

Bachelor Thesis
on organic agriculture in Kenya.

Focus on:

Current approaches in chemical ecology using
regionally adapted sustainable methods.

BSc-Candidate: Eva Winter

2013

Professur für ökologischen Landbau Justus-Liebig-
Universität Giessen

Supervisors: Prof. Dr. Hans E. Hummel and Prof. Dr.
Günther Leithold



Table of contents

Table of graphs.....	4
Table of abbreviations.....	5
Abstract.....	6
Aim of study.....	6
General overview on Kenya.....	6
The agricultural sector in Kenya.....	9
Organic farming and its impact on farmers' livelihoods in Kenya.....	10
1) Case study: An attempt to practise sustainable farming: The push-pull project	16
2) Practical research in push-pull technology: Does <i>Desmodium intortum</i> induce a defense in <i>Zea mays</i> varieties against stem borer moths? Chemical ecology.....	24
Abstract.....	24
Introduction.....	24
Materials and Methods.....	28
Plants and insects.....	28
Experimental set up.....	30
Oviposition test.....	31
Entrainment of volatile plant compounds.....	33
Elution of volatile plant compounds.....	33
Bioassay with four-armed olfactometer.....	33
Chemical analysis.....	34
Statistical analysis.....	35
Results and Discussion.....	35
Conclusion and Outlook.....	39
3) Excursion: Can the Western corn rootworm <i>Diabrotica virgifera virgifera</i> expand into Africa?.....	40
Introduction.....	40
Material and Methods.....	43
Plants and insects.....	43
Sampling method.....	43
Set up.....	43
Results and conclusion.....	43
Literature.....	47

Table of graphs

Graph 1: Map of Africa: Kenya (Wikipedia 2013).....	7
Graph 2: Map of Kenya: Provinces (Medical Mission International 2013).....	8
Graph 3: Kenya's organic sector. Enterprises and land per Province.....	12
Graph 4: Organic produce per Province.....	12
Graph 5: Kenya's exported organic goods in categories in metric tons.....	13
Graph 6: Moth larva: Stem borer (ICIPE 2011).....	16
Graph 7: Damage caused by larvae (ICIPE 2011).....	16
Graph 8: Napier grass (<i>Pennisetum purpureum</i>) (ICIPE 2013).....	18
Graph 9: Sudan grass (<i>Sorghum sudanense</i>) (Stablemade.com 2013).....	18
Graph 10: Compounds with kairomonal actions responsible for the 'push' effect.....	19
Graph 11: Molasses grass (<i>Melinis minutiflora</i>) (FAO 2013).....	19
Graph 12: Silver leaf (<i>Desmodium uncinatum</i>) (Weeds.org 2013).....	19
Graph 13: <i>Striga hermonthica</i> (ICIPE 2013).....	20
Graph 14: A farmer's push-pull plot.....	20
Graph 15: Yield increase after push-pull implementation (ICIPE 2011).....	21
Graph 16: Adoption of push-pull technology by farmers (ICIPE 2011).....	22
Graph 17: Distribution of push-pull farmers (ICIPE 2011).....	23
Graph 18: Freshly germinated maize.....	28
Graph 19: <i>Desmodium intortum</i>	29
Graph 20: Gravid <i>Chilo partellus</i> (Lep.: Crambidae).....	29
Graph 21: <i>Cotesia sesamiae</i> (University of Kentucky 2013).....	30
Graph 22: Experimental set up in screen house: Plants in airborne communication.....	31
Graph 23: Control maize plants: Monoculture.....	31
Graph 24: Treated and Control plant in cage.....	32
Graph 25: Egg batches.....	32
Graph 26: Microscope.....	32
Graph 27: Plant during entrainment.....	33
Graph 28: Experimental set up.....	34
Graph 29: The four-armed Patterson's olfactometer.....	34
Graph 30: Results of oviposition test.....	36
Graph 31: Results of olfactometer test.....	37
Graph 32: Control results.....	37
Graph 33: DMNT (Pherobase 2013).....	38
Graph 34: TMTT (Khan 2000).....	38
Graph 35: MeS (Wikipedia 2013).....	38
Graph 36: Distribution pattern of <i>Diabrotica</i> in Europe (Kiss & Edwards 2012).....	42
Graph 37: Male adult <i>Diabrotica virgifera virgifera</i>	42
Graph 38: Pheromone trap (Levine & Metcalf 1988).....	42
Graph 39: Trap with caught insects.....	44
Graph 40: Trapped beetle.....	44
Graph 41: Set up of trap.....	45
Graph 42: Trapped insect.....	46
Graph 43: Trapped beetle.....	46

Table of abbreviations

C. sesamiae – *Cotesia sesamiae*

D. intortum – *Desmodium intortum*

DMNT – (E)-4,8-dimethyl-1,3,7-nonatriene

GDP – Gross domestic product

GS- MS – Gas chromatography-mass spectrometry

ICIPE – International Centre for Insect Physiology and Ecology

IFOAM – International Federation of Organic Agriculture Movements

IMO – Institute for Market Ecology

ITOC – ICIPE -Thomas Odhiambo campus

KIOF – Kenya institute for organic farming

KOAN – Kenya Organic Agriculture Network

KOFA – Kenya Organic Farmers Association

KOPA – Kenya Organic Producers Association

KS – Kenya seed company

MeS – Methyl salicylate

spp. – *Species pluralis*

TMTT – (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene

UNAIDS – United Nations HIV/Aids Programme

UNDP – United Nations Development Programme

WCR – Western corn rootworm

WS – Western seed company

Abstract

Kenya is an African developing country situated at the equator ranking 157th of 177 countries in the human development index. Agriculture is next to tourism the most important economic sector with 24 % of the GDP. Poverty prevalence is highest with small holder farmers. A promising approach to reduce this precarious condition is the implementation of organic agriculture. 1. Establishment of certified organic cash crop production for a domestic and/or an export market and 2. dissemination of common methods in organic agriculture to subsistently living small holder farmers are the two major strategies of the approach. Both have different positive impacts regarding poverty reduction. This Bachelor thesis focuses on the science-based push-pull project of the second strategy: In South western Kenya, the 'push-pull' cropping system for maize and sorghum production in subsistence farming was developed. It is a farming system which exploits the allelopathic defense reactions of indigenous plants against the major pests stem borers and the parasitic Striga weed in Subsaharan Africa.

*The practical part of this thesis strives to better understand one of the interactions within the cropping system. It is investigated if the legume *Desmodium intortum* which is commonly used in the push-pull cropping system induces a defense in *Zea mays* varieties against stem borer moths (*Lepidoptera: Crambidae*).*

Aim of study

This Bachelor thesis focuses on an agricultural research project which has developed the 'push-pull' cropping system in order to tackle poverty in Subsaharan Africa through exploitation of chemical interactions between plants and pests as well as principles of organic agriculture. The aim of this Bachelor thesis is to put this project into a larger context with Kenya's organic agriculture sector. There are many similarities between the principles of organic agriculture and the methods applied in the push-pull cropping system. The practical research part of this thesis investigates if one specific process within the complex system of interactions in the cropping method takes place as a contribution to understand the whole system better.

General overview on Kenya

Kenya is an East-African country situated at the equator bordering Somalia and the Indian ocean in the East, Ethiopia and Southern Sudan in the North, Uganda and lake Victoria in the West and

Tanzania in the South. It holds a population of 37 million people and covers an area of 582.650 km² (comparable to the size of France). The largest cities are Nairobi as the capital, Mombasa as major trade gate way to the Indian ocean and Kisumu as trade hub at lake Victoria. The population distribution pattern shows high density in cities as well as in fertile areas in the Central and Western regions (Library of Congress 2007). 80 % of the population lives in rural areas (Kledal 2009).

Kenya gained its independence 1963 having formerly been a British colony. The current president Mwai Kibaki is the third president of the republic of Kenya whose political system favours a strong presidency. Approximately 40 tribes live in Kenya belonging to three different linguistic families (Bantu, Cushitic and Nilotic). English and Swahili are official languages. The biggest and most influential tribes are Kikuyu, Kalenjin, Luyha and Luo (Library of Congress 2007). Current politics are still dominated by tribal conflicts with a major outbreak 2007/2008 due to voter fraud known as the post election violence (Jones 2008).



*Graph 1: Map of Africa: Kenya
(Wikipedia 2013)*



Graph 2: Map of Kenya: Provinces (Medical Mission International 2013)

Kenya is a tropical country with some large arid areas especially in the North. The weather is determined by a season of short rains and a season of long rains in November and April with an average of 266 mm rainfall per month. The driest month is August with an average of 24 mm. The average temperature ranges between 24 and 26 °C.

There are a number of environmental issues arising in Kenya such as deforestation, soil erosion, desertification, poaching, water shortage and quality degradation as well as domestic and industrial pollution. Approximately 5000 hectares of forest are cleared every year with only three percent still left leading to loss of biodiversity, erosion, soil degradation and flooding. Water hyacinth infestation due to pollution in fresh water lakes has spread and affected fishing output significantly.

Kenya ranks 152th out of 177 countries in the human development index 2006 (parameters: Gross domestic product/capita; life expectancy; adult literacy; school enrollment).

Birth rate has gone down from an average of eight children per woman to approximately four within the last 40 years. The demographic structure shows that the population is very young with a median age of 18,6 years and a low life expectancy at birth of 47 to 55 years.

As most African countries, Kenya has a relatively high HIV/Aids infection prevalence, although estimated figures differ significantly (16 % UNDP; 6,7 % UNAIDS). Additionally, prevalence is about twice as high for women as for men. Other tropical diseases such as malaria and tuberculosis are common.

Looking at education and welfare, literacy rate ranges between 85 percent (men) and 75 percent (women). As primary school education is currently free of charge, attendance is relatively high. However, only 23 % of the relevant age group attends secondary school. There is a growing gap between the poor and rich as well as a decline of income per capita with 55,4 % of the population living below the poverty line (Library of Congress 2007).

The economy is market-based maintaining a liberalised external trade system. Kenya's gross domestic product (GDP) 2011 was in total \$ 71,427 billion and \$ 1746 per capita (International monetary fund 2012). The strongest economic forces are rain-fed agriculture (24 % of GDP) and tourism (63 % of GDP) which are both highly dependent on external factors making the economy vulnerable. Other contributors to the GDP are mining and mineral production, industry and manufacturing although both contribute only little to the GDP (Library of Congress 2007).

The agricultural sector in Kenya

Approximately 15 % of Kenya's landmass is sufficiently fertile and rain-fed to be cultivated and only 8 % is first class land. Nevertheless, agriculture is -next to tourism- the most important economical sector in Kenya. 75 % of the population is employed in agriculture. 50 % of the production is subsistence farming, the rest is commercially traded. Commercial agriculture, fishing and forestry contribute 26 % to the GDP and make up 60 % of Kenya's export earnings (Kledal 2009). The major cash crops destined for export are tea, horticultural produce, flowers and, though declining in importance, coffee (Library of Congress 2007). Food crops are maize, sorghum, millets, horticulture produce like tomatoes, onions, kale and many more with maize being of distinct importance as a staple food. Livestock such as cattle, sheep and goats for meat and milk production has high prevalence in the whole country and is dominant in the semi-arid and arid regions.

Generally, agriculture is practised by peasant farmers on a very small scale highly dependent on weather conditions. 70 % of all smallholder farmers are women (Kledal 2009).

