IMPROVING MANAGEMENT OF POTATO TASTE DEFECT IN COFFEE AND ELUCIDATING ITS MECHANISMS OF OCCURRENCE

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Entomology-Doctor of Philosophy

2019

ABSTRACT

"IMPROVING MANAGEMENT OF POTATO TASTE DEFECT IN COFFEE AND ELUCIDATING ITS MECHANISMS OF OCCURRENCE"

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Potato taste defect (PTD) is a raw potato-like smell and taste found in green and roasted coffee beans and also in brewed cups of coffee. This defect diminishes the flavor experience and the perception of quality of finished coffee, reducing its value or causing it to be rejected by consumers or international buyers. Occurrence of PTD in coffee has been associated with feeding by Antestia bug, *Antestiopsis thunbergii* (Hemiptera: Pentatomidae). The outcome of laboratory bioassays indicated that pyrethroid (Alpha-cypermethrin) and pyrethrins (Pyrethrum EWC) provided 100% mortality and significantly greater mortality than the neonicotinoid (Imidacloprid) twelve hours after sprays. Under field conditions, pruning plus application of Fastac or Pyrethrum EWC or Pyrethrum 5EW provided significantly greater mortality compared to other treatments. Additionally, pruning combined with insecticide application, especially Fastac, significantly reduced PTD incidence up to 1%. An expansive survey of PTD occurrence in Rwanda demonstrated that the defect is distributed throughout the coffee producing regions of the country with the highest incidence in Central Plateau, Granitic Ridges and Eastern Plateau. High incidence of PTD was shown to be strongly associated with high density and damage of Antestia bug. In contrast, coffee berry borer (CBB), *Hypothenemus hampei* (Coleoptera: Scolytidae) damage was not significantly related to the occurrence of PTD. Although CBB bores holes into coffee berries that could allow the bacterium to enter, survival of the pathogen may be impeded by the presence of the beetle. In light of these findings, there is need to improve

Antestia bug control, especially in the areas where the highest PTD incidence was observed. Antestia bug is distributed in all coffee growing regions of Rwanda with the highest density in the Northern region and the lowest in the Eastern region. In a two - year study (2016 and 2017), the economic damage due to Antestia bug ranged from 0 to 92% in 2016 and from 0 to 81% in 2017, underscoring an urgent need for pest control. Temperature and relative humidity were positively related to Antestia bug density and their increase due to climate change may lead to future pest outbreaks. However, wind speed was not significantly associated with Antestia bug density possibly because of the bug's physical structure and behavior, as it is a relatively a large insect that prefers to hide in dense canopies. In a study conducted to elucidate the mechanisms of PTD occurrence and to assess the relationship between cupping method using the Specialty Coffee Association of America (SCAA) cupping protocol and chemical analysis using Gas Chromatography Mass Spectrometry (GCMS) to detect the presence of PTD, findings indicated the highest incidence of PTD was found in coffee beans damaged by Antestia bug and the lowest was found in undamaged control beans. It was demonstrated for the first time that mechanical damage alone in coffee berries can cause PTD and that Antestia bug is not the absolute cause of PTD, but wounds to the berries including feeding damage by this bug allow entry of the bacterium responsible for PTD. The lowest amount of 2-isopropyl -3-methoxypyrazine (IPMP), the main compound associated with PTD, was found in the control beans while the highest levels was in the coffee beans that had been poked with a needle to simulate bug damage. Additionally, there was a positive relationship between the cupping method to detect PTD and IPMP detection using GCMS. Further research is justified to better understand the ecology of *Pantoea coffeiphilia* and to design methods and techniques for early detection and prevention of this bacterium in the effort to supply PTD free coffee.

ACKNOWLEDGEMENTS

I would like to thank the members of my Ph.D. committee: Drs. Larry Gut, Matthew Grieshop, John Wise and Dan Clay for their availability, continued guidance and support not only during my studies and research but also in other aspects of life throughout my entire stay in the United States. The help they availed to me while pursuing this important milestone is greatly acknowledged. I am thankful to Drs. Chris Adams and Juan Huang for their encouragement and inspiration and also to the rest of the Tree Fruit Entomology laboratory. I am also grateful to Dr. Kevin Walker for allowing me to use the research facility in his laboratory. His assistance in chemical analysis using Gas Chromatography Mass Spectrometry is greatly appreciated. I also would like to express my appreciation to the coffee farmers who willingly accepted to provide their farms for data collection and experimentation. Funding of this work was availed in part by the United States Agency for International Development (USAID), grant numbers BFS-S-11-00002 and EEM-G-00-04-00013 through the Borlaug Higher Education for Agricultural Research and Development (BHEARD) and Feed the Future Africa Great Lakes Coffee Support Program, policy award number AID-OAA-LA-15-00006. I also would like to thank Dr. Larry Gut for providing several air tickets from the United States to Rwanda for data collection. Most significantly, I would like to thank my family, particularly my wife Marie Louise and daughters Jessica and Joannah for their patience and prayers especially during my absence at home while pursuing studies in the US. Finally, thanks to any person who provided support or facilitation to achieve this milestone in my life.

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CHAPTER 1: GENERAL INTRODUCTION

Economic importance of coffee

Coffee is one of the most popular beverages worldwide. Globally, coffee is one of the most traded commodities and is crucial to the economy of many countries in Africa, Asia and Latin America (Aerts et al., 2015). In the above continents, it is grown in more than 70 countries and its annual export value exceeds US$ 24 billion (FAO, 2015). In Africa, it is a major source of income for millions of smallholder coffee farmers and their households who are responsible for an estimated 80% of Africa's coffee production (Oduor & Simmons, 2003). In Rwanda, coffee is among the most prominent export commodities and accounts for about 20% of the country's total annual value of agricultural exports (National Bank of Rwanda, 2015). In addition, it is an important source of direct cash income for 355,000 farmers and provides employment to thousands of Rwandan workers involved in the coffee industry (National Agricultural Export Development Board, 2016).

Coffee is a crop very well suited to production in family units and is appropriate for hillside farming where more demanding cash crops cannot be easily grown. Practically most of the coffee in the country is cultivated on smallholdings, with a coffee farm averaging about 0.1 ha with a planting density of around 250 coffee trees per ha (NAEB, 2015). Coffee thus directly impacts a much higher percentage of the population than all other cash crops in the country. Rwanda produces exclusively Arabica coffee, which is renowned the world over for its distinct fine cup quality attributes (Gueule et al., 2014). Although there are several factors affecting coffee productivity and quality, Antestia Bug (*Antestiospis thunbergii*, Hemiptera: Pentatomidae) is among the most important pest threats.

Antestia bug feeds on various parts of the coffee plants including berries at different stages of development and maturation, flower buds, green shoots and leaves (Kirkpatrick, 1937). High infestations by Antestia bug may prevent the coffee tree from flowering (Waller et al., 2007). Antestia bug may cause yield loss up to 40% (Ahmed et al., 2016). Antestia bug feeding has also been associated with potato taste defect (PTD). This is a potato like smell and taste found in green and roasted coffee beans and also in brewed cup of coffee. PTD diminishes the flavor experience of coffee or causes it to be rejected by consumers or international buyers.

Identification and Taxonomy of Antestia Bug

The adult Antestia bug, *Antestiopsis thunbergii* (Hemiptera: Pentatomidae) is black to brownish or greyish black with white to ivory longitudinal stripes. There are orange spots on the dorsal surface. The pronotum and scutellum have striking variable patterns with black, orange and white colors. Adult females are somewhat larger than males. The wing cuticle is orange to white and the membranes are dark brown (Le Pelley, 1968). It is about 8 mm in length and the life span ranges from 94 to 104 days with an average period of 95 days at an average temperature of 24.5°C (Abebe, 1987). The genus *Antestiopsis* includes several other species, most of which were reported in Africa and Asia. These include - *clymeneis* (Kirkaldy)*, intricata (Ghesquire) orbitalis (Westwood)*, *bechuana* (Kirkaldy) and *ghesquierei* Carayon (Greathead, 1966).

Variations in size, intensity and disposition of color patterns of the body of Antestia bug within species suggest the existence of sub species (Greathead, 1966). For example, *A. faceta* has sub-species such as A. facetoides sp.n. confined to East Africa and *A. crypta* sp.n. only limited to Central Africa. The species *Antestiopsis thunbergii* covers the southern part of South Africa to the high lands of Kenya and Ethiopia up to central Africa and this is the main coffee growing region. This is the species also found in Rwanda. The main coffee pest in West Africa is *A. intricata,* (Crayon, 1954). Unlike the above species that feed exclusively on *Coffea arabica*, this species also feeds on *Coffea canephora* and *Coffea liberica* (Crayon, 1954). In Madagascar, *Antestiopsis clymeneis* (Kirkaldy) was reported on *Coffea arabica* and wild Rubiaceae (Greathead, 1966). Two sub species within *A. clymeneis* were distinguished*: A. clymeneis galtiei* (Frappa) which is the most common on coffee and *A. clymeneis frappai*, a new subspecies reported on wild Rubiaceae of the genera *Gaertnera, Saldinia and Mapouria.* In Asia, *Antestiopsis cruciata* was reported in Pakistan, India, Myanmar, Sri Lanka and China (Rider et al., 2002).

Ecological preferences

Antestia bug originated from Africa but unlike Coffee berry borer (*Hypothenemus hampei* Ferrari), it has not spread globally, probably due to its ecological requirements. Antestia bug commonly feeds on Arabica coffee between 1000 and 2100 masl (Kirkpatrick, 1937). Ecological preferences also vary among species: *A. thunbergii* prefers the cool climate of the highest elevations while *A. intricata* is found in warm climate of forest coffee at low and middle altitudes (Abebe, 1987). In Rwanda, *A. thunbergii* was found in coffee farms at all altitudes (Foucart and Brion, 1959). The bug also prefers coffee under shade: high levels of *A. intricata* (more than 9 bugs per tree) infestations were observed in coffee farms with shaded bushes while unshaded coffee bushes at the same altitude of around 1000 meter above sea level (masl) were less affected (Mbondji Mbondji, 1999). Additionally, high infestations by *A. thunbergii* were reported in Kenyan shaded coffee compared to unshaded coffee at altitude of 1300 to 1500 masl (Mugo et al., 2013). At high altitudes (around 2000 masl) however, high population of *A. thunbergii* were observed in coffee grown in the open sun compared to shade coffee (Le Pelley, 1968). It seems that shade does not affect the bug population directly, instead interaction between pest, temperature and moisture favor its high levels of infestations (Kirkpatrick, 1937).

Regarding the population dynamics, *Antestiopsis* populations in East Africa build up around March and reach the peak in May/June period (Abebe, 1987). Higher temperatures substantially shorten the required time for *A. thunbergii* to develop from immature stages to adults and particularly from eggs to the fourth nymphal stage (Gesmalla et al., 2016). Adult females live an average of 93 days, the oviposition period is 57.9 days and the average fecundity per female is 132.8 eggs at optimum temperature of 20^{o}C (Abebe, 1987). The bug prefers unpruned coffee trees where it hides during the day and also the high humidity levels in the coffee plantations.

Antestia bug feeding habits

Most species within the genus *Antestiopsis* have similar feeding habits. Feeding is mainly on berries at different stages of development and maturation and sometimes on flower buds, green shoots and leaves. Both the nymphal and adult stages of the bug feed on the vegetative and fruiting parts of the coffee tree, which leads to poor yield and low quality (Gesmalla et al., 2016). Feeding on immature green beans causes premature fruit fall and necrosis of the mature beans (Cilas et al., 1998). When the bug feeds on immature berries, it creates punctures that allow the fungus *Nematospora spp* to colonize the beans, which results in endosperm rotting (Le Pelley, 1942). Feeding on mature berries results in defective beans, which are lighter and float at the coffee washing station. In the presence of *Nematospora spp*, the coffee beans turn brown or black and they sometimes exhibit a physical defect known as Zebra Coffee Beans (Ribeyre and Avelino, 2012).

Feeding on flower buds results in browning or blackening of buds that impairs fruit set (Le Pelley, 1968; Mugo, 1994). Significant loss of flower occurs at the onset of rains and severe infestation may prevent the tree from flowering (Waller et al., 2007). The bug also feeds on developed leaves, but this only happens when flowers, berries and shoots are absent. When the bug damages the leaves at the growing point, they become scarred and distorted (Le Pelley, 1968). Damages on shoots result in multiple branches, which in severe cases lead to witches-broom (Waller et al., 2007). High infestation of shoots results in growth reduction and shortening of internodes while damaging the growing point results in duplication and multiplication of branches which produces a bunchy or matted growth (Le Pelley, 1968). It is estimated that the yield loss due to Antestia bug averages 30%, but it can be as high as 45% in the presence of a high insect densities (Craves, 2012).

Management of Antestia Bug

Cultural control

Pruning of coffee trees is the main cultural practice recommended for management of Antestia bug populations. As the bug prefers dense foliage possibly because it avoids direct sunlight (Babin et al., 2018), pruning opens up the coffee trees and thus creates unfavorable conditions for the bug; it also improves pesticide penetration and efficacy (Bigirimana et al., 2012). Pruning results in significant reduction of the amount of coffee bean damage and berry loss due to Antestia bug and also assists the trees in maintaining vigor, which enhances resistance to infestations (Abebe, 1987).

Physical control

Hand picking has been widely used to control Antestia bug particularly in East Africa in the period of 1930 - 1950's (Le Pelley, 1968). Hand picking was conducted either by intensively searching for the bug on each tree or the bugs were forced out of coffee plants using smoke from smoldering cow dung. This practice was effective for controlling Antestia bug when the population ranged from 1 to 2 per tree but difficult for large bug populations of up to 50 per tree (Greathead, 1966). Overall, handpicking is effective at reducing Antestia bug damage but it is costly compared to the benefits.

Use of botanicals

Botanicals for pest control mainly include neem extracts and pyrethrum, which are produced locally in East Africa. They are cheap compared to synthetic insecticides, safe to human health and also environmentally friendly. Pyrethrum has been used against Antestia bug since the 1930's and given its high toxicity, researchers developed emulsions and dusts products that were used successfully to control the bug (Crowe et al., 1961). In Ethiopia, extracts of *Millettia ferruginea* were used against Antestia bug in a laboratory study and achieved mortality of 84% and 78% for adults and nymphs of *A. intricata*, respectively while pyrethrum (*Chrysanthemum cinerariaefolium*) killed 78% and 80% of bugs and nymphs, respectively for the above species under similar laboratory conditions (Mendesil and Abebe, 2007). Plant extracts also are toxic to eggs of Antestia bug. In a different study, extracts from *Dysphania ambrosioides, Thymus vulgaris* and *Ruta chalepensis* produced 92.5%, 90% and 87.5% mortality of eggs of Antestia bugs, respectively (Mendesil et al., 2012).

Chemical control

The first attempts to control Antestia bug using synthetic insecticides occurred in the 1940's when broad-spectrum dichlorodiphenyltrichloroethane (DDT) was used on coffee (Thelu, 1946). This insecticide was initially highly effective but a few years later, there were outbreak of secondary pests, especially leaf miners and mealy bugs, which were previously limited by high levels of parasitism (Le Pelley, 1968). In 1970's organophosphates such as chlorpyrifos-ethyl, malathion, fenthion and fenitrothion were used for the control of Antestia bug mainly due to their lower toxicity to humans. Some of these compounds, including chlorpyrifos-ethyl, fenitrothion, diazinon and dimethoate, were still under use in coffee farming until 2010 (Mugo et al., 2011). The toxicity of organophosphorous compounds to humans and their adverse effects on the environment are currently well documented and their use is restricted in Europe (Fryer et al., 2009). New molecules are now available for Antestia control in Africa. These include pyrethroids such as Cypermethrin and Lambda-Cyhalothrin, which are highly effective but toxic to the environment, especially to fish (Mendesil and Abebe, 2007). Neonicotinoids, such as imidacloprid are now recommended for Antestia control in coffee. They are effective and show persistence in coffee fields but should be sprayed well ahead of harvest (Babin et al., 2018).

Biological control

Antestia bugs are hosts for several parasitoids, especially egg parasitoids. Good parasitism rates against the bug indicate these species are promising agents for biological control (Greathead, 1966). Three findings support the potential of biological control for Antestia bug – (i) egg parasitism ranges from 40 to 95% where the most predominant parasitoid is *Asolcus seychellensis*, (ii) *Aridelus spp*. is responsible for up 50% nymphal parasitism but it is only promising in some areas and (iii) Tachinidae flies (*Bogosia spp*) and Strepsiptera (*Corioxenos Antestiae*) are common adult parasitoids (Greathead, 1966). In Ethiopia, 45-50% egg parasitism were caused by three species, Trissolcus (*Asolcus suranus*), Gryon fulviventre (*Hadronotus Antestiae*) and Anastatus Antestiae *A. suranus* was the most important (Abebe, 1999).

Potato Taste Defect and Antestia Bug

Potato taste defect (PTD) is a raw potato – like smell and taste found in green and roasted coffee beans after brewing. The smell is believed to be caused by a bacterium (fig. 1. 1) of the family *Enterobacteriaceae* which develops in coffee beans and produces 3 – isopropyl – 2– methoxypyrazine (IPMP) and 2-isobutyl-3-methoxypyrazine (IBMP), the compounds that are responsible for the off-flavor (Gueule et al., 2014; Becker et al., 1988). The bacterium responsible for PTD is classified in the genus *Pantoea*, which includes seven species, *P. agglomerans*, *P. ananas, P. citrea, P. dispersa, P. punctata, P. stewartii and P. terrea* (fig. 2). Symbiotic bacteria associated with Antestia bug have also been characterized. A midgut associated obligate symbiont and three facultative symbionts representing the bacteria genera *Sodalis, Spiroplasma*, and *Rickettsia* were identified. The gut symbiont was detected from all populations of *A. thunbergii* where Sodalis symbionts, the Spiroplasma symbiont, and the Rickettsia symbiont represent infection frequencies of 51.3%, 52.6% and 24.0%, respectively (Matsuura et al., 2014).

Surface volatile organic chemicals associated with PTD have been identified and include tridecane, dodecane, tetradecane and methylsalicylate (Jackels et al., 2014). Additionally, visual inspection of the chromatographs allowed differentiation between potato taste (PTD) and non-potato taste (non-PTD) samples where PTD chromatograms were longer and more intense for

PTD samples compared to non-PTD samples. Furthermore, samples with PTD had increased levels of dodecane and tridecane, moderately increased levels of tetradecane, 1-octene-3-ol, 2-ethyl-1-hexanol, and limonene slightly decreased levels of 3-methylbutanoic acid (Jackels et al., 2014). Additionally, an intense PTD profile (mainly alkanes) was not observed in the interior of either PTD or non-PTD samples where volatiles such as hexanal, hexanol, hexanol, nonanal, 3-methylbutanoic acid, and hexanoic acid were prominent and similar in intensity in both the PTD and non-PTD chromatograms which were previously observed in other studies of ground green coffee beans (Zambonin et al., 2005). Although the compounds IPMP and IBMP were reported to be associated with PTD, neither one was observed in any of the PTD or the non-PTD chromatograms of surface volatiles when the samples were heated at 60^{o}C (Jackels et al., 2014).

Male and female specimens of Antestia bug were similarly associated with PTD: analysis of male and female specimens produced very similar chromatograms with the same set of volatiles, including hexanal, dodecane, 1-dodecane, tridecane 2,6,10,14-tetramethylpentadecane, hexanoic acid, and phenol (Jackels et al., 2014). Briefly, PTD was associated with surface volatile compounds on green coffee and analysis using these volatiles could distinguish between PTD and non-PTD samples. The most identifiable compounds found at higher levels in PTD coffee surface include dodecane, tridecane, tetradecane, and 2-ethyl-1-hexanol, while 3-methylbutanoic acid was found at decreased levels. Long-chain alkanes have not previously been associated with volatile compounds of ground green coffee (Ribeiro et al., 2013). However, long-chain alkyl compounds are frequently found as constituents of plants and insects, for example, on leaf surfaces and seed surfaces, and are used as pheromones by insects.

Several studies include alkanes such as tridecane, dodecane, tetradecane, and undecane on lists of defensive pheromones secreted by stink bugs related to Antestia (Favaro et al., 2012).

According to Jackels et al. (2014), PTD is associated with Antestia feeding activity and IPMP, which imparts potato taste in roasted coffee, is produced inside the coffee beans, possibly in response to the stress of Antestia damage. Jackels et al. (2014) also showed that IPMP and IBMP are found inside the beans and not at the surface. Presence of both IPMP and IBMP in PTD samples in ground-beans that have insect damage provides additional evidence of an association between Antestia bug and PTD (Toci & Farah, 2008).

