

**VARIATION OF SILICON IN SOILS AND MAIZE  
PLANTS ALONG ALTITUDINAL GRADIENTS AND ITS  
RELATIONSHIP WITH DISTRIBUTION OF  
LEPIDOPTERA STEM BORERS**

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**A Thesis Submitted in Fulfilment for the Requirement of Degree of  
Master of Science in Chemistry of the Jomo Kenyatta University of  
Agriculture and Technology**

**2015**

## DECLARATION

This thesis is my original work and has not been presented elsewhere for a degree or any other awards.

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## **DEDICATION**

This work is dedicated to my loving wife Elizabeth Nyaguthii; my son Victor Njuguna my parents Mr. Samuel Njuguna and Mrs. Mary Wanjiru and to all my friends.

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## TABLE OF CONTENTS

<b>DECLARATION</b> .....	<b>ii</b>
<b>DEDICATION</b> .....	<b>iii</b>
<b>ACKNOWLEDGEMENTS</b> .....	<b>iv</b>
<b>TABLE OF CONTENTS</b> .....	<b>v</b>
<b>LIST OF TABLES</b> .....	<b>vii</b>
<b>LIST OF FIGURES</b> .....	<b>viii</b>
<b>LIST OF ABBREVIATIONS</b> .....	<b>ix</b>
<b>ABSTRACT</b> .....	<b>x</b>
<b>CHAPTER ONE</b> .....	<b>1</b>
<b>INTRODUCTION AND LITERATURE REVIEW</b> .....	<b>1</b>
1.1 Background of the study .....	1
1.2 Previous studies .....	3
1.2.1 Chemistry of silicon.....	3
1.2.1.1 Soluble silicon.....	3
1.2.1.2 Fixed silicon.....	4
1.2.2 Role of silicon in plant growth and development .....	5
1.2.3 Effect of silicon on abiotic stresses.....	7
1.2.3.1 Drought and salinity.....	8
1.2.3.2 Heat.....	8
1.2.3.3 Aluminium toxicity.....	8
1.2.4 Effect of silicon on biotic stresses .....	9
1.2.4.1 Influence of plant silicon on the survival and growth of insect pests.....	10
1.2.4.2 Trophic relationship among silica levels in maize plants and stem borer feeding .....	11
1.2.5 Effect of temperature on silicon accumulation in maize .....	11
1.2.6 Distribution of Lepidoptera stem borers along the altitudinal gradients .....	12
1.3 Analytical techniques .....	13
1.3.1 Inductively-coupled plasma an optical emission spectroscopy (ICP-OES).....	14
1.3.1.1 Principle .....	13
1.3.2 UV-Visible Spectroscopy .....	14
1.3.2.1 Principle .....	14
1.4 Statement of the problem .....	15
1.5 Justification.....	16

1.6 Hypothesis.....	17
1.7 Objectives .....	17
1.7.2 Specific objectives .....	17
<b>CHAPTER TWO.....</b>	<b>18</b>
<b>MATERIALS AND METHODS.....</b>	<b>18</b>
2.1 Research design .....	18
2.2 Study area.....	18
2.2.1 Machakos Hills transect.....	18
2.2.2 Taita Hills transect.....	19
2.2.3 Mount Kilimanjaro transect .....	20
2.3 Preliminary plant sampling experiments .....	24
2.4 Sampling .....	24
2.4.1 Soil sampling for silicon analysis .....	24
2.4.2 Sampling of maize plant leaves for silicon analysis .....	25
2.5 Composition of maize stem borer species along altitudinal gradients.....	25
2.6 Reagents and Chemicals.....	26
2.7 Determination of soil silicon levels .....	26
2.8 Extraction and analysis of maize plant silicon.....	27
2.9 Influence of altitude on silicon absorption by the maize plants.....	27
2.10 Larval development on potted plants treated with soluble Si.....	28
2.12 Statistical analysis.....	29
<b>CHAPTER THREE.....</b>	<b>30</b>
<b>RESULTS AND DISCUSSION.....</b>	<b>30</b>
3.1 Preliminary experiments for plant sampling.....	30
3.2 Levels of silicon in soils along the gradients .....	32
3.3 Silicon levels in maize .....	35
3.4 Influence of altitude on Si absorption by maize plants.....	37
3.5 Composition of maize stem borer species along altitudinal gradients.....	39
3.6 Larval development on potted plants treated with soluble Si.....	40
3.7 Larval development on artificial diets amended by Si.....	42
3.8 Conclusions.....	45
3.9 Recommendations.....	46
<b>REFERENCES.....</b>	<b>47</b>

## LIST OF TABLES

<b>Table 2.1:</b> The three different transects (Machakos Hills, Taita Hills and Mount Kilimanjaro) studied characterized by altitudinal gradients. ....	22
<b>Table 2.2:</b> Monthly temperature (°C), monthly relative humidity (%) (means $\pm$ SE) and annual mean rainfall (in mm) along the three different transects (Machakos Hills, Taita Hills and Mount Kilimanjaro). ....	23
<b>Table 3.1:</b> Silicon level (mean <sup>a</sup> $\pm$ SE, n=10) according to the maize plant organ and silicon treatment. ....	31
<b>Table 3.2:</b> Silicon level (mean <sup>a</sup> $\pm$ SE, n=5) of mature leaves according to the age of maize plants. ....	31
<b>Table 3.3:</b> Percentage of remaining live larvae (mean <sup>a</sup> $\pm$ SE, n=10) and relative growth rates (RGR, mg d <sup>-1</sup> , mean <sup>a</sup> $\pm$ SE) of <i>Busseola fusca</i> , <i>Sesamia calamistis</i> and <i>Chilo partellus</i> larvae after 15 days of infestation on control and Si-treated maize plants. ....	43
<b>Table 3.4:</b> Percentage of mortality (mean <sup>a</sup> $\pm$ SE, n=10) and relative growth rates (RGR, mg d <sup>-1</sup> , mean <sup>a</sup> $\pm$ SE) of <i>Busseola fusca</i> , <i>Sesamia calamistis</i> and <i>Chilo partellus</i> larvae after 30 days of infestation on control and Si-treated artificial diets. ....	44



## LIST OF FIGURES

<b>Figure 1.1:</b> Beneficial effects of silicon on plant growth in relation to biotic and abiotic stresses (Ma and Yamaji, 2006). .....	6
<b>Figure 1.2:</b> Major components and layout of a typical ICP-OES instrument.....	14
<b>Figure 1.3:</b> Major components and layout of a typical UV-Visible Spectroscopy.....	15
<b>Figure 2.1:</b> Machakos Hills transect. ....	19
<b>Figure 2.2:</b> Taita Hills transect.....	20
<b>Figure 2.3:</b> Mount Kilimanjaro transect .....	21
<b>Figure 3.1:</b> Standard curve for Si determination in maize plant organs (leaves, stems and roots).....	30
<b>Figure 3.2:</b> Levels of total soil Si (in ppm) according to the depth and the altitude in Machakos Hills, Taita Hills and Mount Kilimanjaro transect.....	32
<b>Figure 3.3:</b> Levels of soluble soil Si (in ppm) according to the depth and the altitude in Machakos Hills, Taita Hills and Mount Kilimanjaro transects. ....	33
<b>Figure 3.4:</b> Levels of Si of maize leaves (in ppm) according to the plant status (infested or not) and the altitude in Machakos Hills, Taita Hills and Mount Kilimanjaro transects.....	35
<b>Figure 3.5:</b> Levels of Si of maize leaves (in ppm) according to the treatment realised (control, 10 g of $\text{Ca}_2\text{SiO}_4$ or 10 g of $\text{Na}_2\text{SiO}_3$ ) in Taita Hills transect.....	37
<b>Figure 3.6:</b> Variation in the density of three stem borer species, <i>Busseola fusca</i> (Bf), <i>Chilo partellus</i> (Cp) and <i>Sesamia calamistis</i> (Sc) found along the altitudinal gradient of Machakos Hills, Taita Hills and Mount Kilimanjaro transects. ....	39

## LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of variance
CHIESA	Climate Change Impacts on Ecosystem Services and Food Security in Eastern Africa
HAS	Hydroxyaluminosilicate
<i>icipe</i>	International Centre for Insect Physiology and Ecology
ICP	Inductively Coupled Plasma
IRD	Institut de Recherche pour le Développement
KENCCA	Kenyan Climate Change and Adaptation
m.a.s.l	Meters above the sea level
NSBB	Noctuid Stem Borer Biodiversity
RGR	Relative Growth Rate
RTZ	Root Zone Temperature
SAR	Systemic Acquired Resistance
SD	Standard Deviation
SE	Standard Error
SIDA	Swedish International Development Cooperation Agency

## ABSTRACT

Maize is the main staple crop of most communities of the East African mountains but the yield is affected by both biotic and abiotic factors. While soil nutrients increase the maize yields, infestation of maize fields by Lepidopteran pests such as, *Chilo partellus* (Crambidae) and *Busseola fusca* (Noctuidae) generally reduces the yields. In addition to enhancing maize yields, some soil elements such as silicon (Si) aid plants in overcoming abiotic and biotic stresses. Hence, any factor that induces Si deficiency in soil might be detrimental to the plant growth and its resistance to insect pests and diseases. Understanding the variation of Si level in soil and maize and their relationship with stemborer is crucial in promoting maize production in East Africa. This study evaluated the Si levels in both soil and maize plants cultivated along three altitudinal gradients of East African mountains (Machakos Hills, Taita Hills and Mount Kilimanjaro) characterized by graded changes in climatic conditions. The study also linked the Si level in maize plants to lepidopteran pests' distribution. The amount of silicon in maize plant was analyzed using UV-spectrophotometer while the amount of silicon in soil was analyzed using Inductively-Coupled Plasma (ICP). One-way and two-way ANOVA tests were conducted for data analyses. The means were separated by multiple mean comparison tests following ANOVA using R 2.15.3 (R Development Core Team, 2013). Silicon levels in both soil and maize decreased significantly with a concomitant rise in altitude. This result was not only attributed to soil characteristics but also to environmental conditions. Low temperatures at higher altitudes negatively affected Si assimilation by maize plants. *Chilo. partellus* and *Busseola fusca* significantly dominated lower and higher altitudes respectively. Apart from temperature variations, differences in distribution of insect pests along altitudinal gradient could be attributed to variation in maize Si levels. *Chilo partellus* appeared more tolerant to increasing maize Si levels than *Busseola fusca*.

## CHAPTER ONE

### INTRODUCTION AND LITERATURE REVIEW

#### 1.1 Background of the study

Maize (*Zea mays*) is an extremely important crop for millions of people in Africa where it is mainly cultivated by subsistence farmers for human consumption while the surplus is used as animal fodder (Minja, 1990; Kfir *et al.*, 2002). Many countries in sub-Saharan Africa have remained net importers of maize mainly due to the stagnating yields over the years (FAO, 1999) and the rapidly expanding population. Furthermore, it is forecasted that by the year 2020, the global demand for maize will have grown by 45% of which 72% will be in developing countries while only 28% in the industrialised nations (James, 2003). In order to forestall the surging demand, new methods of production need to be sought while reinforcing the existing ones to better manage the complex problems facing maize cultivations in tropical Africa (FAO, 2002).

In the densely populated areas of eastern Africa that have a high maize yield potential, the crop is grown on the same plot continuously year after year due to population pressure and land constraints. This has led to a steady decline in soil fertility and a net reduction in yields in most of these areas (FEWS, 2008). For example, an estimated 1.4 million hectares of maize was reported to have been under cultivation in Kenya, between 1994 and 1998, with an average annual grain production of 2.5 million tonnes. During this period, the average grain yield was approximately 1.8 tonnes per hectare (FAO, 1999) although yields in some areas were often below 1 tonne per hectare (Grisley, 1997). In Kenya, only about 2% of arable land is farmed under irrigation systems while the rest of the farming is rainfall dependent. This over-reliance on rainfall for maize production poses a major hindrance to sustainable maize production because the rains are often low and unreliable (FAO, 2004). This has been further aggravated by factors such as lack of farm inputs like seed and fertilisers, outbreak of diseases, inability to control weeds and crop losses due to damage by insect pests (Minja, 1990; Grisley, 1997; Bornhof, 2000).

Insect pests constitute one of the major constraints of maize production in Africa. Of the various insect pests attacking maize in Africa, Lepidopteran stem borers are

generally geographically widespread and most destructive causing severe damage to the crop (Ingram, 1958; Youdeowei, 1989; Kfir *et al.*, 2002). Estimates of crop losses vary greatly in different regions and agro-ecological zones. In Kenya alone, losses due to stem borer damage fluctuate around 14% on average (De Groot, 2002). Therefore, the abundance of these pest species in a region presents a major constraint to maize production (Youdeowei, 1989). Therefore, due to their widespread distribution and destructive nature, stem borers have been the subject of extensive research in Africa (Calatayud *et al.*, 2006).

In most parts of Africa, maize is usually grown on small plots, often surrounded by land occupied by wild graminaceous plants. For many decades, these wild plants were considered as natural hosts of stem borer pests (Bowden, 1976, Kfir *et al.*, 2002). Recently, however, these plants were found to host much diverse cereal stem borer species though with few economically important species (Le Ru *et al.*, 2006a; b; Ong'amo *et al.*, 2006) contrary to those earlier reported (Khan *et al.*, 1997).

Cereal stem borers pests have been classified into three families; Crambidae, Pyralidae and Noctuidae (Bleszynski, 1969; Harris, 1990). In East Africa, there is a complex of 12 major species of stem borers pests for cereal crops. The crambids *Chilo partellus* (Swinhoe) and *Chilo orichalcociliellus* (Strand), the noctuids *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson and the pyralid *Eldana saccharina* (Walker) are among the economically most important and widely distributed species (Youdeowei, 1989).

Moreover, climatic conditions under which the current farming systems are practiced are envisaged to change in future (Reid, 2006) and are likely to affect the abundance and distribution of crop pests. Climatic projections *via* modelling studies suggest that by the end of the 21<sup>st</sup> century, climate change could have had substantial impact on agricultural production given the high levels of poverty coupled with low adaptive capacity of human populations in the region (Reid, 2006). In East Africa, the main consequences of climate change as predicted by the global climate change scenarios include a rise in daily temperature with either an increase or a decrease in rainfall patterns. This projected climate changes is envisaged to impact heavily on the

ecosystem. For example, in terms of food crop production, projected climate change is likely to affect stem borer pest species diversity and their dynamics and the general tritrophic interaction between plants-pests and associated natural enemies. Together, changes in these interactions would have significant impact on farming.

## **1.2 Previous studies**

Silicon (Si) is a relatively inert element that rarely occurs freely in nature. In the literature, silicon element, the second most abundant element on earth is frequently designated as ‘Silica’, the oxidised form of silicon,  $\text{SiO}_2$ , mostly present in that form in plants. In soils, Si is mainly present as quartz, alkaline earth or aluminium silicates (Datnoff *et al.*, 2007). Although these forms of silicon are chemically or biologically inert, they are reported to have significant influence on the physical properties of soil such as soil texture, soil water holding capacity and soil fertility (Conley, 2002). Silicon content in soils ranges from between 20-35% for clay or silt to between 40-44% for sandy soils (Essington, 2003). Although silicon is found enormously in most soils its presence in weathered tropical soils is however limited.