As mentioned above, forestry and fishing used to be important segments in agricultural production. However, output in these areas has decreased tremendously due to deforestation and pollution (Library of Congress 2007).

Organic farming and its impact on farmers' livelihoods in Kenya

New attempts and innovations in Kenyan small scale agriculture would be beneficial for a very high percentage of the population. To give a short summary of the important facts:

- 80 % of Kenya's population works in agriculture
- Over 50 % lives below the poverty line, over 35 % is malnourished
- Agriculture is practised mostly by smallholders (75 %; farm size not bigger than 2 ha)
- Small holders are mostly women who are often socially and economically disadvantaged

(Kledal 2009; Library of Congress 2007; Nations encyclopedia 2013; Haerlin and Busse 2009)

One attempt which has shown to be suitable for smallholder farmers is organic farming as principles focus on low input methods. It can also increase yield significantly, especially in tropical countries. Badgley et al. 2007 show that conversion from traditional smallholder agriculture to organic agriculture in subsistence can increase yield to 180 %. Furthermore, world wide conversion to organic agriculture would increase the world yield average to 132 % according to this study. Other studies support this evidence (Gänz 2011; Piras 2011). In addition to an increasing yield, there are other benefits which will be addressed below.

The International Federation of Organic Agriculture Movements defines organic agriculture as the following:

'Organic agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved' (IFOAM 2009).

In other and more pragmatic words, organic agriculture is a matter-cycle oriented, nature conserving form of agriculture. Input of synthetic fertilizers and pesticides is prohibited. Conservation and promotion of natural soil fertility and preventive pest control are key points. Ideal upkeep of the nutrient circulation includes ruminant livestock and fodder crop production. Practical methods are the integration of legumes into the crop rotation either as intertillage or as main crop due to their ability to fix nitrogen (Piras 2011) and mobilise phosphorus (Leithold 2010) in the soil enhancing soil fertility. Furthermore, a wide crop rotation well adapted to the location and considering incompatibilities is essential in order to prevent pests and decreasing soil fertility. Soil erosion is prevented by intertillage or a mixed cropping system including perennials. The major challenges in tropical agriculture are poor soil fertility, high risk of erosion, insect pests and

resulting high input costs for control. Mixed cropping systems specifically address these issues. A good example is agroforestry, an approximation of a forest ecosystem. Taller plants like bananas or coconuts produce shade, legumes provide nitrogen. Perennials as well as annuals prevent erosion, annuals and legumes also provide tillage material enhancing soil fertility. This additionally promotes biodiversity and provides a large variety of foods for the farmer (Piras 2011). This study will focus on a mixed cropping system called '**push-pull system**' in the next chapters.

Before coming to this, it needs to be clarified that there are two ways organic agriculture can be implemented to promote farmers in developing countries. Firstly, it is certified cash crop production qualified for the domestic as well as the export market. Secondly, it is teaching subsistent small holders the principles of organic farming in order to increase their production efficiency.

Production of organic cash crops can improve the situation of smallholders in other ways than the pure implementation of its principles can:

Witnessed was the development of social and political structures and therefore a new level of organisation through certification and cooperatives which can address issues like insufficient payment for labour and gender inequality. Furthermore, an awareness raising for the benefits of an organic diet can create a domestic demand leading into health benefits for the whole population.

However, production for export often means that farmers do not understand the benefits of organic agriculture but tend to see it as a Western fashion. The export orientation is also in conflict with matter cycle management and makes the farmers dependent on the world market. Nevertheless, a domestic market for the produce could conquer these issues (Hesse et al. 2009).

In Kenya, a medium sized organic export market in comparison to other African countries and also a small domestic market have established themselves. The following information on certified organic cash crop production is extracted from the survey 'The world of Organic Agriculture - Statistics and Emerging Trends 2009' (Kledal 2009). The movement started in the 1980s with the foundation of the Kenya Institute of organic farming (KIOF) and was carried by Non-Governmental Organisations. In the 1990s, the Kenya Organic Farmers Association (KOFA) was formed. Through this, the movement became more target-orientated as individual forces started to work closer together. Organic farming standards by International Federation of Organic Agriculture Movements (IFOAM) and the European Union for members of KOFA were published. Additionally, bigger companies and commercial farmers founded the Kenya Organic Producers Association (KOPA).

The latest development was the foundation of the umbrella network Kenya Organic Agriculture Network (KOAN) in 2005 promoting the further growth of the organic sector. However, there are still no official policies on organic agriculture in Kenya in spite of the establishment of an organic desk by the Ministry of Agriculture following this purpose.

Thus, certification is carried out by international organisations such as the Soil Association (UK),

Ceres (USA), EcoCert (France), IMO (Germany) and Bio Suisse (Switzerland).

Since 2007, the East African Organic Products Standard tries to establish the organic brand 'Kilimohai' throughout East Africa in an attempt to standardise the criteria but faces problems due to the lack of national rules.

Nowadays, most of Kenyan organic farming is practised by a small number (35) of big farming enterprises with more than half situated in the highly productive Central Province. The overall certified land covers 78438 ha. Only 4535 ha of this land is agricultural land, most is under extensive use or considered as wild area. To put these numbers into a context, the countries having the largest certified areas such as Australia, Argentina and Brazil cover land from 1.77 to 12 Million hectare. Africa in its whole is the continent with the smallest percentage of certified land in the world (Willer 2010). In the following tables the number of enterprises, certified land and produce per Province are listed.

Graph 3: Kenya's organic sector. Enterprises and land per Province

Province	Number of farm enterprises + supply organisations	Number of outgrowers	Agricultural land (ha)	Wild area/ extensive use (ha)	Total (ha)
Central	19	819	3023	40500	48861
Coast	2	474	1543	-	2017
Eastern	4	334	324	-	658
Nairobi	2	-	16	-	16
North eastern	-	-	-	-	-
Nyanza	-	-	-	-	-
Rift Valley	5	154	276	32640	32599
Western	3	100	251	-	351
Total	35	1811	4535	73851	78338

KOAN, Encert, Kledal, field data (Kledal 2009)

Graph 4: Organic produce per Province

Province	Produce
Central	Beans, peas, sweet corn, chillies, avocados, baby salad, baby vegetables, spinach, potatoes, leeks, indigenous vegetables, cucumbers, passion fruit, pears, oranges, bananas, raspberries, essential oils, lemon grass, rosemary, ground-, macadamia- and cashew nuts, tea, coffee
Coast	Coconut oil, avocado oil

Eastern	Chamomile, carcade, lemongrass, mangoes, guava, sweet bananas, honey and wax, indigenous vegetables, tomato, kale, spinach, onions, pepper, grains
Nairobi	Indigenous vegetables, tomatoes, kales, milk, probiotic yoghurt
North eastern	-
Nyanza	-
Rift Valley	Tea, paprika, birds eye chillies, <i>Tagetes</i> , <i>Echinacea purpurea</i> , coriander, <i>Calendula</i> , borage, safflower, strawberries, milk
Western	Pineapples, chillies, onions

KOAN, Encert, Kledal, field data 2008 (Kledal 2009)

Looking at the domestic versus the export market, the domestic market is rather small and concentrates on the capital city Nairobi and its outskirts addressing mostly the upper class and expatriates. There are a few supermarket chains like Nakumatt and Uchumi as well as some restaurants selling organic produce. Common products are coffee, tea, honey, sunflower oil, flour, macadamia nuts, and various health products. Additionally, at the coast tourism creates a demand for organic produce within hotels and restaurants.

The export market mainly offers fresh vegetables, tropical fruits, essential oils, herbs, nuts, coffee and tea. The following table shows the amounts in export categories in metric tons.

Graph 5: Kenya's exported organic goods in categories in metric tons

Export categories	Metric tons
Vegetables	700
Coconut oil	15
Herbs	150
Nuts	860
Coffee/Tea	400/200
Total	2325

Kledal, August September 2008. Company information collected by interviews (Kledal 2009)

In comparison to Kenya's neighbouring country Tanzania, Kenya has a relatively big organic export market. Tanzania exports 1494 metric tons in 2009 (Willer 2010).

Very recent developments have been either large scale land certification for one specific export crop or small scale certification for the local market. Generally, local growth is still highly dependent on the numerous international organisations operating in Kenya which employ foreigners interested in organically produced goods. Thus, with the insecurity of the post election violence 2007/2008 driving foreigners out of the country, the organic sector suffered. Additionally, discussions on

carbon emissions produced by Kenyan organic export goods in high-demand countries presents problems to growth. In order to help the market grow significantly, considerable investments and policies are needed. If this was the case, organic agriculture in Kenya could contribute to a great extent to poverty prevention, food security, farm environments, gender and social equality as it has done in many other countries (Kledal 2009).

However, as briefly mentioned above, organic agriculture can also have a positive impact on subsistently living farmers who produce on a scale too small to consider a certification and who struggle to feed their families with what their land produces. As pointed out beforehand the methods applied in organic agriculture in the tropics often increase yield in subsistent farming significantly (up to 180 %) (Badgley et al. 2007). This is because tropical small holder farmers often lack the knowledge and the resources to exploit the full production potential of their land. While working in a farmers' empowerment project in Kenya I have witnessed that poor small holder farmers do not have a basic understanding of the most common pests infesting their food crops and therefore cannot sufficiently protect them. Thus, low input methods like legume intercropping or an organic pesticide made out of local plants can have an immediate positive effect on the yield. They are also much easier to access and afford than high input methods like synthetic fertilisers and pesticides or commercially produced seeds. For example, a study on maize in Mexico has shown that agriculture using synthetic external input and organic agriculture are economically comparable as lower yields in organic production and the higher costs in input intensive production even out (Gänz 2011). Moreover, organic agriculture accounts for a reliable and stable harvest in the long term as well as positive impacts on the environment and the farmers' and general population's health (Hesse et al. 2009).

There are also examples of farmers forming political resistance against external input reliant agriculture promoted by the governments of many countries, such as on the Philippines. There, farmers have founded a cooperative for organic production destined for the domestic market. Within the cooperative, farmers work together to push for health regulations in farming, fair land distribution and other related topics to empower themselves in order to improve their livelihoods (Piras 2011). This shows that organic agriculture has the potential to make a significant difference in the lives of tropical small holder farmers, especially if it leads into an alliance of farmers with the same interest.

The main part of this study focuses on a project of the second approach on how organic agriculture can be implemented to promote farmers in developing countries: Teaching subsistent small holders the principles of organic farming in order to increase their production efficiency and to generally improve their living conditions.

The push-pull project addresses the needs of small-scale farmers (down to 0,25 ha) in Subsaharan Africa focusing on the major staple food crops maize and sorghum. Using the technology, farmers

double, sometimes triple their yield, improve soil fertility and produce a high quality animal fodder (ICIPE 2011).

1) Case study: An attempt to practise sustainable farming: The push-pull project

Professor Z. R. Khan and his team started to teach farmers the push-pull technology in South western Kenya in 1997 as part of the International Center for Insect Physiology and Ecology (ICIPE) (ICIPE 2011).

ICIPE was founded in 1970 with the mission to fight poverty in developing countries through research on insects. Many disastrous diseases of humankind are transmitted by insects such as malaria, dengue and kala-azar. Additionally, insects are one of the main causes in the tropics for enormous yield losses while crops are growing and after harvest (ICIPE 2013).