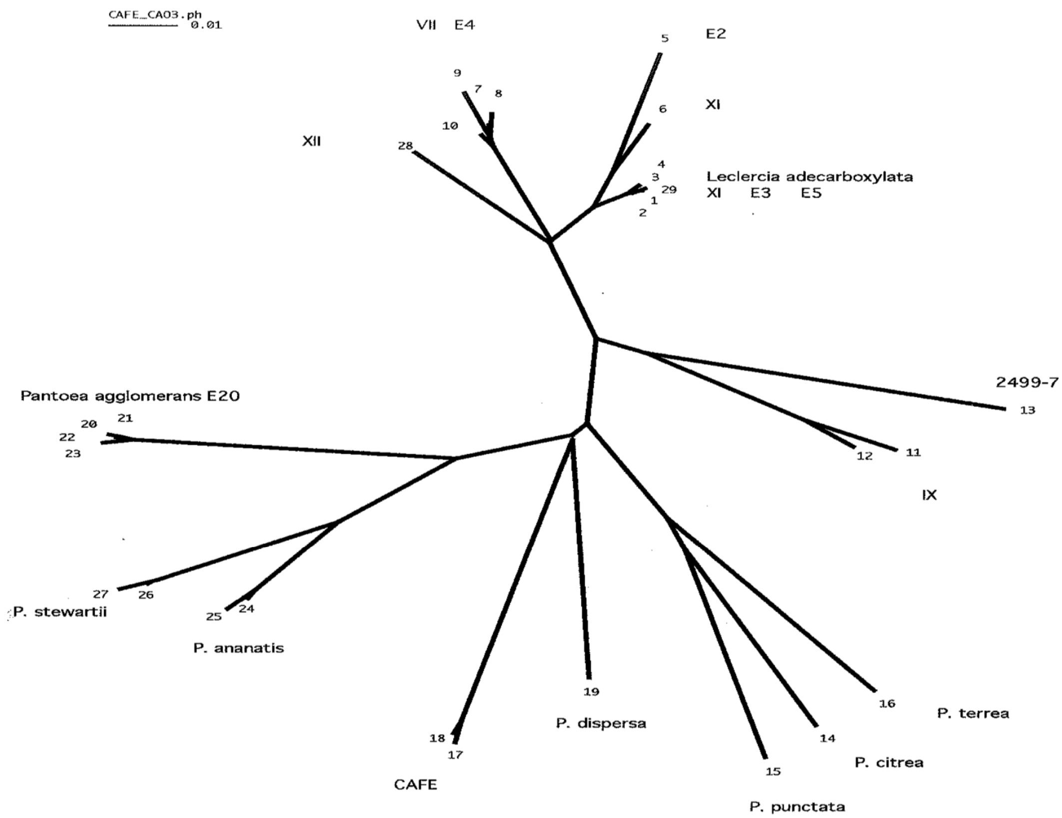

Figure 1. 1 Classification of bacterium responsible for PTD in the genus Pantoea

Problem statement and justification of the study

Coffee from East Africa was classified as grade C (low grade) in the 1990s, especially coffees from Tanzania, Burundi, DRC and Rwanda. It was therefore difficult to sell it on the global market, fetching low prices. Rwanda and other Eastern African countries were unknown in the specialty coffee market. But in the early 2000s, Rwanda placed an emphasis on producing high quality fully washed coffee resulting in the country being recognized as a producer of high-value coffee. The percentage of fully washed coffee has increased from 1% in 2002 to 27% in 2012, owing to an increase in the number of coffee washing stations in the country (NAEB, 2014).

As the coffee quality improved substantially the annual average price for green coffee also increased from about \$2.4 per kilo in 2005 to about \$5.0 per kilo in 2011 (NAEB, 2014). In recent years, potato taste defect has been mentioned by coffee buyers as a major threat to this quality gain, significantly affecting the Rwanda coffee industry. It has become difficult for exporters to guarantee importers a product that does not have the defect, and subsequently for importers to guarantee their consumers a defect-free product. Potato taste defect diminishes the quality perception of Rwandan coffee and confidence in buying coffee from Rwanda. The IPMP and IBMP volatiles responsible for PTD are detected by humans at very low concentration (2.10^{-9} g) (Toci & Farah, 2008). The PTD is mostly found in damaged coffee beans and is generally mitigated by coffee processors who rigorously sort out beans with signs of insect damage, but this method does not entirely eliminate PTD (Jackels et al., 2014). It is therefore hypothesized that the microbes causing PTD enter into coffee cherries that have been damaged by insects. However, not all coffee beans with physical defects exhibit PTD. Also, some coffee beans with no noticeable physical damage exhibit PTD, hence making elimination of PTD by sorting out visibly damaged coffee beans not always effective.

Presence of PTD has been associated with insect damage, especially damage from Antestia bugs (*Antestiopsis thunbergii*). The bugs are suspected of facilitating entry of microbes into the cherries that cause potato taste in roasted coffee beans (Cilas et al., 1998). Antestia bugs are some of the most important insect pests in coffee, especially in the East Africa Great Lakes region (Nyambo et. al., 1996, Cilas et al., 1998). Available research results estimate that Antestia bugs can cause as high as 40% yield loss (Ahmed et al., 2016). It has also been reported that protection of coffee plantations against insect pests decreases the occurrence of the "potato taste" (Bouyjou et al., 1999).

The occurrence of PTD in coffee continues to increase. For example, assessment of occurrence of PTD using coffee lots entered into the Cup of Excellence competitions revealed an increase of PTD close to 51% occurrence in 2013 as opposed to 18% in 2012. While the economic impact of the defect is on the rise, there continues to be insufficient information on the prevalence and prevention of the defect needed to mitigate the problem. This is especially the case in the African Great Lakes regions. Some of the key questions that need to be addressed include:

1) What is the distribution and incidence of PTD across the major coffee growing regions of Rwanda?
2) What are the mechanisms of PTD occurrence? Is it through mechanical damage or is it insect vectored or a combination of both?
3) What is the relationship between Antestia density and the occurrence of PTD in coffee?
4) What is the relationship between management practices and PTD?

5) How effective are the various options for managing Antestia bug?
6) How do climatic variables such as temperature, rainfall, humidity, etc. influence the distribution and population density of Antestia bug?

Research goals

The goals of this dissertation research are (i) to better understand the relationship between PTD and the distribution and density of Antestia bug, as well as the various options for management of this important coffee pest, (ii) to investigate the distribution and incidence of PTD and its relationship with other factors including management practices and insect damage and (iii) to clarify the mechanisms of PTD infection in order to make better informed management decisions aimed at eliminating PTD in coffee.

Specific objectives

Objective 1: To determine the effectiveness of integrated pest management tactics including pruning and the use of several commercially available insecticides against field populations of Antestia bug;

Objective 2: To determine the relationship between Antestia bug density and the occurrence of PTD in coffee and to assess the distribution and incidence of PTD in Rwandan coffee and how it relates to management practices;

Objective 4: To assess the effect of climatic variables on the distribution and population density of Antestia bug;

Objective 5: To evaluate the mechanisms of PTD infections in coffee bean**s.**

CHAPTER 2: OPTIONS FOR MANAGING *ANTESTIOPSIS THUNBERGII* (HEMIPTERA: PENTATOMIDAE) AND THE RELATIONSHIP OF BUG DENSITY TO THE OCCURRENCE OF POTATO TASTE DEFECT IN COFFEE

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Published in: Florida Entomologist (2018) 101(4): 580-586.

ABSTRACT

Potato taste defect is a potato-like smell found in green and roasted coffee beans. PTD reduces the flavor experience of finished coffee and causes it to be rejected by consumers. The presence of PTD has been associated with insect damage, especially damage from the Antestia bug, *Antestiopsis thunbergii* (Gremlin) (Hemiptera: Pentatomidae). A 2-yr study was conducted to evaluate the effectiveness of IPM using pruning alone or in combination with several commercially available insecticides to control a field population of Antestia and to assess the relationship between these treatments and the occurrence of PTD in coffee using laboratory and field tests. Field trials were conducted in 3 major coffee growing regions of Rwanda: Rubona, Gakenke, and Kirehe. In laboratory bioassays, significant differences were found between insecticides on the percentage mortality of adult bugs. The percent mortality was higher for pyrethroid, Fastac (Alpha-cypermethrin), and pyrethrins (Pyrethrum 5EW, Pyrethrum EWC + Sesame and Agroblaster) than for the neonicotinoid, imidacloprid. In the field, the highest mean mortality was achieved with Pyrethrum 5EW, Fastac, and Pyrethrum EWC applied to pruned coffee trees. Multiple logistic regression analysis indicated that PTD was positively associated

with the bug density and the extent of damage caused by Antestia bug. The study suggests that pruning combined with insecticide application, especially Fastac, provides better control of Antestia bug and significantly reduces potato taste defect compared to either pruning or insecticide application alone.

INTRODUCTION

Coffee is an important export crop and a major foreign exchange earner for many countries in Africa, Asia, and Latin America. It is estimated that coffee cultivation, processing, trading, transportation, and marketing provide employment for millions of people worldwide (Phiri et al. 2009). Particularly for Rwanda, coffee accounts for a significant proportion of the country's total annual agricultural export and is an important source of direct cash income for about a half million families (Moss et al. 2017). As most of the coffee in the country is cultivated on smallholdings, it impacts a much higher percentage of the population than all other cash crops in the country.

Despite its importance, coffee suffers yield loss from several factors including insect pests and diseases, amongst which Antestia bug, *Antestiopsis thunbergii* (Gmelin) (Hemiptera: Pentatomidae) is the most important (Gesmalla et al., 2016). Antestia bug feeds on various parts of the coffee plants ranging from flower buds, berries at different stages of development and maturation, and green shoots and leaves (Kirkpatrick 1937). Cilas et al. (1998) reported that Antestia bug moves throughout the coffee plantations and sometimes has an aggregative distribution possibly due to its reproductive behavior. Feeding on flower buds results in browning or blackening of buds that impairs fruit set (Le Pelley 1968; Mugo 1994). When high infestation by Antestia bug occurs during the onset of rains, loss of flowers may be significant and severe infestation may prevent the tree from flowering (Waller et al. 2007). It is estimated that the yield loss due to Antestia bug averages 30% but can be as high as 38% if the insect population reaches 15 bugs per tree (Ahmed et al. 2016). Antestia bug is native to Africa, but also has spread to other coffee growing countries of Asia such as Pakistan, India, Myanmar, Sri Lanka, and southern China (Rider et al. 2002).

Antestia bug also is suspected to facilitate the entry of microbes that are associated with potato taste defect in coffee (Bouyjou et al. 1993). Potato taste defect is a potato-like smell found in green and roasted coffee beans, and also in roasted coffee beans after brewing (Gueule et al. 2015). The smell is believed to be caused by a bacterium of the family *Enterobacteriaceae* that develops in coffee beans and produces isopropyl-2-methoxyl-3-pyrazine (IPMP) and 2-isobutyl-3-methoxypyrazine (IBMP), the compounds that are responsible for the off-flavor (Becker et al. 1988; Gueule et al. 2013, 2015). It is also possible that one of the off-flavor compounds, IPMP, is produced by the plant itself as a stress response to Antestia damage (Jackels et al. 2014). Notwithstanding, it has been reported that protection of coffee plantations to control Antestia bug decreases the occurrence of potato taste defect in roasted beans (Bouyjou et al. 1999).

In studies conducted by Jackels et al. (2014), Bressanello et al. (2017), and Iwasa et al. (2015), the coffee quality was evaluated using the Cupping Protocol of the Specialty Coffee Association of America. Experienced Licensed Q Graders evaluated the cup qualities of samples including the defects, and assigned scores that provided international standards for cup evaluation that were consistent with other analytical techniques, such as Gas Chromatography-Mass Spectrometry, Head-space-Solid phase micro extraction, and Liquid Chromatography-Mass Spectrometry (Jackels et al. 2014; Bressanello et al. 2017; Iwasa et al. 2015). The aim of the current study was twofold: (1) to examine the effectiveness of integrated pest management tactics including pruning and the use of several commercially available insecticides to control field populations of Antestia bug, and (2) to evaluate the relationships between these treatments and the occurrence of potato taste defect in coffee.

MATERIALS AND METHODS

Laboratory bioassays

Adult Antestia bugs were field collected from coffee trees by handpicking in the principal Coffee Research Station situated in Rubona (02.4842°S, 29.7661°E), at altitude 1,673 masl, and held in a 3 L plastic container with holes on all sides to facilitate air entry and circulation. Identity of *Antestiopsis thunbergii* was confirmed by comparing collected insects with preserved specimen of this species. Five insecticides were used in the experiment: (1) Confidor (17.8% imidacloprid, Bayer East Africa, Nairobi, Kenya); (2) Fastac (10% alpha-cypermethrin, BASF Corporation, Nairobi sub-office; Nairobi, Kenya); (3) Pyrethrum 5EW (5% pyrethrins, Agropy Ltd, Kigali, Rwanda; (4) Pyrethrum EWC (2.19% pyrethrins and 10% sesame, Agropy Ltd, Kigali, Rwanda); and (5) Agroblaster (8% pyrethrins, SOPYRWA, Musanze, Rwanda).

All of the insecticides were diluted as 1:1,000 except imidacloprid, where the dilution was 133 ppm to match the recommended concentration and serve as a positive control. Collected adult bugs were sprayed with insecticides or water only as a control on the same day they were collected from the field. Treatments were applied topically to groups of 15 adult bugs placed in a Petri dish using a hand sprayer (Multi-Purpose 1 L Hand Pump Sprayer, Hudson #79142, Zoro, Chicago, Illinois, USA) at the above concentrations. Each treatment was replicated 3 times in a completely randomized experimental design. Insects were counted as dead if they could not move their wings, stand, and fly. Mortality of Antestia bug was assessed at 5 min, 1 h, and 12 h after spraying.

Field trials

Field trials were conducted in Rwanda from 2015 to 2016. Pruning, an agricultural practice used to shape the tree canopy, facilitates field operations and satisfies the needs of crop production and fruit quality (Jiménez-Brenes et al. 2017), and used either alone or in combination with insecticides, was evaluated for the control of Antestia bug. Pruning was carried out 1 month prior to insecticide sprays. Trials were established in the 3 principal coffee growing regions of Rwanda: Rubona, Gakenke, and Kirehe. Rubona, already referred to earlier, is located in the southern province of Rwanda. Gakenke is in the northern province (01.6908°S, 29.7955°E) and at an elevation of 1,792 masl, whereas Kirehe is in the eastern province (2.2633°N, 30.6413°E) and at an altitude of 1,530 masl. These 3 sites were located in different agro-ecologies where high populations of Antestia bug were reported (Bigirimana et al. 2012). In all 3 sites, the variety used was Bourbon Mayaguez 139 (BM 139) and the coffee plantations were between 6 to 8 yr old. There were 2 criteria for farm selection: the farm was not sprayed with insecticides for at least 1 yr prior to the experiment and was large enough to accommodate all the treatments. Treatments consisted of (i) control (plots were not pruned and treated with insecticides), (ii) pruning only, (iii) pruning plus Fastac sprays, (iv) pruning plus sprays with pyrethrum 5EW, (v) pruning plus sprays with Pyrethrum EWC, (vi) pruning plus sprays with Agroblaster, and (vii) pruning plus sprays with imidacloprid.

In addition, all of the insecticides were diluted as 1 : 1,000 except imidacloprid where the dilution was 133 ppm because it was a recommended concentration. Control options were evaluated in a randomized complete block design with 7 treatments replicated 3 times each in the

3 production regions. The experimental plot in each region was comprised of 42 coffee trees, 7 rows by 6 trees per row. The coffee trees were spaced 2 m between rows and 2 m within rows. Experimental plots were isolated from each other by 5 m. The 6 central trees (2 rows of 3 trees) in each plot of 42 trees were used for data collection to provide a 2-tree buffer on all sides of the sampled trees.

Before insecticides were applied, plastic sheeting was used to cover the ground to facilitate catching Antestia bugs that fell out of the canopy following the spray. Similar to the laboratory bioassays, bugs were considered dead if they were not able to move their wings, stand, and fly. Sprays were applied 2 weeks after flowering using a knapsack sprayer (Cooper Pegler Sprayers, Gandhinagar, Gujarat, India), where a plot was sprayed with 15 L of insecticides and water for the control. We sprayed once, 2 weeks after flowering, because the crop was in a most susceptible stage and the period when Antestia bug was most vulnerable (Le Pelley 1942). Dead insects including nymphs and adults were counted 5 min after sprays, then after 1 h, and 12 h post-treatment. On the day of harvest, cherry infestation by Antestia bug was determined by selecting 3 bearing branches (1 at the bottom, 1 in the middle, and the last at the top) of the 6 central trees used for data collection, counting the number of infested and clean berries, and calculating the percent infested.

Testing samples from the study

Three kg of ripe cherries were collected from each test plot 6 months after spraying. The cherries were collected weekly over a 1 month period and hand pulped at the washing station of the principal Coffee Research Station in Rubona. These coffee beans were wet processed and dried. Each sample (500 g of green coffee) was roasted using a laboratory-scale roaster (Suntory Laboratory and Suncafe Laboratory, Kanagawa, Japan). The roasting degree was adjusted to a luminosity (L, brightness) value of 22 to 23 for all samples. Cupping of the samples was performed in accordance with the Specialty Coffee Association of America cupping protocol (Iwasa et al. 2015). All evaluations were conducted by experienced Licensed Q graders, certified by the Coffee Quality Institute. Cupping was arranged in such a way that 15 cups per treatment were tested. Three Licensed Q graders cupped all the samples, and each grader was considered as a replicate. Every grader performed a standard session cupping of all the samples in a randomized order as blind analyses. The cupping objective was not to give the overall cupping score, but to detect whether the sample had or did not have the potato taste defect. The percentage of cups with potato taste defect was calculated for each treatment and then averaged across replicates.

Statistical analysis

Data from laboratory bioassays were corrected for mortality using Abbott's formula (Abbott 1925). Data were arcsine square root transformed to fit normality and homogeneity of variance assumptions prior to being subjected to the repeated measurements analysis of variance (ANOVA) test. Field data were log transformed (x + 1) and also were subjected to repeated measurements ANOVA test. A combined analysis of 3 sites and 7 treatments over time was conducted. For both laboratory bioassays and field experiments, treatment means were separated using the Tukey's test. In addition, the percent occurrence of potato taste defect was calculated for each treatment, and was tested for normality using the Shapiro Wilk test and equality of variance using Brown and Forsythe's Test. This data then was subjected to 1-way ANOVA, followed by Tukey's multiple comparison procedure at $P = 0.05$. The multiple logistic regression analysis was used to assess the relationship between the bug density, the berry density, and the effect of pruning to the occurrence of potato taste defect, a response variable modelled as present or absent. The multiple logistic regression model used can be written as:

$$Logit\ (p) = log\ [\frac{p}{1-p}]$$

or

$$Logit\ (p) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3$$

where p is the probability that potato taste defect occurs; α, the intercept; β_1, the coefficient of bug density (X_1); β_2, the coefficient of berry damage density, i.e., the percentage of damaged cherries ($X2$); and β_3, the coefficient of pruning ($X3$) (Rosner 2011). In this study, independent explanatory variables were Antestia bug density, and the percentage of cherries damaged by Antestia bug. Pruning was a binary variable, because plots were either pruned and the variable was coded 1, or unpruned and the variable was coded 0. The probability value was then expressed as:

$$p = \frac{\exp\ (\alpha+\beta 1X1+\beta 2X2+\ \beta 3X3)}{1+\exp\ (\alpha+\beta 1X1+\beta 2X2+\ \beta 3X3)}$$

Odds ratio was used to facilitate model interpretation. Odds ratio indicates how much likely or unlikely potato taste defect will occur for a set of values of independent variables (Rosner 2011). To fit the model, we used the stepwise regression technique and the model with the smallest Akaike's Information Criterion was selected (Aho et al. 2014). All statistical analyses were conducted using SAS software (Version 9.4, SAS Institute, Cary, North Carolina, USA).

RESULTS

Laboratory bioassays

There were significant differences in percent mortality of Antestia bug following treatments with insecticides ($F = 163.48$; df = 4,12; $P < 0.0001$). Five min after sprays, Fastac and all the pyrethrins (Pyrethrum 5EW, Pyrethrum EWC, and Agroblaster) caused a higher percent mortality of Antestia bug than that of the neonicotinoid insecticide, imidacloprid (Table 2.1). The highest percent mortality was observed with Agroblaster and Fastac, 83 and 86%, respectively. The same trend was recorded 1 h after spraying except that the percent mortality due to imidacloprid sprays increased from 0 to > 26%. Twelve h after treatment, Fastac and Pyrethrum EWC provided 100% mortality and significantly greater mortality than imidacloprid. Antestia bug mortality rates of 94% and 90% were recorded in the Pyrethrum 5EW and Agroblaster treatments, respectively, which was statistically different than the 71% mortality provided by the imidacloprid treatment.

Insecticides effectiveness under field conditions

Analysis of variance did not show significant effects of sites on the bug mortality ($F = 1.16$; df = 2,36; $P > 0.05$); hence, all sites were combined in the analysis of efficacy. Significant differences in mortality of Antestia bug following pruning and the various insecticide treatments under field conditions were observed ($F = 100.2$; df = 6,36; $P < 0.0001$). Time effects also were significant ($F = 66.73$; df = 2,36; $P < 0.0001$). Five min after treatment, pruning, and all treatments combining pruning plus an insecticide provided significantly higher mortality than the no pruning and no pesticide treatment (Table 2.2). Across all time intervals, the highest mean mortality was recorded in plots that were pruned and treated with Pyrethrum 5EW. This treatment provided significantly greater mortality compared to all other treatments. Pruning plus application of Fastac or Pyrethrum EWC resulted in significantly higher mortality than all other treatments except pruning plus Pyrethrum 5EW treatment. A lower level of mortality was recorded in plots that were not pruned and not treated with insecticide. Plots that were pruned and not treated with insecticide had a mean mortality of Antestia bug equivalent to that of plots that were pruned and treated with imidacloprid or Agroblaster. Although mortality increased slightly across all treatments at 1 h and 12 h after sprays, the relative effectiveness of the various treatments was the same as that recorded 5 min after treatment.