In Africa, for example, about 70% of the soils are considered either deficient or highly deficient in accessible Si (Laing *et al.*, 2006).

### **1.2.1 Chemistry of silicon**

Silicon originates from soil and is formed by the process of weathering parent rock material *via* climate, biota, topography and time (Brady and Weil, 2004). Silicate clays are created by altering the physical or chemical properties of the primary minerals, thus decomposing and releasing various forms of silicon into the biosphere (Farmer, 1986; Brady and Weil, 2004). Silicic acid is biochemically and geochemically highly active (Iler, 1979; Matichenkov *et al.*, 2000; Ma, 2003). The formation and release of silicic acid depends on the rate and amount of organic acid leaching from the various soil horizons, the soil characteristics and its chemistry (Farmer, 1986). Silicon exists in two forms, soluble and fixed.

#### **1.2.1.1 Soluble silicon**

Monosilicic and polysilicic acids are the principal soluble forms of Si in soil, which are directly absorbed by plants (Matichenkov *et al.*, 2008). Monosilicic acid occurs in a

weakly adsorbed state in the soil (Matichenkov, 1990) with a low capacity for leaching down the soil profile (Khalid and Silva, 1980). The chemical similarity between the silicate anion and the phosphate anion results in a competitive reaction between the various phosphates and monosilicic acid in the soil. Increasing monosilicic acid concentration in the soil solution causes transformation of the plant-unavailable phosphates into the plant-available ones (Matichenkov, 1996). Monosilicic acid interacts with aluminium, iron, manganese and heavy metals to form slightly soluble silicates (Horiguchi, 1988; Lumsdon and Farmer, 1995). Polysilicic acids whose mechanism of formation is not clearly understood, mainly affect soil physical properties. Unlike monosilicic acid, polysilicic acid is chemically inert and basically acts as an adsorbent, forming colloidal particles (Hadson and Evans, 1995). Polysilicic acids are readily absorbed by minerals and form siloxane bridges (Chendwick, 1987). Since polysilicic acids are highly water saturated, they may have an effect on the soil water-holding capacity. Polysilicic acids have been found to be important for the formation of soil structure (Matichenkov *et al.*, 1995).

#### **1.2.1.2 Fixed silicon**

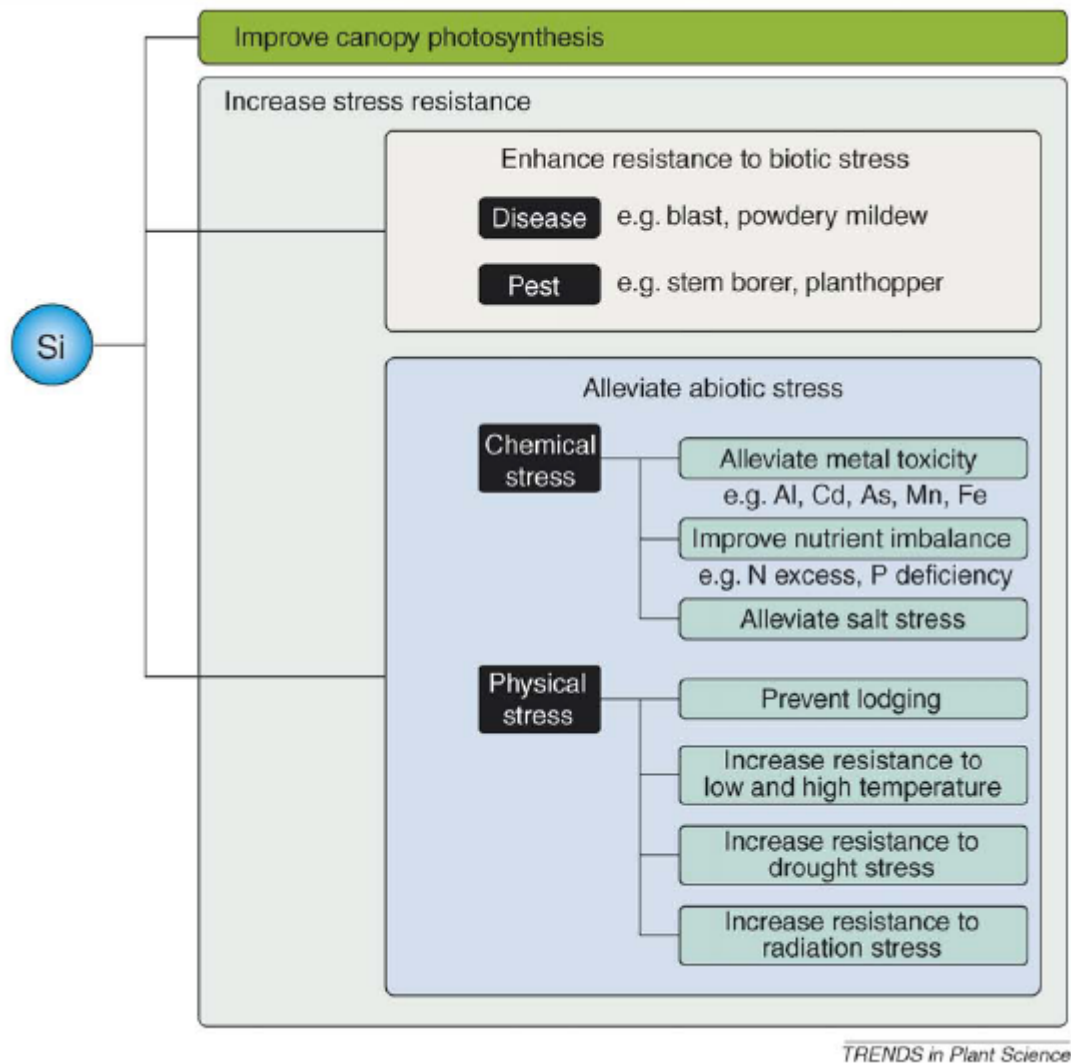
Fixed silicon provides a balance between the plant available silicon and unavailable one. Despite the abundance of soluble Si in most of the world's mineral soils, its deficiency may occur as a consequence of its depletion as a result of continuous cultivation of crops such as rice that have a high requirement of silicon. Rice crop can absorb up to between 230 and 470 kg ha<sup>-1</sup> of Si (Rodrigues *et al.*, 2003). Due to the intensity with which rice is grown, Si can be removed from the soil more rapidly than can be naturally replaced (Savant *et al.*, 1997a,b). Similarly, in regions with high rainfall, different processes that mediate removal of silica from soil such as filtration and desilification have been reported (Savant *et al.*, 1997a;b; Datnofft *et al.*, 2007). Histosols (which are soil consisting primarily of organic materials) have lower amounts of Si available to plants due to its high content of organic matter (80%) and low mineral content, while Entisols (which are soils of recent origin) has a high content of quartz (SiO<sub>2</sub>) in the sand, but the Si is just slightly soluble and unavailable to plants (Datnofft *et al.*, 2007)

### 1.2.2 Role of silicon in plant growth and development

Silicon is absorbed from soil by plant roots as monosilicic acid [ $\text{Si}(\text{OH})_4$ ], transported throughout the plant *via* transpiration and deposited in plant epidermal cell walls as silica deposits (Sangster *et al.*, 2001). The ability of plant tissues to accumulate silica varies considerably among plant species and has been documented to be higher in Poaceae and Cyperaceae families (Ma and Takahashi, 2002). Silica can constitute between 0.1 to 10% weight of the dry matter of most plant species (Ma and Takahashi, 2002). Following its absorption from the soil by roots silicon is translocated to the shoot through xylem where it polymerizes to form silica gel ( $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ ) when silicic acid exceeds 2 mM (Casey *et al.*, 2003; Mitani and Ma, 2005).

In the shoot, silicic acid is further concentrated through transpiration and becomes polymerized. During silicon polymerization, silicic acid is converted to colloidal silicic acid and finally to silica gel with increasing acid concentration in the plant tissue (Ma and Takashi, 2002). It is finally deposited in the cell wall materials as a polymer of hydrated amorphous silica, forming both silica-cuticle and silica-cellulose double layers in the shoots (Ma and Takahashi, 2002). Deposition of Si in the plant tissue enhances the strength and rigidity of cell walls and thus increases the resistance of plants to various stresses. The silicified cell also provides useful palaeoecological and archaeological information known as plant opal or phytoliths (Hodson, 2005). Silicon in plants has been reported to enhance tolerance of plants to both biotic and abiotic stresses in several crops (Figure 1.1).





**Figure 1:1 Beneficial effects of silicon on plant growth in relation to biotic and abiotic stresses (Ma and Yamaji, 2006).**

Several studies have revealed that silicon plays multiple roles in plant life and crop performance. For example, Savant *et al.* (1999) reported increase in sugar content in sugarcane following silicon amendment, while Seebold *et al.* (2000) observed a higher rice yield following silicon amendments to the rice fields. Silicon amendments have been reported to also improve the quality of fruits and vegetables, vitamin C content, and shelf life of avocados (Yanishevskaya and Yagodin, 2000; Kingston, 2008; Kaluwa *et al.*, 2010). Besides the beneficial roles of this element in plant growth, development, yield and quality, silicon has also been reported to increase plant resistance to biotic

stress such as disease and pest and abiotic stresses including metal toxicity – Fe, Mn, Cd -, nutrient imbalance, salt stress, extreme temperature, radiation and drought. Silicon is also reported to improve mechanical properties of the plant such as stature, soil penetration by roots, exposure of leaves to light, resistance to lodging among others). These have been demonstrated in a wide variety of plant species (Ma, 2004; Hattori *et al.*, 2005; Liang *et al.*, 2005a;b).

The presence of silicon in soils is reported to impact positively on soil physical properties such as texture, soil water holding capacity and soil fertility. Silicon has also been reported to increase the weight and volume of roots of drought resistant plants, thereby reducing their water requirement (Bocharnikova and Matichenkov, 2008). When supplied as a fertilizer, silicon interacts with the absorbed nitrogen (Filho and Abdalla, 2008; Mobasser *et al.*, 2008) and regulate the rate of nitrogen absorption. For example, soil amendment with both Si and N based fertilizers is reported to have a negative correlation during absorption by rice (Mobasser *et al.*, 2008). Due to this, silicon is reported to confer considerable relevance in agriculture (Ma, 2004; Ma and Yamaji, 2006), particularly in the face of envisaged climate change.

Plants take up dissolved Si from the soil to form biogenic opal that is Si particles (phytoliths), into plants, which return to the topsoil through organic residues. These phytolith particles exert a strong imprint on the soil-plant cycle, which is important in terms of hydrological output of Si and beneficial effects for plants (Matichenkov *et al.*, 2008). However in the forest ecosystems, the impact of tree species on the uptake and restitution of Si is poorly known. Trees that make Si largely mobile in soil-tree cycles, influencing the solubility of phytolith particles and availability of Si in the soil-plant system have been reported (Cornelis *et al.*, 2008)

### **1.2.3 Effect of silicon on abiotic stresses**

Although silicon is the second most abundant element both on the surface of the Earth's crust and in soils, it has not yet been listed among the essential elements for higher plants. However, the beneficial role of Si in stimulating the growth and development of many plant species has been generally recognized. Silicon is known to effectively mitigate various abiotic stresses such as manganese, aluminium and heavy metal toxicities, and salinity, drought, chilling and freezing stresses (Liang *et al.*, 2006).

However, mechanisms of Si-mediated alleviation of abiotic stresses in plant growth and development remain poorly understood. The key mechanisms of Si-mediated alleviation of abiotic stresses in higher plants include: stimulation of antioxidant systems in plants, complexation or co-precipitation of toxic metal ions with Si, immobilization of toxic metal ions in growth media, uptake processes, and compartmentation of metal ions within plants.

#### **1.2.3.1 Drought and salinity**

Silicon has been reported to alleviate plant water stress by improving leaf photosynthesis and root activity, reduce transpiration and enhance water retention by plants. Biochemically, silica in plants is reported to reduce lipid peroxidation, stimulate antioxidant production and maintain plasma membrane function (Liang, 2008). For example, silicon has been reported to significantly increase drought tolerance in sorghum, thereby improving plant physiological parameters such as water use efficiency and uptake ability (Ahmed *et al.*, 2011). Under saline conditions, Si restricts sodium uptake to the plant's shoot by partial blockage of the transpiration bypass flow (Liang *et al.*, 1996; Yeo *et al.*, 1999).

#### **1.2.3.2 Heat**

Silicon in plants has been reported to increase the plants tolerance to heat. For example, Agarie *et al.* (1998) observed that electrolyte leakage caused by high temperature was less pronounced in the leaves of plants grown on soil amended with Si than in those grown in silica un-amended soils. It has been suggested that Si might be involved in the thermal stability of lipids in cell membranes although the mechanism involved has not been elucidated.

#### **1.2.3.3 Aluminium toxicity**

Aluminium toxicity in plants is one of the major problems both for agriculture on naturally acidic soils and for forest areas affected by acidic rain. The effects of Si on aluminium (Al) toxicity in plants has been extensively reported (Hodson, 1999). It has been shown that under some conditions soil added Si can ameliorate Al toxicity in hydroponic culture (Hodson, 1995; Hodson, 1999). Although the relationship of silicon and aluminium at both acidic and basic pH on plants is unknown, at neutral pH, Al and

Si form hydroxyaluminosilicates (HAS), a complex reported to reduce Al toxicity in plants. The formation of HAS at pH of 4.0 and below has been shown to be negligible, and formation gradually increases as pH increases to pH 5.0 and beyond. These findings have considerable importance for agriculture on acidic soils.

#### **1.2.4 Effect of silicon on biotic stresses**

The role of silicon (Si) as nutrient enabling plants to ameliorate the effects of a range of biotic environmental stressors has become the subject of research. Silicon has been reported to enhance plant defence mechanisms in several crops such as rice and wheat, cucumber, and tomato (Basagli *et al.*, 2003; Rodrigues *et al.*, 2004; Liang *et al.*, 2005a; Diogo and Wydra, 2007). According to previous studies, enhanced resistance of host plants to diseases and to insect herbivores including stem borers, and phloem and xylem feeders have been shown (Laing *et al.*, 2006; Kvedaras and Keeping, 2007; Kvedaras *et al.*, 2007; Keeping *et al.*, 2008). An induction of defence compounds by Si such as phenolic compounds, pathogenesis-related proteins, peroxidase, polyphenoloxidase and chitinase has been reported in several plant species (Fawe *et al.*, 2001; Liang *et al.*, 2005a; Rodrigues *et al.*, 2005; Kurabachew, 2011). These has been contributed by deposition of amorphous silicon ( $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ ), and also by the bioactivity of monosilicic acid. While silicon deposition in plant epidermal cells as amorphous silicon may create a barrier against diseases and pests, it is now thought that silicon induces resistance by a priming effect (Dannon and Wydra, 2004; Diogo and Wydra, 2007; Ghareeb *et al.*, 2011a;b). It catalyzes expression of physiological resistance through production of compounds such as tannins, phenolics and defence related enzymes (Laing *et al.*, 2006; Schacht, 2009). Silicon acts as a signal in triggering plant defence mechanisms similar to systemic acquired resistance (SAR) (Fauteux *et al.*, 2005; Cai *et al.*, 2009). Silicon may also increase leaf abrasion, which subsequently increases wearing of insects' mandibles, and may physically deter larval feeding (Raupp 1985; Massey *et al.*, 2006; Kvedaras *et al.*, 2009). These make the larvae to take time before they establish in the maize stem and thus exposing them to external predictors.