ICIPE and Rothamsted Research (UK) have started research on stem borers in 1994. Originally, stem borers fed only on wild grasses, but with the introduction of maize in Africa, it has become a major food source for them. Maize is nowadays Africa's most important food crop and stem borers are one of the biggest threats to the yield as they on average cause 20 – 40 % yield loss, even up to 80 %. Stem borers lay eggs onto the host plant where the hatched larvae feed on the leaves causing considerable damage to them before entering into the maize stem. The population of the two regions in which the programme started relies on maize as a staple food resulting periodically in a lack of food security. Additionally, the prevalence of livestock ownership is high with a shortage of animal fodder (ICIPE 2011).



Graph 6: Moth larva: Stem borer (ICIPE 2011)



Graph 7: Damage caused by larvae (ICIPE 2011)

Push-pull is based on principles which are compatible with organic agriculture, although the production is mostly developed for subsistently living small holder farmers and thus the produce is

(so far) not part of the certified organic market in Kenya. It is a pest management system based on a mixed cropping system including the main food crop, a legume and a grass. The two latter ones are both suitable as organic manure and good quality animal fodder which is scarce in the addressed areas. There are a number of advantages addressing the agricultural challenges in South western Kenya and other tropical regions. The mixed cropping system has a positive impact regarding pest pressure reduction, land use efficiency and soil fertility. In addition, both legume and grass are perennial plants, therefore a large part of the soil is protected from erosion all year and soil moisture is conserved (ICIPE 2011).

As mentioned before, the legumes (Family: Fabaceae) used in push-pull are in symbiosis with the soil bacteria of the genus rhizobia which fix nitrogen from the air in the soil (Fischinger 2012). Legumes also mobilise phosphorus through the emission of root exudates (Leithold 2010). Furthermore, being designed as a low input system to keep costs low for the farmer, there is generally very little or no external synthetic input such as mineral fertilisers or pesticides safeguarding human health, promoting biodiversity and beneficial soil organisms as well as preventing crop, soil and ground water pollution.

Apart from the positive impact on pest reduction of any mixed cropping system due to its approximation to a natural ecosystem, the push-pull system specifically works with chemical interaction between the three different crops, pests, parasites and beneficial insects. The system is based on two mechanisms, the 'pull' and the 'push'.

The 'pull' part consists of three rows of particular grasses which surround the food crop. The grasses are natural hosts of stem borer moths, thus they draw them away from the maize or sorghum plants. The most suitable ones are Napier grass (*Pennisetum purpureum*) and Sudan grass (*Sorghum sudanense*). Sudan grass does not only attract the moths and distract them from the food crop. It also attracts stem borer parasitoids such as the African parasitic wasp *Cotesia sesamiae* which inject their eggs into the stem borer larvae. The freshly hatched wasps then eat the larvae. Napier grass produces a sticky sap to trap the borer and to prevent the majority of the larvae from developing. Both grasses attract other enemies of stem borers like ants, spiders and cockroaches.



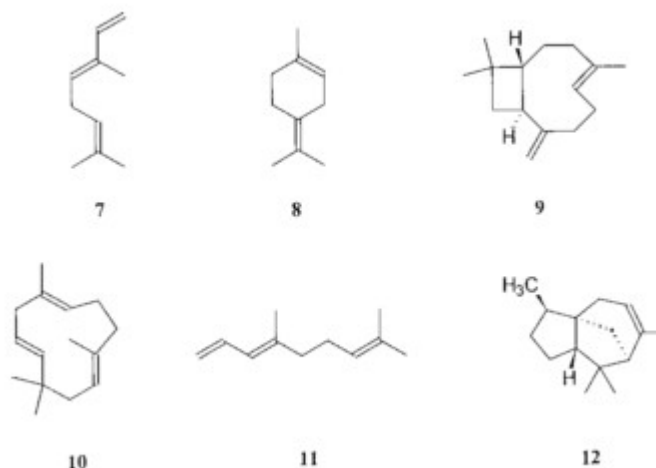
*Graph 8: Napier grass (Pennisetum purpureum)
(ICIPE 2013)*



*Graph 9: Sudan grass (Sorghum sudanense)
(Stablemade.com 2013)*

The 'push' part works as the following: The food crop maize or sorghum is intercropped either with a legume of *Desmodium spp.* or a grass. Molasses grass (*Melinis minutiflora*) has the strongest repellency effect on stem borer moths and the greatest attraction for parasitoids. Push-plants prophylactically produce a blend of volatile compounds which is generally produced by plants

under feeding stress. Low concentrations of this blend attract more herbivores whereas high concentrations drive them away making them believe that the plant is already fully exploited (ICIPE 2011). There are a few key compounds creating this effect: (E)- β -ocimene (7); α -terpinolene (8); β -caryophyllene (9); humulene (10), (E)-4,8-dimethyl-1,3,7-nonatriene (11) and α -cedrene (12) (Khan and Pickett 2000).



Graph 10: Compounds with kairomonal actions responsible for the 'push' effect



Graph 11: Molasses grass (Melinis minutiflora) (FAO 2013)



Graph 12: Silver leaf (Desmodium uncinatum) (Weeds.org 2013)

Desmodium spp. have the additional and extraordinary advantage of acting as a natural defense against *Striga* parasitic witch weeds such as *Striga hermonthica* and *Striga asiatica*. *Striga spp.* are

a major challenge in many parts of Kenya and other Sub-Saharan countries (Khan and Pickett 2000). One plant produces approximately 50000 seeds which can stay dormant in the soil for up to 20 years, awaiting a chemical cue from a potential host (Estabrook and Yoder 1998). An infestation causes yield losses of 30 – 100 %, in very severe cases the land has to be abandoned. Conventional methods are application of nitrogen fertiliser in high amounts, crop rotation, chemical germination stimulants, herbicide application, hoeing, hand-pulling and the use of resistant or tolerant crop varieties. These methods do not prove to be very effective, are too expensive for most small holders or very labour intensive (ICIPE 2011).



Graph 13: Striga hermonthica (ICIPE 2013)



Graph 14: A farmer's push-pull plot

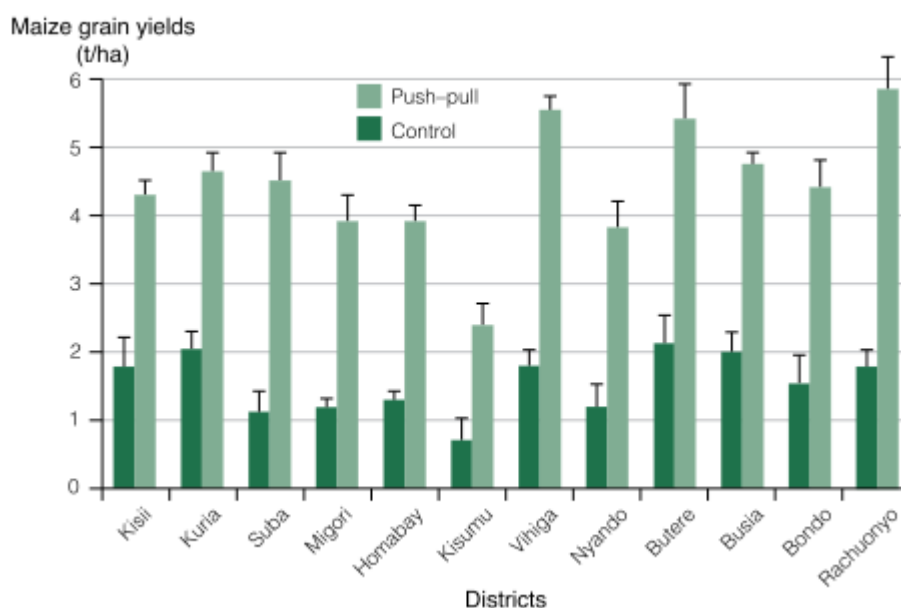
Desmodium spp. emit root exudates which promote germination of *Striga* seeds but then interfere with the haustoria development of the parasite. Through this, *Desmodium spp.* do not only effectively protect themselves from the parasite, they also decrease the number of seeds in the soil (Khan and Pickett 2000). Chemical compounds responsible for these effects are isoflavanone, namely uncinanone A, B, C and genistein. A study has shown that push-pull technology using local

farmers' varieties and no fertiliser has the best cost-benefit ratio in comparison to other *Striga* control methods such as resistant breeds or herbicide application (ICIPE 2011).

All in all, due to pest control, improved soil fertility, erosion and wind lodging prevention adopting the push-pull technology on average increases maize yields by over 100 %. Oftentimes, farmers having formerly struggled to produce enough for their families become self-sufficient and can sometimes sell the surplus. With enough profit, farmers can afford to buy a dairy cow, the milk being an additional source of protein and income. Others focus on push-pull plants seed production for other farmers.

In this manner, many farmers manage the transition from subsistence to income acquiring agriculture which improves their lifestyle and helps them gather further qualifications.

One of the disadvantages is that the adaptation of the technology is very labour intensive in the first months. Push-pull's social scientists have carried out a cost-benefit analysis to verify if the output of the cropping system justifies this input. It has been recorded that farmers efficiently and consistently get a good return using push-pull with a benefit:cost ratio of 2,5 and a positive long term trend. Best results are produced when both *Striga* and stem borers are severe constraints to the assessed area.



Graph 15: Yield increase after push-pull implementation (ICIPE 2011)

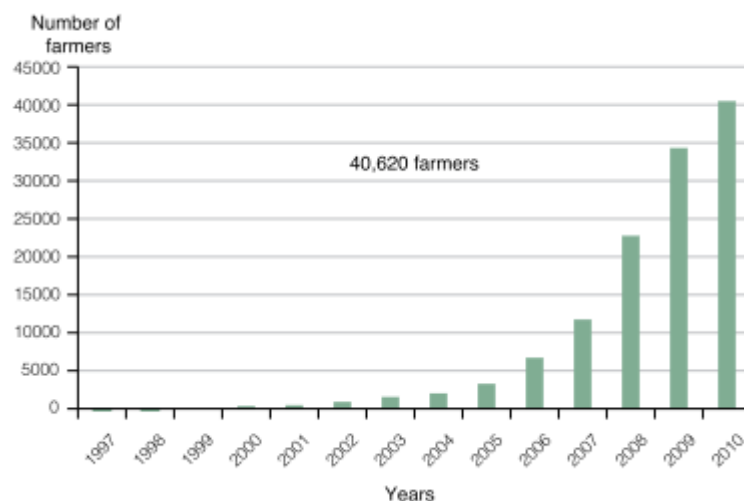
Additionally, push-pull technology has shown to be suitable for different agro-ecologies. It has been established in the regions at the shore of lake Victoria where two maize or sorghum crops a year can be grown due to two rainy seasons with *Striga* being the biggest threat to food security. It has

however also been successfully adopted in the highland region of Trans Nzoia where the main restraints are stem borers and soil fertility. Moreover, the technology improves yields and reduces pests also when used with millets and rice crops which are more drought resistant than maize and therefore more resilient facing the challenges of climate change. This knowledge has amongst others been obtained within the EU funded climate change adaptation research programme of the push-pull project.