Table 2. 1. Mean* (± SEM) percent mortality of Antestia bug in laboratory bioassays at 5 min, 1 hour and 12 hours after spray following insecticide sprays

	Time after spray		
Treatment	5 min	1 h	12 h
Fastac	86.1 ± 2.7 a	83.3 ± 2.7 a	100.0 ± 0.0 a
Pyrethrum 5EW	58.3 ± 2.7 a	58.3 ± 3.7 a	94.4 ± 3.8 ab
Pyrethrum EWC + Sesame	68.1 ± 2.6 a	73.6 ± 3.6 a	100.0 ± 0.0 ab
Agroblaster	86.1 ± 2.7 a	86.1 ± 2.6 a	90.3 ± 3.9 ab
Imidacloprid	0.0 ± 0.0 b	26.4 ± 2.6 a	70.8 ± 2.7 b

*Means in a column followed by the same lowercase letters are not statistically different ($P \leq 0.05$, ANOVA and Tukey's test).

Occurrence of potato taste defect in tested samples

There were significant differences between treatments on the occurrence of PTD in coffee ($F = 261.06$; df = 6,14; $P < 0.0001$). Treatment with Fastac in pruned coffee trees resulted in the lowest PTD and the control had the highest incidence (about 12 times Fastac spraying in pruned plots) (Fig. 2. 1). Pruned plots treated with Fastac or Pyrethrum 5EW had the lowest PTD incidence on average, but plots sprayed with Pyrethrum 5EW had twice PTD incidence than those treated with Fastac. Additionally, the control had twice PTD incidence than pruning alone, which had the same PTD incidence as pruned plots treated with Imidacloprid. Overall, occurrence of PTD in all tested samples was about 6%, i.e, about 6% of tested samples had PTD.

Table 2. 2. Mean* (± SEM) mortality of Antestia Bug in field trials five minutes, one hour and 12 hours following insecticide sprays

Time after sprays	5 minutes	1 hour	12 hours
Treatments			
Pruning & Pyrethrum 5EW	13.1 ± 1.7a	14.4 ± 1.5a	15.8 ± 1.5a
Pruning & Fastac	12.3 ± 1.3a	13.7 ± 0.9a	15.0 ± 1.2a
Pruning & Pyrethrum EWC	8.7 ± 1.7b	10.0 ± 1.6b	11.6 ± 2.3b
Pruning & Agroblaster	5.2 ± 2.2c	6.4 ± 2.2c	8.1 ± 1.8c
Pruning & Imidacloprid	2.1 ± 0.8d	4.6 ± 1.3c	6.9 ± 1.6c
Pruning & no Pesticide	3.1 ± 1.9cd	4.9 ± 1.9c	6.2 ± 1.2c
No Pruning & no Pesticide	0.3 ± 0.8e	1.4 ± 0.8d	2.7 ± 0.8d

*Means in a column followed by the same lowercase letters are not statistically different ($P \leq 0.05$, ANOVA and Tukey's test)

Multiple logistic regression analysis showed that each of the variable was significantly related with the incidence of PTD in coffee (Table 2.3). The odds in favor of PTD incidence for unpruned plots were 1.48 (i.e $e^{0.39} = 1.48$) times as great as for pruned plots, holding all other variables constant. The odds ratio for Antestia bug density was 2.1 ($e^{0.74} = 2.1$), which indicated that, for 2 plots that were 1 unit apart on Antestia bug density and were comparable on all other variables, the odds in favor of PTD incidence for the plot with higher Antestia bug population were 2.1 times as great as the plot with lower bug population. Similarly, the odds ratio for berry damage density was 1.36 (i.e., $e^{0.31} = 1.36$) indicating that for 2 samples that were 1% berry damage density apart, the odds in favor of PTD incidence were 1.36 times as great as for a sample with a higher berry damage compared with a sample with a lower berry damage density. The mean percent-infested berries ranged from 13.1% in pruned plots sprayed with Pyrethrum 5EW to 47.15 % in unpruned plots without insecticides sprays (table 2. 4). It is worth noting that all the variables tested in the analysis were significantly related to PTD occurrence.

Table 2. 3. Stepwise multiple logistic regression analysis using Antestia bug density, berry damage density and pruning as predictors for occurrence of PTD in coffee

Variables/Units	Estimates	Standard error	P > ChSq	Odds ratio	95% Wald Confidence Limits
Intercept	-1.78	0.47	< 0.001		
Antestia bug density (# of bugs per plot)	0.74	0.18	< 0.01	2.10	1.47 - 2.98
Berry damage density (% of damaged cherries)	0.31	0.09	< 0.01	1.36	1.14 - 1.63
Pruning (Pruned versus unpruned plots)	- 0.39	0.17	0.04	1.48	1.02 - 2.14

Model likelihood ratio: $\chi^2 = 14.5851$, df = 4, Pr > $\chi^2 = 0.0056$; AIC = 241.216;
Pseudo – $R^2 = 0.329$; All variables tested in the study (Antestia bug density, berry damage density and pruning) were significantly related with the occurrence of PTD in coffee ($P < 0.05$).

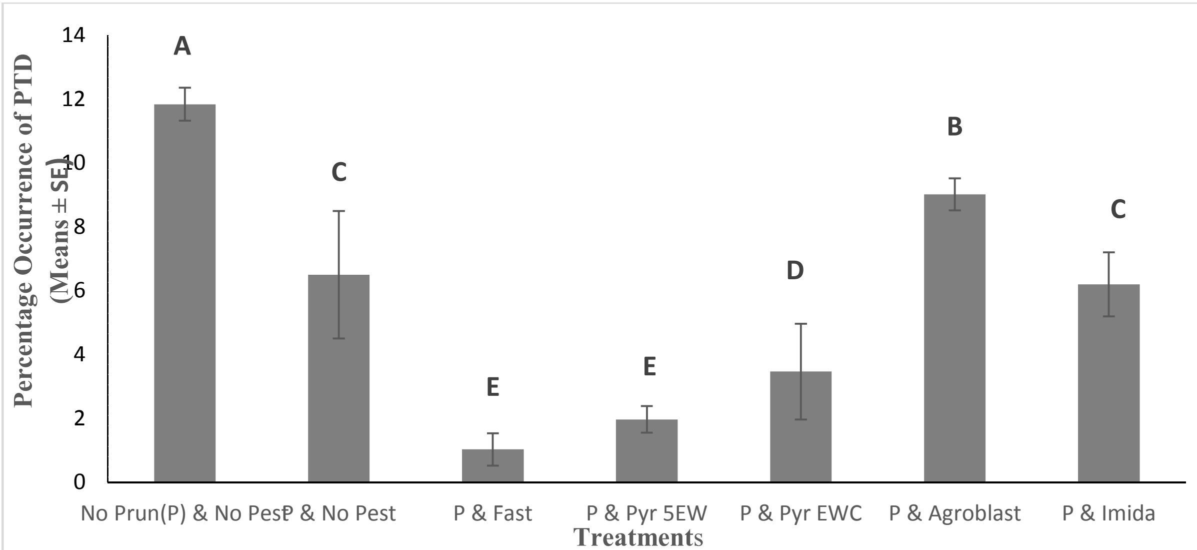

Figure 2. 1 Effects of pest management tactics on the occurrence of PTD in coffee. No Prun(P) & No Pest (No Pruning and No Pesticide), P & No Pest (Pruning and No Pesticide), P & Fastac (Pruning and Fastac), P & Pyr 5EW (Pruning and Pyrethrum 5EW), P & Pyr EWC (Pruning & Pyrethrum EWC), P & Agroblast (Pruning and Agroblast) and P & Imida (Pruning and Imidacloprid). Fastac sprayed in pruned plots had the lowest levels of PTD while the control had the highest. Bars represent the standard error of means.

Table 2. 4. Mean percentage of infested berries in plots that received application of various treatment

Treatment	N	Percentage infested berries* (Mean ± SE)
Pruning & Pyrethrum 5EW	3	13.12 ± 1.02 e
Pruning & Fastac	3	24.62 ± 2.33 d
Pruning & Pyrethrum EWC	3	25.68 ± 1.59 cd
Pruning & Imidacloprid	3	29.86 ± 0.87 cd
Pruning & No insecticide	3	31.44 ± 0.89 c
Pruning & Agloblaster	3	39.16 ± 0.57 b
No Pruning & no pesticide	3	47.15 ± 1.19 a

Means in a column followed by the same lowercase letters are not statistically different ($P \leq 0.05$, ANOVA and Tukey's test).

DISCUSSION

Overall, pruning and all treatments combining pruning and insecticide application provided a higher mortality of Antestia bug compared to pruning alone, no pruning, and no pesticide application. Combining insecticide application and cultural practices is fundamental to Integrated Pest Management programs of many agricultural pests (Alyokh-in 2009; Larson et al. 2017). This strategy not only facilitates insecticide sprays, increases insecticide control efficacy, and reduces the cost of insecticide application, but also helps in reducing the dependency on chemical insecticides, thereby slowing down the development of insecticide resistance and in preventing negative effects on humans and the environment (Freitas et al. 2011).

The highest mortality of Antestia bug was recorded in plots that were pruned and treated with pyrethrins, such as Pyrethrum 5EW with a mean of 15.8 ± 1.5 bugs compared to plots sprayed with imidacloprid with a mean of 6.9 ± 1.6 bugs at 12 h post-treatment. On average, spraying with Pyrethrum 5EW provided greater mortality of the bug than all other treatments, including imidacloprid, with a mean mortality of 15.8 ± 1.5 and 6.9 ± 1.6 bugs for Pyrethrum 5EW and imidacloprid, respectively. In addition, pruning combined with Fastac or Pyrethrum EWC sprays caused significantly higher mortality of the bug than all other treatments except pruning plus Pyrethum 5EW. Pruned plots not treated with insecticides had a mean mortality of Antestia bug equivalent to those that were pruned and treated with imidacloprid, with a mean mortality of 6.9 ± 1.6 and 6.2 ± 1.2 Antestia bugs for pruned plots treated with imidacloprid and pruning plots not sprayed with insecticide, respectively. As shown in this study, pyrethrins provided greater mortality of the bug; however, they are reported to break down quickly, and are thus less toxic to the environment, especially when they are exposed to UV light and other weather conditions (Cecchinelli et al. 2012). However, pyrethrins also kill other non-target organisms. In this study, we did not evaluate the potential impact of pyrethrins and pyrethroids on non-target organisms.

This study also showed that the results of the laboratory bioassays were consistent with those of the field efficacy trial. Bruck et al. (2011) observed that insecticides that performed well in the laboratory bio-assays also performed well in the field, an indication that laboratory screening of new pesticides is a worthwhile activity. An exception to this was Agroblaster, which performed well in the laboratory bioassays but failed to give satisfactory results in the field. This could be attributed to its degradation due to the environmental conditions, but given the lack of published documentation of degradation, this would require further investigation. In addition, results indicated that mortality due to imidacroprid sprays increased gradually over time, and this may be attributed to its slow rate of inducing mortality. Imidacloprid belongs to the neonicotinoid group of insecticides, which are systemic in nature, and with a high potential for long-term protection to control major insect pests (Mota-Sanchez et al. 2009; Simon-Delso et al. 2015).

Our results also showed that pruning alone provided a significant increase in the mean mortality over plots not pruned. These results agree with Bigirimana et al. (2012), where it was reported that pruning opens up the coffee bushes and, thus, creates unfavorable conditions for the bug, while also improving pesticide penetration and efficacy. Additionally, Kirkpatrick (1937) confirmed that Antestia bug rarely can be found on exposed parts of unshaded bushes because it prefers warm conditions, especially when the outer parts of the coffee bush are warmer than the interior. Additionally, Hargreaves (1936), when conducting his research in Uganda, observed that shade was probably responsible for attracting Antestia and that with the removal of shade conditions, the pest disappears. More recently in Kenya, Mugo et al. (2013) confirmed that severity of Antestia spp. on coffee was significantly higher under shade conditions compared to coffee grown in the open. The above evidence suggests that pruning should be considered as an important component for an effective IPM program of perennial high value coffee trees. It is worth noting that in this study, pruning was carried out 1 month prior to pesticide sprays.

This study indicated that about 6% of all tested samples had potato taste defect. It is worth noting that harvested cherries were not subjected to sorting at either the field or coffee washing station level. We also did not subject the coffee cherries to flotation. The effects of sorting or floating coffee cherries on the occurrence of potato taste defect needs further investigation.

Our results also showed the odds in favor of potato taste defect incidence were 1.48 × greater for unpruned plots compared to pruned plots, holding all other variables constant. This could be expected because both the Antestia bug population and the damage were higher in unpruned coffee bushes than in pruned coffee trees. Bouyjou et al. (1999) also observed that coffee plots that received up to 7 annual applications of insecticides could produce beans with less than 1% potato taste defect-contaminated cups, and that with proper processing and sorting of damaged beans could significantly reduce the incidence of potato taste defect in coffee beans.

To conclude, given that Antestia bug is widely distributed in various coffee producing countries and causes huge losses in terms of yield and quality, managing this insect pest is of critical importance. Pruning creates unfavorable conditions for Antestia bug and improves pesticide penetration and, therefore, efficacy, and it should be an integral part of Integrated Pest Management programs. Where only imidacloprid is being used continually to control Antestia bug in coffee plantations, it should be alternated with other contact insecticides because as a systemic insecticide, the likelihood for the bug to develop resistance is high (Prabhaker et al. 1997).

Pyrethrins currently offer the best alternative because they are inexpensive and are produced locally in the eastern African region, and as documented in this study, they are fairly effective. Alpha-cypermethrin (Fastac) provides excellent control of the Antestia bug. However, pyrethroid activity on non-target organisms should be evaluated if one would like to make a sustainable recommendation of pyrethroids in coffee farming. Finally, as coffee coming from the African Great Lakes Region is among the best in the world (Gueule et al. 2013), and yet the place where potato taste defect has been reported, the distribution and the severity of this defect as well as the other factors that may be responsible for the occurrence of this defect should be fully investigated. Additionally, efforts should be made to understand the mechanisms of potato taste defect infection in order to eliminate it in coffee.

CHAPTER 3: OCCURRENCE OF POTATO TASTE DEFECT IN COFFEE AND ITS RELATIONS WITH MANAGEMENT PRACTICES IN RWANDA

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Published in Agriculture, Ecosystems and Environment: (2019) 269: 82–87

ABSTRACT

Coffee production is a critical export for Rwanda and a direct income source for many rural farmers. An undesirable raw potato-like smell found in parchment, green and roasted coffee beans and in brewed cups of coffee referred to as “potato taste defect” (PTD) affects coffee across the Africa Great Lakes Region. Two main insect pests of coffee, Antestia bug (Antestiopsis thunbergii) and coffee berry borer (Hypothenemus hampei) occur in the same region and may be directly or indirectly responsible for the spread of PTD. The objectives of this study were to determine the distribution of PTD in Rwanda and to evaluate how crop management practices influence the occurrence of this defect in coffee. A stratified random sample of 338 coffee farms was selected and the density of Antestia bug and damage to coffee berries attributed to Antestia bug or coffee berry borer (CBB) were quantified. Management practices such as pruning, intercropping and insecticides application were recorded. A random sample of three kg of ripe cherries was collected from each farm, hand pulped, fermented for 24 h, washed, dried and tested.

Processing did not include sorting by floatation or removal of insect - infested cherries. Potato taste defect was detected in about 5% of the samples. The PTD was distributed throughout the coffee producing regions of Rwanda with the highest incidence in the Central Plateau, Granitic Ridges and Eastern Plateau. Occurrence of PTD was significantly correlated with Antestia bug density and damage but not to CBB infestation. Pruning significantly reduced the occurrence of PTD but intercropping did not affect the occurrence of this defect in coffee. This study suggests that PTD could be significantly reduced through proper control of Antestia bug, but its elimination would also require understanding its mechanisms of infection.

INTRODUCTION

Coffee is a critical export commodity worldwide and is crucial to the economy of 70 countries in Africa, Asia and Latin America (Aerts et al., 2015). In Rwanda, coffee is the major foreign-exchange earner and significantly contributes to the country's total annual value of agricultural exports (Murekezi et al., 2014). Coffee is the main source of direct income for a considerable proportion of rural farmers and provides employment for thousands of workers in the coffee industry (Moss et al., 2017). Coffee from the Africa Great Lakes Region, including Rwanda, has been ranked among the best in the world (Gueule et al., 2015). However, the region is constrained by Potato Taste Defect (PTD), an undesirable raw potato like-smell found in parchment, green and roasted coffee beans and in brewed cups of coffee. Potato taste defect diminishes the flavor experience and the perception of quality of finished coffee, reducing its value or causing it to be rejected by consumers.

Potato taste defect was first detected in the 1940s (Stolp, 1960) in the eastern part of the Democratic Republic of Congo, near the Rwandan border and therefore represents a threat to Rwanda. However, there is currently no information available on the occurrence and distribution of this defect in this country. Gueule et al. (2015) believe PTD is caused by a bacterium of the Enterobacteriaceae family that develops in coffee beans and produces Isopropyl–2–methoxyl–3–pyrazine (IPMP) and 2-isobutyl-3-methoxypyrazine (IBMP), the compounds that are responsible for the potato taste flavor. It has been proposed that this defect may be produced by the coffee plant as a stress response to feeding damage by Antestia bug, *Antestiopsis thunbergii* (Hemiptera: Pentatomidae) (Jackels et al., 2014). Antestia bug is native to Africa but has spread to the coffee producing countries of Asia such as Pakistan, India, Myanmar, Sri Lanka and Southern China (Rider et al., 2002). When Antestia bug infests the coffee plantation during the onset of rains, it causes significant loss of flowers and severe infestations may prevent the tree from flowering (Waller et al., 2007). Antestia bug infestations are ubiquitous in coffee plantations but occur in patchy distributions possibly due to the insect's semio-chemical mediated re-productive behavior (Cilas et al., 1998). Greathead (1966) observed that Antestia bug moves throughout coffee plantations to find more attractive trees for oviposition. Yield loss due to Antestia bug has been estimated at up to 40% (Ahmed et al., 2016). Although protection of coffee plantations against insect infestations decreases the occurrence of PTD in coffee (Bouyjou et al., 1999), the relationship between cherry damage by Antestia bug and the occurrence of PTD in coffee is unknown.

Coffee berry borer (CBB), Hypothenemus hampei Ferrari (Coleoptera: Scolytidae) is another major coffee pest worldwide (Murphy and Moore, 1990; Damon, 2000). This pest, which is native to Africa, threatens coffee production in the Eastern African region (Jaramillo et al., 2011). According to Damon (2000), CBB affects mature and immature coffee berries, causes significant yield losses and diminishes the final quality of coffee. Though CBB does not affect stem, leaves or branches, it causes premature fall of coffee cherries, arrested berry development or decay (Damon, 2000). Coffee berry borer infests coffee trees, especially Arabica coffee at all altitudes in the major coffee growing areas but higher infestations generally occur at lower altitudes (Rojas et al., 1999). Feeding by CBB also creates holes into mature berries that serve as an entry point for secondary infections by bacteria and fungi, the pathogens responsible for wet rot of coffee berries (Damon, 2000). Although it is possible that the holes also serve as entry points for bacterium that causes PTD in coffee, the relationship between CBB damage and occurrence of PTD has not been investigated.

Pruning and insecticide application are important agricultural practices in coffee farming. Pruning shapes the tree canopy, facilitates field operations and satisfies the needs of crop productions and fruit quality. Pruning also opens up the coffee bushes and creates unfavorable conditions particularly for Antestia bug while also improving insecticide penetration and efficacy (Bigirimana et al., 2012). Previous research has shown that monthly applications of insecticides against Antestia bug combined with visual sorting of infested cherries and floating at the coffee washing station in Burundi, significantly reduce the percentage of PTD contaminated cups (Bouyjou et al., 1993). While pruning and insecticides sprays are known to reduce insect pest density and berry damage, their relationship to the occurrence of PTD in coffee is not well understood.

Intercropping in coffee plantations also can influence Antestia bug and CBB density and damage to coffee plants. For instance, growing coffee under shade of Agroforestry species such as *Leucaena leucocephala*, *Calliandra calothrusus* and *Albizzia sp.* increases Antestia bug severity and the amount of berry damage and losses in Cameroun (Mbondji, 1999). Similarly, intercropping coffee with food crops increases damage caused by Antestia bug in Kenya (Mugo et al., 2013). However, presence of banana plants in coffee farms improves populations of

Corioxenos Antestiae, a natural parasitoid of Antestia bug which in turn reduces the bug population and its damage (Mbondji, 1999). Furthermore, coffee grown under shade of avocado (*Persea americana*) or grevillea (*Grevillea robusta*) reduces infestations of cherries by CBB and it was proposed to introduce such shade trees into sun coffee plantations to mitigate the effects of climate change on CBB and the coffee plant itself (Jaramillo et al., 2013). However, there is no information available on the effects of intercropping on the occurrence of PTD in coffee.

While PTD threatens coffee quality from the African Great Lakes Region, there is currently no information available on the distribution and incidence of PTD in Rwanda and how it relates to the key coffee management practices of pruning, insecticide use and intercropping. The objectives of this study were (i) to evaluate the distribution of PTD in the coffee growing regions of Rwanda and (ii) to determine the relationship between the occurrence of PTD, Antestia bug and CBB damage and key management practices. GPS coordinates were recorded to map the occurrence of PTD in Rwanda and to identify the areas of highest incidence for the defect.

METHODS

Study sites

Data were collected from all coffee growing regions of Rwanda. Three hundred and thirty-eight coffee farms were selected using stratified random sampling (Fig. 1) from a list of coffee growers held by the Rwanda National Agricultural Export Development Board (NAEB). The study was conducted from March 2015 to November 2016, and covered the main agricultural zones in Rwanda, notably Central Plateau and Granitic Ridges, Kivu Lake Side, Congo Nile Watershed Divide, Bu-beruka Highlands, Mayaga and Bugesera and Eastern Plateau.