#### **1.2.4.1 Influence of plant silicon on the survival and growth of insect pests**

The acceptance or rejection of plants by herbivorous insects depends on their behavioural responses to plant features, which may be physical (such as spines and trichomes) or chemical (such as phenolics and tannins) (Epstein, 1999). The protective effect of silica to plants against insect herbivores, pathogens or abiotic factors is reported to relate to the level of its accumulation and polymerization in the plant tissues with highest levels positively being correlated with highest resistance (Bélanger *et al.*, 2003; Meyer and Keeping, 2005; Laing *et al.*, 2006). Mitigating effects of silica against borers attack has been observed in barley, rice wheat, sugarcane, maize and sorghum (Epstein, 1999; Schoonhoven *et al.*, 2005; Kvedaras *et al.*, 2007a).

Elucidation of the exact mechanism of silica mitigating effects is however still limited although most studies point to both physical and/or chemical mechanisms (Ma, 2004; Fauteux *et al.*, 2006). Mechanically, deposition of silica in plant epidermal cells provides a physical barrier against borer probing and feeding or pathogen penetration into plant tissues. For example, silica mediated borer resistance to *Eldana saccharina* (Walker) (Lepidoptera: Crambidae) on sugarcane or *Chilo suppressalis* Walker (Lepidoptera: Crambidae) on rice is partly due to delayed stalk penetration by larvae as a result of leaf and stalk silification (Djamin and Pathak, 1967; Peterson *et al.*, 1988; Keeping and Meyer, 2002; Keeping and Meyer, 2006; Kvedaras and Keeping, 2007a; Kvedaras *et al.*, 2007b). Silica may also alter the relative palatability of leaves by increasing leaf abrasion, which increases wearing of insects' mandibles and therefore physically deter larval feeding (Raupp, 1985; Massey *et al.*, 2006).

In addition, silica modulates the accumulation of herbivore defensive allelochemicals including phytoalexins, lignin and phenolics in plant tissues (Cherif *et al.*, 1994; Fawe *et al.*, 2001; Bélanger *et al.*, 2003; Rodrigues *et al.*, 2004; Remus-Borel *et al.*, 2005). Similarly, silica is also reported to elicit the production of plant defensive enzymes including peroxidase, polyphenoloxidase and phenylalanine ammonia lyase which are induced in response to plant damage by herbivorous insects (Keeping and Meyer, 2002; Correa *et al.*, 2005; Gomes *et al.*, 2005). These enzymes have been implicated in a number of plant defenses processes such as lignification and/or production of antiherbivore plant metabolites (Felton *et al.*, 1994). Nevertheless, the effects of plant

tissue silification as a defense mechanism against insect herbivores seem not universal. For example, high silica levels in turf grass had no influence on feeding and development of *Herpetogramma phaeopteralis* Guenée (Lepidoptera: Pyralidae), nor on growth, survival, feeding preference or mandibular wear of *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) (Redmond and Potter, 2007).

#### **1.2.4.2 Trophic relationship among silica levels in maize plants and stem borer feeding**

The addition of Si in maize can increase water use efficiency by reducing leaf transpiration and water flow rate in the xylem vessel (Gharineh and Karmollachaab, 2013). Silicon benefits in maize have been related to its effect on the improving of population quality, effective leaf area, and photosynthetic efficiency as well as the delay of leaf senescence (Gao *et al.*, 2004; Zou *et al.*, 2007). Photosynthesis is a determinant factor for crop growth and development as maximum photosynthesis contributes toward more yield and production crop yield potential are reported to increase by 50% by raising photosynthetic capacity (Covshoff and Hibberd, 2012). With respect to maize growth, photosynthesis is reported as the most basic and critical physiological process directly related to maize yield, especially at late developmental stages (Ahmed *et al.*, 2012; Covshoff and Hibberd, 2012). Moreover, Si-mediated plant resistance to insect herbivores has largely addressed the dominant hypothesis of a Si barrier providing mechanical resistance to insect feeding and/or plant penetration (Hanifa *et al.*, 1974; Kvedaras and Keeping, 2007a). Sétamou *et al.* (1993) found that Si applied to maize reduced larval survival, percentage pupation and adult emergence in Lepidoptera stem borer like *S. calamistis*.

#### **1.2.5 Effect of temperature on silicon accumulation in maize**

It is well reported that the mean annual Earth's surface temperature increases at a rate of 0.2°C per decade (Hansen *et al.*, 2006). In plant physiology, root-zone temperature (RZT) is an important growth factor (Pregitzer and King, 2005). It may affect rate related physicochemical and biological processes of nutrient availability in soil (Hussain *et al.*, 2010). The soil temperature influences water and nutrient uptake, metabolic processes, roots and shoot growth (McMichael and Burke, 1998; Toselli *et*

*al.*, 1999). The rates of organic matters decomposition and nutrient in the soils diffusion are also directly related to soil temperature. Hence root-zone temperature (RZT) is a major factor controlling plant growth and development. The RZT changes with the season of the year and geographical location of the area. Moreover, plant response to the expected global temperature will most probably change this synchronized interaction between roots and shoots (Farrar and Jones, 2000). It is also reported that in many plant species, uptake of nutrient like silicon, potassium, and phosphorus by roots decreases at low RTZ (Toselli *et al.*, 1999; Weih and Karlsson, 1999). Therefore decrease in temperature reduces the amount of silicon absorbed by the plants.

#### **1.2.6 Distribution of Lepidoptera stem borers along the altitudinal gradients**

Lepidoptera stem borers are generally considered the most damaging insect pests of maize in sub-Saharan Africa (Seshu Reddy, 1998; Overholt *et al.*, 2001). Maes (1997) reported 20 economically important Lepidoptera stem borer pests whose distribution and pest status vary with environmental conditions (Megenasa, 1982; Songa *et al.*, 1998; Ndemah *et al.*, 2001).

For example, *S. calamistis*, *B. fusca* and *E. saccharina* are found throughout sub-Saharan Africa (Polaszek and Khan, 1998) but their pest status on plants varies depending on the region. *Sesamia calamistis* and *E. saccharina* are the major pests of maize in West Africa whereas *B. fusca* and *C. partellus* are the dominant pests of maize in East Africa (Overholt *et al.*, 2001). In Kenya, the distributions of *B. fusca*, *C. partellus*, *S. calamistis* and also another crambid *C. orichalcociliellus* are different but often overlap in space and time (Seshu Reddy, 1983; Khan *et al.*, 1997; Overholt *et al.*, 2001). Moreover, it was generally reported that *B. fusca* is the dominant stem borer species in high potential zones (highland tropics, moist transitional zone and moist mid-altitude) while *C. partellus* dominates smallholder farms in low potential zones (dry mid-altitude, dry transitional and lowland tropical zone) (Kfir, 1997; Kfir *et al.*, 2002). Harris and Nwanze (1992) argued that temperature, rainfall and humidity were the main factors that affect their distribution, with temperature being the most important.

### **1.3 Analytical techniques**

#### **1.3.1 Inductively-coupled plasma optical emission spectroscopy (ICP-OES)**

### 1.3.1.1 Principle

The solution to analyse is conducted by a peristaltic pump through a nebulizer into a spray chamber. The produced aerosol is lead into an argon plasma. Plasma is the fourth state of material, next to the solid, liquid and gaseous state. In the plasma is generated at the end of a quartz torch by a water-cooled induction coil through which a high frequency alternate current flows. As a consequence an alternate magnetic field is induced this accelerated electrons into a circular trajectory. Due to collision between the argon atom and the electrons ionisation occurs, giving rise to stable plasma. the plasma is extremely hot, 6000-7000 K . In the induction zone it can even reach 10000 K. In the torch desolvation, atomisation and ionisation of the sample takes place. Due to the thermal energy taken up by the electrons, they reach a higher "excited" state. When the electrons drop back to ground level energy is liberated as light (photons). Each element has an own characteristic emission spectrum. By means of an Echelle grating, a prism, and a focussing mirror these emitted photon in various frequencies are captured simultaneously.

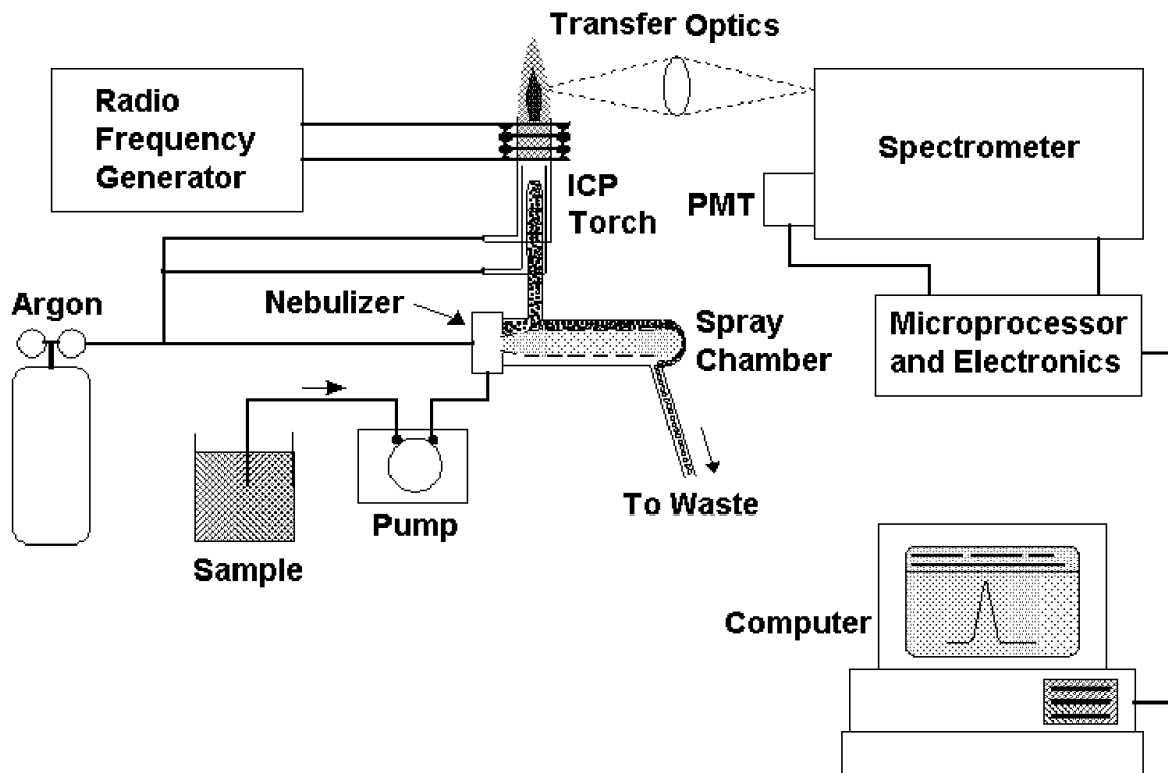


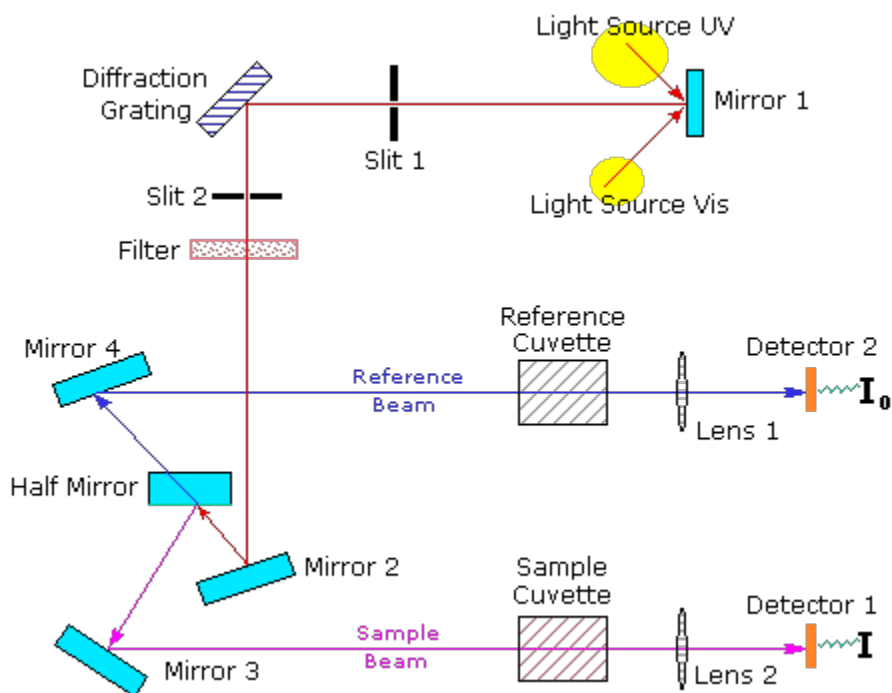
Figure1.2: Major components and layout of a typical ICP-OES instrument.



## 1.3.2 UV-Visible Spectroscopy

### 1.3.2.1 Principle

A diagram of the components of a typical spectrometer is shown in the following diagram. The functioning of this instrument is relatively straight forward. A beam of light from a visible and/or UV light source (colour red) is separated into its component wavelengths by a prism or diffraction grating. Each monochromatic (single wavelength) beam in turn is split into two equal intensity beams by a half-mirrored device. One beam, the sample beam (colour magenta), passes through a small transparent container (cuvette) containing a solution of the compound being studied in a transparent solvent. The other beam, the reference (colour blue), passes through an identical cuvette containing only the solvent. The intensities of these light beams are then measured by electronic detectors and compared. The intensity of the reference beam, which should have suffered little or no light absorption, is defined as  $I_0$ . The intensity of the sample beam is defined as  $I$ . Over a short period of time, the spectrometer automatically scans all the component wavelengths in the manner described. The ultraviolet (UV) region scanned is normally from 200 to 400 nm, and the visible portion is from 400 to 800 nm.



**Figure1.3: Major components and layout of a typical UV-Visible Spectroscopy**

#### 1.4 Statement of the problem

Most plants grow by absorbing nutrients from the soil depending on the nature of the soil. Losses of nutrients through leaching by rain water depend on how strong the nutrient is bound to the soil constituents. When nutrients leach into the soil, they are not available for plants to use thus affecting the plant health. Changes in soil properties with altitude are influenced strongly by microclimate and topography. Generally cooler, wetter condition at higher altitude reduces biological activity and increase leaching. Steeper profile encourage run off and subsurface movement of water down slope. This manifests in the reduction of nutrients susceptible to weathering such as potassium and silicon in the higher altitudes (Proctor *et al.*, 2007; Bendix *et al.*, 2008; Gerold *et al.*, 2008; Wilcke *et al.*, 2008).

The soils accessible Si, such as monosilicic acid, is rapidly lost by leaching. This form of silica is also lost from the soils farming practices that involve a systematic removal of crop residues from farms following the previous cropping season (Tréguer *et al.*, 1995). Hence in order to maintain the level of available Si to plants silicon losses due to

leaching must be replenished. This is because if the available Si is repeatedly drawn from the soils by either leaching or cropping, then the soil will ultimately become depleted in Si, decreasing plant resistance to Lepidoptera stem borers and other abiotic and biotic stresses. This could have a strong impact on the interactions between the crops and the Lepidoptera stem borers. Therefore, there is a need to determine the level of Si in soil and maize plants in different agro-ecological zones of East Africa that are characterized by different temperature and rainfall patterns; and relate these levels to Lepidoptera stem borers distribution.