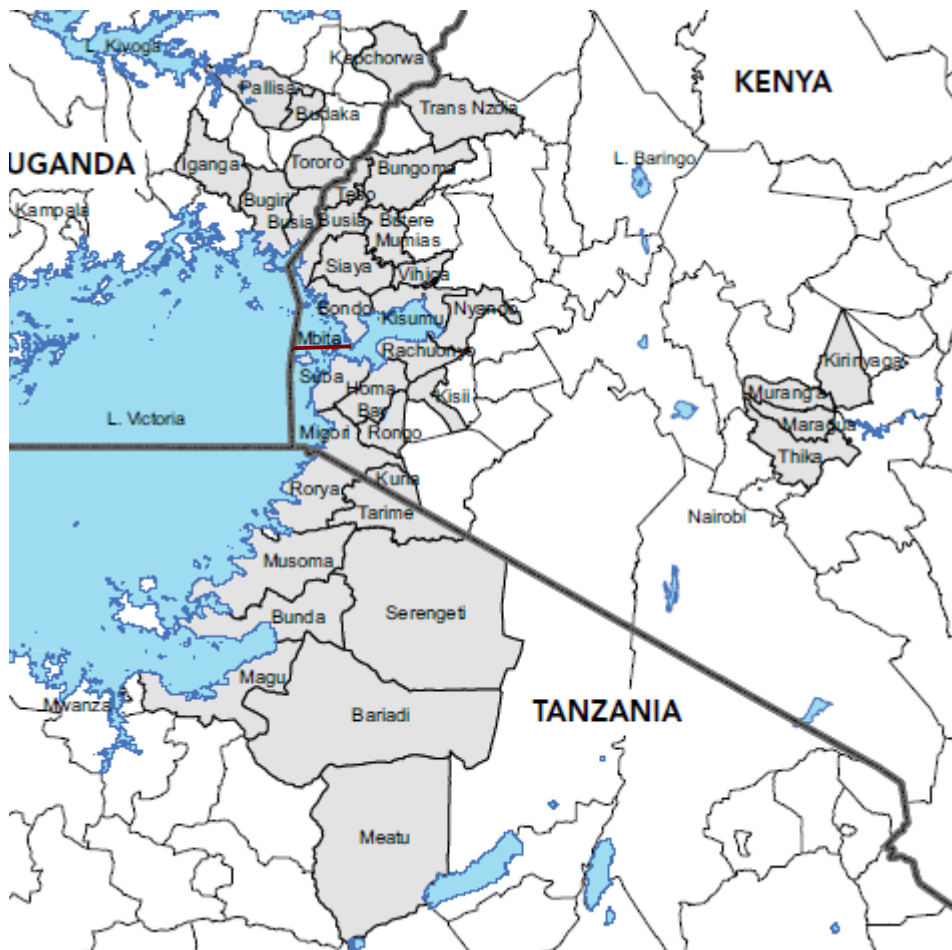
Besides from the natural science research, the push-pull also focuses on the social science aspects of the technology and has therefore developed several educational methods on how to spread the knowledge as effectively as possible.

For example, according to experience, farmers are most likely to understand and adopt the technology if they see a functioning field. Therefore, several demonstration plots have been established at Thomas Odhiambo Campus in Mbita point.

Another factor having led the project into success is training so called farmer-teachers as multipliers. These are farmers who have successfully adopted push-pull on their own land and who then teach other farmers how to do it advising them throughout the process of adoption as well as afterwards. 40.000 farmers have adopted the technique so far (2011). The technology has spread as far as Tanzania and Uganda. Farmers having adopted the technology often identify highly with it and the improvements it has brought to their lives. They solidarise with other push-pull farmers which empowers them to attract more resources than individuals could (ICIPE 2011).



Graph 16: Adoption of push-pull technology by farmers (ICIPE 2011)



Graph 17: Distribution of push-pull farmers (ICIPE 2011)

A future target is to increase the number of farmers applying push-pull up to a million. Nevertheless, there are a few challenges push-pull researchers and farmers have to face.

One of the restraints yet to be overcome is post-harvest loss. Currently, farmers are forced to sell their surplus directly after harvest for a relatively low price instead of being able to store it for times of food shortage. Research on post-harvest diseases and pests is planned for the future by ICIPE. Another threat is the development of diseases and pests towards the plants used in push-pull. This increases with the number of farmers growing them monoculturally for seed production. An example for this is the Napier grass stunt disease caused by a phytoplasma bacterium transmitted by the leaf hopper *Recilia banda*. An effective remedy has not yet been discovered. *Desmodium spp.* are threatened by pollen beetles and a pod borer (ICIPE 2011).

Ever since the discovery of the defense ability of the indigenous plants used in push-pull many research projects are carried out in order to understand the complex mechanisms and interactions within the push-pull cropping system. In the following we will look at the interaction between the nowadays commonly used intercrop plant *Desmodium intortum* and several *Zea mays* varieties.

2) *Practical research in push-pull technology: Does Desmodium intortum induce a defense in Zea mays varieties against stem borer moths? Chemical ecology.*

Abstract

In some cases, plants communicate with each other through volatile chemical compounds emitted by leaves or flowers. In this way, defense mechanisms against herbivores can be induced. This study was designed to investigate if the legume Desmodium intortum known to produce repellent volatiles against stem borer moths induces defense into Zea mays varieties. We looked at two open-pollinated farmers' varieties and two commercial hybrid varieties suspecting the farmers' varieties to be responsive rather than the hybrids. The study was carried out under screen house conditions at ICIPE Mbita Point Campus. Here, the 'push-pull' cropping system for maize and sorghum production was developed, a farming system which exploits the allelopathic defense reactions of indigenous plants against the major pests stem borer moths and Striga spp. in Sub-Saharan Africa. Desmodium spp. are used as stem borer repellent intercrops. A defense induction into maize would have proven the farming system to be even more effective than originally expected when using farmers' varieties. However, no defense induction was detected in this study. This could be explained by an insufficient production of defense inducing volatiles in the Desmodium intortum leaves whereas D. intortum flowers might produce a sufficient amount to induce the response. Consequently, further study is needed to better understand the apparent lack of a response.

Introduction

In chemical ecology research, it is well established that interaction occurs between 1) neighbouring plants, 2) plants and herbivores, 3) plants and the natural enemies of herbivores, and 4) plants and soil organisms. This interaction is mediated by signaling molecules exchanged either via atmosphere or rhizosphere, an effect generally referred to as 'allelopathy' (Molisch 1937, Muller 1970, Rice 1984, Grodzinsky as edited in English by Narwal 2006).

There are many possible elicitors for these interactions: One may be a stress situation of the plant due to attack or illness warning neighbouring plants and/or attracting predators or parasitoids of the attacker (Pickett et al. 2006). Furthermore, some plants have developed a prophylactic defense strategy keeping away enemies by sending out repellents. This can be a chemical blend seriously damaged plants would commonly produce to make the attacker believe its target plant is already

exploited (Khan and Pickett 2000; Pickett et al. 2003). Other plants may also 'notice' the defense strategy and adopt it. This warning system has been witnessed intraspecifically as well as interspecifically (Bruin and Sabelis 2001). These interactions are helpful for the signal receiving plants. The cue induces the expression of one or several genes leading to a defense reaction (Farmer and Ryan 1990). Defense can be induced locally, but also systemically when intact plant parts produce volatile chemicals in order to recruit natural enemies of a pathogen infesting parts of the plant (Guerrieri et al. 2002).

There are two common defense strategies, the direct and the indirect defense mechanism. Both mechanisms are often used simultaneously by a plant. Direct defense refers to a response to the attacker or pathogen itself such as the production of a repellent semiochemical whereas indirect defense is defined by responding to an attack by 'calling for help' from the natural enemies such as predators or parasitoids of the attacker (Dicke et al. 2003).

There are also the competitive interactions which harm neighbouring plants. Aerial allelopathy can influence biomass allocation in different barley cultivars in order to make them superior to their competitors (Ninkovic 2003). Another example is that legumes in the genus *Desmodium* have been found to produce root exudates to promote the germination of parasitic weeds in the genus *Striga*, and to then inhibit further development of the parasite (Khan et al. 2008).

As mentioned above, there are several ways of plant communication through signalling chemicals which are also called semiochemicals. Two interaction methods take place in the rhizosphere. Firstly, plants communicate with other organisms in the soil through root exudates. These can have repellent or attractant effects and have a significant influence on the whole soil flora and fauna (Estabrook and Yoder 1998). For example, intact broad bean plants (*Vicia faba*) become more attractive to the aphid parasitoid *Aphidius ervi* when grown in the same pot as the same species infested with the pea aphid *Acyrtosiphon pisum* due to root exudate communication (Guerrieri et al. 2002).

Secondly, fairly recently it has been found out that plants also communicate through mycorrhiza fungi networks in the rhizosphere. In this manner, pathogen infested tomato plants induce a defense reaction into nearby growing healthy tomato plants (Song et al. 2010).

Additionally, plants communicate via volatile chemicals through the atmosphere. There are a few typical signalling chemicals. One group contains the common green leaf volatiles such as (*Z*)-3-hexenol, (*E*)-2-hexenal, and (*Z*)-3-hexenyl acetate which have been found to induce defense against spider mites in lima bean leaves (Arimura et al. 2001). Often mentioned in the literature for inducing indirect defenses are also for example methyl jasmonate, cis-jasmone and methyl salicylate (Farmer and Ryan 1990; Pickett et al. 2003; Khan et al. 2008). Signalling through green leaf volatiles can also be carried out by producing the same substances but changing the

concentration of some compounds of the composition (Pickett et al. 2006).

In South western Kenya, the 'push-pull' cropping system for maize and sorghum production was developed, a farming system which exploits the allelopathic defense reactions of indigenous plants against the major pests, stem borers and the parasitic *Striga* weed in Subsaharan Africa.

The cropping system comprises a plot of maize or sorghum intercropped with plants producing semiochemicals which repel stem borer moths ('push'). Additionally, the plot is surrounded by plants producing semiochemicals which attract stem borer moths ('pull'). A few plants qualify for the push or the pull part. However, current practise is to use Napier grass (*Pennisetum purpureum*) as attractant and *Desmodium spp.* or molasses grass, *Melinis minutiflora*, as repellent components. *Desmodium spp.* have several advantages over molasses grass. In addition to being repellent to stem borer moths, it is a high protein animal fodder, and being legumes they improve soil fertility through nitrogen fixation. Additionally and most remarkably, *Desmodium spp.* suppress the growth of *Striga hermonthica* through root exudates (Khan et al. 2000).

This study focused on *Desmodium intortum* in stem borer control which is superior to its close relative *Desmodium uncinatum* in biomass production (Kifuko-Koech et al. 2012) and in drought resistance making it more adaptable to climate change (field observation by *ICIPE* push-pull project).

Stem borers belong to the order Lepidoptera (moths) and include the maize stalk borer *Busseola fusca* and spotted stem borer *Chilo partellus*. Their life cycle is approximately 45 days (from oviposition to oviposition). The larvae cause considerable damage to the maize or sorghum plant by shredding their leaves and then entering into the plant stem as third instar. As fifth instar the larvae pupate. The adults are able to identify the right host plants (three to four weeks old plants) to oviposit on their leaves. A biological control method is the use of stem borer parasitoids such as the indigenous parasitoid *Cotesia sesamiae* and the introduced parasitoid *Cotesia flavipes* (Khan et al. 2000). These parasitoids parasitise the 4th stem borer instar entering through the same hole into the stem as the larva did.

The aim of this study was to compare airborne interaction between *Desmodium intortum* and four different varieties of *Zea mays*: Open-pollinated landrace varieties Jowi-red and Nyamula and Hybrid varieties 505 (Western seed company) and PH4 (Kenyan seed company). A repellent effect of *Desmodium spp.* on common stem borer moths is proven, as mentioned above (Khan et al. 2006). Chemicals responsible for this were identified to be ocimene, nonatriene and other sesquiterpenes, such as α -cedrene (Khan et al. 2000). We sought to find out if the maize varieties pick up on this prophylactic defense strategy of the legume when intercropped with *Desmodium intortum* and if there is a difference between the maize varieties with regards to this trait. This would imply that the cropping system is even more effective in its repellency against stem borers

and its attraction to stem borer parasitoids than expected.

