skills using terms such as worms, moths, and hoppers while some farmers targeted beneficials' (BANDONG et al., 2002). Additionally, farmers might think all insects are harmful; insecticides are effective for control and very convenient to use (PALIS, 1998).

Believe in the chemical industry and usage of pesticides is sometimes the only way of reducing pest infestation. Representatives of big agro-chemical corporations do their part to convince farmers what might be best for their crops: 'farmers are afraid not to use insecticides as a result of propaganda from chemical companies and dealers that heighten the fear of pest outbreaks' (BANDONG et al., 2002). Implementation of IPM programs and training of farmers has shown in countries where it is practiced that pesticide application can be reduced (SMIT et al., 2003; HRUSKA and CORRIOLS, 2002) and farmers profit not only from better health (ANTLE and PINGALI, 1994). For instance, MAUMBE and SWINTON (2002) list costs caused by pesticides on the health of Zimbabwean farmers, which reach up to 80 % of the annual household expenditures for pesticides. Integration of pheromones in an IPM program, or direct control with pheromones therefore should beside an idealistic aspect, be convincing regarding positive future effects of the usage. MULLEN et al. (1997) presented a method for assessing environmental benefits of IPM programs, with emphasis of the willingness of the society to pay for such programs for reduction of hazardous substances in agriculture. CUNYO et al. (2000) described the implementation of IPM programs as potential win-win situation, i.e. they may solve pest problems and reduce environmental damage at the same time.

Additionally, economic benefits were estimated for farmers in the Philippines when IPM programs were followed. PALIS (1998) stated that farmers' perception about insects, damage caused by insects and their control could be changed by conducting simple experiments on farms (learning by doing) and through IPM training courses over the season (learning by using) in farmer-field-schools (FFS). Increase in knowledge through FFS conducted for IPM education in the Philippines could cause better pest management behaviour of farmers (PRICE, 2001).

KONRADSEN et al. (2003) reported that if IPM should be more widely used, incentives for pesticide use in developing countries must be removed. Subsidy of pesticides by governments or donor agencies in many developing countries limits IPM (FARAH, 1994) and makes chemical plant protection economically possible and preferable to non-chemical methods.

It is unthinkable to stop the usage of pesticides, particularly in high-infested areas, like Castellano in San Leonardo. Most crops would be completely destroyed due to the high abundances of different pest species, particularly *P. xylostella* and *H. undalis*. However, a decrease in whatever amount would be of great importance though, e.g. reduction of the costs for the chemicals, reduction of exposure to the mist sprayed by farmers and field aids, and certainly reduction of residues on treated plants for the consumers.

Farmers provide pest species in many observed ways the possibility to remain in high populations. The mode of cultivation is certainly one problem. Seeds are broadcasted with uneven plants stand directly in contact to others favouring the spread of plant diseases and dispersal of pests. Another example are the manners of leaving old, by pests destroyed crops in the field until preparation of the area for a new planting, but also with piles of cut leaves and debries of harvested plants. Plant material is decaying fast under the tropical sun but development of larvae can be completed (Fig. 54) or larvae can reach new host plants. Radish is one of the crops in Castellano, which is frequently grown all year round. A reason, which makes it so popular to cultivate, is the part to sell that is not affected directly by pest species. Quantity of plants harvested depends upon prices and is not completed usually within one day in a field. Farmers cut off the leaves and pile them in the field. *H. undalis* larvae were found in such piles (Fig. 54). Larvae were observed to leave the decaying leaves, migrating to adjacent plants. Underneath decayed piles were pre-pupae and pupae (Fig. 54) in rotten leaves and buried in the soil. The extra labour to destroy or burrow the leaves is seen as too much effort and rejected, when the farmers were asked. KEHRLI and BACHER (2003) examined the influence of emergence of *Cameraria ohridella* on horse chestnut trees (*Aesculus* *hippocastanum*) after diapause and the influence of leaf litter removal. Removing reduced the population significantly the following year.

Figure 54: Larva (left, white arrow) found in pile of decaying radish leaves left in the field after harvest. Pupa (right, white arrow) found underneath such a pile buried in the soil (lower arrow).

7.6 Conclusive Remarks and Future Prospects

What is the outcome of three years research in the Philippines?

New insights of the ecology and biology of *H. undalis* were found. The major component of the sex pheromone was tested successfully for monitoring and basic information regarding the use of the synthetic pheromone for future studies. Decreased attraction of males with E,E-11,13-Hexadecadienal revealed the necessity to re-identify the sex pheromone, possibly from populations where *H. undalis* is an important pest to reveal differences in the composition of the sex pheromone. Although attraction of the synthetic pheromone is not as high as the natural attractant it could be demonstrated that monitoring was possible. When *H. undalis* is present but not regularly abundant, the synthetic pheromone could be used for monitoring and decision taking when to apply pesticides.

After all, it is difficult to keep pests, like *H. undalis*, under control in tropical lowlands such as Central Luzon. Governmental restrictions of pesticide use, particularly what kinds of pesticides are permitted, approximation to reach conditions of ecological balance and ecological education of farmers could ease the current situation.

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9 Appendix

9.1 Curriculum vitae

Name:	Sebastian Kalbfleisch
Geburtsdatum:	22.11.1969
Geburtsort:	Marburg, Deutschland
1976-1980	Grundschule Marbach
1980-1990	Gymnasium Martin-Luther Schule, Marburg
1990	Abitur
1991-1992	Zivildienst Klinikum Lahnberge/Marburg in der Orthopädischen Poliklinik
1992-1995	Studium der Biologie in Marburg im Diplomstudiengang
1995	Vordiplom
1995-1996	Freiwillige Mitarbeit im Institut für Zoologie bei Prof. Dr. P. Götz an der Freien Universität Berlin
1996-2000	Wechsel an die Humboldt Universität zu Berlin zum Hauptstudium
1999-2000	Diplomarbeit im Museum für Naturkunde Berlin; Morphologische und Anatomoische Veränderungen bei *Conomelus anceps* (Germar, 1821) (Homoptera: Fulgoromorpha: Delphacidae) hervorgerufen durch den Parasiten *Elenchus tenuicornis* (Kirby, 1815) (Strepsiptera: Elenchidae)
2000	Diplom
2000	Aufenthalt in Neuseeland und Australien
2001-2003	Wissenschaftlicher Mitarbeiter an der Technischen Universität München (TUM) am Lehrstuhl für Gemüsebau im Rahmen eines von der GTZ geförderten Projektes mit Sitz auf den Philippinen
2001-2005	Bearbeitung der vorliegenden Dissertation am Lehrstuhl für Gemüsebau unter der Leitung von Prof. Dr. W. H. Schnitzler
2006	Promotion