### **1.5 Justification**

Although much have been achieved in the last 5 years challenges still remain in achieving food security, poverty reduction, transformation of agriculture from subsistence to commercial farming and efficient use of input and agricultural credit. Enhancing host resistance by using elicitors such as Si amendment in solution form can be an effective strategy to enhance plants' ability to cope with biotic and abiotic stresses. Silicon has been reported to enhance plant defence mechanisms against insect pests and diseases in several crops such as rice, wheat, cucumber, and tomato (Basagli *et al.*, 2003). These defense properties of silicon are attributed to the deposition of amorphous silicon ( $\text{SiO}_2, n\text{H}_2\text{O}$ ) in the plant stem leaves and roots as well as due to the bioactivity of monosilicic acid. While it has long been suggested that silicon deposition in plant epidermal cells as amorphous silicon creates a barrier against pests and diseases, it is now believed that silicon induces resistance to biotic factors through a priming effect (Dannon and Wydra, 2004).

Soil Si amendment has been widely used by farmers in Asia and Brazil, and also in South Africa to improve crop performance (Kerstin Wydra, Personal communication). However, this strategy has been little demonstrated in East African countries. This study aims at understanding the level silicon in soil and maize plants which will allow the establishment of the relationships between silicon levels and the distribution of Lepidoptera stem borer pests under natural field conditions. This will allow interrogation of any association between stem distribution and the levels of Si in both abiotic (soil) and biotic (plants).

## **1.6 Hypothesis**

There is no relationship between the variations of Si content in soils and maize plants with the Lepidoptera stem borers' distribution along the altitudinal gradients.

## **1.7 Objectives**

### **1.7.1 General objective**

The general goal of this study was to determine the variations of Si in soils and maize plants grown along altitudinal gradients and relate these variations to Lepidoptera stem borers' distribution.

### **1.7.2 Specific objectives**

- i. To determine the levels of Si in soils and maize plants tissues and maize along altitudinal gradients.
- ii. To determine the variation of Si absorption by maize plants along altitudinal gradients.
- iii. To relate the variation of Si in maize plants with the stem borer pests distribution along the altitudinal gradients.

## CHAPTER TWO

### MATERIALS AND METHODS

#### 2.1 Research design

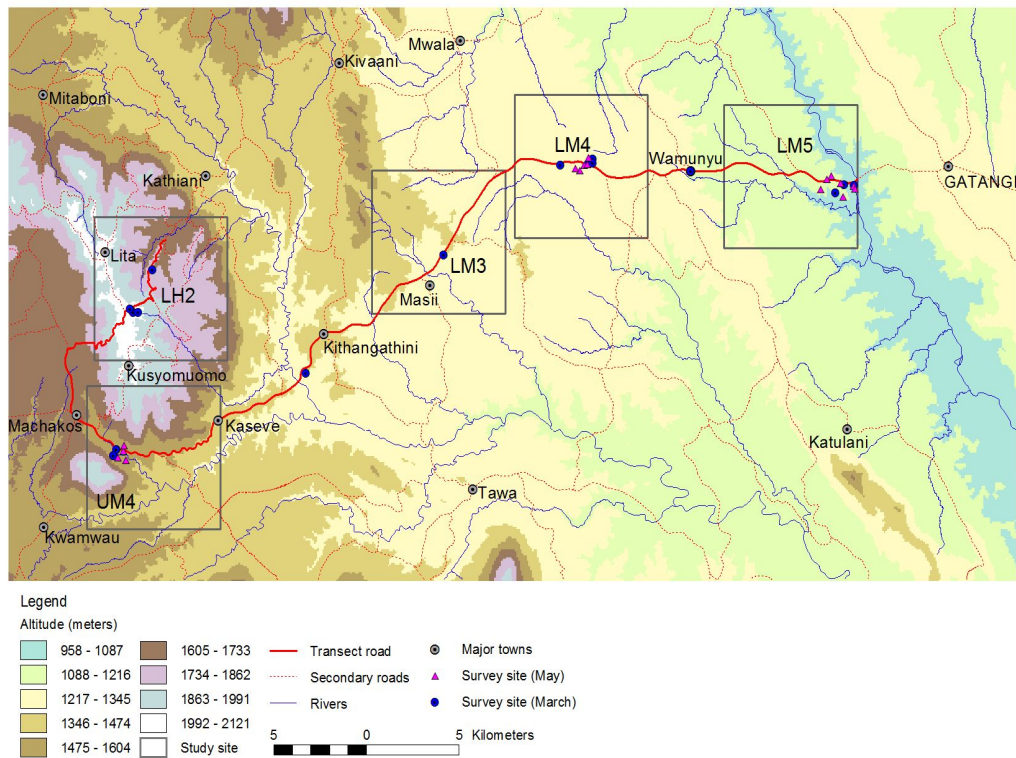
This study was part of the Noctuid Stem Borer Biodiversity project (a joint IRD – *icip*e project). The project focused on the analysis of Si content in maize plants leaves and soils along altitudinal gradients with variable climatic transects. This involved sampling of both maize leaves and soils for Si content and simultaneously, surveying Lepidoptera stem borer species distribution along the same transects across the year. This allowed for the establishment of the relationships between the level of Si in both soil and maize plants as well as the distribution of Lepidoptera stem borer pests under natural field conditions. To interrogate any association between stem borers diversity/density and the levels of Si in both abiotic (soil) and biotic (plants) a greenhouse based study was carried out to test the influence of increasing levels of Si in maize plants via soil amendments on the survival and development of different stem borer species found in the fields.

#### 2.2 Study area

The study was carried out on three climatic transects: Machakos Hills, Taita Hills and Mount Kilimanjaro. These transects are characterised by rapid change in altitudinal gradients and a significant variation in climatic condition along the gradients (Tables 2.1 and 2.2). The donor (CHIESA) wanted people who will do research related to climate within this areas. The sample size was determined by the funds donated for this project. This study interrogated variations of Si content in soils and maize plants with the Lepidoptera stem borer pests' distribution along the altitudinal gradients characterized by different rainfall and temperature patterns.

##### 2.2.1 Machakos Hills transect

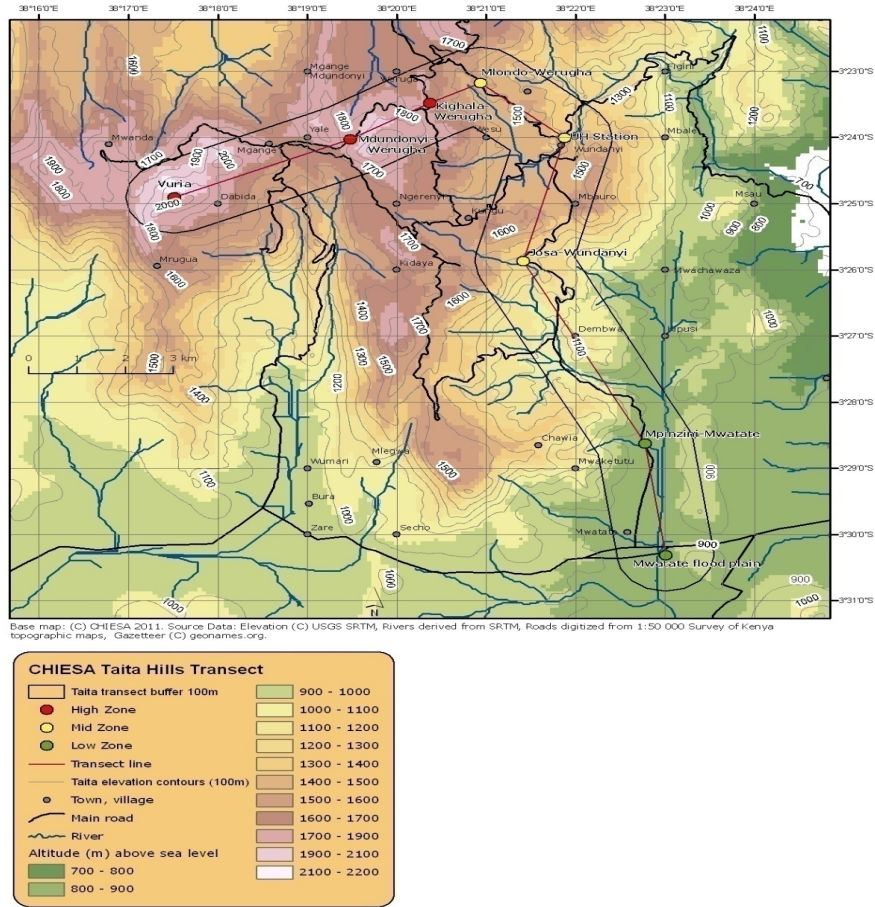
This transect is found in Machakos county, that lies about 64 km south of Nairobi. The Machakos Hills transect involved five benchmark sites between latitude 1°35'37" to 1°20'16" S and longitude 37°13'59" to 37°38'44" E (Figure 2.1). In this study this transect covered an area with an altitudinal gradient starting at Athi river bridge (1,049 m.a.s.l.) along Machakos-Kitui road to Iveti hills (2,124 m.a.s.l.) covering about 60 km.



**Figure 2.1: Machakos Hills transect.**

### 2.2.2 Taita Hills transect

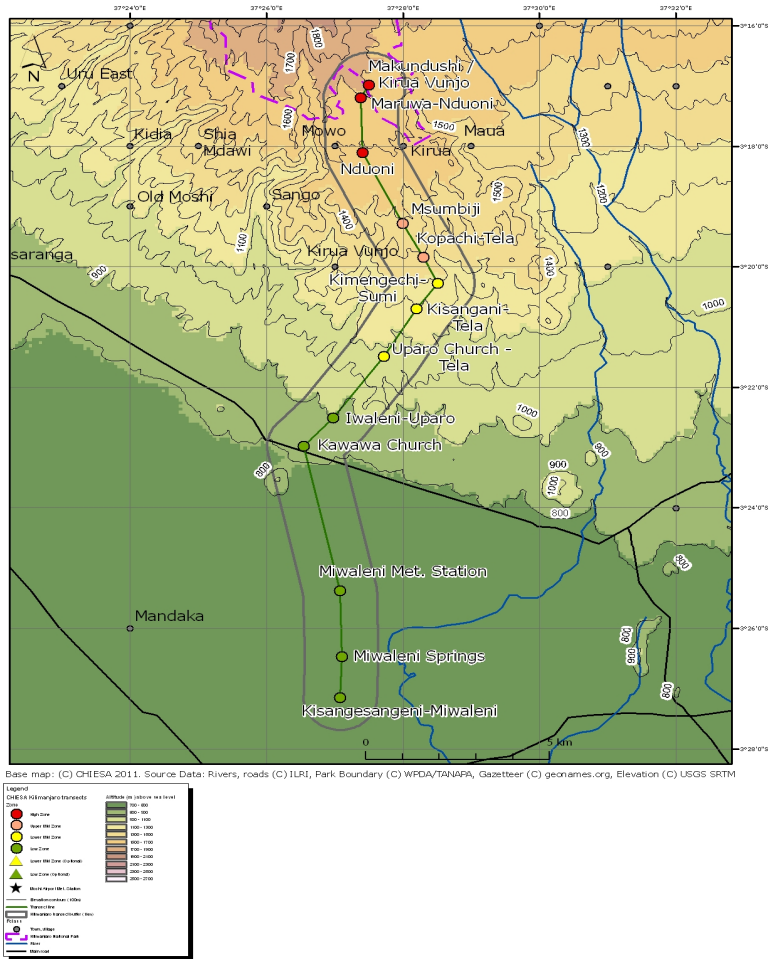
Taita Hills are a precambrian mountain range in the south-west of Kenya in Taita-Taveta county. The hills consist of three massifs namely Dabida, Sagalla in the southern side of Voi township and Kasigau in the south near the border of Tanzania. The Dawida massif is the largest and tallest of the three, with an altitude of 2,228 m.a.s.l. at its highest peak, Vuria. The transect had six benchmark sites lying between latitude 3°23'49" to 3°28'70" S and longitude 38°17'56" to 38°22'72" E (Figure 2.2). This transect covered an altitudinal gradient starting at Mwatate (700-900 m.a.s.l.) to Vuria (1,800-1,900 m.a.s.l.).



**Figure 2.2: Taita Hills transect.**

### 2.2.3 Mount Kilimanjaro transect

Kilimanjaro is one of the 30 regions of Tanzania, a home of Mount Kilimanjaro with Moshi being the region's capital. The region is bordered to the north and east by Kenya, to the south by the Tanga region, to the southwest by the Manyara region, and to the west by the Arusha region. The Kilimanjaro transect had six benchmark sites between latitude 3°17'14" to 3°25'16" S and longitude 37°26'86" to 37°28'24" E (Figure 2.3). This transect covered an altitudinal gradient starting at Miwaleni (700-800 m.a.s.l.) to Marua (1,600-1,800 m.a.s.l.).



**Figure 2.3: Mount Kilimanjaro transect.**

For each transect, the GPS coordinates were read in the middle of each farmer’s plot for each altitude. For each altitude, the temperature and relative humidity (Table 2) were recorded hourly during the study period using <sup>TM</sup>HOBO data loggers while rainfall was recorded daily using GENERAL<sup>R</sup> wireless rain gauges placed permanently in one of the farmer’s plots. The soil characteristics were also evaluated according to Jaetzold and Schimdt (1983) for Machakos Hills, Jaetzold *et al.* (2012) for Taita Hills and Samki (1977) for Mount Kilimanjaro transects (Table 2.1).