The criteria for farm selection was two-fold: the field was bearing at the time of the survey, and had sufficient land area to obtain 30 plants along two transects. Surveyed farms were at least 7 m distant one from the other.

Assessment of pest densities, damage and preparation of samples

At each farm, 30 coffee bushes were assessed along two transects (15 plants each) for the density of Antestia bug and berry damage attributed to Antestia or CBB. For each selected coffee tree, Antestia density was assessed by observing the tree for 5 min and counting the number of Antestia bug present. To determine density of berry infestation, three bearing branches (one from the bottom, middle and top of the tree) were sampled. The total number of berries per branch along with the number of those infested by Antestia bug and CBB were recorded. Coffee beans infested by Antestia bug had lateral holes on the berries whereas those damaged by CBB had galleries at the bottom of berries where eggs were mostly deposited (Bigirimana et al., 2018; Damon, 2000).

At each farm, information was collected on the kinds and frequency of fertilizer or pesticides applied and whether the plantation was weeded. We also recorded whether bushes were pruned, and if and what kinds of intercrops were planted in the coffee plantation. Altitude in meters above sea level (masl) was recorded using a Global Positioning System (Thommen Altitronic) at a central point for each farm surveyed.

Evaluation of PTD in coffee samples

Coffee berries from branches near those sampled for pest density and damage were harvested in each field, and a random sample of 3 kg was collected for wet processing, on the basis that it gives enough coffee beans for organoleptic quality test, to determine if they were affected with PTD. Ripe berries were processed through hand palping the day of harvest at six different coffee washing stations – Gakenke, Muhanga, Karongi, Rusizi, Huye and Rwamagana. The coffee washing stations were selected based on proximity to the coffee plantations sampled in the study. We transferred hand palped berries to plastic basins (15 cm diameter and 10 cm height) for fermentation (12 h), after which the coffee beans were dried. During processing, there was no sorting by floatation or removal of insect-infested cherries. Drying was done in the open sun until the moisture content was at 12–15%; humidity was measured using a moisture meter (Tramex mep, Japan). We obtained approximately 500 g of green beans for each sample, of which a random sample of 200 g was roasted using a laboratory-scale roaster (Suntory laboratory and Suncafe laboratory, Kanagawa, Japan). We adjusted the roasting degree to a luminosity (L) value of 22 −23 for all samples. Cupping samples was performed in accordance with the Specialty Coffee Association of America (SCAA) cupping protocol (Iwasa et al., 2015). Professional cuppers tasted the samples, and each cupper was an experienced Licensed Q grader, certified by the Coffee Quality Institute (CQI). Cupping was arranged in such a way that a maximum of 6 cups per sample were compared at a time. Three Licensed Q graders cupped all the samples, each grader was considered as a replicate. Every grader performed a standard session cupping of all the samples in a randomized order as blind analyses. The cupping provided an accurate assessment of whether the sample had or did not have PTD, rather than provide an average cupping score.

Data analysis

Berries damaged by insects were calculated as percentages attributed to Antestia bug or CBB. The percentage of berries damaged by Antestia bug or CBB were averaged for each farm surveyed. Two-tailed t-tests were used to compare insect damages between cropping systems (monocrop versus intercropping), between pruning types (pruned versus unpruned), and between pesticide sprays (sprayed versus un-sprayed). The Chi Square test of independence was used to compare occurrence of PTD among agricultural zones. We used a multiple logistic regression (MLR) model to evaluate the relationship between the occurrence of PTD, Antestia bug density, percentage of berries affected by Antestia bug and CBB and management practices (spraying and intercropping). The MLR estimates parameters of a multivariate explanatory model in situations where the dependent variable is dichotomous and the independent variables are continuous or categorical (Serneels and Lambin, 2001). In this study, the dependent variable, occurrence of PTD in coffee, was binary; the defect was either present or absent. Quantitative explanatory variables were (i) the number of Antestia bug per tree (Antestia bug density), (ii) the percentage of berries affected by Antestia bug and (iii) the percentage of berries damaged by CBB. Categorical variables were spraying (farms were either sprayed or unsprayed) and intercropping (other crops were mixed with coffee or coffee was grown as a monocrop). Sprayed farms were coded 1 and unsprayed 0. Similarly, intercropped farms were coded 1 and monocrop systems 0. If we assume X as a vector of explanatory variables, p the response probability to be modelled will be

$p = \Pr(Y = 1|X)$ for the case of a dichotomous dependent variable, with Y = 0 indicating absence of PTD and Y = 1 meaning presence of PTD.

The MLR model can be written as

$$logit\ (p) = \log\ [\frac{p}{1-p}]$$

or

$$logit\ (p) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5$$

Where α is the intercept, β_1 the coefficient of Antestia bug density, β_2 the coefficient of berry damage by Antestia bug, β_3 the coefficient of berry damage by CBB, β_4 the coefficient of spraying and β_5 the coefficient of intercropping (Rosner, 2011). The probability value was thus estimated as:

$$p = \frac{\exp(\alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5)}{1 + \exp(\alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5)}$$

To facilitate model interpretation, odds ratios were used. This is a measure of association which indicates how much more likely or unlikely it is for the outcome to be present, here the occurrence of PTD, for a set of values of independent variables (Serneels and Lambin, 2001; Rosner, 2011). If there was a change in 1 unit of explanatory variable, for instance if there was increase by one unit in the number of Antestia bug per tree, how much more likely or unlikely would PTD occur? To fit the model, we used the stepwise regression technique and the model with the smallest Akaike's Information Criterion (AIC) was selected (Burnham et al., 2011). Analyses were conducted using SAS (version 9.4) and maps were generated using ArcGIS (version 9).

RESULTS

Site and management characteristics

Altitudes of surveyed farms ranged from 1381 masl to 2135 masl with a mean of 1730.6 ± 7.1 masl (Mean ± SE). Both Antestia bug and CBB infested coffee berries at altitudes ranging from 1381 masl to 2135 masl. Potato taste defect was detected in coffee beans grown at altitudes ranging from 1494 masl to 2135 masl. Coffee was inter-cropped in 29.3% of the farms. Common intercrops were banana (*Musa spp*), silky oak (*Grevillea robusta*), pineapple (*Ananas comosus*), avocado (*Persea americana*), Cedrela spp, colocase (*Colocasia esculenta*), fig tree (*Ficus spp*), papaya (*Carica papaya*) and beans (*Phaseolus vulgaris*). Twenty-one percent of intercropped farms had more than one intercrop (i.e. two or more crops grown with coffee). Sixty-two percent of farms were sprayed with insecticides. The main insecticide used was Imidacloprid (17.8% Imidacloprid, Bayer East Africa, Nairobi, Kenya) and was sprayed at recommended uniform concentrations of 133 ppm. Sprays were conducted three months prior to the survey.

Incidence and distribution of PTD in Rwanda

Potato taste defect was found in 4.9 ± 0.1% of the samples. The defect was distributed throughout the coffee growing areas with the highest concentration in Central Plateau and Granitic Ridges and Eastern Plateau (Fig. 3.1). Incidence of PTD ranged from 0 to 12% ± 0.2% countrywide. The northern part of the country had the highest PTD incidence, averaging 10.11 ± 1.24%. The western province, the widest region growing coffee in Rwanda had the lowest PTD incidence, averaging 1.2 ± 0.1%. The agroecological zone in Central Plateau and Granitic Ridges accounted for 43.2 ± 2.8% of all observed PTD samples in the country. The test of association between agro-ecological zone and occurrence of PTD in coffee indicated a highly significant relationship between agro-ecological zone and occurrence of PTD ($\chi^2 = 14.32$, $P < 0.01$, $DF = 4$). However, occurrence of PTD was not significantly related to altitudes ($\chi^2 = 0.59$, $DF = 3$, $P > 0.05$).

Antestia bug density and damage

Antestia bug was detected on 98.7% of the farms and it was distributed throughout the whole country. The bug density ranged from 0 to 6 per tree with a mean of 0.5 ± 0.1. Damage caused by Antestia bug ranged from 0 to 81% with a mean of 11.43 ± 0.64%. Pruning did not significantly affect the density of Antestia bug in coffee (*T* = 0.95, *P* = 0.34, *DF* = 334) with a mean of 0.54 ± 0.25 and 0.58 ± 0.06 Antestia bug in pruned and unpruned farms respectively. However, unpruned coffee bushes had significantly higher damage (*T* = 5.8, *P* < 0.001, *DF* = 334) due to Antestia bug than pruned coffee bushes, with an average of 11.2 ± 0.3% and 9.5 ± 1.2% damage (Table 3.1), respectively. Similarly, sprayed farms had significantly lower Antestia bug damage than unsprayed farms (*T* = 3.04, *P* < 0.01, *DF* 332), the former with a mean of

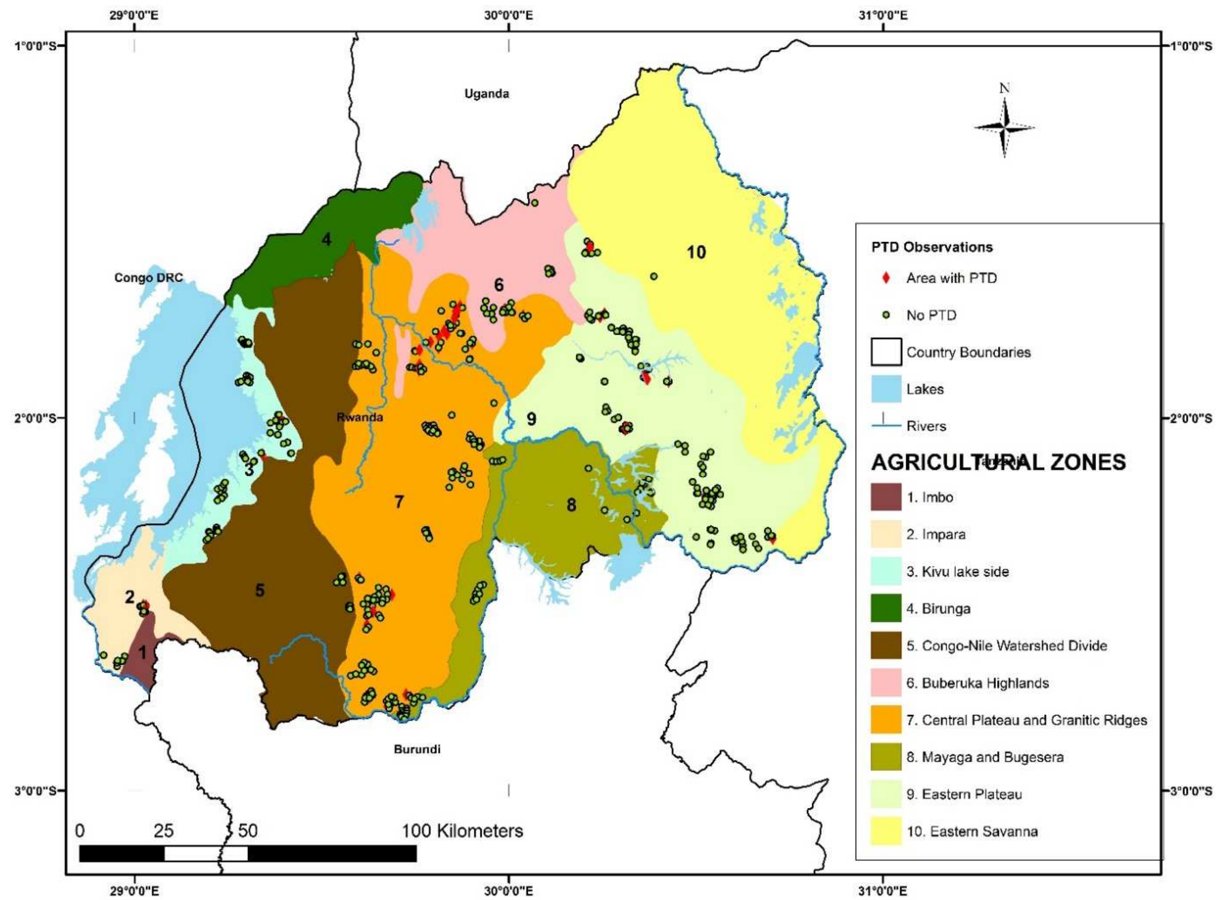

Figure 3. 1 Map showing areas sampled and the distribution of PTD in the coffee growing regions of Rwanda. The defect is distributed throughout the coffee growing regions of the country with the highest incidence in Central Plateau and Granitic Ridges (Zone 7) and Eastern Plateau (Zone 9).

15.71 ± 1.07% and the latter with a mean damage of 11.35 ± 1.25%. Significantly higher infestation by Antestia bug was observed in monocrop systems, with an average of 11.7 ± 0.4% damage compared to 9.5 ± 0.5% in intercropped coffee bushes (*T* = 6.37, *P* < 0.0001, *DF* = 334).

Coffee berry borer density and damage

Coffee berry borer was recorded in 25% of surveyed farms. The extent of damage from the beetle ranged from 0 to 27%, with a mean of 2.41 ± 0.21%.

Table 3. 1. Effect of pruning, intercropping and insecticide treatment on Antestia bug damage

Management practices	Mean damage (%)	Standard error (SE)	Sample size (n)	P value
Pruned	9.5	1.2	249	< 0.0001
Unpruned	11.2	0.3	87	-
Intercropped	9.1	0.5	98	0.0002
Non-Intercropped	11.7	0.4	238	-
Insecticide treated	10.2	0.9	208	< 0.0001
No insecticides	10.4	0.3	128	-

Pruning did not influence CBB severity, pruned and unpruned coffee trees had a mean damage of 2.41 ± 0.22% and 2.39 ± 0.17%, respectively (*T* = 0.9, *DF* = 334, *P* > 0.05). Similarly, spraying did not significantly influence CBB damage, sprayed and un-sprayed farms had a mean damage of 2.15 ± 0.27% and 1.97 ± 0.14%, respectively (*T* = 1.12, *DF* = 334, *P* > 0.05).

Table 3. 2. Effect of insecticides treatment, intercropping and pruning on coffee berry borer infestation

Management practices	Mean damage (%)	Standard error (SE)	Sample size (n)	P value
Pruned	2.41	0.22	249	< 0.05
Unpruned	2.39	0.17	87	-
Intercropped	1.57	0.25	98	0.01
Non-Intercropped	2.75	0.15	238	-
Insecticide treated	2.15	0.27	208	< 0.05
No insecticides	1.97	0.14	128	-

However, intercropping significantly influenced damage due to CBB, intercropped farms had significantly less damage by CBB than monocrop systems with a mean damage of 1.57 ± 0.25% and 2.75 ± 0.15%, respectively (T = 8.79, DF = 334, P < 0.01). Damage due to CBB was not the same across the co□ee growing regions; the northern part had the least da-mage and the western the highest, with a mean damage of 1.25 ± 0.02% and 3.72 ± 0.13%, respectively (Table 3. 2).

Insects damage in relations to the occurrence of PTD in co□ee

There was a significant relationship between insect damage, specifically damage from Antestia bug and the occurrence of PTD in co□ee. The probability that PTD occurs, increased as the damage density due to Antestia bug increased (Fig. 3. 2). An increase in the density of Antestia bug per tree also increased the probability of PTD occurring in co□ee samples (Fig. 3. 3). However, having a higher number of berries damaged by CBB did not increase the probability of PTD occurrence (Fig. 3. 2).

Management practices in relation to the occurrence of PTD

Preliminary analysis showed low levels of multicollinearity between independent quantitative variables (Antestia density, berry damage by Antestia bug and percentage cherries infested by CBB). The correlation between density of Antestia bug and damage caused by this insect was weak and not significant ($r = 0.06$, $P > 0.05$). Similarly, the correlation between Antestia bug density and co□ee bean damage by CBB was very weak ($r = -0.00144$, $P > 0.05$) and also not significant. Finally, the correlation between damage caused by CBB and the one attributed to Antestia bug was also low and not significant ($r = 0.09$, $P > 0.05$).

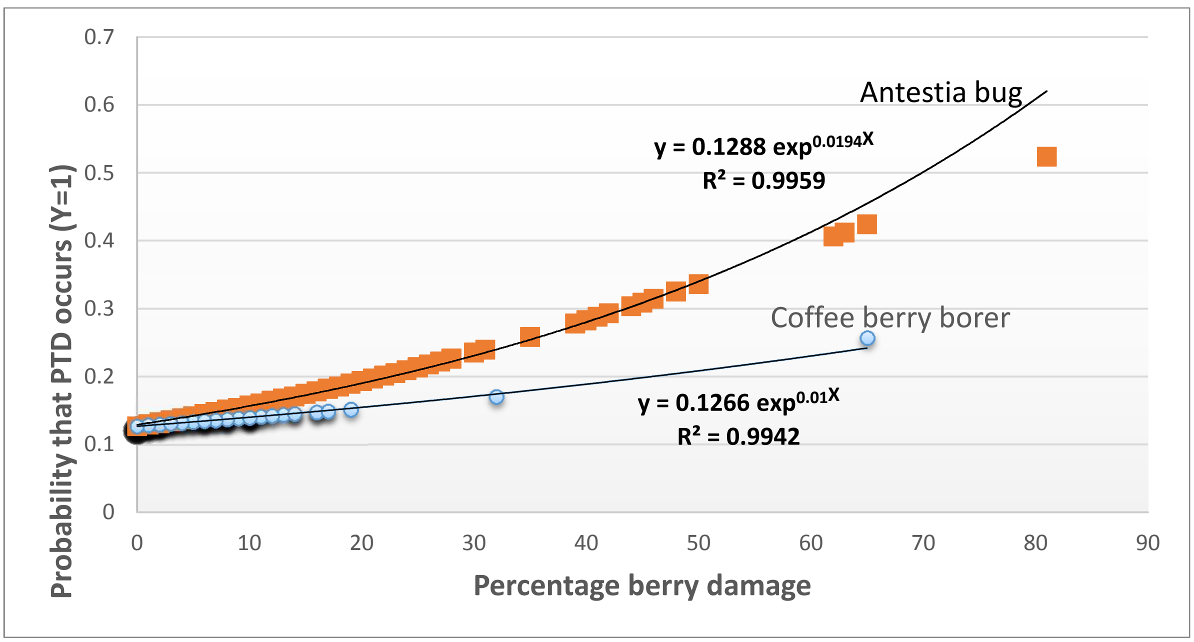

Figure 3. 2 The relationship between Antestia bug damage and occurrence of PTD controlling for other factors and the relationship between CBB damage and the occurrence of PTD in coffee controlling for other factors.

PTD increased as the % berries affected by Antestia increased. However, increase in the number of berries damaged by CBB did not significantly increase the probability that PTD occurs ($Y = 1$).

Table 3. 3. Stepwise multiple logistic regression results using intercropping, Antestia damage, pruning and Antestia density as predictors for occurrence of PTD in coffee

Variable	Para. estimate	Standard error	Wald χ^2	Pr > χ^2	Odds ratio
Intercept	-1.816	0.523	12.06	0.0005*	
Antestia density	0.3085	0.135	5.23	0.0222*	1.36
Intercropping	0.5516	0.552	2.27	0.1322	
Pruning	-1.0453	0.499	4.39	0.0361*	0.35
Antestia damage	0.0268	0.012	4.85	0.0277*	1.03

Model likelihood ratio: $\chi^2 = 14.5851$, df = 4, Pr > $\chi^2 = 0.0056$; AIC = 241.216; Pseudo – R^2 = 0.329, Coefficients with P values marked with * indicate significance at $P < 0.05$.

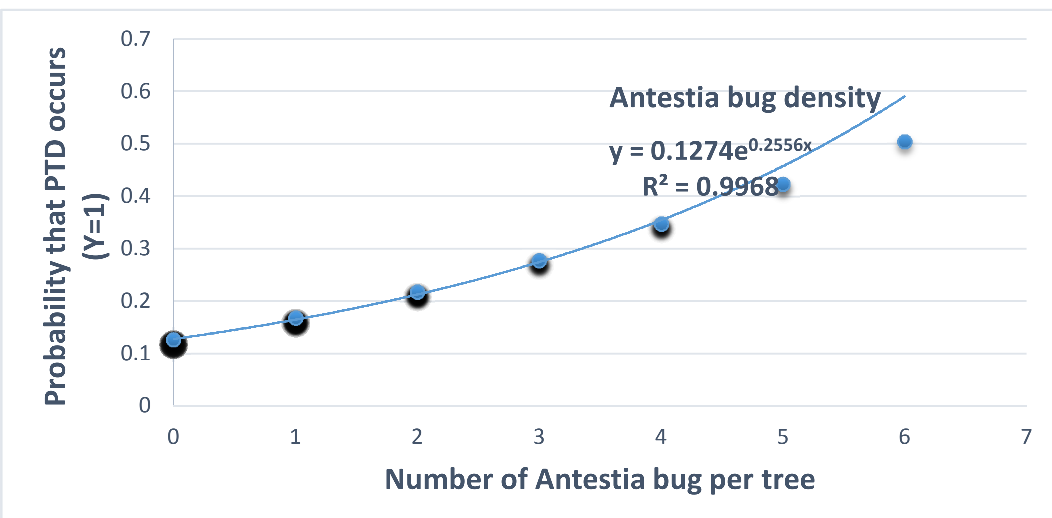

Figure 3. 3 The relationship between the Antestia bug density and the occurrence of PTD in coffee controlling for other factors.

