

**RATE AND TIMING OF MEAT AND BONE MEAL (TANKAGE)  
APPLICATIONS INFLUENCE GROWTH AND YIELD OF SWEET CORN  
(*ZEA MAYS* VAR. *SACCHARATA*) AND SOIL WATER NITRATE  
CONCENTRATIONS IN TWO HAWAIIAN SOILS**

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## ABSTRACT

Meat and bone meal (MBM) is a locally available organic waste derived from animal by-products that has been used as a high-nitrogen organic fertilizer (9-10% N). Currently, it is being evaluated as a local input to produce food for a growing population in an environmentally and economically viable way. Despite efforts by previous researchers, the data needed to make a full range of fertilizer recommendations using MBM is limited. The two main objectives of this thesis were to determine effects of MBM application rates (0, 112, 224, 336, 448, 672 kg N ha<sup>-1</sup>) and timing on: 1) sweet corn growth, yield and quality and 2) soil water nitrate concentrations within and below the root zone.

The study was conducted at two agriculture research stations of the University of Hawaii, at Waimanalo Experiment Station from June to August 2015, and Poamoho Experiment Station from April to July, 2016 on the island of Oahu, Hawaii, USA. The experiment was arranged in a split-plot randomized complete block design (RCBD) with four replications. Treatments of timing included preplant (100% applied before planting) and split (50% preplant with 50% applied one month after planting). Treatments of rates (0, 112, 224, 336, 448, 672 kg N ha<sup>-1</sup>) were randomly distributed in the sub-plots sized 3.05 × 3.05 m<sup>2</sup>. Suction cup lysimeters were installed at two depths in each plot, 30 and 60 cm, to collect soil pore water on a weekly basis for nutrient analysis. A Minolta-502 SPAD leaf chlorophyll meter was used to monitor leaf greenness as an indicator of N status on a weekly basis. Marketable yield of sweet corn and data on plant growth parameters were collected. Corn growth, yield and leaf chlorophyll content increased with increasing application rate ( $P < 0.05$ ). At Waimanalo, MBM application rates significantly affected nitrate (NO<sub>3</sub>-N) concentrations within and below the root zone ( $P <$

0.10). Conversely, at Poamoho, timing but not rate of MBM application had significant effects on NO<sub>3</sub>-N concentrations within and below the root zone ( $P < 0.10$ ). Results of these field trials suggested that MBM is a viable organic N fertilizer for sweet corn. N rates of 224 kg ha<sup>-1</sup> or above were not different from each other, suggesting that application rates of 224 kg ha<sup>-1</sup> are sufficient. When MBM was applied in split applications, corn yield can be increased with an application rate of 336 kg N ha<sup>-1</sup> while keeping nitrate leaching levels below the root zone comparable to that in the N rate of 224 kg ha<sup>-1</sup>. Split of application of MBM reduced NO<sub>3</sub>-N concentrations below the root zone by 20% and 40% at Waimanalo and Poamoho, respectively. The results provide some support for benefits of split application of MBM to reduce NO<sub>3</sub>-N leaching. However, more research is needed to further investigate the nutrient release patterns from split applications of MBM in field sites under various conditions and soil types and for longer periods of study.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....ii

ABSTRACT.....iii

LIST OF TABLES.....vii

LIST OF FIGURES.....ix

LIST OF ABBREVIATIONS AND SYMBOLS.....x

CHAPTER 1- INTRODUCTION AND LITERATURE REVIEW

Introduction.....1

Meat And Bone Meal.....2

Sustainable Agriculture In Hawaii.....3

Nitrogen Dynamics.....4

Challenges of Organic Fertilizers.....5

Water Management.....6

Nitrogen Use Efficiency.....8

Monitoring Tools.....8

Previous Research.....10

Goals and Objectives.....11

CHAPTER 2- RATE AND TIMING OF MEAT AND BONE MEAL (TANKAGE)

APPLICATIONS INFLUENCE GROWTH AND YIELD OF SWEET CORN (ZEA  
MAYS VAR. SACCHARATA) AND SOIL WATER NITRATE CONCENTRATIONS

Abstract.....13

Introduction.....	14
Materials and Methods.....	16
Study Area.....	16
Experimental Design.....	17
Fertilizer Application Rate and Timing.....	18
Lysimeter Installation and Collection .....	19
Soil Solution Analysis.....	19
Plant Growth, Yield, and Quality Measurements.....	20
Statistical Analysis.....	21
Results.....	22
Precipitation and Temperature Data.....	22
Growth, Yield, and Quality.....	23
SPAD.....	24
Nitrate Concentrations.....	25
Discussion.....	26
CHAPTER 3- SUMMARY AND CONCLUSION	
Extending Our Knowledge Of MBM.....	30
Key Themes.....	31
Limitations to the Research.....	32
Direction for Future Research.....	32
TABLES AND FIGURES.....	34
LITERATURE CITED.....	65