**Table 2.1: The three different transects (Machakos Hills, Taita Hills and Mount Kilimanjaro) studied characterized by altitudinal gradients.**

	<b>Localities</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Altitude (m.a.s.l.)</b>	<b>Soil characteristics</b>
<b>Machakos Hills</b>	Miondoni	S 01°24.981' - 01°40.673'	E 37°37.708' - 37°63.369'	1,083 - 1,144	nito-rhodic ferralsols and nito-chromic cambisols
	Ghetto	S 01°24.296' - 01°40.959'	E 37°30.563' - 37°51.670'	1,134 - 1,307	ferralochromic / orthic / ferric acrisols with luvisols and ferralsols
	Masii	S 01°32.001' - 01°53.548'	E 37°17.230' - 37°28.695'	1,521 - 1,592	ferralochromic / orthic / ferric acrisols with luvisols and ferralsols
	Mwanyani	S 01°32.115' - 01°53.664'	E 37°17.223' - 37°29.035'	1,513 - 1,792	acrisols with arenosols
	Mutito	S 01°28.438' - 01°49.575'	E 37°17.678' - 37°30.464'	1,701 - 2,048	humic cambisols
<b>Taita Hills</b>	Kipusi	S 03°47.870' - 03°47.936'	E 38°36.726' - 38°38.001'	818 - 871	rhodic ferralsols
	Dembwa	S 03°44.140' - 03°44.721'	E 38°36.051' - 38°36.446'	1,083 - 1,102	rhodic ferralsols, with ferralic arenosols and ferralochromic luvisols
	Josa	S 03°42.988' - 03°43.306'	E 38°35.610' - 38°35.801'	1,340 - 1,358	humic cambisols, with humic nitisols, dystic regosols
	Mbengonyi	S 03°41.736' - 03°42.046'	E 38°36.210' - 38°36.601'	1,467 - 1,490	humic cambisols, with humic nitisols, dystic regosols
	Kighala	S 03°39.086' - 03°39.265'	E 38°33.803' - 38°33.896'	1,678 - 1,709	chromic acrisols, with cambisols and ferralsols
	Vuria	S 03°40.275' - 03°40.475'	E 38°29.575' - 38°29.656'	1,797 - 1,814	rankers and cambisols
<b>Mount Kilimanjaro</b>	Miwaleni	S 03°41.721' - 03°42.823'	E 37°45.738' - 37°45.945'	714 - 765	luvic xerosol
	Uparo-Iwaleni	S 03°36.675' - 03°37.460'	E 37°04.501' - 37°46.638'	891 - 987	eutric nitosol
	Uparo-Church	S 03°34.406' - 03°34.821'	E 37°46.030' - 37°45.988'	1,158 - 1,188	eutric nitosol
	Kopachi-Tela	S 03°33.008' - 03°32.916'	E 37°47.063' - 37°47.245'	1,365 - 1,402	eutric nitosol
	Nduoni	S 03°30.805' - 03°31.003'	E 37°46.266' - 37°46.451'	1,550 - 1,561	eutric nitosol
	Marua	S 03°28.615' - 03°28.821'	E 37°45.265' - 37°45.685'	1,667 - 1,683	dystic cambisol

**Table 2.2: Monthly temperature (°C), monthly relative humidity (%) (means ± SE) and annual mean rainfall (in mm) along the three different transects (Machakos Hills, Taita Hills and Mount Kilimanjaro).**

	Localities	Altitude (m.a.s.l.)	Temperature over the day	Temperature over the night	Relative humidity over the day	Relative humidity over the night	Annual rainfall
<b>Machakos Hills<sup>1</sup></b>	Miondoni	1,083 – 1,144	nd	nd	nd	nd	744
	Ghetto	1,134 – 1,307	27.2 ± 0.7	18.7 ± 0.3	57.2 ± 3.1	87.4 ± 4.0	854
	Masii	1,521 – 1,592	25.2 ± 0.1	19.2 ± 0.4	67.9 ± 0.9	95.2 ± 1.2	845
	Mwanyani	1,513 – 1,792	26.1 ± 0.8	18.3 ± 0.3	56.0 ± 3.7	84.5 ± 3.0	788
	Mutito	1,701 – 2,048	22.4 ± 0.9	15.3 ± 0.4	65.7 ± 3.4	93.5 ± 2.1	1192
<b>Taita Hills<sup>2</sup></b>	Kipusi	818 - 871	27.3 ± 0.5	20.5 ± 1.4	56.7 ± 2.2	83.7 ± 1.7	1010.4
	Dembwa	1,083 – 1,102	25.6 ± 0.7	18.5 ± 0.3	62.8 ± 1.7	92.7 ± 1.5	1387.2
	Josa	1,340 – 1,358	21.4 ± 0.6	18.1 ± 0.5	71.5 ± 2.5	91.7 ± 0.9	1526.4
	Mbengonyi	1,467 – 1,490	21.3 ± 0.5	16.6 ± 0.4	74.5 ± 2.4	90.4 ± 2.1	1544.4
	Kighala	1,678 – 1,709	20.1 ± 0.5	15.8 ± 0.4	81.0 ± 2.1	95.1 ± 1.8	1648.8
	Vuria	1,797 – 1,814	19.4 ± 0.7	15.1 ± 0.4	76.4 ± 2.0	94.6 ± 1.7	1706.4
<b>Mount Kilimanjaro<sup>2</sup></b>	Miwaleni	714 - 765	28.3 ± 0.8	22.1 ± 0.4	52.5 ± 2.6	74.9 ± 1.8	865.2
	Uparo-Iwaleni	891 - 987	25.2 ± 1.2	20.3 ± 1.1	62.2 ± 2.0	85.5 ± 3.0	1054.8
	Uparo-Church	1,158 – 1,188	23.3 ± 0.4	18.3 ± 0.2	66.8 ± 2.3	80.9 ± 3.2	1641.6
	Kopachi-Tela	1,365 – 1,402	21.8 ± 0.8	17.7 ± 0.4	72.9 ± 3.4	90.7 ± 1.1	2158.8
	Nduoni	1,550 – 1,561	21.0 ± 0.7	16.3 ± 0.4	74.3 ± 3.1	92.4 ± 1.9	2198.4
	Marua	1,667 – 1,683	20.0 ± 0.6	15.0 ± 0.3	78.4 ± 2.0	96.5 ± 0.7	1852.8

nd: not determined; for Machakos Hills the average annual rainfall was derived from WorldClim (interpolations of observed data, representative of 1950-2000) data set. Further information can be found at <http://www.worldclim.org/>

<sup>1</sup>from January to March 2013;

<sup>2</sup>from June 2012 to June 2013.

## **2.3 Preliminary plant sampling experiments**

The following laboratory experiments were carried out to ascertain the maize plant tissue with invariable accumulated Si levels between plant developmental stages to be sampled in the field. The following experiments were undertaken:

1 - Maize variety hybrid 513 provided by Simlaw (Kenya Seeds Company, Nairobi) was used. The seeds were planted in plastic pots (diameter 14 cm, height 15 cm) using soil obtained from *icipe*. Two weeks after germination, the potted plants were treated with two concentrations of calcium silicate, 10.0g or 20.0g, while the control plants were left untreated. The pots were placed over plastic trays to prevent loss of silica through leaching during watering. Young leaves (1<sup>st</sup> leaves from the apex), mature leaves (3<sup>rd</sup> - 4<sup>th</sup> leaves from the apex), stem and roots of each plant were collected separately after 4 weeks of growth. All plant parts were separately sliced, dried at 60°C for 7 continuous days, and then ground to a fine, homogenous powder using a Bolmill Grinder (type MM40) prior to the extraction and analysis of Si from plant tissues.

2 - In another experiment, seeds of the same maize variety were directly planted in *icipe* fields. Maize plants of the following developmental stages were used: young (9-11 leaves; 40-50 cm height); middle-aged (13-15 leaves; 65-90 cm height) and old (15-16 leaves; 140-145 cm height). For each growth stage, the third leaf from the top (i.e. mature leaf) was collected and subjected to the above aforementioned tests.

## **2.4 Sampling**

### **2.4.1 Soil sampling for silicon analysis**

Soil samplings were done in 10 cultivated plots in each transect and altitudinal zone considered. Each of the cultivated plots represented one replicate per altitudinal zone. For each plot, 3-4 sub-samples were randomly selected, sampled and grouped to finally represent the entire farmer's plot. Prior to soil sampling, 1-2 cm upper surface soil layers were first scooped off from each sampling area to avoid contamination of the samples resulting from foreign material such as crop residues present on the soil surface. Two types of soil sample were then collected, first at a depth of between 0-25 cm and another at 25-50 cm, using a soil augur. Sampling was done in February 2013 in the Machakos Hills transect, and in October 2012 in the Taita Hills and Mount

Kilimanjaro transects, during a period that coincided with either the off-season or beginning of the planting season, depending on the altitude. For each plot, soil sub-samples were mixed and homogenized with respect to sampling depths to finally provide two samples in each cultivated plot.

#### **2.4.2 Sampling of maize plant leaves for silicon analysis**

Plant leave samples were collected in 10 cultivated plots for each transect and altitudinal zone considered. Each cultivated plot represented one replicate per altitudinal zone. For each plot, seven healthy lepidoptera stem borers' uninfested maize plants were randomly selected, sampled and grouped. A similar sampling process was performed for lepidoptera stem borers' infested maize plants. During sampling an entire third leaf from the apex of each plant (i.e. mature leaf) was collected for Si analyses. The mature plant leaf was found to constitute invariable high levels of maize plant Si than other plant tissues considered throughout the plant developmental stage (see preliminary results). Plant samplings were done at different periods of the year depending on planting season in each transect. For Machakos Hills, samplings occurred from January to March 2013 only while for Taita Hills and Mount Kilimanjaro sampling occurred monthly between June and December 2012 and between February and June 2013 respectively. All leaves collected were pooled and sliced together into small pieces, dried at 60°C for 7 continuous days and thereafter ground to a fine, homogenous powder using a Bolmill Grinder (type MM40) prior to silica analysis.

#### **2.5 Composition of maize stem borer species along altitudinal gradients**

Stem borers were sampled from maize plants in 10 cultivated plots in each of the three transects and altitudinal zones. For each plot, six plants were inspected for the presence of the stem borers 5 meters on a straight line from the previous plant. Plants inspected were either on the left or right along a straight line. The plants were inspected for external signs of stem borer attack (dead heart, leaf damage, bored holes and exit holes). A maximum of 60 plants were randomly selected from each altitude and cut at ground level and transported to the laboratory at *icipe* for destructive sampling in order to recover the stem borers. These plants were dissected and all stem borers recovered sorted according to stage and species. The total number of stem borers obtained was

established and each was placed in a glass vial containing artificial diet until the adult stage when the species identity was confirmed. For each altitudinal zone, the density of a species was expressed as the total number of individual of this species collected from the 60 plants of the 10 plots.

## 2.6 Reagents and Chemicals

- Acetic acid  $\text{CH}_3\text{COOH}$
- Ammonium nitrate  $\text{NH}_4\text{NO}_3$
- Ammonium fluoride  $\text{NH}_4\text{F}$
- Benzoic acid  $\text{C}_7\text{H}_6\text{O}_2$
- Boric acid  $\text{H}_3\text{BO}_3$
- Citric acid  $\text{C}_6\text{H}_8\text{O}_7$
- Concentrated Sulphuric  $\text{H}_2\text{SO}_4$
- Ethylenediaminetetraacetic acid (EDTA)
- Hydrochloric acid  $\text{HCl}$
- Sodium Molybdate  $\text{NaMoO}_4$
- Nitric acid  $\text{HNO}_3$

## 2.7 Determination of soil silicon levels

Soil samples obtained along the transects were air dried and sieved prior to their analysis. The free Si content was extracted from the soil using the soil 1:2 extraction procedure that is used to determine the available soil nutrients. It is based on an extraction procedure using a wet/field moist soil volume to water (deionized volumes; i.e. one part soil and two parts water). The Mehlich 3 extraction method was used to estimate the total Si content of the soil samples. The method uses 0.2N  $\text{CH}_3\text{COOH}$  + 0.25N  $\text{NH}_4\text{NO}_3$  + 0.013N  $\text{HNO}_3$  + 0.015N  $\text{NH}_4\text{F}$  + 0.001M EDTA a useful reagents to extract Si as well as other micro-elements in the soil. To estimate the total silica content two grams of an air dried soil sample (<2mm) was weighed and placed in extraction cup and a 20ml of Mehlich 3 extracting solution added using an oxford dispenser. The content was thoroughly mixed using the orbital shaker for 5 min at 200 rpm. The sample solutions

were then filtered using medium porosity (Whitman no 42. filter paper) and filtrate transferred into disposable poly-propylene test tubes.

All samples were analysed within 24 hrs using inductively-Coupled Plasma Optical Emission Spectroscopy (ICP-OES), method at Crop Nutrition Laboratory Services at Nairobi.

## **2.8 Extraction and analysis of maize plant silicon**

The molybdenum-yellow method proposed by Saito *et al.* (2005) was adapted for the analysis of silicon levels in maize plant tissues with amendment in preparation of stock solution. The silica stock solution was prepared by digesting 214mg of silica (SiO<sub>2</sub>) (Sigma-Aldrich) in 2 ml of 1.5M HF-0.6M HCL solution and then topped to 10 ml with distilled water to give a stock solution of 10 mg/ml concentration. This stock solution was used to prepare standard solutions of concentration range between 0 to 1.5 mg/ml. Absorbance of both samples and standard solutions were measured in a 5 ml cell on a Beckman DU 650 spectrophotometer at 400 nm after 4-10 minutes following the addition of citric acid. The amount of silica in the samples was calculated from a calibration curve generated using silica standard solutions. All reagents and solutions for this experiment were prepared in silica free polypropylene plastic ware soaked in 0.1% HF solution prior to the analyses.

## **2.9 Influence of altitude on silicon absorption by the maize plants**

The purpose of this experiment was to test if altitude with variable temperature, atmospheric pressure and humidity characteristics can influence maize plant Si absorption and to also validate the aforementioned estimation of Si in leaves from transects with different altitudes. This experiment was done from mid-October to end of November 2013 in the six sites of Taita Hills transect.

A maize variety (hybrid 513) provided by Simlaw (Kenya Seeds Company, Nairobi) was grown for all the altitudinal zones earlier indicated. The seeds were planted in 30 plastic pots (diameter 14 cm, height 15 cm) using soil obtained from Mbengonyi area situated in the mid-altitudes of the Taita Hills transect to minimize the logistics of transporting the soil to the low or high altitudinal study sites. They were then placed in

one farm of each altitudinal zone of this transect. Watering was done by each farmer to avoid any water stress for plant growth during the experiment. Four weeks following plants germination, 10 pots were each amended with 10.0 g of calcium silicate, 10 other pots were amended with 10.0 g of sodium silicate and the remaining 10 pots were left as the controls. All the potted plants were then placed on plastic plates to prevent loss of silica through leaching during watering. The experiment was terminated after two weeks of plant growth by collecting maize leaves (all leaves for each potted plant). The Si levels of leaves of each potted plant was then determined using the aforementioned spectrophotometric molybdenum-yellow method.

### **2.10 Larval development on potted plants treated with soluble Si**

The aforementioned maize variety (513 hybrid) was planted in plastic pots in the green house (diameter 14 cm, height 15 cm) (one plant per pot) using soil obtained from *icipe* fields. Two weeks after maize germination, the plant pots were each treated with two different levels of calcium silicate 10.0g and 20.0g per pot except the control pots, which were instead treated with calcium carbonate. As aforementioned, all the potted plants were placed over plates to prevent loss of silica through leaching. Potted plants were carefully and randomly assigned in a greenhouse avoiding contact among the neighbouring plants. For each stem borer species, each potted plant was infested with thirty neonates using a fine camel hair brush. Nets with tiny holes were used to cover the plant so as to prevent migration of larva to adjacent plants

Evaluation of larval performance (growth and survival) was done after 14 days of infestation. The weight gain and the number of surviving larvae recovered per plant were recorded and the percent larval recoveries per plant calculated. The relative growth rates (RGR) were calculated by subtracting the initial larval weight from the final weight and dividing the difference by the number of days of infestation according to Ojeda-Avila *et al.* (2003). Thereafter, each dissected plant was used for Si content analyses as aforementioned in order to determine whether Si-amended plants exhibited higher Si contents than non-amended plants.