Such an effect has for example been witnessed with barley, *Hordeum spp.*, becoming less attractive to aphids through airborne chemical interaction with couch grass (*Elymus repens*) or quack grass (*Agropyron repens*). Some barley cultivars themselves can also induce aphid repellency into other barley cultivars (Pickett et al. 2003). Generally, the capability of plants to react to 'warning' signals has been reported numerous times and has therefore been in the focus for agricultural exploitation (Khan et al. 2008).

In this experiment, we suspected to find the effect of 'smart plants' listening to the cues of others in farmers' varieties rather than in hybrid varieties. In the work of Tamiru et al. 2011 studying host plant reactions to stem borer oviposition, a defense mechanism was only found within the open-pollinated maize landraces in contrast to commercial hybrid varieties.

The four different maize varieties were placed with *D. intortum* before flowering under screen house conditions for 21 - 28 days after germination and were then compared to maize of the same variety grown without exposure to *D. intortum* (control). Analysis took place in three steps.

Firstly, treated and untreated maize plants were exposed to gravid female stem borer moths of the species *Chilo partellus*. Then the number of eggs laid on each plant were counted. Here we suspected to witness the direct defense mechanism of the farmers' maize varieties induced by volatiles of *D. intortum*.

Secondly, volatile organic compounds from the immediate surrounding of the upper part of the maize plants were entrained and analysed using gas chromatography-mass spectrometry (GC-MS). The expected result was to identify volatile semiochemicals responsible for a defense against stem borer moths.

Thirdly, the attracting effect of the volatile composition of treated and untreated maize plants of all varieties on stem borer larvae parasitoids *Cotesia sesamiae* were tested in an olfactory bioassay using a four armed olfactometer. A significant attraction of the parasitoids to volatiles of the farmers' varieties induced by *D. intortum* volatiles would show an indirect defense. However, it has been reported beforehand that attractiveness of *Desmodium* to parasitic wasps does not take place until its flowering (Midega et al. 2009). Sufficient induction of defence by *D. intortum* would open up opportunities for synthetical production of semiochemicals which would then be used to induce defences in maize. This would however call for further studies to identify the gene(s) responsible for expression of the defense. This knowledge may then be used to exploit the possibilities of genetic engineering to develop maize varieties with the smart inducible trait.

Materials and Methods

Plants and insects

The maize varieties used in this study were open-pollinated local farmers' varieties Jowi red and Nyamula, and hybrid varieties PH4 of Kenya seed company and WH505 of Western seed company. The emitter of inducing semiochemicals was the legume *Desmodium intortum*, also known as Greenleaf, from Kenya seed company.

All plants were grown individually in pots filled with fertilised soil in an insect-proof screen house at ICIPE - Thomas Odhiambo campus (ITOC), Mbita Point in western Kenya. Environmental conditions at the ITOC field station are as described by Khan et al. (2006).

For the oviposition test, maize plants were exposed to the stem borer moth *Chilo partellus* obtained from the insect rearing unit at ITOC. Only gravid female adults were used. The olfactometer bioassays were carried out with the hymenopteran stem borer larval parasitoid *Cotesia sesamiae* (Braconidae), also obtained from the same facility at ITOC. Only freshly hatched gravid female adults were used. The insects were fed on a 20 % honey solution to increase their longevity.



Graph 18: Freshly germinated maize



Graph 19: Desmodium intortum



Graph 20: Gravid Chilo partellus (Lep.: Crambidae)



Graph 21: Cotesia sesamiae (University of Kentucky 2013)

Experimental set up

Each maize variety was planted in 20 (19 cm diameter x 20 cm height) pots filled with fertilised soil. The pots were placed in a screen house (7x11x2,6 m) with an average temperature of 28 °C and average humidity of 60 %. Three seeds were placed in each pot leaving them for six days to germinate and develop the first leaf. 60 *D. intortum* plants were planted as vines in pots and left to sprout for approximately 30 days. After this, ten pots of each maize variety were transferred to another screen house with an average temperature of 28 °C and average humidity of 76 %. They were placed in between two rows (in a whole 15) of potted *D. intortum* plants for 21 to 28 days. The leaves of maize and *D. intortum* did not touch each other.



Graph 22: Experimental set up in screen house: Plants in airborne communication

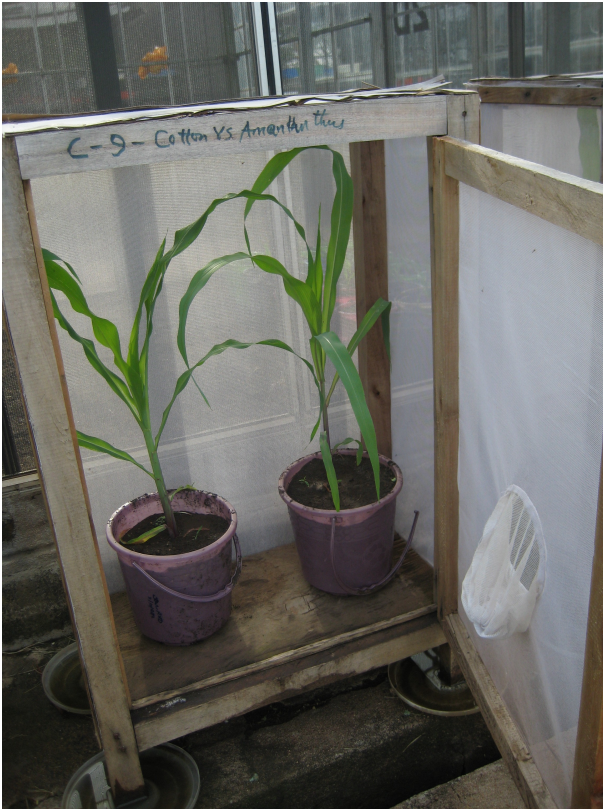


Graph 23: Control maize plants: Monoculture

Oviposition test

Exposure to stem borer moths: After 21 – 28 days, four treated maize plants and four control plants of each variety were placed in 75x63x33 cm cages in pairs of one treated and one control maize plant. Approximately two hours before the end of the photophase, ten female adults of *C. partellus* which had just mated were released in these cages for 12 hours.

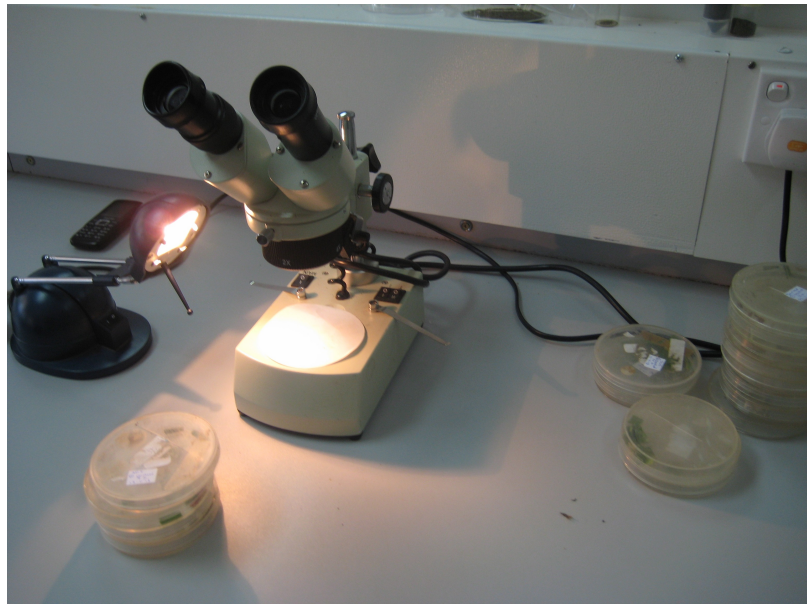
Egg counting: Batches of eggs laid on treated and control plants were identified and counted. Furthermore, the number of eggs in each batch was recorded. For this, the plant parts with eggs attached to them were collected and left for four days in order for the eggs to develop black heads. The eggs were then counted under a light microscope (Zeiss 475022).



Graph 24: Treated and Control plant in cage



Graph 25: Egg batches



Graph 26: Microscope

Entrainment of volatile plant compounds

Using Entrainment kits (manufactured by B. J. Pye, Hertfortshire, UK), the headspace compounds emitted by the four upper leaves of the maize plants were collected and trapped onto a porapak containing a high surface organic substance which adsorbs organic volatile compounds from the plants. Each entrainment set up was left to run for 48 hours.



Graph 27: Plant during entrainment

Elution of volatile plant compounds

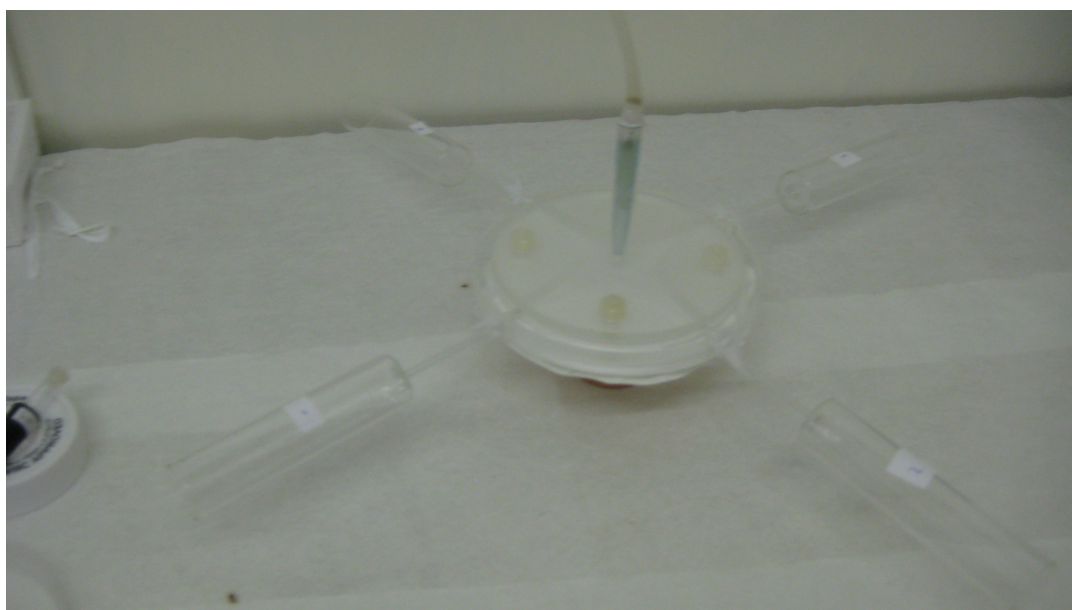
The volatile compounds trapped on the porapak Q were then eluted by dichloromethane, a colourless volatile liquid often used as a solvent; two mL per single porapak Q run were taken.

Bioassay with four-armed olfactometer

A four-armed Patterson's olfactometer was used to evaluate the behaviour of *C. sesamiae*. Data documentation was supported by the Olfa software (F. Nazzi, Udine, Italy). In one trial, 2 μ L of a treated plant sample were placed on a piece of filter paper in one arm whereas 2 μ L of the control plant sample were placed opposite to it. In the two remaining arms 2 μ L of the solvent were placed. One female *C. sesamiae* was released inside of the olfactometer and its entries into different areas of the olfactometer were recorded for 12 minutes. Each parasitoid was only used once, a trial with one set of samples was repeated three times with different individuals, giving a total of 48 runs.