The model selected for multiple logistic regression had the smallest AIC value, with AIC = 241.216 in the stepwise regression analysis (Table 3. 3).The results showed that occurrence of PTD increased with increasing density of Antestia bug and the extent of damage caused by this pest. The likelihood of detecting PTD decreased in pruned plots compared to unpruned coffee farms. The estimated odds to find PTD were 35% higher in unpruned plots compared to pruned farms. Results also indicated that an increase of 1 damaged coffee bean increased the probability of detecting PTD by 97% (the inverse of odd ratio of 1.03), with the threshold ranging from 94.7% to 99.4%. Similarly, an increase of 1 Antestia bug per tree increased the probability of detecting PTD by 73% (the inverse of odd ratio of 1.36), with the threshold ranging from 56.3% to 95.6%. Intercropping did not affect the occurrence of PTD; the likelihood of detecting PTD was not significantly different between intercropped farms and monocrop systems (Table 3.3).

DISCUSSION

Potato taste defect was distributed throughout the coffee producing regions with the highest incidence in the Central Plateau and Granitic Ridges and the Eastern Plateau. These agro-ecological zones are characterized by an altitude ranging from 1500 to 1900 m, 1400 to 1700 m and 1400 to 1800 m, respectively and a rainfall of 1200 to 1300 mm, 1050 to 1200 mm and 900 to 1000 mm, respectively (Verdoodt and Van Ranst, 2003). Additionally, these are the areas with the highest temperature among the coffee growing regions in Rwanda (Verdoodt and Van Ranst, 2003). Such climatic characteristics favor a high population of the Antestia bug; which prefers a warm climate where it causes high infestation in the field, as a result of the interaction between the pest, temperature and the moisture (Le Pelley, 1968).

This study also showed a strong association between Antestia bug damage and PTD. The relationship between Antestia bug and PTD has also been reported by Bouyjou et al. (1993) and

Cilas et al. (1998). Jackels et al. (2014) observed Isopropyl–2–methoxyl–3–pyrazine (IPMP) and 2-isobutyl-3-methoxypyrazine (IBMP), the compounds responsible for PTD inside ground beans with visible damage of Antestia bug in the samples that were already detected with PTD. The results of this study were based on 6 cups per sample, tested each by 3 different graders. Every grader was considered as a replicate. Further research is needed to confirm adequate coffee sample size and number of cups.

The study also showed that CBB damage was not significantly related to the occurrence of PTD in coffee. Despite the absence of association with PTD, CBB causes massive yield losses through weakening affected mature berries and making them vulnerable to infection and further pest attack (Mariño et al., 2017). This pest creates holes in the attacked green berries and is also responsible for significant reduction in quality of the final coffee product (Fernandes et al., 2011). Two species within the Enterobacteriaceae family, namely *Erwinia stewartii* (Smith) and *E. salicis* (Day) are responsible for wet rot in the mesocarp of immature berries infested by CBB (Damon, 2000). Although CBB creates lesions on the coffee berries that serve as an entry site for secondary infection by bacteria and fungi, it does not appear to be a causal factor for PTD. The mechanisms of PTD infections in coffee beans need further investigations.

Some management practices were associated with the occurrence of PTD in coffee. For example, samples from unpruned farms had more PTD incidence than samples from farms where pruning was practiced. This is likely related to the higher Antestia bug damage in unpruned coffee farms than in pruned coffee trees. Pruning opens up the coffee bushes, hence creates unfavorable conditions for the bug while also improving pesticide penetration and efficacy (Bigirimana et al., 2012; Ratnadass et al., 2012).

Occurrence of PTD was not significantly different in intercropped coffee bushes compared to the monocrop systems. Though several intercrops were recorded in the study, the main ones were banana (*Musa spp*) and Agroforestry species (silky oak (*Grevillea robusta*), avocado (*Persea americana*), *Cedrela spp*, fig tree (*Ficus spp*) and papaya (*Carica papaya*)). As most coffee in Rwanda is produced on smallholdings, this offers an opportunity for efficient use of land resources. Since banana is the primary intercrop in coffee farming systems, potential benefits of this practice should be evaluated in Rwanda. It is likely that coffee-banana intercropping improves yield of both crops, increases coffee quality by increasing size and weight of coffee beans and also reduces proportion of imperfect berries (Muschler, 2001). Intercropping coffee with bananas was also reported to be more profitable than coffee mono-cropping (Van Asten et al., 2011).

Intercropping coffee with agroforestry species may offer benefits as well. According to Nesper et al. (2017), shade coffee maximizes yield and quality through maintaining balance between optimal temperature in shaded environments and optimal photosynthetic rates in unshaded environments. Shade coffee plantations are also valued for biodiversity conservation and provision of ecosystem services (Tscharntkeet et al., 2011). Because intercropping was not associated with occurrence of PTD in coffee, benefits of this practice to producing high quality coffee need further investigation.

Antestia bug density in Rwanda was quite high, ranging from 0 to 6 bugs per tree. The economic threshold of this insect pest is as low as 1 - 2 bugs per tree in East Africa, due to the importance of damage and also depending on the country (Gesmalla et al., 2016). In the study, the damage due to Antestia bug ranged from 0 to 81%, indicating the urgent need for pest control. Although the correlation between density of Antestia bug and damage caused by this insect was weak and not significant, this insect has aggregative behavior and moves around to find the most suitable bushes for oviposition (Greathead, 1966). Given PTD concentration in Central Plateau, Granitic Ridges and Eastern Plateau and its association with Antestia damage, it is important to intensify control of Antestia bug in this region aimed at eliminating PTD or significantly reducing it to non-problematic levels.

CONCLUSION

PTD was distributed throughout most of the coffee growing regions of Rwanda with the highest incidence in Central Plateau, Granitic Ridges and Eastern Plateau. Occurrence of PTD was significantly related to Antestia bug density and damage but not to CBB infestations. There is need to effectively control Antestia bug in coffee plantations and to understand mechanisms of PTD infection in order to reduce or eliminate its occurrence.

CHAPTER 4: POPULATION DISTRIBUTION AND DENSITY OF ANTESTIA BUG, *ANTESTIOPSIS THUNBERGII* (GMELIN, 1970) (HEMIPTERA: PENTATOMIDAE) IN THE COFFEE GROWING REGIONS OF RWANDA IN RELATION TO CLIMATIC VARIABLES

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Submitted to: In revision for publication to Crop Protection (March 4th, 2019)

ABSTRACT

Antestia bug, *Antestiopsis thunbergii* (Gmelin, 1970) (Hemiptera: Pentatomidae) is one of the most damaging coffee pest worldwide, except in central and south America. A two – year study was conducted to assess the distribution and the density of Antestia bug in the coffee growing regions of Rwanda, and to determine the effects of climatic variables on density of Antestia bug. Over 200 farms were surveyed in 2016 and 190 farms in 2017. The density and damage of Antestia bug were quantified and climatic variables including temperature, rainfall, wind speed and relative humidity were recorded. Antestia bug was distributed in all coffee growing regions of Rwanda, during both study periods. Over both years, the highest density was recorded in the northern region of the country while the lowest was recorded in the eastern region. The economic damage due to Antestia bug ranged from 0 to 92% and from 0 to 81% in 2016 and 2017, respectively. Stepwise multiple linear regression analysis indicated that temperature and rainfall significantly influenced the pest density. However, wind speed was not significantly associated with the density of Antestia bug. Principal component analysis (PCA)

indicated three distinct groupings. Coffee regions that received higher, regular rains had a higher population of Antestia bug than where rains were erratic. Similarly, the areas where temperature was consistently high had the highest densities of Antestia bug. Wind speed was highly and negatively related to principal component one. This study indicated the need to intensify control measures against Antestia bug. Furthermore, the impact of climate change on density and damage of Antestia bug and other coffee pests need further research.

INTRODUCTION

Coffee production is critical to the economy of several countries in Africa, Asia, and tropical America (Teodoro et al., 2008). Although there are several constraints to coffee production, including prolonged periods of water stress, low soil fertility in many areas, diseases, and insect pests, Antestia bug is probably the most important (Ahmed et al., 2016). Antestia bug, *Antestiopsis thunbergii* (Gmelin, 1970) (Hemiptera: Pentatomidae) is a direct pest of coffee worldwide, except in central and south America (Babin et al., 2018). Antestia bug is native to Africa but has spread to the coffee growing regions of Asia; such as China, Sri Lanka, Myanmar, Pakistan, and India (Rider et al., 2002). Both the nymph and adult stages of the bug feed on vegetative and fruiting parts of the coffee plant, thereby causing yield loss and negatively affecting quality of coffee beans (Ahmed et al., 2016). When Antestia bug feeds on flower buds, they become black or brown; which impairs fruit set (Mugo, 1994). Feeding on leaves at the growing point results in leaves that are scarred and distorted (Abebe, 1987). Waller et al. (2007) report that Antestia bug causes a significant loss of flowers when it is present in coffee plantations during the onset of rains and severe infestations may prevent the tree from flowering. The economic threshold for Antestia bug is as low as one to two bugs per tree, depending on the damage levels and the country (Mugo et al., 2013). Yield loss due to Antestia bug has been estimated at up to 40% (Ahmed et al., 2016).

Antestia bug damage has also been associated with the potato taste defect (PTD) in coffee (Becker et al., 1988; Gueule et al., 2014; Jackels et al., 2014). Potato taste defect is a potato – like smell and taste found in green and roasted coffee beans and in brewed cups of coffee. This defect is an economic concern for producers, because it reduces the coffee prices paid to farmers and cooperatives. Additionally, international buyers may generally reduce prices they are willing to pay for coffee coming from regions suspected to have PTD, whether or not there is specific evidence of PTD in a given lot of coffee. Although the PTD mechanisms of infection into the coffee beans are not yet known, it has been reported that protection of coffee plantations against Antestia bug reduce the incidence of PTD (Bouyjou et al., 1993).

Climatic variables may vary from one region to another and this may affect the density and damage of the pest differently, not only during some parts of the year but also in different agro-ecological zones (Bellard et al. 2012). Climatic variables affect insect pests directly, through modification of the physiological or behavioral systems, or indirectly through modification of other factors such as the host plant and natural enemies (Bale et al., 2002). Hodkinson (1999) believes there is a link between climate and pest distribution, specific to every species and dependent on the ecophysiology; and which explains its temporal and spatial distribution in the given agro-ecological zone. Amongst the climatic variables, temperature plays a direct role in pest distribution and density. For example, Antestia bug survives and develops within a temperature range of 14.6 – 32.9^{o} C, but the best growth and reproduction rates are obtained within a temperature range of 19 – 25^{o} C (Ahmed et al., 2016).

Similarly, rainy periods influence the distribution and density of insect pests. Liebhold et al. (1993) reported a positive correlation between rainfall levels and grasshopper densities; however, in the areas that received above-average rainfall, grasshopper populations decreased. Furthermore, rainfall increased psyllid population in the Eucalyptus forests to the extent of causing outbreak of senescence – inducing leaf feeders (Gherlenda et al., 2016). Relative humidity also influences density of insect pests such that, for coffee berry borer for example, it accounted up to 9.5% of the pest density variations (Teodoro et al., 2008). Additionally, (Kirkpatrick, 1937) reported that Antestia bug infestations increase with humidity levels in the coffee growing regions of Kenya.

Wind speed and direction also affect the distribution pattern of insects and may cause pest outbreak in some parts of the world (Moser et al., 2009). William et al. (2007) suggest that upwind migrations and wind directions may help in pest dispersal. The pollen beetle for example (*Meligethes aeneus*) was shown to be influenced by the wind speed; it explained about 75% of the variance in the pest density (Moser et al., 2009). Lewis (1969) also showed that the density of pollen beetle (*Meligethes aeneus*), aphids, and syrphids increased two-to three times in the areas where the wind speed exceeded 2.5 km/h.

Despite the availability of climatic data and massive yield losses and coffee quality issues that Antestia bug causes, particularly through the association with the potato taste defect, there is currently no information available on Antestia bug density and distribution in Rwanda; and how they relate to climatic variables. The objectives of this study were twofold: (i) to evaluate the density and the distribution of Antestia bug in the coffee growing regions of Rwanda, (ii) to determine the effects of climatic variables on the density of Antestia bug.

MATERIALS AND METHODS

Study sites

This study was conducted in all the coffee growing regions of Rwanda, i.e Lake Kivu side, Impala, Congo Nile Watershed Divide, Central Plateau and Granitic Ridges, Mayaga and Bugesera, Eastern plateau and Eastern savannah. Surveyed farms were selected using stratified random sampling from the list of coffee growers held by the Rwanda National Agriculture Export Development Board (NAEB). The altitude of surveyed farms ranged from 1381 to 2135 meters above sea level (masl). Over 200 farms were surveyed in the first year and nearly 190 in the second year. The study was conducted from March to July 2016 and was repeated from May to July 2017. In the study, we collected information about the age of the coffee plantations, whether the plantation was weeded or whether pesticide was applied and at which dates. We also recorded if shade trees or intercrops were used and whether the coffee bushes were pruned. The requirements for farm selection were threefold: (1) the farm was bearing at the time of the survey, (ii) the field was sufficiently large to obtain 30 plants along the diagonals and (iii) farms were at least 7 km distant one from the other, to avoid the problem of spatial auto-correlations (Overmars et al., 2003).

Assessment of pest density and damage

At each farm, 30 coffee bushes were assessed along two transects (15 plants each) for the presence or absence of Antestia bug. For each selected coffee tree, we assessed Antestia bug density through direct count, by observing the coffee tree for 5 min and counting the number of Antestia bug present. This method of assessing Antestia bug density through direct count has been previously used by Bigirimana et al. (2019) and by Azrag et al. (2018) and is effective because Antestia bug is relatively a big insect.

To determine infested berry density, three bearing branches (one at the bottom, middle, and top of the tree) were sampled. Assessment of damage density was done using damage symptoms where coffee beans infested by Antestia bug had lateral holes on the berries. The total number of berries per branch along with those infested by Antestia bug were counted. Altitude in meters above sea level (masl) was recorded using a Ground Positioning System (Thommen Altitronic) at a central point for each farm surveyed.

Climatic variables

Climatic variables, i.e. temperature, rainfall, wind speed, and relative humidity were obtained from weather stations across the coffee growing regions of Rwanda. The number of weather stations in the coffee growing areas varied between agricultural zones. In the Eastern region, we used climatic data from Kirehe and Ngoma weather stations. Kirehe is located in Kirehe district, at latitude of -1.968, longitude of 30.137 and altitude of 1481 masl while Ngoma is located in Ngoma district, at latitude of -1.968, longitude of 29.53 and altitude of 1602 masl.

In the Southern region, we used data from Rubona and Byimana weather stations. Rubona is located in Huye district, at a latitude of -1.58, longitude of 29.89 and altitude of 2396 masl while Byimana is in Ruhango district, at latitude of -1.968, longitude of 30.137 and an altitude of 2396 masl. In the Western region, we used data from Kamembe, Nyamasheke and Rubengera weather stations. Kamembe is located in Rusizi district, at latitude of -1.68, longitude of 29.26 and altitude of 1556 masl, Nyamasheke weather station is in Nyamasheke district, at latitude of -2.34, longitude of 29.09 and altitude of 1471 masl while Rubengera is in Karongi district, at an altitude of 1700 masl, latitude of -2.07 and longitude of 29.42. Climatic data from the weather stations closest to the farms surveyed were used in the study. In most cases, we used climatic data on surveyed farms from weather stations located in the same regions. Because variations in climatic data among weather stations were small, we used average monthly data per each variable for each year of the study in both 2016 and 2017.

Statistical analysis

The percentage berries infested by Antestia bug along with the bug density were averaged for each farm surveyed. Mean climatic data were also calculated for monthly measurements of temperature, rainfall, relative humidity and wind speed. To test whether Antestia bug density and damage were significantly different between agricultural zones, we used one-way analysis of variance (ANOVA) for both variables. To this end, the percentage infested berries and the bug density were subjected to the normality test using Shapiro Wilk test and the homogeneity of variance test using Levene's test. The one-way ANOVA test was followed by Tukey's multiple comparison procedure at $P = 0.05$.

We also used the stepwise multiple regression analysis to assess the influence of climatic variables on the density of Antestia bug in the coffee growing regions of Rwanda using the following model:

$$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \epsilon$$

Where Y is the bug density, α : the intercept, β_1 the coefficient of temperature (X1), β_2 the coefficient of rainfall (X_2), β_3 the coefficient of windspeed (X_3) and β_4 the coefficient of relative humidity (X_4) and ϵ is the error term. Model selection was done using Bayesian Information Criterion and the model with the smallest (BIC) was selected (Aho et al., 2014).

Given that climatic variables were inter-related, a phenomenon that could be predicted because of the nature of the climatic data, for example rainfall and relative humidity, we sought to identify the ones that explained the most variations. To this end, we used the Bartlett's test of sphericity to test the null hypothesis that the correlations among the climatic variables were zero or that the correlation matrix was an identity matrix (Raykov and Marcoulides, 2008) for both the climatic data and Antestia bug density in both 2016 and 2017. We observed that the Bartlett's test was significant (χ^2 = 577.603, DF = 10, P < 0.000) which indicated that the climatic variables and the Antestia bug density in 2016 were related. We also subjected the same data set to Keiser – Meyer – Olkin (KMO) measure of sampling adequacy and observed that KMO was equal to 0.627. Both the Bartlett test and KMO index indicated that the data set in 2016 was appropriate for Principal Component Analysis (PCA).

We also used the Bartlett's test and KMO index for the data set in 2017 and similar to 2016, we observed that the Bartlett test was significant (χ^2 = 707.694, DF = 10, P < 0.000) and that the KMO was equal to 0.664. Both the Bartlett's test and KMO index indicated that the data set in 2017 was also appropriate for PCA. Both data sets were then analyzed using PCA and relationships between climatic variables, locations, and Antestia bug densities were visualized using the PCA score plots. Statistical analyses were conducted using SAS (Version 9.4, SAS Institute, Cary, North Carolina, USA).

RESULTS

Antestia bug distribution across the coffee growing regions of Rwanda

In both 2016 and 2017 when the surveys were conducted, Antestia bug was found in all the coffee growing regions of Rwanda. In 2016, the density ranged from 0 to 8 with a mean density of 0.75 ± 0.19 (Mean ± SE) Antestia bugs per tree. We found significant differences between locations for Antestia bug density ($F = 9.36$, $DF = 3, 20$, $P < 0.01$) during the first year of the study, 2016. The highest density was recorded in the North with the mean of 1.46 ± 0.28 bugs per tree and the lowest in the East with the mean density of 0.27 ± 0.04 bugs per tree (Fig. 4. 1).

Similarly, significant differences were observed between locations in 2017, which was the second year of the study ($F = 27.09$, $DF = 3, 20$, $P < 0.01$). Consistent with the first year, the highest density of Antestia bug was recorded in the North, with a mean density of 1.72 ± 0.11 Antestia bugs per tree and the lowest in the East with a mean density of 0.58 ± 0.04 Antestia bugs per tree. The exception was in the West where the density was 0.84 ± 0.15 Antestia bugs per tree in 2016 and decreased to 0.43 ± 0.16 Antestia bugs per tree in 2017. Additionally, in 2017, the bug density ranged from 0 to 9 Antestia bugs per tree with an overall mean density of 0.67 ± 0.24.

Although efforts were made to sample the same coffee farms, it was not possible in most cases, hence, neighboring farms were sampled. We therefore assumed that data on bug density in 2016 were independent from those in 2017.

Antestia bug damage across the coffee growing regions of Rwanda

Results indicated that Antestia bug damage was consistently higher in 2017 compared to 2016 in all coffee growing regions of Rwanda except in the East (Fig. 4. 2). In 2016, all surveyed farms across the coffee growing regions of Rwanda had varying levels of berry damage by Antestia bug. Significant differences were observed between regions in berry damage by Antestia bug ($F = 10$; $DF = 3, 8$; $P < 0.01$).

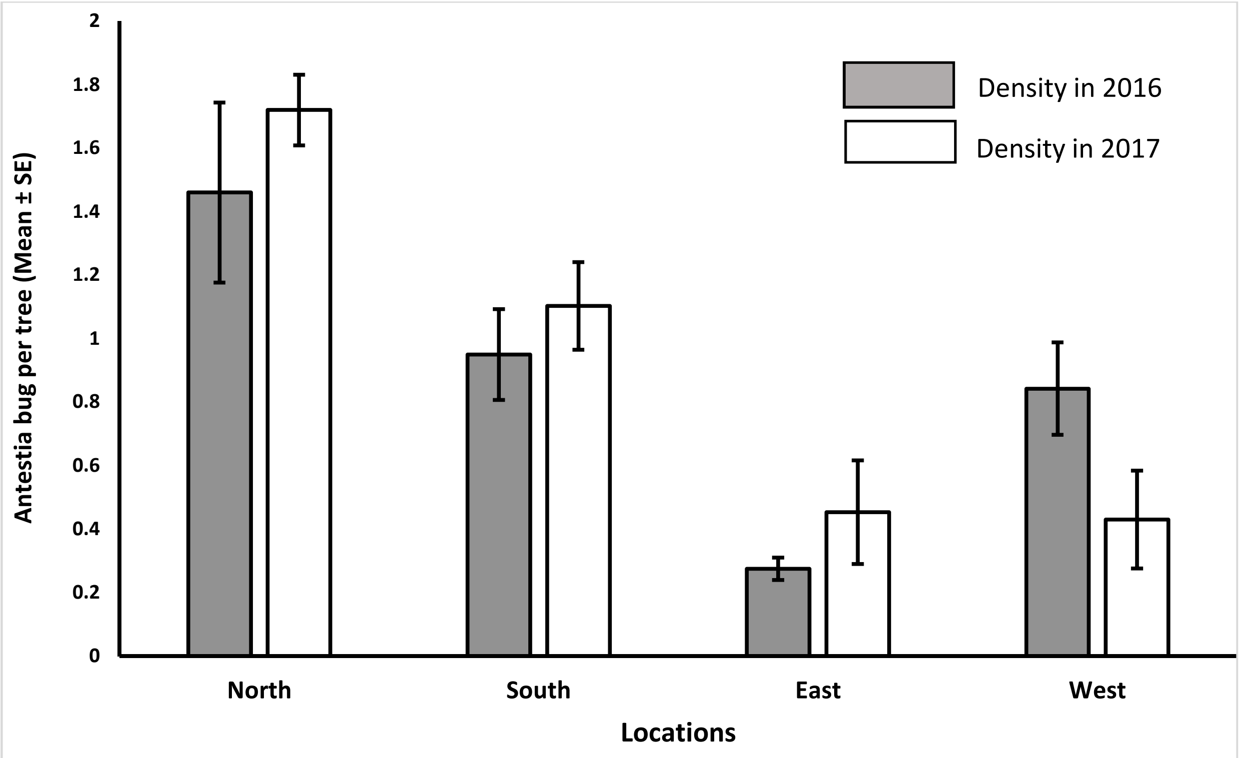

Figure 4. 1 Antestia bug density in different coffee growing regions of Rwanda. Bars represent the standard error of means.