### **2.11 Larval development on artificial diets amended by Si**

To evaluate *B. fusca*, *S. calamistis* and *C. partellus* survival and larval growth rates to increasing dietary silica levels, the artificial diet prepared by Onyango and Ochieng-Odero (1994) was amended with varying levels of aforementioned calcium silicate powder. The levels of silica in the artificial diet ranged between 0 to 250 mg/ml of the artificial diet. Once prepared, 20 mls of each diet fraction (with different silica levels) was dispensed in each heat-sterilised glass vials (7.5 cm long x 2.5 cm diameter), 10 vials per each silica level. Preweighed *B. fusca*, *S. calamistis* and *C. partellus* neonates were then inoculated into each vial containing the diet fraction (5 neonates per vial) twenty four hours following their hatching. For all experiments, vials were tightly fitted with a cotton wool following each inoculation and kept in 80% relative humidity on a 12:12 hr (L/D) photoperiod. Larvae were allowed to feed *ad libitum* during the experimental period and the respective diet fractions replaced as necessary. Thereafter, surviving larvae were enumerated and weighed after 30 days of development and the percent larval recoveries per vial calculated. The relative growth rates (RGR) were calculated by subtracting the initial larval weight from the final weight and dividing the difference by the number of days of larval development according to Ojeda-Avila *et al.* (2003).

### **2.12 Statistical analysis**

All data were log-transformed or arcsin transformed (for percentage data) to fit to normal distribution as well as for homoscedasticity of the variance. Untransformed data is presented in the results. Analyses of variance (ANOVAs: one-way and two-way analyses of variance) were used to indicate significant factors to separate means and if significant interactions between factors existed. In case of significant F-values of ANOVA, the data were subjected to post hoc Tukey's contrast tests. All statistics were done using R 2.15.3 (R Development Core Team, 2013).

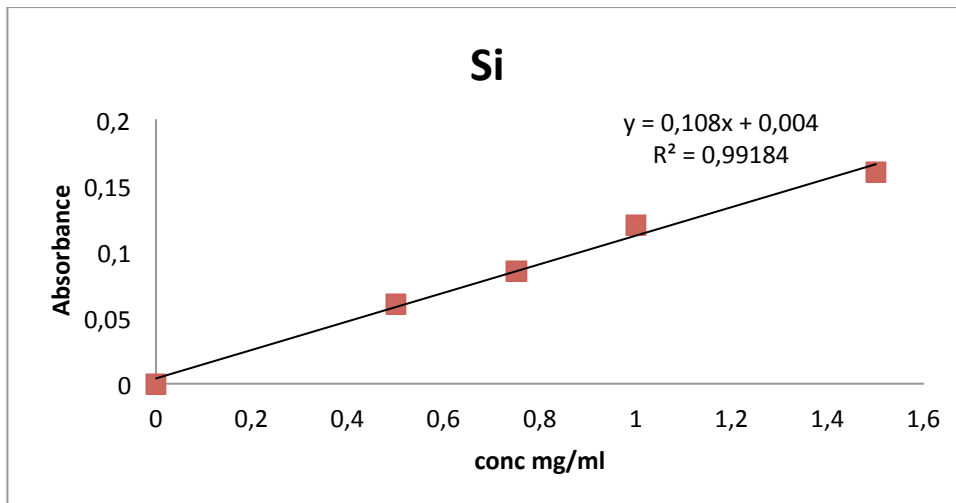


## CHAPTER THREE

### RESULTS AND DISCUSSION

#### 3.1 Preliminary experiments for plant sampling

Mature leaves turned out to be the most suitable plant parts for field samplings because their Si contents significantly responded to soil Si treatments (Table 3.1) possibly due the complete silification of leaves by silicon. Also the Si concentration of the mature leaves did not vary with plant growth stage (Table 3.2). Moreover, they are easier to sample than, for example, the roots. The calibration curve of (Figure 3.1) was used to determine the Si levels in plant organs.



**Figure 3.1: Standard curve for Si determination in maize plant organs (leaves, stems and roots).**

**Table 3.1: Silicon level (mean<sup>a</sup> ± SE, n=10) according to the maize plant organ and silicon treatment.**

Organ	Treatment	Si (ppm)
Root	Control	1,200 ± 0.8a, B
	10g Si	1,600 ± 0.7ab, C
	20g Si	1,860 ± 0.6b, C
Stem	Control	470 ± 0.4a, A
	10g Si	540 ± 0.4a, A
	20g Si	600 ± 0.6a, A
Young leaf	Control	640 ± 0.4a, AB
	10g Si	690 ± 0.4a, AB
	20g Si	610 ± 0.5a, A
Mature leaf	Control	810 ± 0.6a, AB
	10g Si	1,020 ± 0.6ab, B
	20g Si	1,130 ± 0.5b, B
2-way ANOVA	Organ (A)	F <sub>3,100</sub> = 41.7428 ; p < 0.0001
	Treatment (B)	F <sub>2,100</sub> = 4.8048 ; p = 0.01015
	A x B	F <sub>6,100</sub> = 1.4581 ; p = 0.20038

<sup>a</sup>Means within a column followed by different letters are significantly different at 5% level (Tukey's contrasts test following ANOVA); a, b: comparisons of Si levels between treatments within each plant organ; A, B: comparisons of Si levels between plant organs within each treatment.

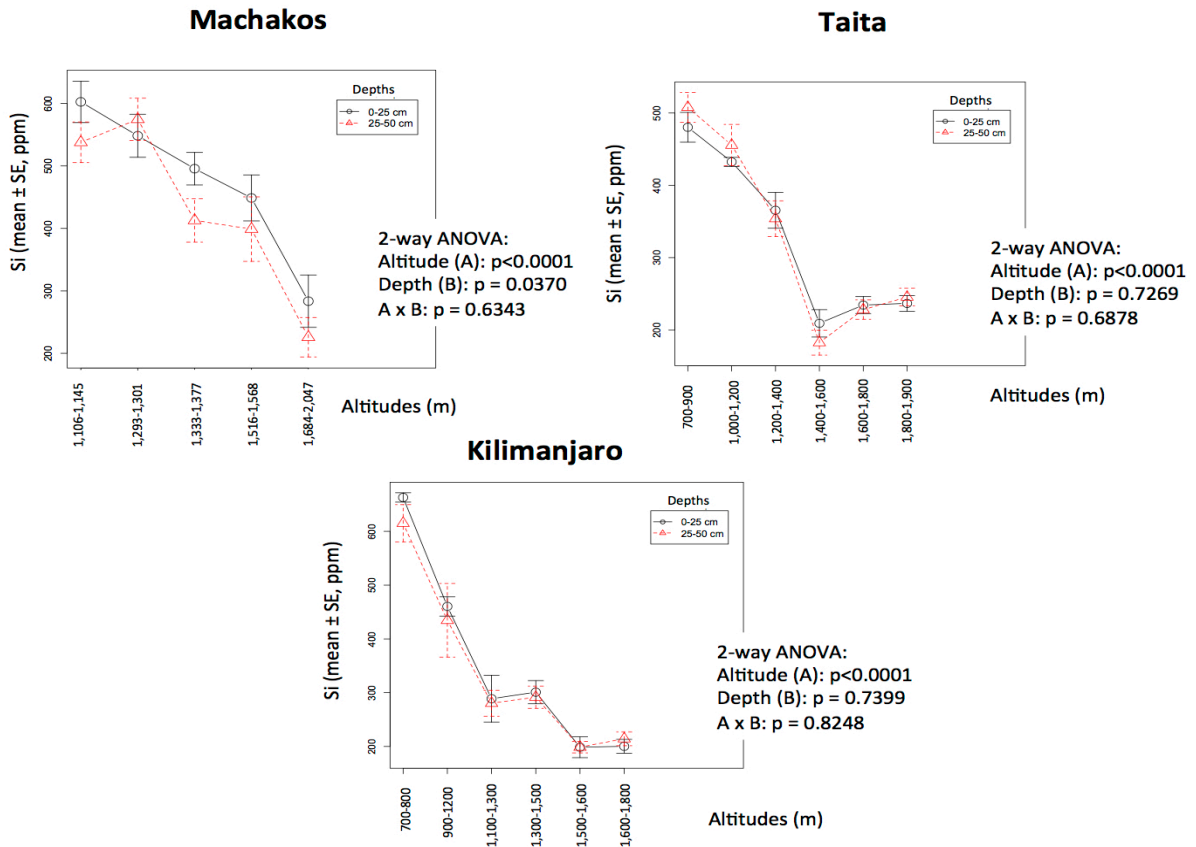
**Table 3.2: Silicon level (mean<sup>a</sup> ± SE, n=5) of mature leaves according to the age of maize plants.**

Age	Si (ppm)
Young	1,020 ± 0.6a
Middle-aged	1,060 ± 0.8a
Old	980 ± 0.8a

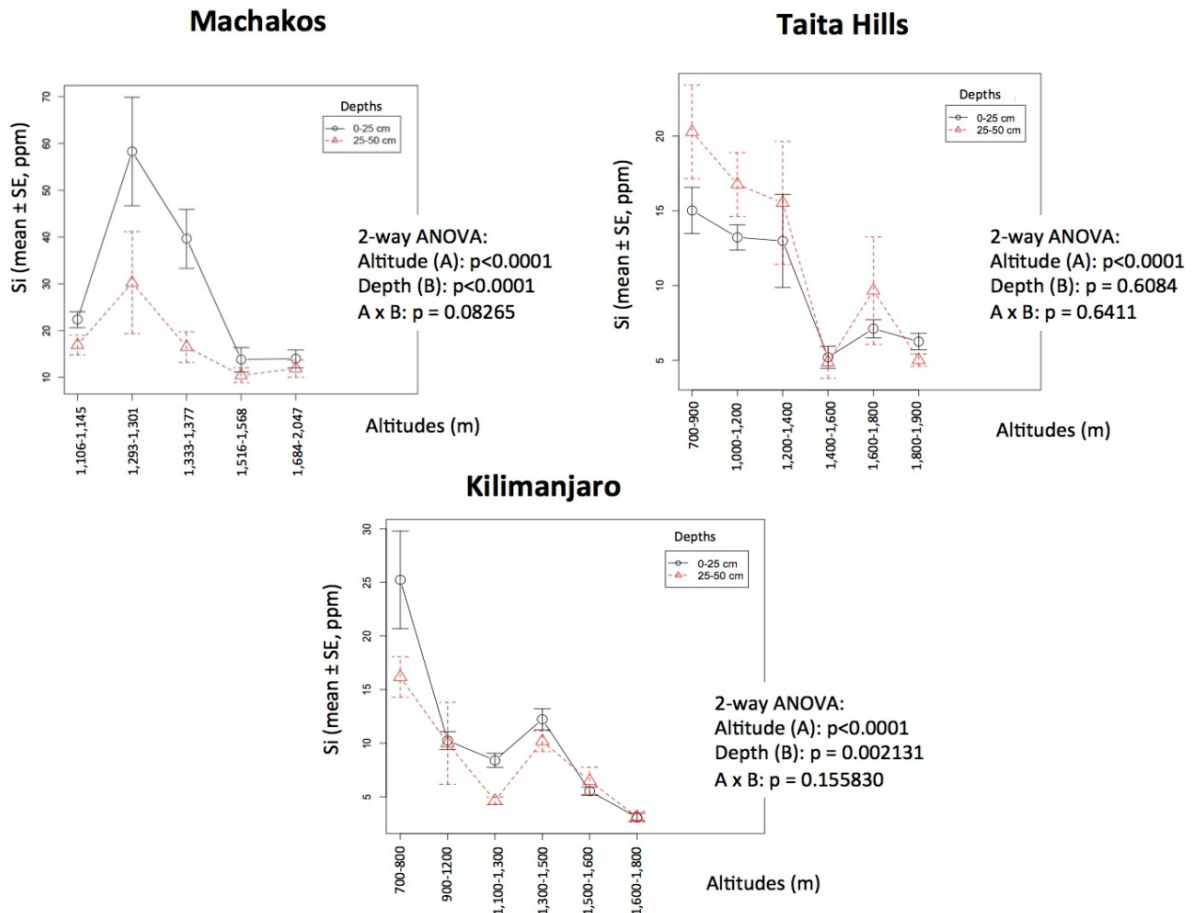
<sup>a</sup>Means followed by the same letter are not significantly different at 5% level (Tukey's contrasts test following ANOVA).

### 3.2 Levels of silicon in soils along the gradients

For all transects the soluble and total Si levels in soil decreased significantly with increase in altitude (Figures 3.2 and 3.3). Except for the Machakos Hills, where Si levels were significantly lower in the deeper than the upper soil layers, Si levels did not vary significantly between the two soil depths.



**Figure 3.2 Levels of total soil Si (in ppm) according to the depth and the altitude in Machakos Hills, Taita Hills and Mount Kilimanjaro transect.**

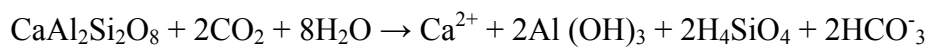


**Figure 3.3: Levels of soluble soil Si (in ppm) according to the depth and the altitude in Machakos Hills, Taita Hills and Mount Kilimanjaro transects.**

In all transects, soil Si concentrations at both depths decreased significantly with altitude (Figure. 3.3); results of F-test and 2-way ANOVA for altitude [A] factor:  $F=28.7960$ ,  $df=4$ ,  $P<0.0001$  for Machakos Hill;  $F=71.1230$ ,  $df=5$ ,  $P<0.0001$  for Taita Hills;  $F=62.0988$ ,  $df=5$ ,  $P<0.0001$  for Mount Kilimanjaro). Except for the Machakos Hills, where Si concentrations were significantly lower in the deeper than the upper soil layers (result of 2-way ANOVA for depth [B] factor:  $F=4.5265$ ,  $df=1$ ,  $P=0.0370$ ), Si concentrations did not vary significantly between the two soil depths (results of 2-way ANOVA for depth [B] factor:  $F=0.1226$ ,  $df=1$ ,  $P=0.7269$  for Taita Hills;  $F=0.1108$ ,  $df=1$ ,  $P=0.7399$  for Mount Kilimanjaro). There was no significant interaction between altitude and soil depth in all transect (results of 2-way ANOVA for A x B:  $F=0.6421$ ,