Graph 28: Experimental set up



Graph 29: The four-armed Patterson's olfactometer

Chemical analysis

Gas chromatography (GC). Volatiles were analysed on a Hewlett-Packard 5890A gas chromatograph equipped with a cold on-column injector, a flame ionization detector (FID) and a 50 mm x 0.32 mm i. d. HP-1 bonded phase fused silica capillary column. The oven temperature was maintained at 40 °C for two minutes and then programmed at 10 °C/min to 250 °C. The carrier gas was hydrogen.

Coupled gas chromatography-mass spectrometry (GC-MS). A capillary GC column (50 mm x

0.32 mm i. d. HP-1) fitted with an on-column injector was directly coupled to a mass spectrometer (VG Autospec, Fisons Instruments). Ionization was by electron impact at 70 eV, 250 °C. The oven temperature was maintained at 30 °C for five minutes and then programmed at 5 °C/min to 250 °C.

Statistical analysis

Data of the olfactometer bioassay were compared by analysis of variance (ANOVA). For this, data were converted from time spent in each arm in minutes into proportions and a logratio transformation was carried out. In case of significant differences, means were compared by Tukey test (SAS version 9.2). Oviposition data were compared by Student's t-test.

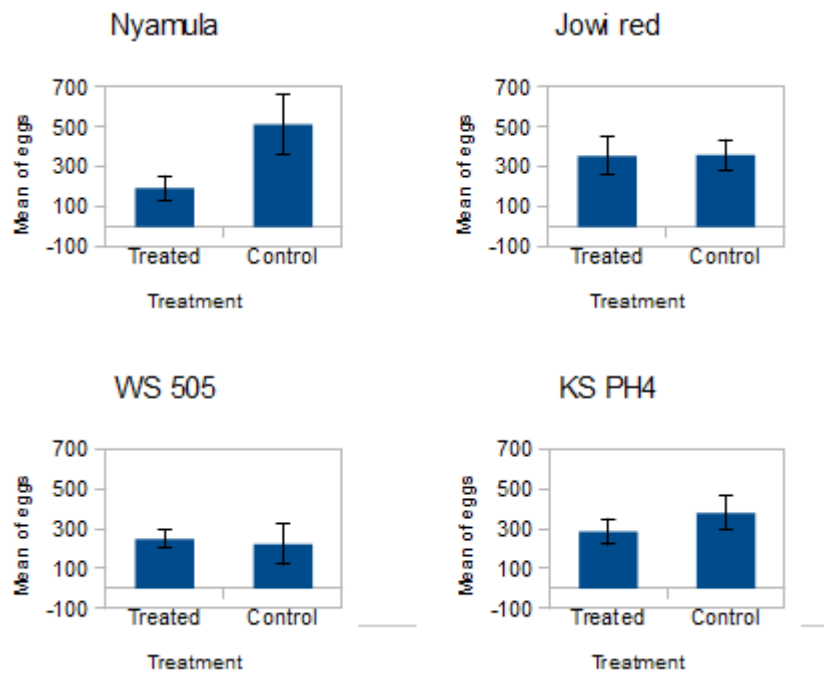
Results and Discussion

Oviposition behaviour of mated Chilo partellus female adults to maize varieties exposed to Desmodium intortum

In case of the maize variety Nyamula, t-test mean comparison of eggs laid on the treated and the control plants did not show a statistically significant difference at $P = 0,05$ ($t = 1,96$; $DF = 6$; $P = 0,0981$). However, this might be due to a relatively small sample size of four treated and four control plants, as the means were far apart from each other ($\bar{y}_{\text{Nyamula}/D. \text{intortum}} = 195$ eggs, $\bar{y}_{\text{Nyamula}} = 514$ eggs), indicating that the moths did prefer plants not exposed to *D. intortum* in favour of those exposed to *D. intortum*.

The moths did also not show any preference with regards to the other three maize varieties (Jowi red: $t = 0,04$; $DF = 6$; $P = 0,971$; PH4: $t = 0,9$; $DF = 6$; $P = 0,405$; 505: $t = -0,23$; $DF = 6$; $P = 0,827$).

Oviposition behaviour of C. partellus towards the maize varieties:

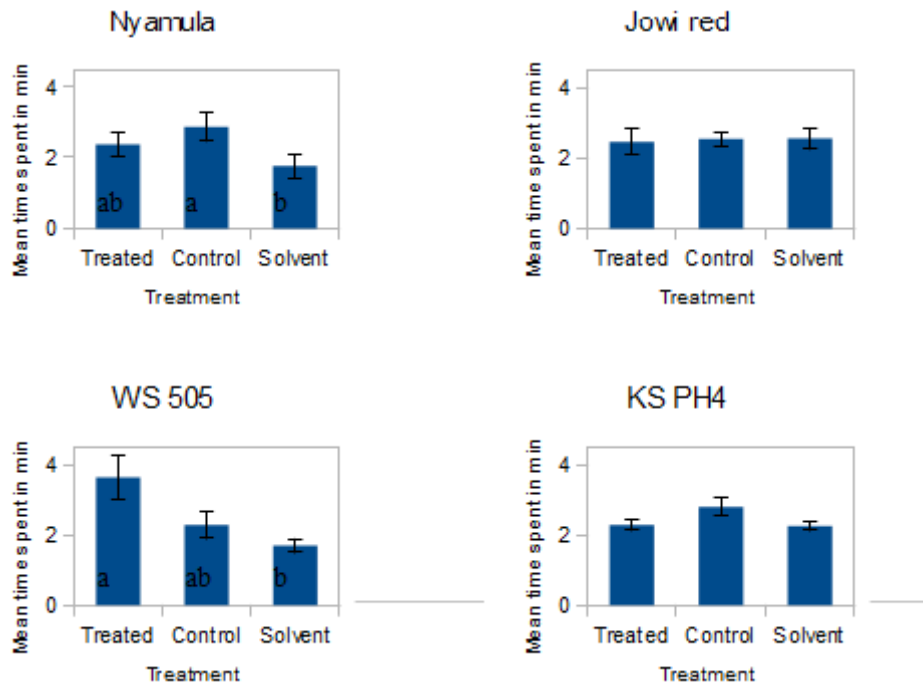


Graph 30: Results of oviposition test

Behavioural responses of larval parasitoid Cotesia sesamiae to headspace samples of maize varieties exposed to Desmodium intortum

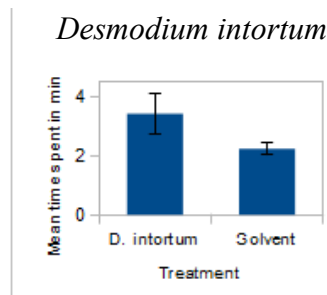
There were no significant preferences of the larval parasitoids. Volatile compounds of the farmers' maize varieties Nyamula and Jowi red did not represent a significantly higher attraction to *Cotesia sesamiae* when grown with *D. intortum*. Moreover, *C. sesamiae* were not significantly attracted to volatile compounds of hybrid varieties PH4 ($F_{2,33} = 1,91$; $P = 0,164$) and WS 505 ($F_{2,33} = 2,16$; $P = 0,131$) grown with *D. intortum*. A control trial showed that *C. sesamiae* were likewise not significantly attracted to *D. intortum* volatiles ($t = -1,23$; $DF = 22$; $P = 0,23$).

Responses of C. sesamiae to headspace samples of the maize varieties:



Graph 31: Results of olfactometer test

Graphic 18: Responses of C. sesamiae to headspace samples of D. intortum:



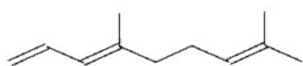
Graph 32: Control results

Chemical analysis of headspace samples

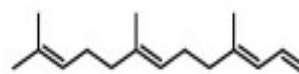
Chemical analysis via GC-MS showed no evidence of a defense induction in maize plants intercropped with *D. intortum*. Neither the treated landrace headspace samples nor the treated hybrid headspace samples contained significantly higher amounts of chemical compounds potentially responsible for a defensive reaction than the non-treated samples. On the contrary, the headspace samples taken from landrace maize plants in monoculture sometimes contained higher amounts of these compounds. The key active volatile compounds found in some of the headspace

samples included (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), methyl salicylate (MeS) and (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT).

DMNT and TMTT are semiochemicals belonging to the group of volatile terpenoids (Arimura et al. 2001) typically emitted by plants in a stress situation. They are also found in the blend emitted by *Desmodium spp.* which is responsible for the repellency against stem borer moths suggesting a high infestation of the plants thereby inhibiting pest attack (Khan et al. 2000). Moreover, both are proven to induce both a direct and an indirect defensive reaction against spider mites in healthy lima bean plants by activating a number of defensive genes (Arimura et al. 2001).

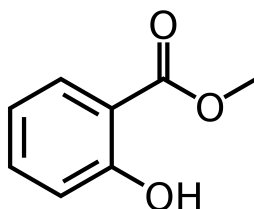


Graph 33: DMNT (Pherobase 2013)



Graph 34: TMTT (Khan 2000)

MeS is a phytopheromone which has similar characteristics as the above with regards to herbivore repellency and defense induction (Ruther and Kleier 2005). Additionally, studies suggest that it plays a role in indicating non-hosts to insects and in indirect defense mechanisms as it is attractive to a large range of insect families such as Braconidae and Empididae (Pickett et al. 2003; Khan et al. 2008).



Graph 35: MeS (Wikipedia 2013)

All three chemicals have also been shown to be attractive to *C. sesamiae* (Tamiru et al. 2011).

From the current study, only one Jowi red sample of plants intercropped with *D. intortum* contained significant amounts of all three volatile plant compounds (DMNT, MeS and TMTT) and only two had significant amounts of MeS.

Samples from untreated Jowi red plants contained significant amounts of TMTT in all four, significant amounts of MeS in three out of four and a large amount of DMNT in one of the samples.

The Nyamula samples showed similar results although the amounts of compounds were relatively less concentrated, with no increases in the treated plants.

Two of the four treated WS 505 samples contained TMTT, similar to three of the control samples. In general, treated samples contained fewer volatile compounds than control samples.

Overall, PH4 samples by far showed the smallest amounts of volatile plant compounds. Only one sample of the treated plants contained DMNT and TMTT. None of the control samples contain any volatile plant compounds.

The analysis of four *D. intortum* headspace samples showed that there were relatively small amounts of the chemical compounds DMNT and TMTT responsible for the repellency against stem borer moths. This is a possible explanation for the results obtained. A yet unpublished study from ICPE has shown that a defense induction in maize landraces takes place when high amounts and a wide variety of semiochemicals are present. Thus, there might not have been enough semiochemicals for the induction in the set up of the current study. Further investigation is needed to clarify why the *D. intortum* plants used emitted such small quantities of semiochemicals. Possibly the amount and/or the blend of volatiles needed to create the indirect defense mechanism induce a defense in neighbouring plants. It has been shown beforehand that stem borer parasitoids are not significantly attracted to *Desmodium spp.* leaf volatiles but to the ones emitted by the flowers (Midega et al. 2009). Consequently, a similar experiment carried out with flowering *D. intortum* plants might show a defense induction in maize landrace varieties.