## LIST OF TABLES

Table 1. Analyses of soil from field trials at two locations in Oahu, Hawaii analyzed by the ADSC at the University of Hawaii.....	34
Table 2. Nutrient analyses of meat and bone meal fertilizer used in field trials at two locations in Oahu, Hawaii analyzed by the ADSC at the University of Hawaii.....	34
Table 3. Mean (SE) growth and yield components of sweet corn grown in field trials receiving meat and bone meal at various application rates and timing at Waimanalo.....	35
Table 4. Mean (SE) growth and yield components of sweet corn grown in field trials receiving meat and bone meal at various application rates and timing at Poamoho.....	36
Table 5. Analyses of sweet corn tissue at the V8 growth stage taken at Waimanalo on July 31, 2015 in field trials receiving meat and bone meal fertilizer at various application rates and timing and analyzed by the ADSC at the University of Hawaii.....	37
Table 6. Mean (SE) biomass weights of sweet corn grown in field trials receiving meat and bone meal at various application rates and timing at Waimanalo.....	38
Table 7. Mean (SE) biomass weights of sweet corn grown in field trials receiving meat and bone meal at various application rates and timing at Poamoho.....	39
Table 8. Analyses of sweet corn tissue at the V8 growth stage taken at Poamoho on June 14, 2016 in field trials receiving meat and bone meal fertilizer at various application rates and timing and analyzed by the ADSC at the University of Hawaii.....	40
Table 9. Mean (SE) SPAD measurements of sweet corn grown in field trials receiving meat and bone meal at various application rates and timing at Waimanalo.....	41
Table 10. Mean (SE) SPAD measurements of sweet corn grown in field trials receiving meat and bone meal at various application rates and timing at Poamoho.....	42
Table 11. Sufficiency Index (S.I.) of leaf relative chlorophyll content from SPAD measurements over 7 weeks. Weekly readings were averaged and converted to a percentage sufficiency as compared to a high N rate (448 kg ha <sup>-1</sup> ) plots at the Waimanalo and Poamoho on the island of Oahu, Hawaii.....	43
Table 12. Mean (SE) nitrate-N concentrations (mg/L) within the root zone (30 cm depth) in field trials receiving meat and bone meal at various application rates and timing at Waimanalo Research Station in Oahu, Hawaii.....	44

Table 13. Mean (SE) nitrate-N concentrations (mg/L) below the root zone (60 cm depth) in field trials receiving meat and bone meal at various application rates and timing at Waimanalo Research Station in Oahu, Hawaii.....	45
Table 14. Mean (SE) nitrate-N concentrations (mg/L) within the root zone (30 cm depth) in field trials receiving meat and bone meal at various application rates and timing at Poamoho Research Station in Oahu, Hawaii.....	46
Table 15. Mean (SE) nitrate-N concentrations (mg/L) below the root zone (60 cm depth) in field trials receiving meat and bone meal at various application rates and timing at Poamoho Research Station in Oahu, Hawaii.....	47
Table 16. Analysis of variance (ANOVA) table for nitrate-N concentrations (mg/L) within and below the root zones as affected by rates and timing of meat and bone meal on in two locations in Oahu, Hawaii.....	48
Table 17. Analysis of variance (ANOVA) table for experiment studying the impact of meat and bone meal application rate and timing on NH <sub>4</sub> -N concentrations (mg/L) within and below the root zones in two locations in Oahu, Hawaii.....	49
Table 18. Analysis of variance (ANOVA) table for experiment studying the impact of meat and bone meal application rate and timing on soil water pH within and below the root zones in two locations in Oahu, Hawaii.....	49
Table 19. Analysis of variance (ANOVA) table for experiment studying the impact of meat and bone meal application rate and timing on electrical conductivity (EC) within and below the root zones in two locations in Oahu, Hawaii.....	50



## LIST OF FIGURES

Figure 1. Daily mean precipitation over the study period at Waimanalo (A) and Poamoho (B) Research Stations in Oahu, Hawaii.....	51
Figure 2. Daily mean air temperatures over the study period at Waimanalo (A) and Poamoho (B) Research Stations in Oahu, Hawaii.....	52
Figure 3. Regression analysis between meat and bone meal application rates and yield of sweet corn in Waimanalo (A) and Poamoho (B).....	53
Figure 4. Regression analysis between meat and bone meal application rates and (A) fresh weights (FW) and (B) dry weights (DW) of sweet corn ears in Poamoho.....	54
Figure 5. Regression analysis between meat and bone meal application rates and shoot fresh weights of sweet corn in Waimanalo (A) and Poamoho (B).....	55
Figure 6. Regression analysis between meat and bone meal application rates and shoot dry weights of sweet corn in Waimanalo (A) and Poamoho (B).....	56
Figure 7. Regression analysis between meat and bone meal application rates and root fresh weights of sweet corn in Waimanalo (A) and Poamoho (B).....	57
Figure 8. Regression analysis between meat and bone meal application rates and root dry weights of sweet corn in Waimanalo (A) and Poamoho (B).....	58
Figure 9. Regression analysis between meat and bone meal application rates and plant heights of sweet corn in Waimanalo (A) and Poamoho (B).....	59
Figure 10. Regression analysis between meat and bone meal application rates and leaf areas of sweet corn in Waimanalo (A) and Poamoho (B).....	60
Figure 11. Regression analysis between meat and bone meal application rates and soil water nitrate-N concentrations (mg/L) at a depth of (A) 30 cm and (B) 60 cm in Waimanalo.....	61
Figure 12. Weekly means of NO <sub>3</sub> -N concentrations by rate (mg/L) (A) within the root zone (30 cm) and (B) below the root zone (60 cm) at Waimanalo.....	62
Figure 13. Weekly means of NO <sub>3</sub> -N concentrations by timing (mg/L) (A) within the root zone (30 cm) and (B) below the root zone (60 cm) at Poamoho.....	63
Figure 14. Regression analysis between nitrate readings by Agricultural Diagnostic Service Center laboratory and Nitrate ion-selective electrode for samples from Waimanalo (A) and Poamoho (B).....	64