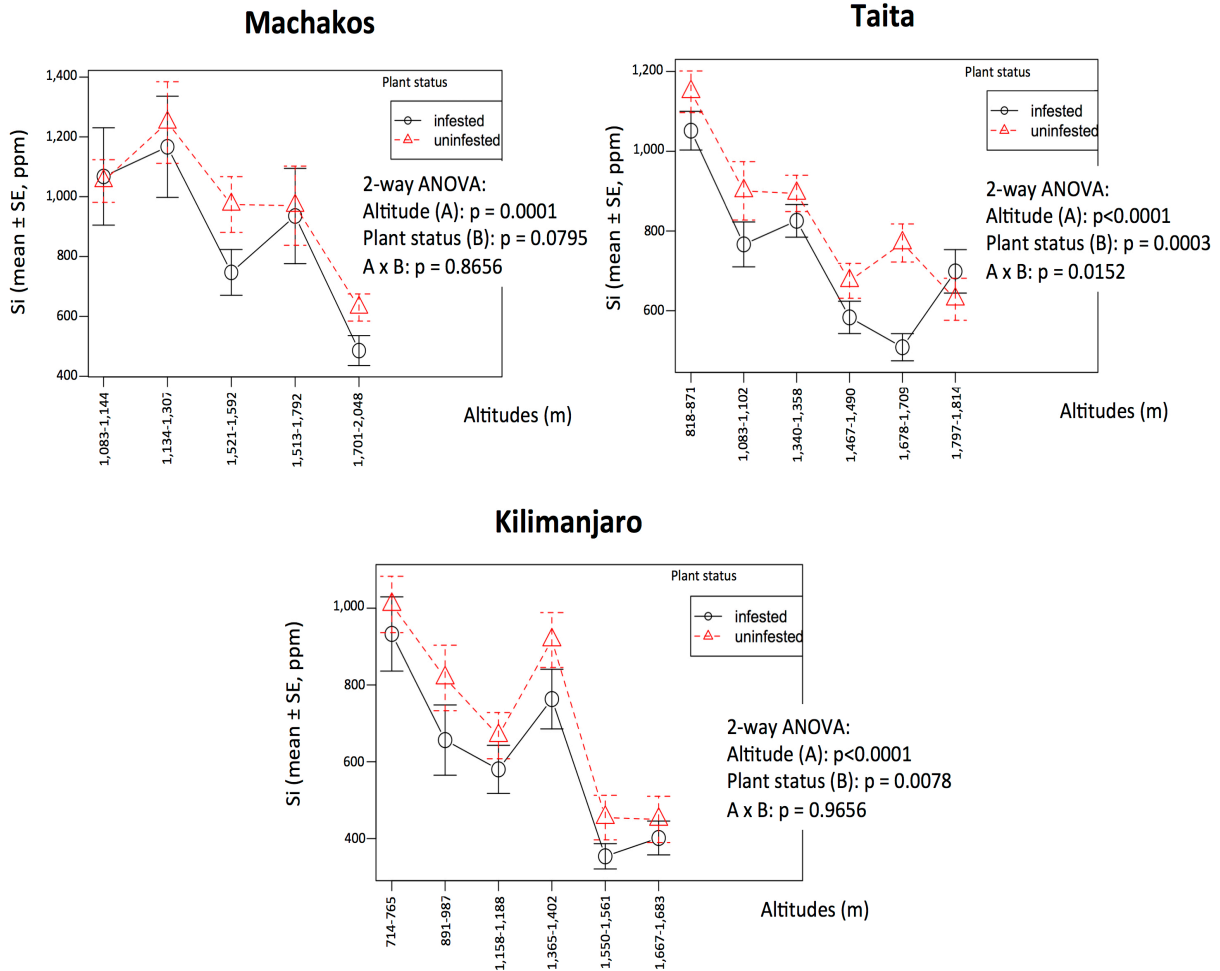
df=4, P=0.6343 for Machakos Hill; F=0.6161, df=5, P=0.6878 for Taita Hills; f=0.4329, df=5, P=0.8248 for Mount Kilimanjaro).

Such decreased levels of Si with the altitude can be explained by both the leaching phenomenon caused by the rain along the altitudinal gradient and by the level of organic carbon, which was found to increase with the altitude in all the transects considered ([www.chiesa.icipe.org](http://www.chiesa.icipe.org)). The organic carbon is involved in the synthesis of primary silicates in the soil, whereby CO<sub>2</sub> is consumed according to the following reaction (Berner, 1995; Kump *et al.*, 2000):



This results in the formation of silicic acid (H<sub>4</sub>SiO<sub>4</sub>), which is the main highly soluble component of soil solution (Dove, 1995). Due to high level of precipitation in higher altitudes and the sloppy nature of the gradient, H<sub>4</sub>SiO<sub>4</sub> is easily leached from the top to the bottom part of the hill, reducing the level of Si at higher altitudes. Such leaching is followed thereafter by a flux from the soils to the rivers strongly impacting the oceanic biogeochemical cycle of Si. It is reported that the land-ocean flux can contribute to more than 80% of the input of Si in the oceans (Tréguer *et al.*, 1995).

### 3.3 Silicon levels in maize



**Figure 3.4: Levels of Si (in ppm) of maize leaves according to the plant status (infested or not) and the altitude in Machakos Hills, Taita Hills and Mount Kilimanjaro transects.**

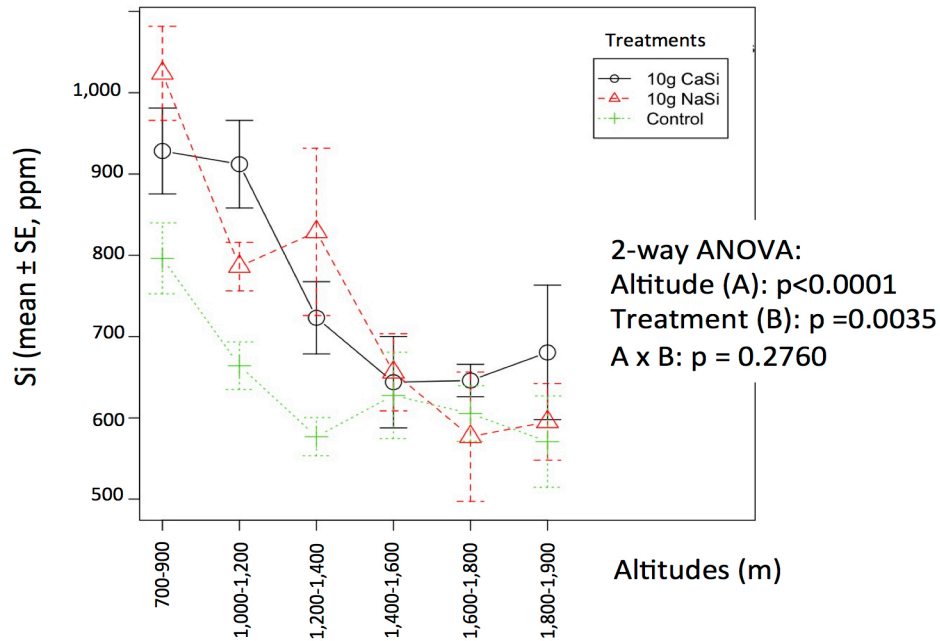
Silicon concentrations decreased significantly with altitude in both infested and uninfested plants (Figure 3.4; results of 2-way ANOVA for altitude [A] factor:  $F=7.4745$ ,  $df=4$ ,  $P<0.0001$  for Machakos Hill;  $F=27.2954$ ,  $df=5$ ,  $P<0.0001$  for Taita Hill;  $F=24.2989$ ,  $df=5$ ,  $P<0.0001$  for Mount Kilimanjaro). Leaves of infested plants exhibited lower Si concentrations than uninfested ones in most study sites (altitudes) of all transects (results of 2-way ANOVA for plant status [B] factor:  $F=3.1607$ ,  $df=1$ ,  $P=0.07959$  for Machakos Hill;  $F=13.4892$ ,  $df=1$ ,  $P=0.0002607$  for Taita Hill;  $F=7.1538$ ,  $df=1$ ,  $P=0.007801$  for Mount Kilimanjaro). A significant interaction between

altitude and plant status was obtained only for Taita Hills (results of 2-way ANOVA for A x B:  $F=0.3170$ ,  $df=4$ ,  $P=0.86572$  for Machakos Hill;  $F=2.8381$ ,  $df=5$ ,  $P=0.0152079$  for Taita Hill;  $F=0.1919$ ,  $df=5$ ,  $P=0.965572$  for Mount Kilimanjaro).

A similar trend between the Si level and the altitude was obtained for each period of the year when the maize was sampled for each of the transect. This necessitated the data obtained to be grouped as shown in the results section in Figure 3.4. Within the year of maize cultivation, the Si levels of mature maize leaves decreased with increase in altitude in all transects considered. This is not surprising since the level of Si accumulation in plants is due to the ability of the roots, the main tissue in plants involved in soil water and nutrient/element uptake, to absorb this element from the soil (Takahashi *et al.*, 1990). An increase of Si in soils can therefore contribute to an increase in the desorption of this element into the plant tissues, explaining why, in our study, the maize plants cultivated on soil with highest Si levels possessed highest Si levels as well.

Moreover, Si level was generally significantly lower in leaves of infested plants as compared to uninfested ones. This trend was well observed in Taita Hills as well as in Mount Kilimanjaro transects. This corroborated the hypothesis that Si enhances maize resistance to insect pests, as reported by (Ma and Takahashi, 2002), and particularly to Lepidoptera stem borers since mostly maize plants with low Si levels are greatly infested by such type of insects (Ma and Takahashi 2002). Differences in silica content among plants are frequently associated with plant resistance to insect attacks (Keeping and Meyer, 2002; Kvedaras *et al.*, 2005; Keeping and Meyer, 2006). Therefore, during host selection process herbivorous insects may discriminate between high- and low-silica plants and feed preferentially on the later. However, maize stem borers infestation can influence the physiology of the plant and thereby reduce the ability of the plant to absorb nutrients and different elements such as Si; explaining also the lower levels of Si generally found in maize infested plants.

### 3.4 Influence of altitude on Si absorption by maize plants



**Figure 3.5: Levels of Si of maize leaves (in ppm) according to the treatment realised (control, 10 g of  $\text{Ca}_2\text{SiO}_4$  or 10 g of  $\text{Na}_2\text{SiO}_3$ ) in Taita Hills transect.**

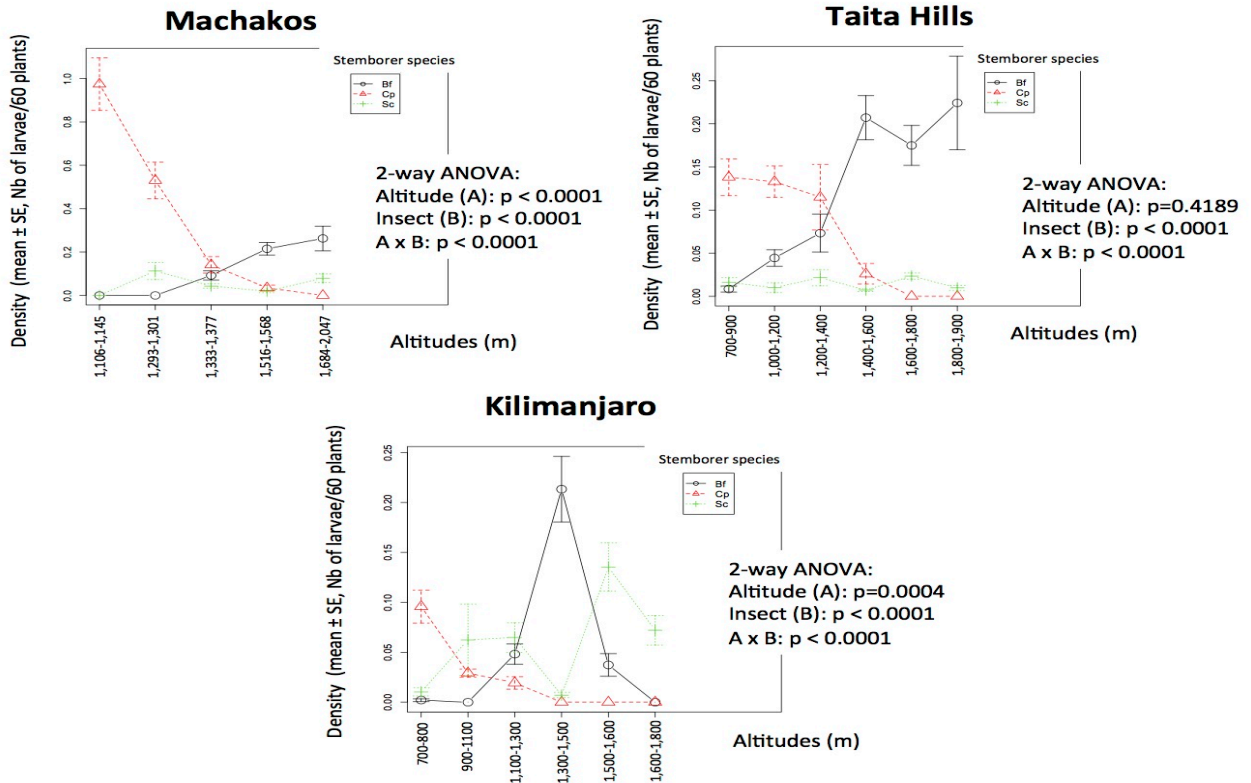
In the pot experiment, where the same soil was used in all sites, leaf Si concentrations decreased significantly with altitude (Fig. 5; results of 2-way ANOVA for altitude [A] factor:  $F=7.7014$ ,  $df=5$ ,  $P<0.0001$ ). Although the Si treatment had a significant influence on leaf Si concentration (results of 2-way ANOVA for Si treatment [B] factor:  $F=14.5778$ ,  $df=1$ ,  $P=0.0003855$ ); it had a significant effect only in the lower three altitudes but not the upper three ones (ANOVA done only for the lower 3 altitudes  $F=14.01$ ,  $df=1$ ,  $P=0.000832$ ; ANOVA done only for the upper 3 altitudes:  $F=1.701$ ,  $df=1$ ,  $P=0.203$ ). No significant interaction between altitude and Si treatment was obtained (results of 2-way ANOVA for A x B:  $F=1.0526$ ,  $df=5$ ,  $P=0.3982489$ ).

Soil Si amendment of potted maize plants induced different maize plant Si absorption characteristics relative to the altitude (Figure 3.5). The leave Si levels decreased significantly with increasing altitude. Moreover, potted Si amendment was significantly efficient only at lower altitudes but did not have any significant effect in leave silification at higher altitudes. Therefore, the absence of Si amendment response in



maize plants at higher altitudes is more likely to be related to a decreased capacity of plants to absorb Si from soil at high altitudes partly due to low temperatures. Among the climatic parameters that characterize different altitudes temperature is an important component that decreases with an increasing altitude (Table 3.2). Temperature can influence the plant physiology in term of Si absorption (Hussain *et al.*, 2010). It is well known that the root-zone temperature has a pronounced role in nutrient uptake from soil and their use by plants and hence on plant growth (Pregitzer and King, 2005; Hussain *et al.*, 2010). This root-zone temperature influences significantly the root physiology because the enzymes and cell membrane activities linked to such nutrient uptake are directly related to soil temperature (Taiz and Zeiger, 1991; Larcher, 1995). Although it is well described that crop genotypes generally differ in nutrient utilisation efficiencies (Maqsood *et al.*, 2009; Aziz *et al.*, 2006) and that the soil type displays different Si supplying capacity (Savant *et al.*, 1999), since in this experiment we used the same maize genotype and the same type of soil, the variations in Si absorption in leaves in relation to the altitude could only be attributed to variations in environmental conditions like temperature. This aspect is very important to take into account particularly when Si amendment is required. Moreover, there was no significant difference between the types of silicate used for amendment regardless of the altitude.