The percent berry damage ranged from 0 to 92% damaged coffee beans with a mean of 4.39 ± 0.69. The highest berry damage was recorded in the East with the mean damage of 7.51 ± 0.83 percent-damaged coffee beans and the lowest in the South with the mean damage of 2.93 ± 0.40 percent-damaged coffee beans (Fig. 4. 2).

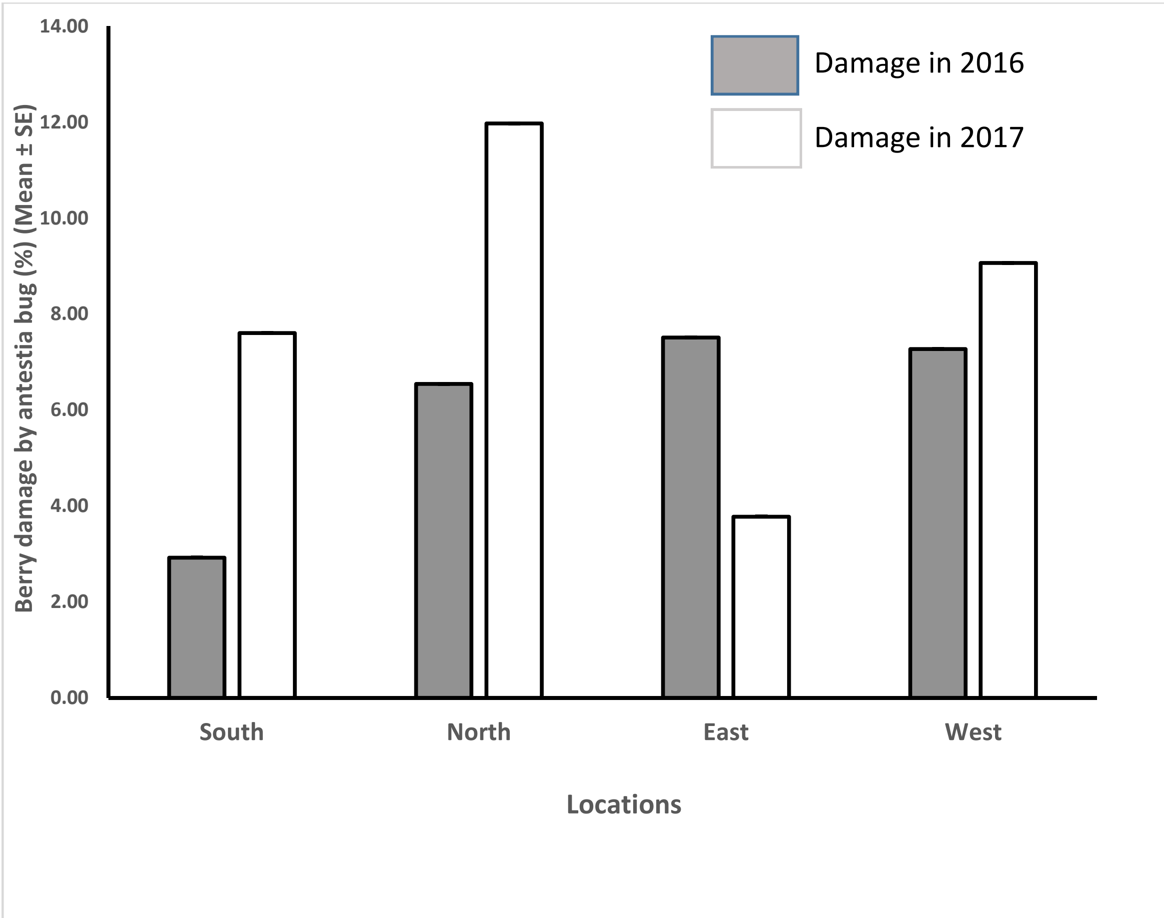

Figure 4. 2 Percent berry damage by Antestia bug in different coffee growing regions of Rwanda. Bars represent the standard error of means.

Significant differences in berry damage caused by Antestia bug were also observed between locations in 2017 ($F = 10.65$, $DF = 3, 8$, $P < 0.01$). The highest berry damage was recorded in the North, with the mean of 11.97 ± 0.45 percent - damaged coffee beans and the lowest in the East, with the mean of 3.78 ± 0.67 percent - damaged coffee beans. The percent – damaged beans ranged from 0 to 81% with the mean of 11.05 ± 0.16 across the country. Consistently with the first year of study, the percent - berry damage by Antestia bug was higher in 2017 compared to 2016 in all the three locations except in the East where it was low in 2017 compared to 2016, with a mean of 3.71 ± 0.67 compared to 7.51 ± 0.83 percent – infested berries in 2016.

Table 4. 1 Stepwise regression of climatic variables on density of Antestia bug in the coffee growing regions of Rwanda

		Year 1		
Variable	**Par. estimate**	**Stand. error**	**t Value**	**Pr > F**
Intercept	124.367	36.734	11.46	0.0009**
Wind speed	1.136	0.594	3.66	0.0571^{ns}
Temperature	6.636	1.943	11.66	0.0008**
Rainfall	0.187	0.048	15.28	0.0001**
Relative humidity	1.122	0.141	63.02	<.0001**
		Year 2		
Variable	**Par. estimate**	**Stand. error**	**t Value**	**Pr > F**
Intercept	6.854	7.555	-0.91	0.3655^{ns}
Temperature	6.034	2.696	2.24	0.0264*
Rainfall	0.021	0.007	-3.07	0.0024**
Wind speed	0.560	0.407	-1.38	0.1706^{ns}
Relative humidity	0.046	0.017	2.71	0.0073**

Parameters whose P values are marked with * indicate significance at P < 0.05 and those marked with ** indicate significance at P < 0.01.

Relationships between climatic variables and Antestia bug density

The model selected for multiple linear regression had the smallest Bayesian Information Criterion (BIC) with BIC = 814.879 in 2016. The stepwise multiple linear regression model accounted for 31.5% of the variability in Antestia bug density as a function of climatic variables. In this model, variables including temperature ($P = 0.0008$), rainfall ($P = 0.0001$) and relative humidity ($P < 0.0001$) significantly influenced Antestia bug density in the coffee growing regions of Rwanda (Table 4.1) in 2016.

In 2016, only wind speed did not significantly influence bug density in the coffee growing regions of Rwanda ($P = 0.0571$). Increase in temperature and relative humidity also increased Antestia bug density. However, rainfall was negatively related to the Antestia bug density.

Similar to 2016, the model selected for multiple linear regression in 2017 had the smallest BIC, with BIC = 715.18. The model selected in stepwise multiple linear regression accounted for 29.6% of the variability in Antestia bug density as a function of climatic variables. In 2017, variables including temperature ($P < 0.05$), rainfall ($P < 0.01$) and relative humidity ($P < 0.01$) significantly affected the density of Antestia bug in the coffee growing regions of Rwanda (Table 4. 1). Consistently with the previous year, wind speed did not significantly affect the bug density ($P > 0.05$). Results also indicated that increase in temperature and relative humidity increased the bug density. However, rainfall was negatively related to Antestia bug density.

The principal component analysis indicated that the first and the second principal components (PC) accounted for approximately 52.74% and 22.88% of the variance respectively (Fig. 4.3). Wind speed was highly and negatively related to the PC1. However, temperature was positively and highly correlated with PC1 (table 4.2). Other climatic variables such as rainfall and relative humidity were positively and highly correlated with PC2 (table 4.2). The correlation between Antestia bug density and wind speed was low (r = 0.15) but significant ($p < 0.05$). However, rainfall, temperature and relative humidity were positively related to Antestia bug density to varying degrees. The correlation between Antestia bug density and rainfall was low (r = 0.23) but significantly different from 0 ($p < 0.05$). Similarly, the correlation between temperature and Antestia bug density was low (r = 0.17) but significantly different from 0 ($p < 0.05$). In contrast, the correlation between relative humidity and Antestia bug density was moderate (r = 0.47) and also significantly different from 0 ($p < 0.01$). Climatic variables and Antestia bug density in different regions were grouped differently using PCA. This could be confirmed by our observation at the field level.

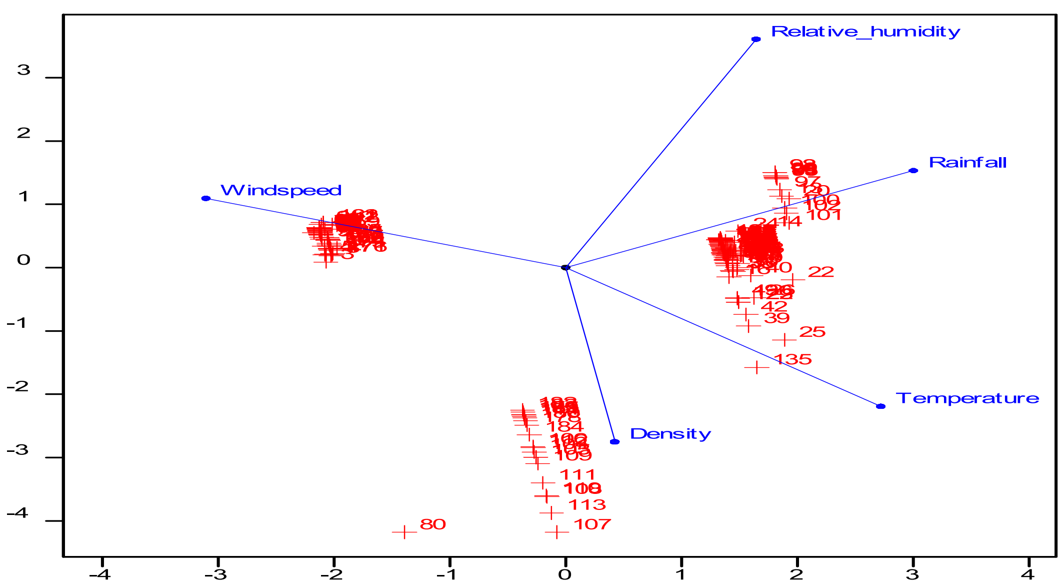

Figure 4. 3 Biplot from principal component analysis of 205 samples collected across the coffee growing areas of Rwanda. The first and the second component accounted for 52.74% and 22.88% of the total variation, respectively.

For example, coffee regions that received higher, regular rains had a higher population of Antestia bug than where rains were erratic. Similarly, the coffee growing areas where temperature was consistently higher had higher densities of Antestia bug. There were three clear distinctions in the data set (Fig. 4. 3). Regions where the wind speed was high tended to have a high density of Antestia bug.

During the second study year, the principal component analysis showed 57.79% of the variations were explained by PC1 and 29.25% by PC2 (Fig. 4. 4). Consistent with year 1 of the study, wind speed was highly and negatively related to PC1 and temperature was highly but positively related to PC1. Other climatic variables such as rainfall and relative humidity were positively related to PC2. Density was highly correlated with PC2 (table 4. 2)

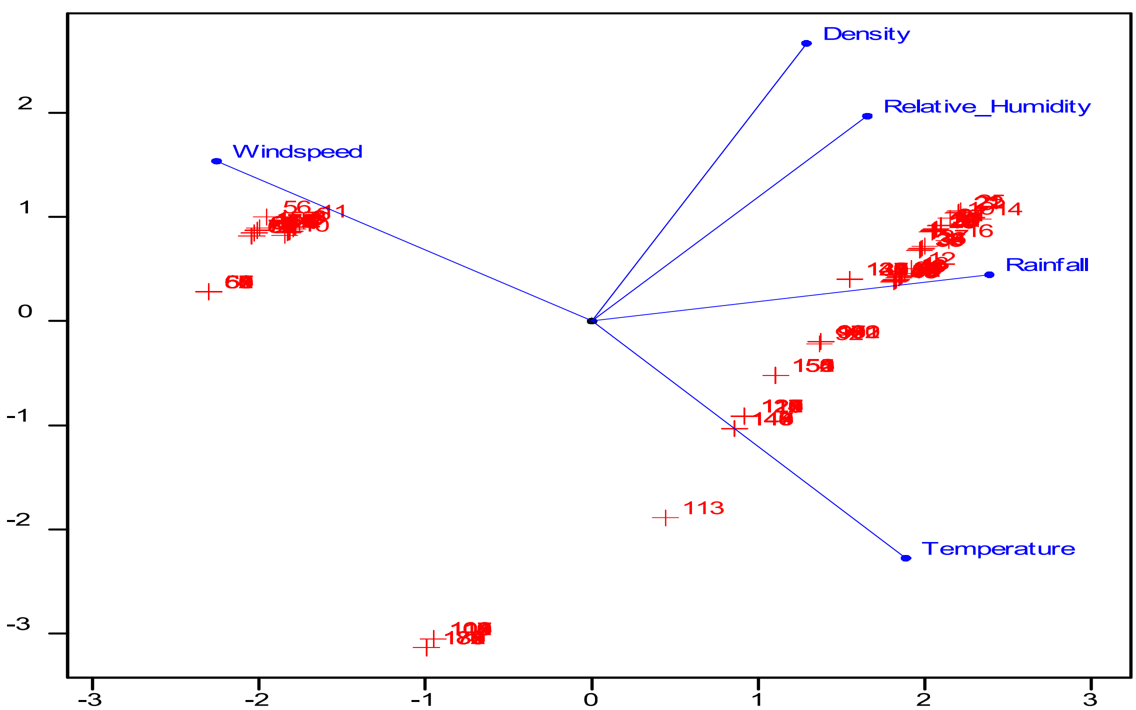

Figure 4. 4 Biplot from principal component analysis of 191 samples collected across the coffee growing areas of Rwanda. The first and the second component accounted for 57.79% and 29.25% of the total variation, respectively.

The correlations between climatic variables and Antestia bug densities varied to some degree. The correlation between Antestia bug density and relative humidity was high, with a magnitude of r = 0.57 and was significantly different from 0 ($p < 0.05$). Similarly, the correlation between rainfall and Antestia bug density was high (r = 0.59) and also significantly different from 0 ($p < 0.05$). However, the correlation between the wind speed and Antestia bug density was weak and negative (- 0.18) but significantly different from 0. Principal Component Analysis showed two distinct groupings – (i) the regions that received higher rains and with higher temperatures had high density of Antestia bug and (ii) the regions where the wind speed also was high had high Antestia bug density (Fig. 4. 4).

Table 4. 2 Principal component loadings for the first two principal components for both study years. The correlation between the variable and the principal component is indicated in bold.

	Year 1		**Year 2**	
Climatic variables	**PC1**	**PC2**	**PC1**	**PC2**
Temperature	**0.922**	0.191	**0.961**	-0.082
Rainfall	0.448	**0.845**	0.66	**0.698**
Wind speed	**-0.817**	-0.502	**-0.936**	-0.245
Relative humidity	-0.166	**0.908**	0.169	**0.848**
Density	**0.426**	-0.161	-0.052	**0.901**

DISCUSSION

The study showed that Antestia bug is distributed throughout the country with the highest densities in the Northern region. It has been reported in Rwanda since 1959 (Foucart and Brian, 1959, Babin et al., 2018) and it appears to be an established coffee pest in the country because the climatic conditions are favorable. This is the first study conducted to assess the distribution and density of this pest in the country. The highest density of Antestia bug in the North may be explained by the prevailing climate in the region. Most coffee growing areas in the North are located in the Central Plateau and Granitic Ridges, an agro-ecological zone with the highest temperature among the coffee growing regions of Rwanda (Verdoodt and Van Ranst, 2003, Bigirimana et al., 2019). Temperature substantially shortens the time it takes Antestia bug to complete a life cycle and thus higher temperatures contribute to increased generations of this pest (Ahmed et al., 2016).

Antestia bug density was consistently higher in 2017 in all regions than in 2016 except in the West where the pest population was reduced. This can possibly be attributed to the rainfall because this region received more rains than the remaining coffee growing areas. Naronjo and Ellsworth (2005) observed that excessive rains reduced pest density of *Bemisia tabaci* (Hemiptera: Aloydidae), a major pest of many field and horticultural crops. The study also showed that Antestia bug damage was consistently higher in 2017 than in 2016 in all areas except in the East. This can possibly be explained by pest competition. Hughes and McKinlay (1987) reported that pest species with a patchy distribution, such as Antestia bug, sometimes cause irregular losses that decrease with increased pest densities.

Antestia bug density was positively and significantly related to temperature, rainfall and relative humidity. This finding agrees with Ahmed et al. (2016) who reported that optimum temperature for Antestia bug development ranges from 20 to 25^{o}C and that beyond 35^{o}C, pest development slows down. Liebhold et al. (1993) observed a similar positive correlation between temperature and pest population, while investigating variation in grasshopper densities.

Furthermore, Kirkpatrick (1937) reported that Antestia bug density increases with relative humidity in the coffee growing regions of Kenya.

There was no significant relationship between the wind speed and Antestia bug density. Lewis (1969) showed that aphids and pollen beetle densities increase 2 to 3 times with wind speed exceeding 2.5 km/h. In this study, the average wind speed was 2.25 km/h but ranged from 1.99 to 3.78 km/h. The non-significant relationship between Antestia bug density and the wind speed can possibly be explained by its physical structure and behavior, as it is a relatively large insect that prefers dense canopies where it hides (Bigirimana et al., 2012).

In 2017, PCA grouped climatic data and the pest density in two separate clusters – (i) the pest data and climatic variables in the West where rainfall and temperature were generally high, (ii) the pest density and climatic variables in the South and the North combined while the East was scattered in the PCA score plot. Scattering of pest and climate data from the East in the PCA score plot may be explained by low values in Antestia bug density and climatic variables, particularly the wind speed, the rainfall and the relative humidity, possibly as a result of climate change. Ouyang et al. (2014) reported that climate change exacerbates its effects on agriculture, through disturbing the population dynamics and it sometimes causes pest outbreak. The impact of climate change on insect pests in Rwanda needs further research. Principal component analysis grouped pest and climatic data in 2016 into three groups and those in 2017 into two groups based on similar characteristics. Pico (2015) observed that PCA provides deeper insight into the data set and is useful to display patterns that are not always obvious.

The density of Antestia bug in both years of the study was high, ranging from 0 to 8 Antestia bugs per tree and 0 to 9 Antestia bug per tree, in 2016 and 2017, respectively. The economic threshold of this pest is as low as 1 to 2 bugs per tree, depending on the damage and on the country (Bigirimana et al., 2012, Ahmed et al., 2016). In the study, the economic damage ranged from 0 to 92% and from 0 to 81% in 2016 and 2017, respectively. These data suggest that there is an urgent need for Antestia bug control. Although two years is a short time for climate change assessment, temperature rise in East Africa has been reported (Jaramillo et al., 2011). This has benefited coffee berry borer, another worldwide coffee pest, which has increased its damage to coffee crops and expansion in the distribution range (Jaramillo et al., 2011). Expansion of the coffee production area was proposed in Rwanda, based on the spatial distribution of actual and potential production zones for Arabica coffee (Nzeyimana et al., 2014). Antestia bug may also expand its distribution range, following the newly planted coffee areas. Antestia bug prefers feeding on coffee, particularly Arabica coffee and its density increases with altitude (Ahmed et al., 2018). The impact of climate change on density and damage of Antestia bug as well as of other coffee pests need further research.

CHAPTER 5: UNDERSTANDING POTATO TASTE DEFECT IN COFFEE: HOW DOES IT GET INSIDE COFFEE BEANS?

ABSTRACT

Potato Taste Defect (PTD) is a potato like smell and taste found in green and roasted coffee beans and in brewed cups of coffee. This defect makes coffee unpalatable, hence reducing its value or causing it to be rejected by consumers. Occurrence of PTD in coffee has been associated with Antestia bug damage, *Antestiospsi thunbergii* (Hemiptera: Pentatomidae), but the processes that lead to the disorder are not well understood. The objectives of this study were: (1) to evaluate the mechanisms of PTD occurrence in coffee cherries and (2) to assess the relationship between cupping method and Gas Chromatography - Mass Spectrometry (GC-MS) for PTD detection. Treatments consisted of (i) insect damaged beans by caging Antestia bug on coffee branches; (ii) mechanically damaged beans without Antestia bug infestation, and (iii) control beans without any manipulation in the field. At harvest, coffee cherries were wet processed and medium roasted. Detection of PTD was done (i) using organoleptic test following the Specialty Coffee Association of America cupping protocol and (ii) using GC–MS analysis to detect the major compound responsible for the off taste, 3 - isopropyl – 2 – methoxypyrazine (IPMP). High incidence of PTD was found in beans damaged by Antestia bug or mechanically damaged; low PTD incidence was found in control beans. This is the first demonstration that mechanical damage alone in coffee berries can cause PTD in coffee. The lowest amount of IPMP was found in the control beans while the highest was detected in berries with simulated damage. There was a positive relationship between the cupping method to detect PTD and IPMP detection by GC-MS in coffee samples. These results suggest that Antestia bug is not absolutely required to cause PTD but wounds to the berries including feeding damage by this bug would allow entry

of *Pantoea coffeiphilia*, a bacterium responsible for occurrence of PTD in coffee and the subsequent production of IPMP. Elimination of PTD in coffee requires avoiding means of entry for *Pantatoea coffeiphilia* in coffee beans along with proper control of Antestia bug.