Another interesting aspect is that according to the farmers' maize varieties naturally emitted more semiochemicals than the commercial hybrids. However, as has been shown in this study, the amounts of semiochemicals produced by the plants were not large enough to significantly affect *Chilo partellus* or *Cotesia sesamiae*. Thus, this effect is highly unlikely to play a significant role in the field.

Nevertheless, the non-induction of a defense by *D. intortum* can also be seen as a positive effect. After all, a repellency of the maize itself would drive away moths so that the attracted parasitoids would hardly find any hosts on the plants which would make the indirect defense mechanism ineffective and might eventually lead into an indifference towards the semiochemicals by the parasitoids.

Conclusion and Outlook

This study does not show a direct or an indirect defense induction by *D. intortum* in the four maize varieties, possibly as a result of inadequate amounts of inducer chemicals from *D. intortum*. The expected difference in induction between maize varieties could therefore not be proven.

We showed that the farmers' varieties generally contained higher amounts of the three compounds MeS, TMTT, DMNT. These semiochemicals are known to induce direct and indirect defense reactions in plants as well as repelling herbivores and attracting their natural enemies. However, the amounts were not large enough to elicit a reaction in stem borer moths and their larval parasitoid *C. sesamiae*.

Nevertheless, a positive aspect of the non-induction through *D. intortum* leaf volatiles is that stem borer parasitoids will find hosts when attracted by flower volatiles.

Further research is needed to investigate why the defense induction did not occur. Possible reasons for the apparent lack of defense induction include: 1) There generally is no defense induction by *Desmodium* green leaf volatiles or 2) an unknown factor has interfered with the communication between the two plant species or 3) an unknown factor has led to a decreased amount of volatile emission by the used *D. intortum* plants.

Additionally, a similar study with flowering *D. intortum* plants may be carried out to investigate if the amount of volatiles emitted by the flowers is sufficient for a defense induction.

3) *Excursion: Can the Western corn rootworm Diabrotica virgifera virgifera expand into Africa?*

Introduction

The Western corn rootworm *Diabrotica virgifera virgifera* LeConte (Chrysomelidae) is one of the major threats to modern corn production. Due to favourable conditions in East Africa it is probable that it will migrate to this region. This experiment was designed to investigate with the help of sex pheromone traps if *D. virgifera virgifera* has already reached the South western region Nyanza in Kenya.

Since the discovery of pheromones fifty years ago, interactions studied in chemical ecology belong to the most studied subject areas in ecology. More than 25,000 original reports on pheromones are listed in the data base 'Web of science' (Eisinger 2011, Hummel et al. 2012). These advances in chemical ecology provide a gain in theoretical knowledge but are also of enormous practical significance for integrated pest management in many parts of the world.

In an attempt to quantify the presence of pest insects in time and space, the 'monitoring' of adult insects in the field is a widely accepted and very beneficial tool for deciding where, when and how many insects of a given insect species are present.

Diabrotica v. virgifera LeConte (Coleoptera: Chrysomelidae) (WCR) is an important quarantine pest of maize in Europe. First introduced 20 years ago near Belgrade, Serbia, it is still spreading

throughout Central and South eastern Europe (Kiss and Edwards, 2012) and so far resisted all attempts of intervention. It is regarded as established and will not be eradicated any more. With time, it is also quite likely to colonize other regions of the world like Africa and Asia where maize cultivation is common. According to a model calculation by Hongmei Li et al. (2006), East Africa will be a likely target for new colonization attempts by the beetle which is known to follow mankind along its numerous trade routes by air, water, land and railroads.

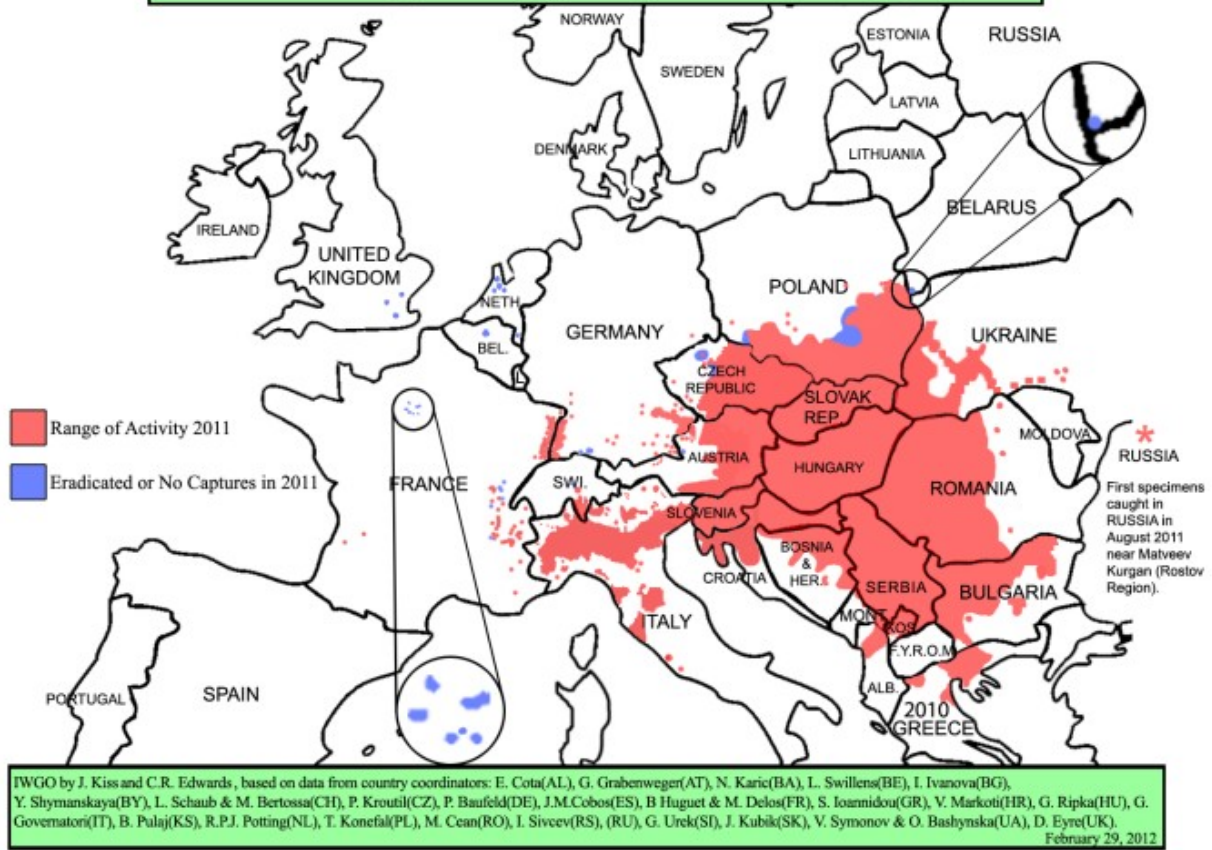
An early confirmation of the actual arrival of WCR would be most important because this would increase the chances for a timely and successful management strategy for this important maize pest. Its importance may become apparent from its nickname 'billion dollar beetle' given to it by frustrated US farmers who were incapable of eradicating it despite all their attempts during the past fifty years in the midwestern US.

Pheromone baited sticky cup traps described first by Levine and Metcalf (1988) are the most sensitive monitoring tool known for WCR today (Hummel 2007). No expensive and intensive field scouting and searching activity on thousands of maize plants is necessary. To the contrary, it is sufficient to establish cheap 'Metcalf traps' and lure the beetles very specifically to strategic locations, usually the margin of a maize field which is conveniently accessible. A tiny amount of 0,5 mg of synthetic sex lure, 8-methyl-decane-2-ol propanoate, will attract the males to the trap where they are easily counted, identified, and registered. This monitoring system is simple, inexpensive, robust and would be eminently suitable for East African purposes.

If monitoring traps established according to well published experience of the past two decades do not catch any males, this result indicates that the beetles are absent from the location.

With this background in mind, trapping experiments were carried out at the Mbita site. The complete absence of WCR during the months of November 2012 to January 2013 can be taken as an indication that WCR, in contrast to the prediction of possible introduction voiced by Li et al. (2006), has not arrived at that location (but may be present elsewhere in Kenya).

Diabrotica virgifera virgifera LeConte in Europe 2012



Graph 36: Distribution pattern of *Diabrotica* in Europe (Kiss & Edwards 2012)



Graph 38: Male adult *Diabrotica virgifera virgifera*

'Metcalf' sticky trap (1988)



Graph 37: Pheromone trap (Levine & Metcalf 1988)

Material and Methods

Plants and insects:

The experiment was carried out in plots of maize in monoculture with three different plot sizes which was due to availability: 50 m x 10 m, 20 m x 3 m and 10 m x 10 m. Sampling took place when the maize plants were between 82 and 120 days old.

The target insect was the western corn rootworm *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae).

Sampling method:

The sex pheromone 10-methyl-decane-2-ol propionat was used in order to attract male *D. virgifera virgifera* in search for a mating partner. These traps were developed by Levine & Metcalf 1988.

Set up:

A total of 50 traps was set up within a time span of 3 months. The three plots were sampled for a week with two, one and one traps in one trial. There is a risk of traps interfering with each other if less than 50 m apart. Traps were attached to corn cobs at a height of 100 -120 m at the borders of the plots. After the sampling period the insects caught were identified and counted. The experiment was carried out at ICIPE -Thomas Odhiambo Campus at Mbita Point in Kenya (see Graphic 17).

Results and conclusion

Diabrotica virgifera virgifera was not found during the sampling period. This implicates that it has not yet reached South western Kenya. It would be beneficial to repeat the experiment near Nairobi and Mombasa as it is well known that the beetle travels long distances via airplane transport.



Graph 39: Trap with caught insects



Graph 40: Trapped beetle



Graph 41: Set up of trap



Graph 42: Trapped insect



Graph 43: Trapped beetle

Literature

- Arimura, G., Ozawa, R., Horiuchi, J., Nishioka, T., and Takabayashi, J.. 2001. "Plant–plant interactions mediated by volatiles emitted from plants infested by Spider Mites." *Biochemical Systematics and Ecology* 29 (10): 1049–61.
<http://linkinghub.elsevier.com/retrieve/pii/S0305197801000497>
Date of insight: 10.10.2012
- Badgley, C., and Perfecto, I.. 2007. "Organic Agriculture and the Global Food Supply." *Renewable Agriculture and Food Systems* 22 (2): 86.
http://www.journals.cambridge.org/abstract_S1742170507001640
Date of insight: 21.02.2013
- Bruin, J., and Sabelis, M. W.. 2001. "Meta-analysis of Laboratory Experiments on Plant–plant Information Transfer." *Biochemical Systematics and Ecology* 29 (10): 1089–1102.
<http://linkinghub.elsevier.com/retrieve/pii/S0305197801000527>
Date of insight: 10.10.2012
- Dicke, M., Agrawal, A., and Bruin, J.. 2003. "Plants talk, but are they deaf?" *Trends in Plant Science* 8 (9): 403–5.
<http://www.ncbi.nlm.nih.gov/pubmed/13678903>
Date of insight: 10.10.2012
- Eisinger, M. T.. 2011. "Nanofasern als Trägermaterial für Pheromone in Konfusionsverfahren und Suche nach weiteren innovativen Dispenser-Systemen in internationalen Fachdatenbanken." 1–99.
- Estabrook, E., and Yoder, J.. 1998. "Plant-plant Communications: Rhizosphere signaling between parasitic Angiosperms and their Hosts." *Plant Physiology*: 1–7.
<http://www.plantphysiol.org/content/116/1/1.short>
Date of insight: 10.10.2012
- FAO. 2013. <http://ecocrop.fao.org/ecocrop/srv/en/cropView?id=1437>
Date of insight: 23.05.2013
- Farmer, E. E., and Ryan, C. A.. 1990. "Interplant Communication: Airborne Methyl Jasmonate induces Synthesis of Proteinase Inhibitors in Plant Leaves." *Proceedings of the National Academy of Sciences of the United States of America* 87 (19): 7713–6.
<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=54818&tool=pmcentrez&rendertype=abstract>

Date of insight: 10.10.2012

Fischinger, S.. 2012. "Anbau von Futterleguminosen und Zwischenfrüchten - Vorlesung 'Nutzpflanzen im Ökologischen Landbau', Justus-Liebig-Universität Giessen."