## LIST OF ABBREVIATIONS AND SYMBOLS

ADSC: Agricultural Diagnostic Service Center

BMP: Best Management Practices

DF: Degrees of Freedom

DW: Dry Weight

FW: Fresh Weight

MBM: Meat and Bone Meal

N: Nitrogen

$\text{NH}_4^+$ : Ammonium

$\text{NO}_3^-$ : Nitrate

$\text{NO}_3\text{-N}$ : Nitrate Nitrogen

NUE: Nutrient Use Efficiency

S.I.: Sufficiency Index

SPAD: Soil Plant Analysis Development

USDA: United States Department of Agriculture

# CHAPTER 1

## INTRODUCTION

Sustainability is using resources with the future in mind. The three pillars of sustainability are often referred as the triple bottom line or the three P's: people, planet, profit. The world's population is growing and it is projected that farmers will need to increase food production by 50% by 2050 (Tilman et al., 2002). In agriculture, the basic challenge is to feed the growing population, in a socially and economically responsible way, without compromising environmental integrity. Farming needs to be profitable so farmers can make a living wage for their families and continue farming to feed the world's population. Farming practices must also be socially acceptable. Agriculture is listed by the U.S. EPA (2015) on the list of contributors of nutrients, chemicals, and sediment to polluted water bodies. Furthermore, transportation of food and agricultural inputs often involves freight by ship, truck or rail and use of fossil fuels that contribute to carbon emissions (Weber & Matthews, 2008). Production practices, water, and nutrients need to be managed to not degrade the environment. Increasing the production of locally grown foods and using locally produced agricultural inputs can reduce the "food-miles" traveled and the carbon footprint. Farmers can also adopt best management practices (BMP's) to increase the efficiency of nutrient and water use. Farming practices that use local resources in an environmentally responsible way can help determine a pathway forward to meet our global food challenges.

### *Meat and bone meal*

Organic waste products are a commonly available local resource valuable to farmers because of their nutrient content. Meat and bone meal (MBM), also called “bone meal” or “tankage”, is an organic waste product derived from inedible animal parts and may be safely used as a fertilizer for organic crop production. Animal rendering is the ancient technique of using heat to physically and chemically transform non-edible animal by-products into a stabilized form (Meeker, 2006; Woodgate & Van der Veen, 2004). A primary product of the rendering process is oil, which is removed once heated and used for biofuel. The remaining product is dehydrated with steam to form a solid that can be used in feed or as fertilizer. The properties of MBM will vary depending on the feedstock, rendering process, and storage time (Zwetsloot et al., 2015). MBM was previously incorporated into animal feed. However, since the outbreak of mad cow disease this substance is prohibited as feed to ruminant animals. Currently, MBM is widely used in feed for fish (Ribeiro et al., 2016), as well as a fertilizer in field production (Nogalska, 2016).

Baker Commodities, Inc. in Kapolei, HI is the facility that produces MBM on the island of Oahu. It recycles the island’s fish and meat by-products into a useful locally available resource and is certified by the National Organic Program (NOP) for use on organic farms. It has been gaining popularity for use as a fertilizer and Hawaii producers are currently using this product, probably because of its high nitrogen content (~10%) and its low cost at about \$0.30 per pound. The N-P-K varies slightly from batch to batch, depending on feedstock, with an average analysis of 9-3-1 and a carbon to nitrogen ratio of roughly 5:1 (Arakaki, 2008). The Oahu facility produces 67 metric tons of MBM per

month (Radovich et al., 2012), which is available for purchase and use as a sustainable local organic fertilizer.

### *Sustainable agriculture in Hawaii*

Hawaii is a remote island chain, vulnerable to a range of disruptions to the shipping supply and highly dependent on imported goods (Leung & Loke, 2008). Increasing the island's food production is one of the State of Hawaii's initiatives as outlined in a recent report by the Office of Planning (2012). In a farmer survey, sourcing local inputs was ranked third as a barrier to increasing local crop production. Imported fertilizer is subject to dramatic changes in price associated with transportation costs that fluctuate with the price of oil (Radovich et al., 2012). In the latest Agricultural Census, farmers in Hawaii reported a 99.5% increase in expenditures for commercial fertilizer, lime and soil conditioners from 2007 to 2012 (U.S. Department of Agriculture & National Agricultural Statistics Service, 2012). As the prices of imported fertilizers increase, there will be a greater demand for local substitutes and lower cost alternatives. Replacing imported fertilizers with local organic inputs was identified as a priority issue by a survey of stakeholders in Hawaii agriculture (Radovich et al., 2009). The use of inexpensive local fertilizers such as MBM can help reduce our dependence on imports, increase farmer profitability and create a more resilient food system in Hawaii. Furthermore, it is important to the health of the islands for these waste products to be used in the most environmental friendly way as improper use or disposal of MBM has the potential to contribute potentially harmful nutrients to the environment, groundwater, and ocean.