INTRODUCTION

Potato Taste Defect (PTD) is a potato like smell and taste found in green and roasted coffee beans and in brewed cups of coffee, making it unpalatable. This defect threatens coffee from the Africa Great Lakes region where it reduces its market value or causes it to be rejected by consumers. Specifically, this defect negatively affects producers because it reduces the coffee prices paid to farmers and cooperatives. Furthermore, international buyers generally reduce the prices they are willing to pay for coffee coming from PTD epidemic regions, regardless of whether or not there is evidence of PTD in a given lot of coffee. Occurrence of PTD in coffee has been associated with insect infestations, particularly damage from Antestia bug, *Antestiopsis thunbergii* (Hemiptera: Pentatomidae) (Becker et al., 1988; Gueule et al., 2015; Jackels et al., 2014; Bigirimana et al., 2018). Furthermore, pruning and the use of insecticides to protect coffee plantations against Antestia bug substantially reduced the occurrence of PTD in coffee (Bouyjou et al., 1999; Bigirimana et al., 2018).

Potato taste defect is believed to be caused by *Pantoea coffeiphilia*, a bacterium of the family *Enterobacteriaceae* which develops in coffee beans and produces 3 - isopropyl–2–methoxylpyrazine (IPMP) and 2-isobutyl-3-methoxypyrazine (IBMP), the compounds that are responsible for the off-flavor (Becker et al., 1988, Czerny and Grosch, 2000; Gueule et al., 2015). Midguts associated with Antestia bug have been identified and three facultative symbionts representing the bacteria genera *Sodalis, Spiroplasma*, and *Rickettsia* with infection frequencies of 51.3%, 52.6% and 24%, respectively were reported (Matsuura et al., 2014).

Whole bean green coffee where PTD was previously identified emitted a distinguished volatile profile compared to the coffee beans where PTD was not detected (Jackels et al., 2014). In the former case, the coffee samples released volatile compounds such as tridecane, dodecane, and tetradecane which could not clearly be found in the latter case (Jackels et al., 2014). Additionally, visual inspection of chromatographs revealed differences between PTD samples and non - PTD samples, with longer and more intense chromatograms for PTD than non PTD samples (Jackels et al., 2014).

The wounds caused by Antestia bug feeding on coffee cherries provides an entrance point for the bacterium to invade the coffee cherries (Becker et al., 1988). Additionally, lack of Antestia bug control programs in coffee plantations favors high infestations of other insects in coffee plantations, such as thrips, leafhoppers, and cercopids (Cilas et al., 1998). It is possible that these insects puncture the coffee berry skin without damaging the beans (Bouyjou et al., 1999), providing an entry point for the bacterium.

After the bacterium enters the coffee beans, it produces 3– isopropyl – 2 – methoxypyrazine (IPMP) and 2 – isobutyl – 3 – methoypyrazine (IBMP) (Jackels et al., 2014). Because of their strong odors, both pyrazines are potent chemical signals. Both IPMP and IBMP can be detected by humans at very low concentrations, approximately 2 ngL^{-1} in water and about a factor of one thousand less in air (Sidhu et al., (2015). They are perceived as musty or earthy odors. These pyrazines can also be produced by the coffee plant itself as a stress response to Antestia bug damage (Jackels et al., 2014).

Although IPMP is strongly associated with PTD, its detection has not been used as a means of identifying the defect in coffee samples. Coffee quality assessment is generally done through cupping using the Specialty Coffee Association of America protocol where experienced licensed Q graders evaluate the cup qualities of samples and assign scores which are consistent with other analytical techniques such as Gas Chromatography - Mass Spectrometry, Headspace – Solid Phase Micro Extraction and Liquid Chromatography - Mass Spectrometry (Bressanello et al. 2017; Iwasa et al. 2015).

Progress has been made in identifying compounds associated with PTD. Dodecane, tridecane, tetradecane and 2-ethyl-1-hexanol are frequently detected on the surface of coffee beans that test positive for the disorder. IPMP is the main chemical found inside contaminated coffee beans and is thought to be responsible for PTD (Jackels et al., 2014). However, understanding the pathways or mechanisms of PTD infection has received little attention. The aims of this study were (1) to elucidate the mechanisms of PTD infection in coffee berries and (2) to assess the relationship between the cupping method for detecting PTD using the Specialty Coffee Association of America protocol and Gas Chromatography - Mass Spectrometry for detection of the chemicals thought to be responsible for PTD in coffee.

MATERIALS AND METHODS

Study design and treatments

This study was conducted in Rwanda at Rubona Agricultural Research Station at latitude: 02˚29'03.2" S, longitude: 29˚45'58.2' E and altitude: 1673 masl, one month after coffee flowering. A randomized complete block design was used with three treatments replicated four times. Treatments consisted of (i) caging Antestia bug on coffee branches to contain Antesia bugs at a known density and keep other insects from attacking the berries; (ii) simulated damage where coffee cherries were artificially damaged in a manner simulating way as feeding by Antestia bug and (iii) a control where coffee trees were left without any manipulation.

The caged treatment consisted of releasing Antesia bugs in sections of a coffee branch that were covered with a sleeve made of gauze screen (1 mm diam., ca.2 × 2 × 2.4 m, Nairobi, Kenya). Sleeves were placed on six branches on each of six central trees, two at the bottom of the canopy, two at mid-canopy and two in the upper canopy. Antestia bugs were collected from within the coffee planting. Twenty bugs were randomly selected and release into each caged branch for a total 120 bugs/tree per replicate. Antestia bugs caged on branches were regularly checked on a weekly basis and the dead ones were removed. Since berries often drop following infestations of Antestia bug, replacement of dead bugs was conducted on a two month interval to mitigate dislodging damaged berries (Kirkpatrick et al., 1937). The number of Antestia bugs within the cage was counted 2, 4 and 6 months after flowering.

For the simulated damage treatment, an approximately 2 mm diameter needle was used to punch a hole in all coffee berries on selected branches that were similar in size to those used for the cage treatment. About 50% of the berry depth were punctured in a manner simulating damage by Antestia bug. Six branches were selected from three canopy locations as in the caged treatment. Holes were similar in size to those created by Antestia bug feeding on coffee berries. Coffee berries with simulated bug damage were protected from subsequent Antestia bug infestations by placing a net sheet (1 mm diam., ca.2 × 2 × 2.4 m, Nairobi, Kenya) over the treated branch. The number of Antestia bugs on the branch within the netting were counted 2, 4 and 6 months after flowering. Uncaged branches from low, mid and high at an approximate distance of 40 cm between low, mid and high levels in the canopy were used as controls. For the control treatment, the number of Antestia bugs was assessed 2, 4 and 6 months after flowering by observing the coffee tree for 5 min and counting the number of Antestia bugs present.

Coffee trees used in the experiment were spaced two meters between rows and two meters within rows. Each experimental plot comprised of 42 coffee trees, i.e seven rows by six trees per row, only six central coffee trees (2 rows by 3 trees per row) were used for data collection. This experiment was repeated 4 times with a total of 12 treatment plots. All the treatment plots were weeded as needed and fertilized as required.

Six months after flowering, each plot was harvested separately. Ripe cherries were collected on a weekly basis, processed at the washing station and sun dried. It is worth noting that the cherry processing did not include floatation at the washing station or removal of insect – infested cherries. The percentage of insect - damaged berries in each treatment was determined by counting the number of berries infested by Antestia bug and dividing by the total number of berries within each treatment. Presence of PTD in coffee samples evaluated through organoleptic tests was recorded as 1 for PTD present and 0 for absence of PTD as described below. The amount of IPMP in coffee samples was measured using Gas Chromatography – Mass Spectrometry and was recorded in μM as described below.

Cupping Procedure

Coffee beans from each treatment were medium roasted using a laboratory scale roaster. The roasting degree was adjusted to a luminosity (L) value of 22−23 for all samples. Samples were roasted in accordance with the Specialty Coffee Association of America (SCAA) cupping protocol (Keiko et al., 2015). Roasted coffee samples were randomly divided into two parts. One part (half) was evaluated by experienced Licensed Q graders, certified by the Coffee Quality

Institute (CQI) and the other half was used for Gas Chromatography – Mass Spectrometry detection as described below. Cupping was arranged in such a way that a maximum of 6 cups were compared at a time. Three Licensed Q graders cupped all samples, each grader was considered as a replicate. Every grader performed a standard session cupping of all the samples in a randomized order as blind analyses. The cupping objective was not to give the overall cupping score but to detect whether the sample had or did not have the PTD. Presence of PTD in coffee samples evaluated through organoleptic tests was recorded as 1 for PTD present and 0 for absence of PTD.

Gas Chromatography - Mass Spectrometry Analysis

Roasted coffee beans were ground to a fine powder for 3 min (particle size < 0.5 mm) using a commercial coffee grinder (Easehold WH-9300). In a 20 mL vial, 5 g of the coffee powder and 5 mL ethyl acetate were added. The vial was stirred for 20 min and centrifuged with a table top centrifuge (IEC Clinical Centrifuge, 3800 g, 2 min). 250 µL of the clarified supernatant was aliquoted and transferred into a 2ml GC vial for GC-MS analysis.

GC-MS analysis was performed with an Agilent 6890N gas chromatograph equipped with a capillary GC column (30 m × 0.25 mm × 0.25 µM; HP-5MS; J&W Scientific) with helium as the carrier gas (flow rate, 1 mL/min). The injector port (at 250 °C) was set to splitless injection mode. A 1-µL aliquot of each sample was injected using an Agilent 7683 auto-sampler (Agilent, Atlanta; GA). The column temperature was increased from 50 – 110 °C at 50 °C/min, then increased by 8 °C/min to 180 °C, followed by increasing it finally at 50 °C/min to 245 °C with a 6 min hold (total run time of 18.30 min).

The gas chromatograph was coupled to a mass selective detector (Agilent, 5973 *inert*) operated in electron impact mode (70 eV ionization voltage). All spectra were recorded in the mass range of 50 – 600 *m/z*. IPMP (2-isopropyl-3-methoxy pyrazine) eluted at 3.91 min. An authentic standard of IPMP (98% purity, Sigma-Aldrich, USA) was diluted to various concentration series from 10 – 1000 μM and injected in GC-MS to generate a standard curve. From the standard curve, an equation Y = 5580.9 X was generated where X was IPMP concentration (μM) and Y was the average ion count (millions) which was used to estimate the amount of IPMP present in coffee samples.

Data analysis

Antestia bug density and the percentage berry damage by Antestia were checked for normality using the Shapiro Wilk test and equality of variance using Brown Forsythe's test prior to being subjected to the Analysis of Variance (ANOVA) test. Similarly, the amount of 3 -isopropyl -2- methoxylpyrazine was checked for normality using the Shapiro Wilk test and equality of variance assumptions using Brown Forsythe's test prior to being subjected to the Analysis of Variance test. A logistic regression analysis was used to determine the relationship between IPMP concentrations in coffee samples and identification of PTD using the organoleptic test (cupping method) where samples which had PTD were recorded as 1 and those without PTD as 0. The logistic regression model used can be written as:

$$Logit\ (p) = \log\ [\frac{p}{1-p}]$$

or

$$\text{Logit (p)} = \alpha + \beta X + \varepsilon$$

where p is the probability to detect potato taste defect using organoleptic test; α – intercept; β - the slope of IPMP and ε – the error term.

RESULTS

Antestia bug density during the cherry development period

Two months after flowering, Antestia densities differed significantly among treatments ($F = 19.46$, $DF = 2, 9$, $P < 0.01$). The highest bug density was recorded in the caged treatment in which bugs were released, with a mean of 8.1 ± 1.1 (Mean ± SE) live individuals (Fig. 5. 1).

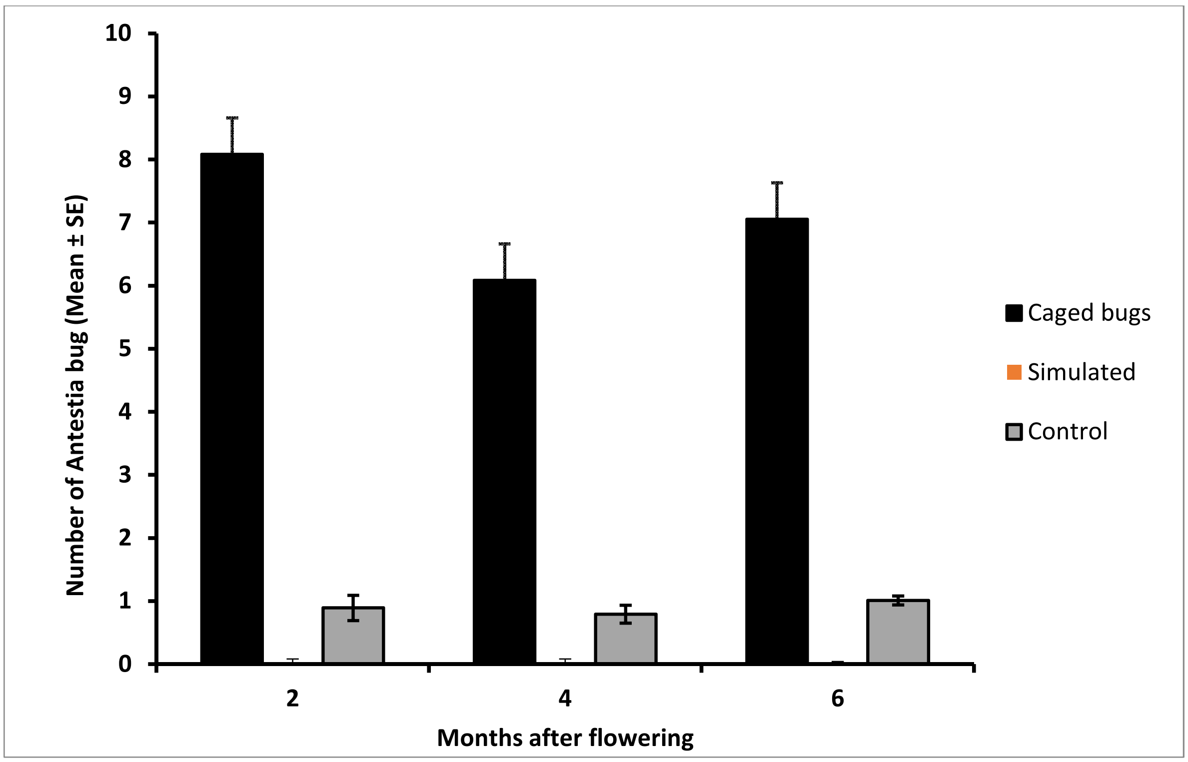

Figure 5. 1 Mean number of Antestia bug recorded two, four and six months after flowering for three treatments, caged branches containing released bugs, branches with coffee berries mechanically damaged and an untreated control. Bars represent the standard error of means.

Around 40% of the Antestia bugs released were alive and feeding on the coffee berries. The mean number of Antestia bugs recorded in the control plots was 0.9 ± 0.2. Coffee berries where simulated damage was carried out were covered, and as expected there were no Antestia bugs recorded on this treatment (Fig. 5. 1). Four months after flowering, significant differences in Antestia bug density among treatments also were observed ($F = 6.69$, $DF = 2, 9$, $P < 0.05$). The highest Antestia density was recorded in the caged treatment with released bugs, with a mean of 6.1 ± 1.1 live bugs (Fig. 5. 1). Thus, about 30% of live Antestia bugs that we caged on the coffee branches were still feeding on coffee berries at this period. The control had a mean number of 0.79 ± 0.14 Antestia bugs per sample, which was about seven times fewer than for the caged treatment. The simulated damage treatment in which bugs were excluded with netting again was devoid of Antestia (Fig. 5. 1).

A final assessment of Antestia bug density was conducted six months after flowering. Consistent with the two previous samples, there were significant differences in bug density among treatments ($F = 12.30$, $DF = 2, 9$, $P < 0.01$) (Fig. 5. 1). The highest density was recorded in the caged treatment with released bugs, with a mean of 7.05 ± 0.68 Antestia. This represented approximately 35% of all bugs that were caged on branches in the period of two months since the previous assessment. The control had a mean of 1.01 ± 0.14 Antestia bugs, which was higher than the economic threshold for this pest. No Antestia bugs were detected on the berries that were artificially damaged and covered with netting.

Berry damage between treatments after harvest

There were significant differences between treatments in coffee berry damage after harvest ($F = 14.21$, $DF = 2, 9$, $P < 0.01$). As expected, the highest damage was recorded for the simulated damage treatment, as the poking of all berries on selected branches was successful and resulted in a mean of 100% injury. In comparison, the caged treatment with released bugs had a mean of 74.32 ± 4.50 percent berry damage and the control had a mean of 30.68 ± 2.0 percent berry damage. Thus, the simulated damage was about 3 times higher than the unmanipulated control and damage to the caged berries was about twice that of the control.

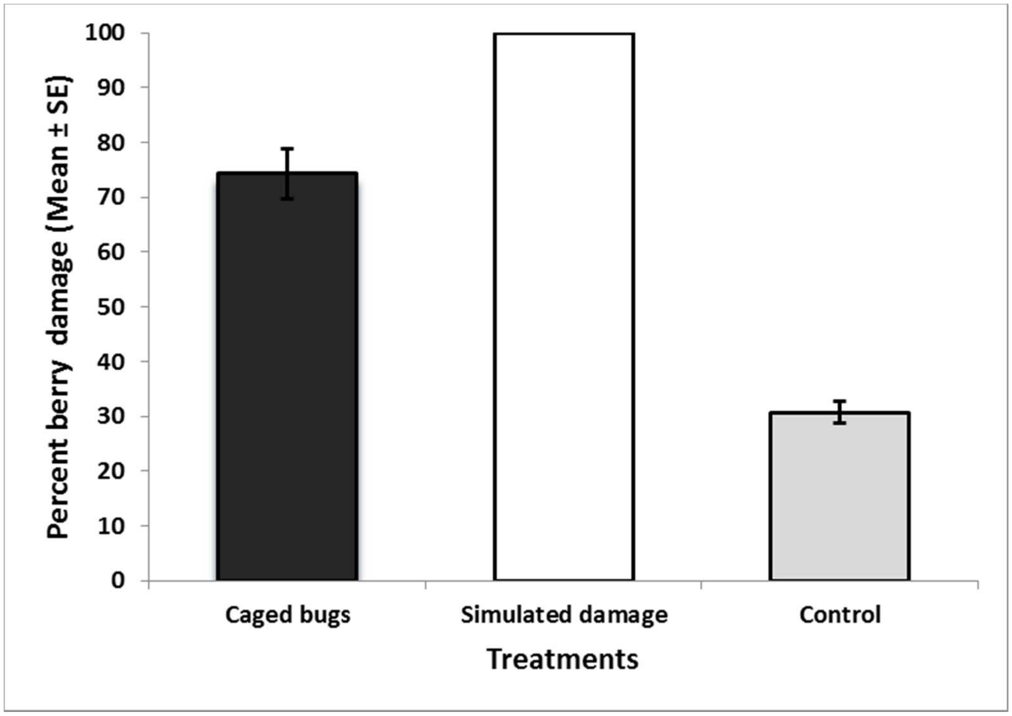

Figure 5. 2 Mean percent berry damage recorded for three treatments, caged branches containing released bugs, branches with coffee berries mechanically damaged and an untreated control. Bars represent the standard error of means.

Presence of IPMP in the treatments

The fragmentation pattern of 3 – isopropyl – 2 methoxypyrazine was the same for the standard compound and the simulated damage. The retention time for both the standard compound and the simulated damage treatment was also the same (about 4 min). However, the intensity of the chromatographs differed among treatments.