Gänz, P.. 2011. "Kulturpflanze Mais: Ökologische Kleinbauernwirtschaft als Beitrag zur Ernährungssicherheit in Mexiko."

<http://www.naturland.org/publikationen.html#c19620>

Date of insight: 12.06.2013

Guerrieri, E., Poppy, G. M., Powell, W., Rao, R., and Pennacchio, F.. 2002. "Plant-to-plant Communication: Mediating in-flight Orientation of *Aphidius Ervi*." *Journal of Chemical Ecology* 28 (9): 1703–15.

<http://www.ncbi.nlm.nih.gov/pubmed/12449500>

Date of insight: 10.10.2012

Grodzinsky, A. M.. 2006 (Original version 1979). "Allelopathy in Soil Sickness." *Scientific Publishers, Jodhpur, International Allelopathy Foundation, HISAR..*

Haerlin, B., and Busse, T.. 2009. "Wege aus der Hungerkrise, Zusammenfassung des Weltagrarberichts." AbL Verlag, Berlin.

Hesse, M., and Hülsebusch, C.. 2009. "Ökologischer Landbau und Fairer Handel in Entwicklungsländern: Status Quo und Potenzialanalyse." DITSL GmbH, Witzenhausen.

Hummel, H. E.. 2007. "*Diabrotica virgifera virgifera* LeConte: Inconspicuous leaf beetle – Formidable challenges to agriculture." *Communications Applied Biological Sciences* 72 (2): 7 – 32. Ghent University.

Hummel, H. E., Eisinger, M. T., Hein, D. F., Breuer, M., Schmid, S., Leithold, G.. 2012. "New dispenser types for integrated pest management of agriculturally significant insect pests. An algorithm with specialized searching capacity in electronic data bases." *Communications Applied Biological Sciences* 77 (4): 639 – 46. Ghent University.

ICIPE. 2011. "Planting for Prosperity Push – Pull: A Model for Africa's Green Revolution."

<http://icipe.org/push-pull/publications>

Date of insight: 10.10.2012

ICIPE. 2013. <http://icipe.org/index.php/about-us/about-us.html>

Date of insight: 22.03.2013.

IFOAM. 2009. "Definition of Organic Agriculture." <http://ifoam.org/sub/faq.html>

Date of insight: 07.02.13.

International Monetary Funds. 2012. "International Monetary Funds – Kenya."

<http://www.imf.org/external/pubs/ft/weo/2012/01/weodata/weorept.aspx?sy=2009&ey=2012&scsm=1&ssd=1&sort=country&ds=.&br=1&pr1.x=44&pr1.y=12&c=664&s=NGDPD%2CNGDPDPC%2CPPPGDP%2CPPPPC%2CLP&grp=0&a=>

Date of insight: 06.02.13.

Jones, B.. 2008. "Ethnic Bloodletting spreads in Kenya." USA today news.

http://usatoday30.usatoday.com/news/world/2008-01-28-kenya-unrest_N.htm

Date of sight: 06.02.2013.

Kiss, J., Edwards, C. R.. 2012. "*Diabrotica virgifera virgifera* LeConte in Europe 2012." *IWGO*.

Khan, Z. R., James, D. G., Midega, C., and Pickett, J.. 2008. "Chemical Ecology and Conservation Biological Control." *Biological Control* 45 (2): 210–24.

<http://linkinghub.elsevier.com/retrieve/pii/S1049964407002939>

Date of insight: 10.10.2012

Khan, Z. R., Pickett, J., Wadhams, L. J., Hassanali, A., and Midega, C.. 2006. "Combined Control of *Striga hermonthica* and Stemborers by maize – *Desmodium spp.* intercrops." *Crop Protection* 25 (9): 989–95.

<http://linkinghub.elsevier.com/retrieve/pii/S0261219406000329>

Date of insight: 10.10.2012

Khan, Z. R., and Pickett, J.. 2000. "Exploiting Chemical Ecology and Species Diversity: Stem Borer and *Striga* Control for Maize and Sorghum in Africa." *Pest Management* 56: 957–62.

[http://onlinelibrary.wiley.com/doi/10.1002/1526-4998\(200011\)56:11%3C957::AID-PS236%3E3.0.CO;2-T/full](http://onlinelibrary.wiley.com/doi/10.1002/1526-4998(200011)56:11%3C957::AID-PS236%3E3.0.CO;2-T/full)

Date of insight: 10.10.2012

Kifuko-Koech, M., Pypers, P., Okalebo, J. R., Othieno, C. O., Khan, Z. R., Pickett, J. A., Kipkoech, A. K., and Vanlauwe, B.. 2012. "The Impact of *Desmodium spp.* and cutting Regimes on the Agronomic and Economic Performance of *Desmodium* – maize Intercropping System in Western Kenya." *Field Crops Research* 137: 97–107.

<http://linkinghub.elsevier.com/retrieve/pii/S0378429012002742>

Date of insight: 10.10.2012

Kledal, P.. 2009. "Organic Food and Farming in Kenya." *The World of Organic Agriculture - Statistics and Emerging Trends 2009*: 127–132. IFOAM, Bonn; FiBL, Frick; ITC, Geneva.

Leithold, G.. 2010. "Bodenfruchtbarkeit im Organischen Landbau und Nährstoffversorgung der Pflanzen - Vorlesung 'Grundlagen des Ökologischen Landbaus, Justus-Liebig-Universität Giessen."

Levine, E., Metcalf, R. L.. 1988. "Sticky attractant traps for monitoring corn rootworm beetles."

Illinois Natural history survey reports: 270: 1-2.

- Li, H., Edwards, C. R., Xue, D.. 2006. "Predicting the possibility for establishment of the Western corn rootworm, *Diabrotica virgifera virgifera*, in different world regions: Based on the CLIMEX model." *IWGO-Newsletter: 28 (1): 30.*
- Library of Congress. 2007. "Library of Congress – Federal Research Division Country Profile: Kenya, June 2007."
<http://lcweb2.loc.gov/frd/cs/profiles/Kenya.pdf>
Date of insight: 21.06.2013
- Medical Mission International. 2013. <http://em3international.org/files/2011/06/Provinces-of-Kenya.png>
Date of insight: 23.05.2013
- Midega, C., Khan, Z. R., Van den Berg, J., Ogol, C. K. P. O., Bruce, T. J., and Pickett, J. A.. 2009. "Non-target Effects of the 'push-pull' Habitat Management Strategy: Parasitoid Activity and Soil Fauna Abundance." *Crop Protection 28 (12): 1045–51.*
<http://linkinghub.elsevier.com/retrieve/pii/S0261219409001963>
Date of insight: 10.10.2012
- Molisch, H.. 1937. „Der Einfluss einer Pflanze auf die andere – Allelopathie“. Fischer Verlag. Jena.
- Muller, C. H.. 1970. „Phytotoxins as habitat variables“ *Recent advances phytochemistry 3: 106–21.*
- Nations Encyclopedia. 2013. "Nations Encyclopedia - Kenya's agriculture."
<http://www.nationsencyclopedia.com/Africa/Kenya-AGRICULTURE.html>
Date of insight: 06.02.2013
- Ninkovic, V.. 2003. "Volatile Communication between Barley Plants affects Biomass Allocation." *Journal of Experimental Botany 54 (389): 1931–9.*
<http://www.ncbi.nlm.nih.gov/pubmed/12815028>
Date of insight: 10.10.2012
- Pickett, J. A.. 2003. "Plant Stress signalling: Understanding and exploiting Plant-plant Interactions." *Biochemical Society Transactions 31: 123–7.*
<http://www.ncbi.nlm.nih.gov/pubmed/12546668>
Date of insight: 10.10.2012
- Pickett, J. A., Bruce, T. J. A., Chamberlain, K., Hassanali, A., Khan, Z. R., Matthes, M. C., Napier, J. A., Smart, L. E., Wadhams, L. J., and Woodcock, C. M.. 2006. "Plant volatiles yielding new ways to exploit plant defence." *Physiology 16: 161–73.* Springer Verlag, Heidelberg.
- Piras, E.. 2011. „Reis ist Leben“ - Wie Öko-Landbau und Fairer Handel zu

Ernährungssouveränität führen". Naturland e. V.
<http://www.naturland.org/publikationen.html#c19620>
Date of insight: 12.06.2013

Rice, E. L.. 1984. „Allelopathy“. *New York, Academic press*, 2nd Edition.

Ruther, J., and Kleier, S.. 2005. "Plant-plant signaling: Ethylene synergizes volatile emission in *Zea mays* induced by exposure to (Z)-3-hexen-1-ol." *Journal of Chemical Ecology* 31 (9): 2217–22.
<http://www.ncbi.nlm.nih.gov/pubmed/16132223>
Date of insight: 10.10.2012

Song, Y., Zeng, R., Xu, J., Li, Jun, Shen, X. , and Yihdego, W. G.. 2010. "Interplant Communication of Tomato Plants through underground common Mycorrhizal Networks." *PLoS One* 5 (10): 13324.
<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2954164&tool=pmcentrez&rendertype=abstract>
Date of insight: 10.10.2012

Stablemade. 2013. http://stablemade.com/horsecare/images2/poison/sudan_grass.jpg
Date of insight: 23.05.2013

Tamiru, A., Bruce, T. J. A., Woodcock, C. M., Caulfield, J. C., Midega, C., Ogol, C. K. P. O., Mayon, P., Birkett, M. A., Pickett, J. A., and Khan, Z. R.. 2011. "Maize Landraces recruit egg and larval Parasitoids in Response to egg deposition by a Herbivore." *Ecology Letters* 14 (11): 1075–83.
<http://www.ncbi.nlm.nih.gov/pubmed/21831133>
Date of insight: 10.10.2012

University of Kentucky. 2013. <http://www.uky.edu>
Date of insight: 23.05.2013

Weeds. 2013. <http://www.weeds.org.au/cgi-bin/weedident.cgi?tpl=plant.tpl&ibra=all&card=V31>
Date of insight: 23.05.2013

Willer, H.. 2010. "The World of Organic Agriculture: Statistics and Emerging Trends 2010."
<http://orgprints.org/id/eprint/2997>
Date of insight: 21.02.2013

Wikipedia. 2013. <http://de.wikipedia.org/wiki/>
Date of insight: 23.05.2013

I hereby declare that I have produced the submitted paper with no assistance from any other party and without the use of any unauthorized aids and, in particular, that I have marked as quotations all passages which are reproduced verbatim or nearby-verbatim from publications. Further, I declare that this thesis has never been submitted before to any other examination board in either its present form or in any other similar version.

Giessen,