### *Nitrogen dynamics*

Nitrogen (N) is the primary nutrient needed for plant growth and most soils do not supply sufficient N for crops, therefore fertilizers are necessary to achieve sufficient yields. Nitrogen management is important not only for crop production, but also from an environmental standpoint. Bruulsema et al. (2011) recommend following the framework of the four R's (right source, right amount, right place, right time) of fertilizer use in best management practices (BMP's) to minimize losses of N from leaching, volatilization, surface runoff, and denitrification. Leaching is the downward movement of N with water percolation through the soil profile and may occur when the excessive fertilizer is lost by over-irrigation or heavy rainfall. Preventing N leaching is important to reduce pollution of water bodies and reduce risk of contaminating drinking water, which can cause a human health condition called baby blue syndrome (Gupta et al., 2008). Nitrogen can be lost into the air, especially if fertilizers are placed directly onto the soil surface and not incorporated. Volatilization, a process where N changes into ammonia gas ( $\text{NH}_3$ ), may result in a net loss of N from the soil system and contributing to air pollution (Zhang et al., 2014). Organic fertilizers release nutrients more slowly as compared to mineral fertilizers, as N in organic form is converted into plant available forms of N including nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) through bio-chemical processes of mineralization and nitrification. Ideally, the rate of conversion from organic N to  $\text{NO}_3^-$  and  $\text{NH}_4^+$  will synchronize with plant N uptake. Nitrate is a highly mobile form of N because of it is a negative charged ion, anion, with limited ability to be held by soil surfaces and is carried to the plants roots with water by mass flow. Ammonium is less subject to leaching because it is a positively charged ion, or cation, and is held by the negative charge on soil

particles. Management options to mitigate N loss from fertilizers include reducing N application rates, synchronizing N supply and plant demand, use of cover crops (Di & Cameron, 2002). Broadening the understanding of MBM's N release pattern with more science-based studies will aid in appropriate use for agricultural production.

### *Challenges of organic fertilizers*

Growers today are faced with a dual responsibility of maintaining crop yields while minimizing negative impact of farming practices to the environment. Many traditional organic inputs such as compost and manure release N slowly and have low (< 5%) N content (Gaskell, 2007). According to Hartz (2006), a NO<sub>3</sub>-N concentration of 25 mg/L in the soil solution in the root zone is adequate for plant growth. However, it would be difficult to meet N demand from heavy feeding agronomic crops from traditional organic fertilizer inputs. A handful of higher N content organic inputs have been identified, mostly animal-based amendments, with N content ranging from 6-14% (Hartz & Johnstone, 2006). Previous research indicates that organic fertilizers can attain comparable yields to synthetic fertilizers (Seufert et al., 2012). However, these high N organic fertilizers still have considerable leaching potential even though N content and release rate is still much lower than synthetic fertilizers such as urea (46% N), or ammonium nitrate (36% N). Similar to these synthetic fertilizers, it is recommended that the high N organic fertilizers be applied in several split applications to improve N availability in the root zone to the crop and reduce potential N leaching to the environment. Split applications of organic fertilizers can be sidedressed and incorporated into the soil and/or applied by fertigation, which involves supplying liquid fertilizers

through the irrigation system. Challenges with both of these methods of split fertilizer application in organic systems include applying the split at the right rate and timing, labor costs, and technical feasibility to sidedress at post plant, or the solubility of organic fertilizers in fertigation lines. Information concerning N mineralization of organic inputs is needed to calculate potential N availability and hence rate and timing of fertilizer application. The first-order kinetic equation has been used to estimate N mineralization rate (Ahmad et al., 2014):

$$N_{\min} = N_0(1 - e^{-kt})$$

where:

$N_{\min}$ : the gross amount of N mineralized

$N_0$ : the potentially mineralizable N

$k$ : mineralization rate coefficient

$t$ : time

Mineralization rate is expected to be higher in field conditions than in laboratory settings where many incubation studies are performed, as it is affected by soil temperature, soil moisture and microbial activity. Therefore, in this thesis project, MBM application rates and timing will be based in part from the MBM mineralization rate studies.

### *Water management*

Efficient irrigation management is a key component of any nutrient management program in agriculture. Water is the driver of where the nutrients, especially N, will be in the soil, within or below the root zone, N, is highly mobile in the soil and can easily leach. While routine watering schedules and touch and feel analyses by farmers are often used to make irrigation decisions, technology has advanced in providing decision making tools for more precision water management (Fares et al., 2004). A variety of soil moisture sensors



and water budgeting procedures can be used to keep a balance of inputs from rainfall and irrigation and outputs lost from evapotranspiration (ET). Evapotranspiration is the combined total of water lost from evaporation from the soil surface and water lost by plant transpiration. The Penman-Monteith method is a robust and commonly used model for estimating reference ET ( $ET_0$ ) (Zotarelli et al., 2010). This method takes into account processes that influence ET such as air temperature, solar radiation, relative humidity, and wind speed (Allen et al., 1998). The Penman-Monteith model is expressed as follows:

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T - 273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)}$$

where:

$ET_0$  = reference evapotranspiration ( $\text{mm day}^{-1}$ )

$R_n$  = net radiation ( $\text{MJ m}^{-2} \text{day}^{-1}$ )

$G$  = soil heat flux ( $\text{MJ m}^{-2} \text{day}^{-1}$ )

$T$  = mean daily air temperature ( $^{\circ}\text{C}$ )

$u_2$  = wind speed at 2 m height ( $\text{m s}^{-1}$ )

$e_s$  = saturation vapor pressure (kPa)

$e_a$  = actual vapor pressure (kPa)

$e_s - e_a$  = saturation vapor pressure deficit (kPa)

$\Delta$  = slope of the saturation vapor pressure curve ( $\text{kPa } ^{\circ}\text{C}^{-1}$ )

$g$  = psychrometric constant ( $\text{kPa } ^{\circ}\text{C}^{-1}$ )

When all model inputs are not available, the Hargreaves model may be used if temperature and solar radiation data are available. The Hargreaves model has been used in Hawaii and showed a high correlation ( $r^2 = 0.90$ ) with other ET models when a 7-day average is used (Wu, 1997). Once ET is calculated, crop characteristics can be accounted for using a crop coefficient ( $K_c$ ) to estimate crop evapotranspiration ( $ET_c$ ) using the following equation:

$$ET_c = ET_0 \times K_c$$

Kisekka (2010) recommends the crop coefficient value be determined by crop growth stages for vegetables. For example, for sweet corn the crop coefficients are reported 0.3, 1.1, 1.0 for initial-, mid-, and late growth stages, respectively. Therefore in this thesis, irrigation practice was closely monitored in an attempt to efficiently provide adequate soil moisture without over-irrigating.