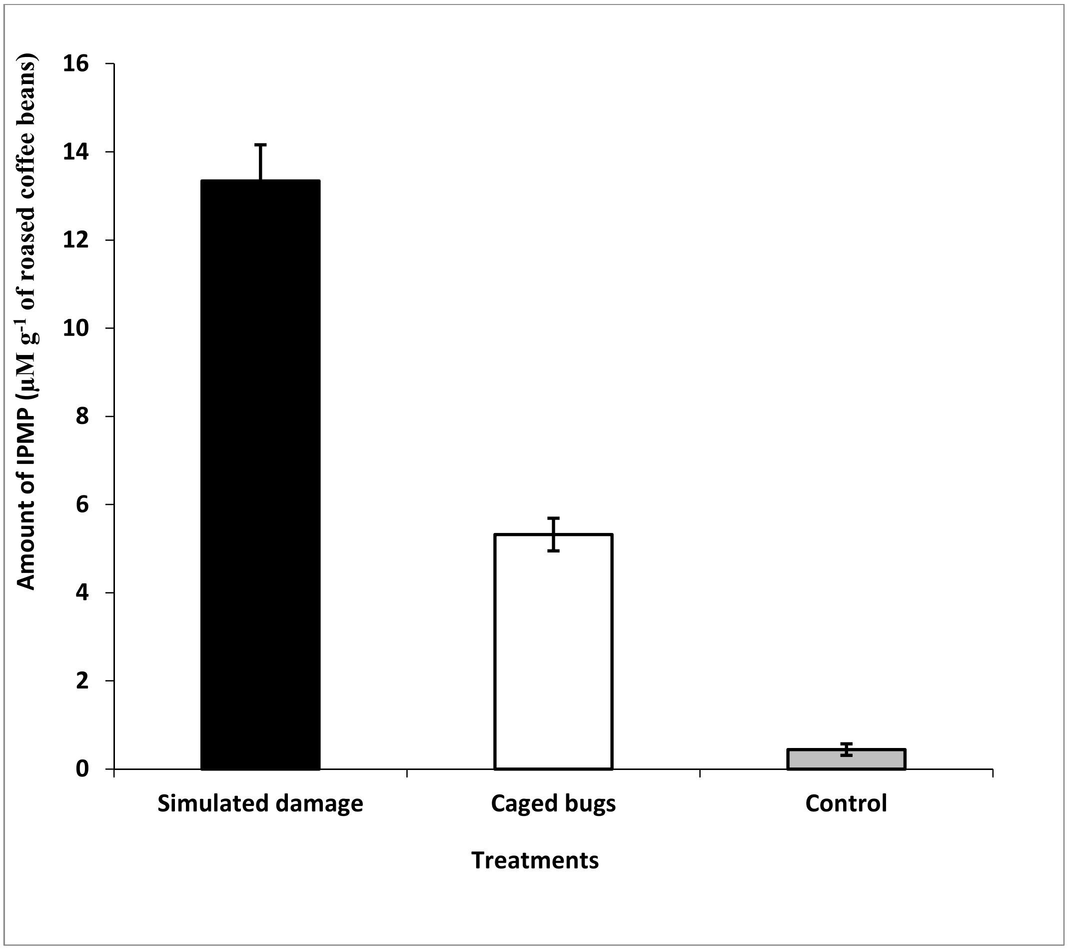

Figure 5. 3 Mean concentration of IPMP (µM per gram of roasted coffee beans) recorded for three treatments, caged branches containing released bugs, branches with coffee berries mechanically damaged and an untreated control. Bars represent the standard error of means.

Samples obtained from the three treatments, control, simulated damage and caged bugs, had varying levels of IPMP. The lowest amount of IPMP was detected in the control, with a concentration of 0.392 µM of IPMP per gram of roasted coffee beans and the highest was found in the simulated damage with a concentration of 14.563 µM of IPMP per gram of roasted coffee beans. Thus, IPMP concentrations were about 37 times higher in the simulated damage treatment compared to the control.

Significant differences in IPMP concentrations were detected among treatments ($F = 158.848$, $DF = 2, 8$, $P < 0.0001$). The highest concentration of IPMP was found in berries that had been mechanically damaged, with a mean of 13.34 ± 0.81 µM per gram of roasted coffee beans. The lowest IPMP concentration was detected in the control, with the mean of 0.44 ± 0.034 µM per gram of roasted coffee beans (Fig. 5. 3). Thus, IPMP was about 30 times higher in mechanically damaged berries compared to the control. The berries caged with bugs had a mean of 5.31 ± 0.37 µM of IPMP per gram of roasted coffee beans, which was about three times less than the level in berries with simulated damage, and about 12 times higher than the control.

PTD detection through cupping

Given that one sample with PTD results in rejecting a lot of coffee beans, we considered the entire sample of coffee to be classified as PTD positive if at least one cup among the six cups was deemed to have PTD. PTD was not detected in the control treatment using the cupping procedure; i.e after cupping six cups from each sample from the control, across all the replications, all the cups were PTD free. In the simulated damage treatment, two of the cupping samples tested positive for PTD. The coffee beans from the caged treatment had the highest PTD occurrence, with three of the four samples, testing PTD positive.

Relationships between cupping and IPMP concentrations

The logistic regression function indicated a positive relationship between higher concentrations of IPMP analyzed by GC-MS and PTD presence detected by the cupping method (Fig. 5. 4). The logistic regression equation was: Logit (p) = - 2.017 + 0.618*X Where p was the probability to detect PTD in the coffee samples and X was the concentration of IPMP in the coffee samples. The logistic regression model accounted for 56.6% of the observations in the study.

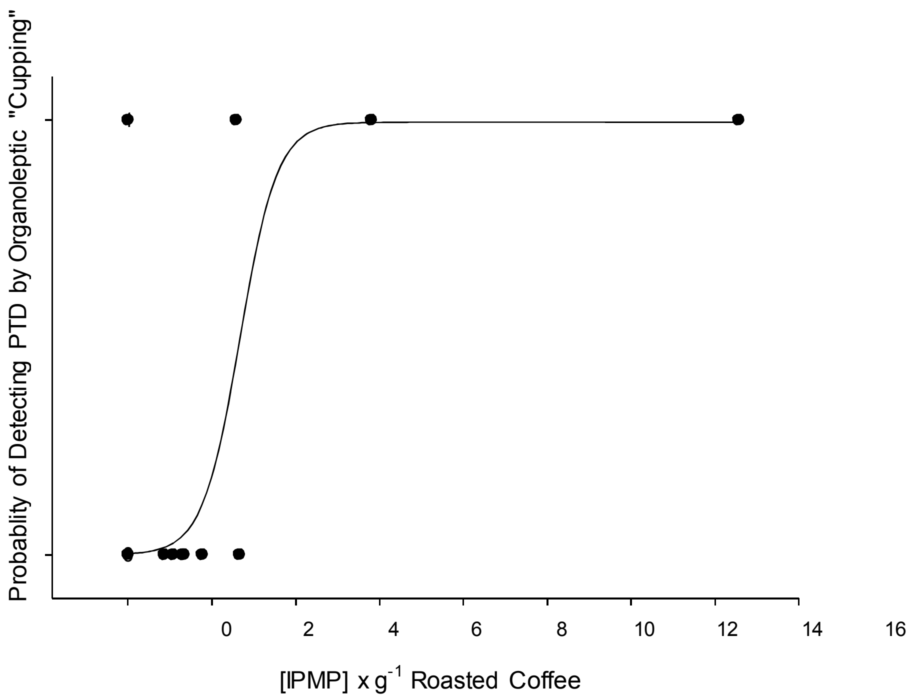

Figure 5. 4 Relationship between PTD detection by cupping and IPMP concentrations determined by GC-MS in the coffee samples. The higher the IPMP concentration, the higher the probability of PTD defect in the coffee samples. Coffee samples were roasted and tested in accordance with the Specialty Coffee Association of America (SCAA) cupping protocol. IPMP concentration was measured by grinding the roasted coffee beans and measuring the concentration by Gas Chromatography Mass Spectrometry.

DISCUSSION

This study demonstrated for the first time that mechanical damage alone in coffee berries can cause PTD in coffee beans. Although Bouyjou et al. (1999) reported that protection of coffee trees against Antestia bug during the fruit development period results in coffee that is largely free from PTD, we detected the defect in 50% of the coffee samples from mechanically damaged berries without Antestia bug feeding. These results are in agreement with Fassi (1957) who hypothesized that wounds created by Antestia bug feeding serve as the entry of bacterium that causes PTD in coffee. However, PTD was also detected in mechanically damaged beans without the presence of Antestia bug, indicating that wounding alone may be enough to allow entry of the bacterium responsible for PTD in coffee. PTD was not detected in coffee beans damaged by coffee berry borer, *Hypthenemus hampei* Ferrari (Bigirimana et al. 2019). In contrast to Antestia bug's feeding on berries from the outside, coffee berry borer feeds and develops inside the berries. Even though the entrance by the borer may allow the bacterium responsible for PTD to enter, its survival may be impeded in the presence of the coffee berry borer (Vega et al., 2009).

Antestia bug feeding on berries creates wounds, providing an entrance for bacterium growing inside and producing pyrazines, especially 3 - isopropyl–2–methoxylpyrazine (IPMP), the compound responsible for the off-flavor (Czerny and Grosch, 2000, Jackels et al., 2014, Gueule et al., 2014, Bigirimana et al., 2019). Geuele et al. (2015) reported that IPMP is produced by *Pantoea coffeiphilia*, a bacterium that grows inside coffee beans. Antestia bugs damage berries by sucking and thereby producing holes in the coffee berries that serve as entry points for *Pantoea coffeiphila*, it appears that the bacterium can enter the coffee beans not only through the wounds caused by Antestia bug as previously reported by Bouyjou et al., 1999; Jackels et al.

2014; Fassi, 1957; Gueule et al., 2015 but through any wound, including those that are artificially induced.

The concentration of IPMP was statistically higher in mechanically damaged beans without the bug than those with the bugs, indicating that IPMP can be produced in the absence of Antestia bug feeding. The high concentration of IPMP in the coffee samples in relation to PTD occurrence has also been reported by Becker al. (1988) who found high amounts of IPMP in PTD coffee samples. This was indeed confirmed by cupping where coffee samples from simulated damage also had PTD without Antestia bug feeding.

This study showed varying concentrations of IPMP in coffee berries that were exposed to Antestia or mechanically damaged. The control in which berries were exposed to endemic populations of Antestia had the lowest concentration of IPMP while berries with simulated damage had the highest concentration. Additionally, there was a positive relationship between cupping and the amount of IPMP in the coffee samples. This is consistent with Jackels et al. (2014) finding that coffee samples with PTD have high levels of IPMP while those without PTD have little concentration of IPMP and that 3-isobutyl-2-methoxypyrazine is found in both types of samples at approximately the same amount. Given that IPMP was extracted from ground coffee beans and that attempts to extract it from the whole bean coffee samples was unsuccessful, these results are consistent with Bouyjou et al. (1999) who hypothesized that IPMP is produced in the coffee beans and not deposited on the surface by a bacterial growth in the fruit. Jackels et al. (2014) found neither IPMP nor IBMP in the surface volatiles. In this study, we did not find IBMP in the interior of the coffee samples probably because our observations were based on ground coffee beans, which were solvent-extracted and subjected to a multistep process before chemical analysis. The lowest amount of IPMP in the coffee samples that corresponds to the sensory detection threshold needs further research.

IPMP is an integral part of various foods and horticultural products. In coffee, it develops as a result of roasting in a very low sensory threshold while at a high level it significantly impacts the coffee quality and aroma (Šuklje et al., 2012). Both IPMP and IBMP also exist in grapes, the former contributing to the aroma and the latter to the green color (Lee et al., 2015). Allen et al. (1995) reported that in wine, IPMP concentration of 9.7 ng/l is comparable to the IBMP concentration of 9.3 ng/L and that IPMP contributes more to the aroma of the wine more strongly than IBMP. Similar to findings for coffee, Allen et al. (1995) observed that IPMP is not endogenous to the wine prior to bottling but comes from an external source, such as microbial contamination.

Despite finding that mechanical damage can lead to PTD, managing Antestia bug remains the best means of reducing the incidence of the disorder. Antestia is a direct coffee pest in the Africa Great Lakes region where PTD is a major problem (Gueule et al., 2013, Bigirimana et al., 2019) and an associated bacterium is responsible for PTD in coffee. Additionally, yield loss due to Antestia bug has been estimated at 40% (Azrag et al., 2016). Management of Antestia bug in the Africa Great Lakes region is largely accomplished using insecticide applications (Foucart and Brion, 1959; Bouyjou et al., 1993; Babin et al., 2018). More recently, Bigirimana et al. (2018) demonstrated that Integrated Pest Management programs incorporating pruning combined with Fastac sprays, provides enhanced control of Antestia bug and significantly reduces the occurrence of PTD compared to either pruning or insecticides application alone. Other promising pest control tactics such as biological control and “attract and kill” also need to be explored as options for managing Antestia bug. More research is needed to evaluate the effectiveness of other options, such as biological control, for managing Antestia bug and preventing the losses that the bug causes to coffee production in the Great Lakes region of Africa.

In addition to controlling Antestia bug, options for reducing the incidence of PTD in coffee include floating at the coffee washing stations, removal of insect – infested beans and grading and sorting (Bouyjou et al., 1993, Bigirimana et al., 2018). Sorting should also include removal of cherries with any holes, as these may carry the bacterium responsible for PTD. Elimination of PTD also requires understanding the sources of the bacterium, i.e identifying whether it comes from the field, water used at the washing station or if entry occurs at the drying site or throughout the roasting process. Methods and techniques need to be designed to identify the bacterium at the very early stage and destroy it or eliminate the coffee sample in the effort to supply PTD free coffee.

CHAPTER 6: CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

The research reported in this dissertation has provided new information on potato taste defect (PTD) in coffee with relevance to better understanding the mechanisms of infection and identifying the most effective tactics for managing this defect. Given that PTD reduces the market value of coffee or causes it to be rejected by consumers, there is need to eliminate this defect or reduce it to non – problematic levels. Although I have contributed to elucidating the mechanisms of PTD entry into coffee berries and proposed options for managing Antestia bug, a coffee pest that creates holes into coffee cherries that serve as entry for *Pantotea coffeiphilia, a bacterium* responsible for the off-flavor; more research is needed to understand this bacterium, which may include for example the sources of inoculum as well as to design methods and techniques for early detection and prevention of the bacterium in the effort to supply PTD free coffee.

Current PTD management efforts include Antestia bug control using insecticides and cultural methods, floating coffee cherries at the coffee washing station (CWS), removal of insect-infested beans and sorting of defective coffee beans. Although these practices reduce the incidence of PTD in coffee samples, the occurrence of this defect is still a challenge to the coffee industry as lots of beans continue to be rejected by consumers and international buyers. Furthermore, defective coffee beans removed through various stages of the processing process constitute an important yield loss. Prior to this research, Antestia bug was controlled by annual Imidacloprid sprays made on a calendar basis, which was unsustainable, detrimental to beneficial enemies and expensive. This research has demonstrated that, through Integrated Pest Management (IPM), the incidence of PTD can be reduced significantly, down to 1%.

I have investigated the effectiveness of IPM using pruning alone or in combination with commercially available insecticides to control field populations of Antestia bug and to evaluate the relationship between these treatments and the occurrence of PTD in coffee using both laboratory and field tests (Chapter 2). In laboratory bioassays, significant differences were found between insecticides on the mortality of adult bugs. Higher mortality was recorded for pyrethroids (Alpha-cypermethrin) and pyrethrins (Pyrethrum 5EW, Pyrethrum EWC + Sesame and Agloblaster) than for the neonicotinoid (Imidacloprid). These findings were also consistent with the field trials where the highest mortality of Antestia bug was achieved with Pyrethrum 5EW, Fastac and Pyrethrum EWC + sesame applied to pruned coffee trees. Additionally, pruning alone registered a statistically higher mortality than unpruned coffee trees without insecticide applications.

This work demonstrated that pruning combined with insecticide application, especially Fastac, better controls Antestia bug and significantly reduces PTD compared to either pruning or insecticide application alone (Chapter 2). This finding is important to the coffee industry because prior to conducting this research, export fees deducted from coffee sales were used to buy Imidacloprid, which was distributed to farmers in Rwanda over years. When this finding was presented, the policy changed and Fastac is now purchased and distributed based on its greater effectiveness on Antestia bug control. The policy also includes implementing pruning campaigns to ensure pruning is completed in time before the next season's spraying. Given that insecticides are expensive and sometimes have negative effects on humans and environment, more research is needed to effectively control Antestia bug and to eliminate PTD in coffee. Future research should explore other options such as biological control, behavioral control, attract and kill, etc.

Additionally, there is need to evaluate the effects of floating and sorting coffee cherries in combination with IPM on the occurrence of PTD in coffee.

This research has demonstrated that PTD is distributed throughout the coffee producing regions of Rwanda with the highest incidence in Central Plateau, Granitic Ridges and Eastern Plateau (Chapter 3). Among six agroecological zones (AEZ) surveyed, the AEZ in Central Plateau and Granitic Ridges accounted for about a half of all observed PTD samples in the country. This AEZ has temperature and rainfall favorable to high populations of Antestia bug, which prefers a warm climate where it causes high infestations in the field, as a result of the interactions between the pest, temperature and the rainfall. High PTD incidence was shown to be strongly associated with high density and damage of Antestia bug. This coffee pest creates holes on coffee berries that serve as entry for *Pantotea coffeiphilia*, a bacterium that produces isopropyl-2-methoxyl-3-pyrazine (IPMP) and 2-isobutyl-3-methoxypyrazine (IBMP), the compounds responsible for the off-flavor (Chapter 5). However, coffee berry borer (CBB) damage was not significantly related to the occurrence of PTD. Although CBB bores holes into coffee berries that could allow the bacterium to enter, survival of the pathogen may be impeded by the presence of the beetle.

While some management practices such as pruning and insecticides application were related to the occurrence of PTD, others like intercropping were not (Chapter 3). Because the main intercrops were banana and Agroforestry species, there is an opportunity for an efficient use of land resources as most coffee in Rwanda is produced on smallholdings. Benefits of intercropping coffee with banana or agroforestry species in Rwanda need more research. Given that Antestia bug density and damage were associated with PTD occurrence and that CBB damage was not related to the incidence of PTD, yet both being major coffee pests in Rwanda, there is need to intensify Antestia bug control, especially in the areas where the highest PTD incidence was observed.

In a study conducted to assess the population density and damage of Antestia bug in the coffee growing regions of Rwanda (Chapter 4), it was found that the pest was distributed in all coffee growing regions of Rwanda with the highest density in the Northern region and the lowest in the Eastern region consistently over two years. The exception was in the Western region where the population density was low in 2016 compared to 2017 possibly because of higher rainfall in this area as this region received more rains compared to the other coffee growing areas of Rwanda. On the other hand, the damage due to Antestia bug was the highest in the Northern region and the lowest in the Southern region consistently over two years except in the Eastern region where the percent berry damage was low in 2017 compared to 2016, possibly as result of pest competition (Chapter 4). The economic damage due to Antestia bug ranged from 0 to 92% in 2016 and from 0 to 81% in 2017, underscoring an urgent need for pest control. Efforts should be concentrated in the Northern region because this is the region where the highest pest density and damage were observed.

Climatic variables such as temperature and relative humidity were positively related to Antestia bug density, indicating that their increase through climate change may lead to pest outbreaks. However, wind speed was not significantly associated with Antestia bug density possibly because of the bug's physical structure and behavior as it is relatively a large insect that prefers dense canopies where it hides. Given that the study was conducted for 2 years, which is a short time for climate change assessment, more research is needed to evaluate the effects of climate change on population density and damage of Antestia bug and other coffee pests. Additionally, as temperature rise has been reported in the study area, introduction of shade trees in sun grown coffee plantations should be further evaluated as an adaptation measure. Such an evaluation should include the best species of shade trees, along with spacing of tree species in the coffee plantation to allow easy adoption by farmers if the practice is promising.

Before this research, occurrence of PTD in coffee was associated with Antestia bug feeding. However, understanding the mechanisms of infection has received little attention. Thus, a study was conducted to evaluate the mechanisms of PTD infection in coffee berries and to assess the relationship between the cupping method using the Specialty Coffee Association of America (SCAA) cupping protocol and chemical analysis using Gas Chromatography Mass Spectrometry (GCMS) to detect the presence of PTD. Findings indicated the high incidence of PTD was found in coffee beans damaged by Antestia bug and the lowest was found in undamaged control beans. The lowest amount of 2 – isopropyl – 3- methoxypyrazine (IPMP) was found in control beans while the highest levels was in coffee berries that had been poked with a needle to simulate bug damage. The research also revealed a positive relationship between the cupping method to detect PTD and IPMP detection using GCMS.

This study demonstrated for the first time that mechanical damage alone in coffee berries can cause PTD and the production of IPMP, the compound responsible for the off-flavor. Feeding by Antestia bug creates wounds that provide entrance for the bacterium that produces pyrazines, especially IPMP. My research showed that the concentration of IPMP was significantly higher in mechanically damaged beans without the bugs than in beans caged with and damaged by Antestia bugs. Thus, IPMP can be produced in the absence of Antestia bug feeding. Findings also revealed that coffee samples with PTD detected by cupping had high concentrations of IPMP, while those without PTD in cupping samples had low levels or no

IPMP. However, the lowest amount of IPMP in coffee samples that corresponds to the sensory detection threshold needs further research.

Throughout this research, the intention was to generate findings that may translate into recommendations to growers and other stakeholders in the coffee industry. I have presented the results of this work in a meeting involving farmers, coffee washing station owners, coffee buyers and policy makers and I will make many more in the near future. Combining knowledge of Antestia bug biology and available methods for pest control, I have developed an IPM method integrating pruning and sprays of alpha- cypermethrin (fastac) in coffee plantations which controls Antestia bug and reduces the incidence of PTD to 1% or less (Chapter 2). The policy makers have adopted this method, which is currently under implementation by farmers. Information provided in Chapter 3 about PTD incidence and distribution, combined with populations of Antestia bug in relation to climatic variables (Chapter 4) should further assist in managing this pest and the defect. Understanding the mechanisms of PTD infection in coffee beans (Chapter 5) provides the basis for developing new approaches to eliminate or reduce PTD to non-problematic levels.

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: _______2018-09____________

Author and Title of thesis:
Joseph Bigirimana
Improving management of potato taste defect in coffee and elucidating its mechanisms of occurrence

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:
.

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Pentatomidae	*Antestiopsis thunbergii*	adult	10 (males)	pinned
Pentatomidae	*Antestiopsis thunbergii*	adult	10 (females)	pinned

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