### 3.5 Composition of maize stem borer species along altitudinal gradients



**Figure 3.6: Variation in the density of three stem borer species, *Busseola fusca* (Bf), *Chilo partellus* (Cp) and *Sesamia calamistis* (Sc) found along the altitudinal gradient of Machakos Hills, Taita Hills and Mount Kilimanjaro transects.**

In all transects, there was a significant effect of the altitude on stem borer proportion of each species on infested plants (Fig. 4; results of 2-way ANOVA for altitude [A] factor:  $F=6.0870$ ,  $df=4$ ,  $P=0.0001913$  for Machakos Hill;  $F=6.3076$ ,  $df=5$ ,  $P<0.0001$  for Taita Hill;  $F=14.3615$ ,  $df=4$ ,  $P<0.0001$  for Mount Kilimanjaro). The insect species factor was not influencing significantly the stem borer proportion of each species found on infested plants at Machakos Hills and Mount Kilimanjaro (results of 2-way ANOVA for species [B] factor:  $F=2.9951$ ,  $df=1$ ,  $P=0.0864552$  for Machakos Hill;  $F=1.5600$ ,  $df=1$ ,  $P=0.2135$  for Mount Kilimanjaro) whereas a significant effect was obtained in Taita Hill (results of 2-way ANOVA for species [B] factor:  $F=20.1790$ ,  $df=1$ ,  $P<0.0001$ ). There was no significant interaction between altitude and insect species only in Taita Hills (results of 2-way ANOVA for A x B:  $F=5.3594$ ,  $df=4$ ,  $P=0.0005750$

for Machakos Hill;  $F=0.3771$ ,  $df=5$ ,  $P=0.8644$  for Taita Hill;  $F=7.7523$ ,  $df=4$ ,  $P<0.0001$  for Mount Kilimanjaro). In all transects, the relative proportion of *C. partellus* in infested plants decreased whereas that of *B. fusca* increased with altitude.

Only three stem borer species, *B. fusca*, *C. partellus* and *S. calamistis* were recovered along the altitudinal gradient of each transect during all surveys made (Figure 3.6). For each transect, since a similar trend between the abundance of each species and the altitude was obtained for each period of time sampled, the data were grouped as shown the results in (Figure 3.6). In general, while the abundance of *C. partellus* decreased significantly with an increase in altitude in contrast, the abundance of *B. fusca* increased relative to the altitude in Taita Hills and Machakos Hills transects. However, in Mount Kilimanjaro transect there was a decrease in the abundance of *B. fusca* but increase in the abundance of *S. calamistis* with an increase in altitude. Moreover, there was no general trend in the variation of *S. calamistis* as compared to *C. partellus* and *B. fusca* along the altitudinal gradients. In the literature, it is well reported that *C. partellus* is more abundant in the lowlands than in the highlands where *B. fusca* is more predominant (Kfir *et al.*, 2002; Ong'amo *et al.*, 2006). This can be associated not only to climatic conditions but to other factors such as the accumulated Si levels in maize plants along the gradients corroborating our study hypothesis.

### **3.6 Larval development on potted plants treated with soluble Si**

Soil Si-amendments to potted plants induced a significant increase in the concentration of Si in maize leaves (Table 3.3; result of ANOVA:  $F=22.43$ ,  $df=3$ ,  $P<0.0001$ ). Concomitantly, the percentage of larvae recovered for each stem borer species decreased significantly (results of ANOVA:  $F=9.491$ ,  $df=3$ ,  $P<0.0001$  for *B. fusca*;  $F=8.514$ ,  $df=3$ ,  $P=0.000682$  for *C. partellus*). RGR of *B. fusca* also decreased significantly (result of ANOVA:  $F=7.868$ ,  $df=3$ ,  $P=0.000452$ ) but not for *C. partellus* larvae (result of ANOVA:  $F=0.264$ ,  $df=3$ ,  $P=0.85$ ), indicating that *C. partellus* was less susceptible to increasing Si in maize than *B. fusca*.

The purpose of this experiment was to determine whether an increase in Si levels in maize plants leaves via soil amendment influenced the development of the three stem borer species largely found in the field. The results (Table 3.3) indicated that soil Si-amendments induced a significant increase in maize plant leaves' Si levels. The accumulated Si in maize plant leaves induced a decrease in the percentage and the number of larvae recovered for each stem borer species considered and a concomitant, decrease in relative growth rates (RGR) of *B. fusca* and *S. calamistis* larvae. This indicated that among the three stem borer species studied, *C. partellus* appeared less sensitive to increase in plant tissue Si content.

This detrimental effect of silicon on *B. fusca* and *S. calamistis* larvae that resulted in the reduction in population and larval growth rates on high silicon containing plants may partly be attributed to the effect of silicon at both nutritional and physical levels. This data is corroborated by Bernays and Barbehenn (1987) who suggested that Si is among the several features of Gramineae that makes them develop resistance against stem borer feeding. It is reported that most of the plant Si occurs in the epidermal layer of leaves and this becomes effective in dislodging young borer larvae before their establishment on the plant stem. Furthermore, plant accumulated silicon is reported to increase hardness of a plant tissue that ultimately interferes with insect larval boring and feeding activities since silicon is reported to negatively infer with insect mandibles. Moreover, as reported by Panda and Kush (1995), elevated levels of silicon in plant cell wall might increase the bulk density of the diet and also discourage larvae from ingesting sufficient quantities of nutrients and water leading to their poor performance on the host plant.

This data is hence important from an applied point of view. Since silica augments the resistance of various plants to insects pest (Ma and Takahashi, 2002; Keeping and Kvedaras, 2008) and that plants have a capacity to accumulate silica (Ma and Yamuji, 2006), is particularly relevant for silica deficient soils of cereal growing regions of East Africa. Hence silica amendments in plant tissues in form of fertiliser or by any other possible means may provide an improved resistance to *B. fusca* in these regions. This has been demonstrated for *S. calamistis* following maize tissue silification (Sétamou *et al.*, 1993) as a result of direct silica amendment to the soils. However, for *B. Fusca* and

*C. partellus* field trials are required to confirm this hypothesis. It would also be imperative to also confirm the quality of the modified crops with regard to adverse effects on human health upon consumption. Moreover, the fact that *C. partellus* was less sensitive to higher levels of Si in plant tissues as compared to *B. fusca* and *S. calamistis*, can clearly explain the variations in distribution exhibited by the three species as reported in (Figure 3.6) Although *C. partellus* was mostly found on maize grown at lower altitudes with the highest Si levels indicating that the *species* was somehow more tolerant to an elevated plant tissue Si content, their overall population was reduced. Hence, this influenced the decision to confirm the variations in larval sensitivity towards Si among *C. partellus*, *B. fusca* and *S. calamistis* using artificial silica amended diets.

### **3.7 Larval development on artificial diets amended by Si**

Si-amendments artificial diet induced a significant increase in the concentration of Si in artificial diet (Table 3.4; result of ANOVA:  $F=32.43$ ,  $df=10$ ,  $P<0.0001$ ). Concomitantly, the percentage of larvae mortality for each stem borer species decreased significantly (results of ANOVA:  $F=36.442$ ,  $df=10$ ,  $P<0.0001$  for *B. fusca*;  $F=33.241$ ,  $df=10$ ,  $P=0.000682$  for *C. partellus*). RGR of *B. fusca* also decreased significantly (result of ANOVA:  $F=2.768$ ,  $df=10$ ,  $P=0.000452$ ) but not for *C. partellus* larvae (result of ANOVA:  $F=2.224$ ,  $df=10$ ,  $P=0.85$ ), indicating that *C. partellus* was less susceptible to increasing Si in maize than *B. Fusca*

The purpose of this experiment was to confirm that *C. partellus* larvae are more tolerant to an increase Si concentrations in their diets than *B. fusca* and *S. calamistis*. Unlike *B. fusca* and *S. calamistis*, which did not tolerate high silicon amended diets (Table 3.4), *C. partellus* were more tolerant and generally exhibited a high survival on Si-amended diets. This mirrored the high tolerance exhibited by the species on plants with highest levels of silica in the fields.

In addition, the results in (Table 3.4) also indicated that *S. calamistis* larvae are less tolerant to elevated levels of Si in the diet as compared to other species studied which exhibited decreased RGR and subsequent increased mortality.

**Table 3.3: Percentage of remaining live larvae (mean<sup>a</sup> ± SE, n=10) and relative growth rates (RGR, mg d<sup>-1</sup>, mean<sup>a</sup> ± SE) of *Busseola fusca*, *Sesamia calamistis* and *Chilo partellus* larvae after 15 days of infestation on control and Si-treated maize plants.**

	<i>Busseola fusca</i>			<i>Sesamia calamistis</i>		<i>Chilo partellus</i>	
	Si in plant leaves (ppm)	% live larvae	RGR	% live larvae	RGR	% live larvae	RGR
Control plants							
10g of Ca	539.9 ± 38.8a	16.3 ± 2.1b	0.22 ± 0.03b	14.0 ± 1.3b	0.11 ± 0.007b	52.2 ± 8.0b	0.10 ± 0.001a
20g of Ca	545.9 ± 32.7a	18.0 ± 1.9b	0.24 ± 0.05b	12.0 ± 1.7ab	0.12 ± 0.01b	48.9 ± 4.0b	0.11 ± 0.003a
Si-treated plants							
10g of Si	797.5 ± 48.3b	10.7 ± 1.9ab	0.09 ± 0.005a	8.5 ± 1.4a	0.08 ± 0.007a	27.0 ± 3.4a	0.10 ± 0.009a
20g of Si	923.2 ± 37.7b	6.7 ± 1.3a	0.06 ± 0.01a	8.0 ± 1.5ab	0.07 ± 0.005a	25.6 ± 3.0a	0.12 ± 0.02a

<sup>a</sup>Means within a column followed by different letters are significantly different at 5% level (Tukey's contrasts test following ANOVA).

**Table 3.4: Percentage of mortality (mean<sup>a</sup> ± SE, n=10) and relative growth rates (RGR, mg d<sup>-1</sup>, mean<sup>a</sup> ± SE) of *Busseola fusca*, *Sesamia calamistis* and *Chilo partellus* larvae after 30 days of infestation on control and Si-treated artificial diets.**

	<i>Busseola fusca</i>		<i>Sesamia calamistis</i>		<i>Chilo partellus</i>	
	% mortality	RGR	% mortality	RGR	% mortality	RGR
Control diets (mg/ml)						
0 of Ca	46 ± 6a	6.2 ± 0.5c	46 ± 7a	1.5 ± 0.4bc	40 ± 9b	4.5 ± 0.4c
15.625 of Ca	22 ± 6a	6.6 ± 1.0c	62 ± 7ab	2.4 ± 0.5bc	22 ± 6ab	4.2 ± 0.3c
31.25 of Ca	54 ± 9a	5.3 ± 0.8c	68 ± 8ab	1.4 ± 0.3b	26 ± 4a	4.6 ± 0.4c
62.5 of Ca	48 ± 7a	6.2 ± 0.9c	70 ± 8b	2.1 ± 0.6bc	40 ± 5b	4.5 ± 0.4c
125 of Ca	44 ± 7a	5.3 ± 0.9c	58 ± 5ab	2.7 ± 0.3c	38 ± 5ab	4.5 ± 0.4c
250 of Ca	54 ± 9a	4.9 ± 0.6bc	44 ± 9a	2.0 ± 0.3bc	24 ± 6ab	4.6 ± 0.6c
Si-treated diets (mg/ml)						
15.625 of Si	40 ± 8a	4.2 ± 0.6b	70 ± 8b	1.6 ± 0.4bc	40 ± 7b	3.6 ± 0.5bc
31.25 of Si	48 ± 7a	4.2 ± 0.3b	88 ± 4bc	0.7 ± 0.1a	44 ± 6b	3.9 ± 0.3c
62.5 of Si	96 ± 3b	1.0 ± 0.3a	96 ± 3c	0.5 ± 0.2a	62 ± 7bc	2.7 ± 0.4ab
125 of Si	96 ± 3b	0.7a	94 ± 4c	0.4 ± 0.1a	88 ± 4c	0.9 ± 0.2a
250 of Si	100b	-	100c	-	96 ± 3c	0.7a

<sup>a</sup>Means within a column followed by different letters are significantly different at 5% level (Tukey's contrasts test following ANOVA)

### 3.8 Conclusions

Silicon contents of both soil and maize plant decreased significantly with a concomitant rise in altitude. This result was not only attributed to soil characteristics but may have also been influenced by environmental conditions. High precipitation in the higher altitude increased leaching of Si in soil thus low silicon was absorbed by plant. However due to higher silicon level in the lower altitude plants absorbed more silicon Si uptake capacity by maize plants also decreased with increase in altitude. These were due to low temperatures at high altitudes which negatively affected Si assimilation by maize plants.

In all transects studied, three species of stem borers *C. partellus*, *B. fusca* and *S. calamistis* were found to have infested maize plants at all levels of the altitudinal gradients: However, *C. partellus* and *B. fusca* significantly dominated the low and the higher altitudes respectively. Although there was a decrease in both plant and soil silica levels with rise in altitude, *Sesamia calamistis* did not exhibit a specific trend as compared to the two other stem borer species that coexisted in the field. Apart from temperature variations, differences in the distribution of these insect pests along altitudinal gradient could be attributed to variation in the accumulated maize Si levels. Results of laboratory experiments strongly indicated that *C. partellus* is a more tolerant species to increasing Si levels of maize and artificial diets than *B. fusca* and *S. calamistis*. From an applied point of view the fact that silica augments the resistance of various plants to insects pest (Keeping and Kvedaras, 2008) and that plants have a capacity to accumulate silica (Ma and Yamuji, 2006), is particularly relevant for silica deficient soils of cereal growing regions of East Africa. Silica amendments in plant tissues may hence provide an improved resistance to stem borer species in these regions.

Therefore all these results indicate that the projected rise in temperature due to envisaged climate change will enhance maize plants' Si assimilation and modify significantly the abundance and the composition of Lepidoptera stem borer pest species in higher altitudes of East African Mountains which are key maize producing regions. Moreover, this research shows clearly that Si applications in soil amendments in form of fertiliser or other forms can contribute significantly to the reduction of stem borer



pest damage (Belanger *et al.*, 1995; Ma and Takahashi, 2002; Meyer and Keeping, 2005). This could be beneficial because it would reduce pest populations, leave no insecticide residues in food or the environment, are relatively cheap, and could easily be integrated with other pest management practices including biological and cultural practices (Ukwungwu, 1990).

### **3.9 Recommendations**

From this research it was observed that the most important species of stem borers are the spotted *C. partellus* found in the warmer and lower areas, and *B. fusca* found in the cooler and higher altitudes. A third, less important species as regards to maize infestation and destruction is *S. calamistis* found in mid-altitude areas. In Kenya alone, losses due to stem borer damage fluctuate around 14% on average (De Groote, 2002). To overcome this, silicon (Si) has received attention as a nutrient capable of providing some measures of defence for plant against stem borers. In this research, the levels of silicon in both maize plant leaves and soils greatly reduced both percentage survival and relative growth rate of the aforementioned stem borer species. Although use of elicitors such as silicon is considered to be an effective strategy to enhance plants' ability to cope with these stresses, 70% of the soils in Africa are generally deficient in accessible silicon for plants (Laing *et al.*, 2006).

- i. Measures to raise the level of soil Si should be encouraged so as to reduce maize infestations and subsequent destruction by the stem borer.
- ii. Additionally farmers should be encouraged to use silicon based fertilizers so as to boost the level of silicon in the soil for plant uptake and accumulation and thus plant resistance to biotic and abiotic stresses.
- iii. Poor farming practices like the removal of plant residual material from farms after harvesting should be discouraged.

Future research into pest management with Si applications to soils could include:

- Identification of good Si sources, and their optimal dosages for effective pest control in different crops
- Integration of Si applications with biological control for ecologically sustainable pest management programmes.

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