### *Nitrogen Use Efficiency*

In farming systems, it is challenging to maximize nitrogen use efficiency (NUE) and it must be evaluated in order to determine the overall efficiency of the system. For example, Agronomic Efficiency is calculated by the increase of crop yield per kg nutrient applied. NUE can also be evaluated by estimating nutrient uptake, such as the Percent Fertilizer Recovery where calculations use differences in N fertilized crops compared to an unfertilized control crop. Unfortunately, recovery of N in crop plants is less than 50% worldwide (Fageria, 2005). Over-irrigation and heavy rainfall events can also contribute to the loss of N to leaching. BMP's seek to maximize NUE by synchronizing supply of plant available N with crop demand. In efforts to find optimal rates of fertilizer application, a compromise is often made between maximizing yield and environmental preservation by limiting N loss. Fertilizer rates that achieve optimal yield are based on the intersection where increase in yield starts to level off and before a sharp increase in nitrate leaching loss occurs. While NUE was not specifically calculated in this study, the overall goal of the study was to maximize yield while minimizing nitrate leaching below the root zone.

### *Monitoring tools*

There have been several tools and strategies to monitor N status in crops that can save farmers money on fertilizer, optimize yields, and protect the environment. Preplant soil tests for nitrogen are generally considered unreliable because of nitrogen's dynamic nature, but are encouraged when application of manure is part of the nutrient management program. Tissue N concentrations may be the most precise measure of N status, however this method is destructive, costly, and time consuming. By the time the results are back from the laboratory it may be too late for in-season N fertilization. Ideally, recommendations for N application are based on real time N availability within the root zone. There are several other methods of measuring  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , including ion selective electrodes, nitrate test strips, and numerous other portable handheld devices that are less or non-destructive and quickly produce results. However, water samples are needed for these analyses so the soil solution must be extracted from the soil before nutrient concentrations can be measured. Methods to extract soil solution may include use of lysimeters or soil:water dilution at various ratios. Petiole sap testing is quick method for on-site analysis, although destructive, that can give farmers an indication of N status (Pena-Fleitas et al., 2015).

Leaf chlorophyll meters are a tool widely used to assess relative greenness of the plant and are quick, non-destructive and do not require soil water sampling. Leaf N content is one of the factors influencing greenness as well as variety differences, leaf age, deficiencies of other nutrients, and insect damage. Since many factors affect leaf greenness, it is recommended that the chlorophyll meter be calibrated to well-fertilized reference strips in each field. Reference strips should receive 25% more N than the

recommended rate at preplant N application (Brouder, 2003). Leaf chlorophyll meters can be used on a variety of crops including agronomic crops, ornamentals, and turf. Leaf chlorophyll meters are widely used in corn production and have been found to be a good predictor of grain yield (Wood et al., 1992).

#### *Previous research on MBM*

Researchers affiliated with University of Hawaii have studied the MBM produced in Hawaii for over 20 years. The earliest published study found in the literature on Hawaiian MBM was using a test crop of jicama (Valenzuela et al., 2000). In this replicated experiment, MBM was compared to synthetic fertilizer, chicken manure, and compost. Results showed MBM was comparable to synthetic fertilizer and yields were greatest at the rate of one kilogram per hectare. Radovich et al. (2012) tested yield response from four increasing application rates of MBM on eggplant and the results showed eggplant maximum yield peaked at an N rate of 300 kg ha<sup>-1</sup> and was slightly decreased at higher rates. Meat and bone meal was studied in combination with invasive seaweed (*Eucheuma sp.*) to determine growth response and yield of sweet potato using these local fertilizers in field trials (Cadby, 2014). In this study, MBM was applied at 155 kg N ha<sup>-1</sup> to compare with synthetic fertilizer (ammonium sulfate) as a source of N along with application rates of invasive seaweed as a source of K<sup>+</sup>. These studies suggest that a rate of 1 to 3 Mg ha<sup>-1</sup> is an adequate rate of MBM application, with rates of 4 Mg ha<sup>-1</sup> and above reducing yields. However, the numbers of crops tested in these field trials were limited in the rates compared, locations tested, and none examined timing of application.

Mineralization rate of Hawaiian MBM (Island Commodities, Kapolei, HI) was characterized in a study using leachate columns in Mollisol and Oxisol soils (Ahmad et al., 2016). The release rate of total plant available nitrogen was reported to be between 50 and 75% within 90 days. This is a wide range and needs to be confirmed with further studies and under field conditions, although is expected to be higher under field conditions with increased microbial diversity and activity. A greenhouse experiment, comparing MBM to urea, and evaluating the effect of MBM incorporated into potting media on corn and bean seedling growth and found fresh weight increased with increasing applications (Arakaki, 2008).

In a review of the available literature, it is clear that there is a dearth of rigorous studies on MBM. According to Radovich et al. (2012), “The need for recommended application rates to guide growers is increasingly apparent”. It is difficult for farmers to use MBM at the correct rates because there are currently no recommended rates from the manufacturer and it is difficult to find recommendation from the literature. More rigorous research studies on rates, timing of application of MBM on different crops and at multiple locations are needed. This research project seeks to fill the gap in knowledge of optimal rates and timing of MBM application on sweet corn while contributing to the previous body of work.

### *Goals and objectives*

The purpose of this study was to gain better resolution of release patterns and plant response to MBM fertilizer in agricultural field systems. Specifically we focused on application rates and timings for crop production in two different Hawaiian soils. Sweet

corn (*Zea mays* L.) will be used for a test crop because of its high demand for nitrogen and its significance as an agricultural crop in Hawaii (James L. Brewbaker, 2003). An estimate of optimal N application of MBM was based on achieving plant N sufficiency for sweet corn yields and minimizing potential harm to the environment through N losses. The research findings will be useful to stakeholders including local farmers who are interested in using MBM as a nitrogen source. These findings will help local farmers to maximize fertilizer efficiency, limit pollution and preventing unnecessary spending associated with excess N application. Specific objectives of this thesis were:

1. To evaluate the response of sweet corn plant growth, yield, and quality to applications (rates and timing) of MBM.
2. To determine the influence of application rates and timing on nutrient availability and leaching potential of nitrate and ammonium within and below the root zone.

It was hypothesized that the optimal rate of application will range from 224 - 392 kg N ha<sup>-1</sup>. This hypothesis was based on the recommended N rate for sweet corn from the literature (150-224 kg N ha<sup>-1</sup>) and the previous work done on mineralization rates of Hawaiian MBM that showed 50 – 75% of N mineralized in the first 90 days. Our second hypothesis was that application of fertilizer in two successive applications at one month apart will result in reduced NO<sub>3</sub>-N leaching below the root zone.

## CHAPTER 2

### **Rate and Timing of Meat and Bone Meal (Tankage) Applications Influence Growth and Yield of Sweet Corn (*Zea mays* var. *saccharata*) and Soil Water Nitrate Concentrations**

#### ABSTRACT

Using local resources and minimizing environmental impacts are two important components of sustainable agriculture. Meat and bone meal (MBM), or tankage, is an attractive locally available organic fertilizer because of its relatively high nitrogen content (9-10%). This experiment was conducted to investigate the response of sweet corn (*Zea mays* L. var. *saccharata* Stuart.) and nitrate concentrations to MBM application in two locations, Waimānalo and Poamoho on the island of Oahu in Hawaii. The objectives were to determine effects of application rates (0 – 672 kg N ha<sup>-1</sup>) and timing (100% preplant and 50% preplant with application of the remaining 50% one month later) of MBM on: 1) sweet corn growth, yield and quality and 2) soil water nitrate concentrations within and below the root zone. Experimental design was a split-plot design with four replications arranged in randomized complete block. Growth, yield and relative leaf chlorophyll content of sweet corn increased with increasing application rates ( $P < 0.05$ ) in both locations. Timing of application did not affect growth or yield parameters measured in Waimānalo. By contrast in Poamoho, yield was 13.6% greater ( $P < 0.05$ ) in preplant versus split MBM application. Although timing of MBM application was not significantly different between preplant and split MBM application for NO<sub>3</sub>-N below the root zone at Waimanalo ( $P < 0.05$ ) and marginally significant at Poamoho ( $P$

<0.10), N losses were numerically reduced by ~20% at Waimānalo and ~40% at Poamoho when applied as split compared to preplant. These findings suggest that MBM is an effective nitrogen source for sweet corn and split application of MBM may reduce potential for groundwater pollution. To the best of our knowledge this is the first report of the independent and interactive effects of rate and split application of MBM in sweet corn.

## INTRODUCTION

Utilizing locally produced inputs to efficiently provide plant nutrition are important components of sustainable agriculture systems (Radovich et al., 2012). Applying organic waste by-products as fertilizer also serves as a strategy to safely recycle nutrients that would otherwise need to be disposed (Fetter et al., 2012). Meat and bone meal (MBM), or tankage, is the solid by-product of animal rendering. After the outbreak of bovine spongiform encephalopathy, or mad cow disease, MBM was banned from animal feed and alternative uses were emphasized. Non-feed uses of MBM that are considered safe include use as soil amendment (Garcia & Rosentrater, 2008), biofuel (Mondini et al., 2008), and feed supplement for fish, poultry, and non-ruminant animals (Song et al., 2016). Meat and bone meal is an attractive choice for organic fertilizer because of its high (9-10%) nitrogen (N) content (Ahmad et al., 2016; Jeng et al., 2006). This is considerably higher than traditional organic inputs such as composts and manures that typically have low (<5%) N content and is comparable to other animal based-fertilizers such as blood and feather meal with a typical N content ranging from 6-12% (Gaskell, 2007). With a low carbon to nitrogen ratio, ranging from 3.7-5:1, MBM has a relatively high N



mineralization rate as compared to other organic amendments. When MBM is applied, crops also benefit from other macro- and micro-nutrients that enhance plant growth (Stepien & Wojtkowiak, 2015), increased microbial population and activity (Mondini et al., 2008), and suppression of plant parasitic pathogens (Lazarovits, 2001). Furthermore, MBM is allowed under the National Organic Program (NOP) and locally available in many regions of the world. Use of MBM to fertilize organic crops can aid in agriculture intensification, which will help meet increasing consumer demand for local and organically grown produce (Meas et al., 2015). There are few scientific studies on MBM even though annual production in U.S. and Canada combined was reported to be 2.5 million metric tons in 2004 (Garcia & Rosentrater, 2008). Previous studies have reported MBM is an effective N source for wheat and rapeseed (Stępień & Wojtkowiak, 2015), cereals (Nogalska et al., 2014), and comparable to mineral fertilizer on barley and oat (Chen et al., 2011). However, no studies from the available literature have investigated the effect of application timing of MBM within a cropping season.

Applying nutrients needed for crop growth while minimizing nutrient losses to the environment is an essential part of sustainable nutrient management (Zhou et al., 2016). However, synchronizing N supply with crop-N uptake is challenging when using organic fertilizers (West et al., 2016). Nitrogen in organic materials must first be mineralized to inorganic form, mainly nitrate ( $\text{NO}_3^-$ ), by soil bacteria. Mineralization is a complex process that depends on factors such as feedstock, temperature, moisture levels, soil type and pH (Hartz & Johnstone, 2006). Many organic amendments have low N content and high C:N ratio resulting in slow mineralization of nitrogen. However, this is not the case with high nitrogen materials. For example, West et al. (2016) reported increased sweet

corn yield attributed to in-season N application of feather meal, which has high N content. Among the studies that have evaluated mineralization of MBM, Ahmad *et al.* (2016) found the N release rate to be up to 75% within 90 days in a leachate column study in Hawaii with two soils. Similarly, Chaves *et al.* (2014) reported 18-37% of the N release in the first two weeks after soil application in Spain.

Split application of rapidly mineralizing, high N fertilizers are encouraged to improve N synchronization of N availability with crop demands (Montemurro & Diacono, 2016). According to guidelines suggested by Bustamante and Hartz (2015), MBM is a good candidate for split application in organic systems because of its high N content and low C:N ratio, which indicates potential for rapid N mineralization and nutrient release (Deenik & Yost, 2008). However, reports of the effect of split application of MBM on crop growth and soil solution  $\text{NO}_3^-$  concentrations were not found in the literature. The full effects of in-season fertilization with organic fertilizers have yet to be studied and refereed literature on the use of MBM fertilizer is limited.

Sweet corn (*Zea mays* L. var. *saccharata* Sturt.) was selected as a test crop because it is agriculturally important worldwide and planted year round in the tropics (J.L. Brewbaker, 2010). Specific objectives of this study were to examine the effect of MBM application rates (0 - 672 kg N ha<sup>-1</sup>) and application timing (1) on sweet corn growth, yield, and quality and (2) nitrate concentrations within and below the root zone.

## MATERIALS AND METHODS

### *Study area*

This study was conducted at two research stations of the University of Hawaii: Waimanalo and Poamoho Agriculture Research Stations, on the island of Oahu. The study was conducted from June 6 to Aug. 31, 2015 at Waimanalo, (21°19'57''N; 157°42'49''W) located at 23 m elevation with a mean annual temperature of 22° C and a 20-year mean annual rainfall of 1397 mm. The soil was a Waialua series (very fine, mixed, superactive, isohyperthermic Pachic Haplustolls). At Poamoho (21°32'38''N; 158°05'17''W), the study was conducted from April 26 to July 12, 2016 on a Wahiawa soil series (very fine, kaolinitic, isohyperthermic Rhodic Haplustox). The elevation at Poamoho station is 140 m, the mean annual temperature is 22° C, and a 20-year mean annual rainfall of 889 mm. Daily rainfall and temperature data for the two sites were collected from weather stations at each site and converted to daily averages.

### *Experimental Design*

The experiment was arranged in a split-plot randomized complete block design (RCBD) with four replicates, except at Waimanalo where the 112 kg N ha<sup>-1</sup> rate treatments were replicated only two times due to logistics. Treatments of application timing included preplant (100% applied before planting) and split (50% preplant with 50% applied one month after planting) were randomly distributed in the main plots sized 6 x 12 m (72 m<sup>2</sup>). Sub plot treatments were randomly distributed in experimental units 3.05 x 3.05 m (9.3 m<sup>2</sup>) in size with treatments of targeted N application rates of 112, 224, 336, 448 kg ha<sup>-1</sup> and an unfertilized control. At Poamoho an additional N rate of 672 was included in the field trial in attempt to reach a yield plateau that was not well defined in Waimanalo the

first year. The N rate of 224 kg ha<sup>-1</sup> used in this experiment is the rate recommended in the literature for sweet corn (Hochmuth & Hanlon, 2000).

#### *Fertilizer application rate and timing*

Meat and bone meal (Baker Commodities, Kapolei, HI) contained an average (n = 12) of 9.7% N, 3.27% P, 0.84% K (Ahmad et al., 2016). Initial soil and MBM samples for each location were submitted to the Agricultural Diagnostic Service Center (ADSC) at the University of Hawaii, Manoa for nutrient analysis (Table 1, Table 2). Field sites were tilled and treatments of MBM were incorporated manually to a depth of 10 cm one week before planting. Preplant fertilization occurred 100% before planting and split application of fertilizer occurred 50% preplant with the remaining 50% one month later. Sweet corn ‘Supersweet #10’ was direct seeded along drip irrigation lines with 19 cm between plants and 75 cm between rows, at approximately 62,500 plants ha<sup>-1</sup>. Corn seeds were sown on June 6, 2015 and April 20, 2016, thinned to one plant per hole after one week, and harvested on August 30, 2015 and July 11, 2016 for Waimanalo and Poamoho, respectively. Pest and weed control was performed at each site as needed. At Poamoho, lime (Microna Agricultural Lime, Crop Protection Services, Kunia, HI) was applied at a rate of 1 Mg ha<sup>-1</sup> to adjust pH from 5.4 to 6.4 according to the liming curve for this soil type (Hue & Ikawa, 1997). For the split application that occurred one month after planting, MBM fertilizer was band applied on the soil surface 7 cm from plants and soil incorporated manually. Field technicians scheduled irrigation using a managed allowable depletion level of 50% of field capacity. A simple water balance was calculated using the sum of rainfall and irrigation for water inputs and subtracting ET losses as water outputs.

When data was not available from the onsite weather stations, climatic data was used from the Rainfall Atlas of Hawaii (Giambelluca et al., 2014).

#### *Lysimeter installation and collection*

Two suction-cup lysimeters (Soil Moisture Corp, Santa Barbara, CA) were installed between the center rows of each plot for weekly collection of soil pore water during the study period. Holes were drilled using an auger at two depths: within the root zone (30cm) and below the root zone (60cm). Hydrologic contact between the soil and lysimeter was achieved by making a slurry (1:2; soil:water) to fill the space between the bottom of the hole and the ceramic cup at the base of the lysimeter. A vacuum was drawn 80 kPa using a hand vacuum pump and the neoprene tubing was clamped to maintain suction. A 50 mL sample from each lysimeter was extracted each week using a collection kit and the remaining soil solution was discarded. To reset for the next sampling period, lysimeters were rinsed with deionized water and a vacuum was drawn to 80 kPa.

#### *Soil solution analyses*

The soil solution samples were collected weekly from lysimeters for eight consecutive weeks during the study periods. After collection, samples were cooled for transport, stored at 4° C to prevent nitrogen transformation prior to analysis, and were analyzed within 36-hours (Crabtree & Seaman, 2006). Vernier ion-selective electrodes (Vernier Software, Beaverton, OR) were used to measure nitrate (NO<sub>3</sub>-N) and ammonium (NH<sub>4</sub><sup>+</sup>-N) ions according to manufacturers recommendations. The electrodes were soaked for at least 30 minutes and calibrated to high (100 mg/L) and low (1 mg/L) standard solutions

before each use. Samples were brought up to ambient temperature (25° C) prior to analyses. Data from the electrodes were collected using LabQuest® interface (Vernier Software, Beaverton, OR) used as a standalone device. For quality assurance of NO<sub>3</sub>-N analysis, 25 random samples from each location over the study period were submitted to the ADSC at the University of Hawaii for analysis using method 353.2 to determine NO<sub>3</sub>-N by automated colorimetry (O'Dell, 1993). Electrical conductivity (EC) and pH were analyzed from each soil water sample with a digital meter (Model 98129, Hanna Instruments, Woonsocket, RI). The meter was calibrated before every session with EC calibration solution of 12.88 mS/cm and pH calibration solutions of 4.01 and 7.01. All sensors were rinsed with deionized water between samples and blotted dry before reading the next sample.

#### *Plant growth, yield and quality measurements*

Data was collected from the two center rows of the four rows per plot for all plant growth, yield, and quality parameters. Relative leaf chlorophyll content was measured weekly for 8 consecutive weeks during the study periods using a Minolta-502 SPAD meter (Spectrum Technologies, Plainfield, IL). One reading was taken from the top collared leaf of each of 3 plants per plot and the average was recorded. Corn plant tissue was sampled from the top fully mature leaf, four per per plot, at the V8 (8<sup>th</sup> leaf with visible leaf collars) growth stage and wiped clean with deionized water (Jones & Case, 1990). Tissue samples were combined by treatment to form a composite sample and submitted for nutrient analysis. Plant growth data included post-harvest measurements of leaf area, plant height, and fresh and dry weights of shoot and root biomass. Leaf area,

plant height, and below ground root biomass was taken from a sub-sample of 3 representative plants per plot, while above ground shoot biomass was taken from a sub-sample of 5 representative plants cut at 10 cm above ground level. Shoot and root biomass fresh weights were recorded, then oven dried at 70° C for 72 h and dry weights were recorded. Yield data included total fresh weight (husk on, untrimmed) of marketable ears per plot, corn ear fresh weights, dry weights, and soluble solids content (SSC). Number of plants per row was recorded and yield was expressed as grams per plant. Ear fresh weights were recorded from a representative sub-sample of 3 ears per plot, then dried at 70° C for 72 h and dry weights were recorded. An additional sub-sample of 3 ears per plot was selected to estimate kernel SSC, as measured in brix units (°Bx) where kernels were cut from a 5 cm cross section in the center of the cob and the composite sample was squeezed with a garlic press onto a digital refractometer (Model 96811, Hanna Instruments, Woonsocket, RI).

### *Statistical Analysis*

Data from each location was subjected to split-plot analysis of variance (ANOVA) using SAS Version 9.4 (SAS Institute Inc., 2013). Soil water NO<sub>3</sub>-N and leaf chlorophyll measurements were subjected to repeated measure analysis using PROC MIXED procedure. All variables were checked for normality using PROC UNIVARIATE and transformed accordingly if needed. Only true means were presented. When treatment effects were significant, means were separated by Waller-Duncan *k*-ratio (*k* =100) *t*-test. Statistical significance was defined as  $P \leq 0.05$  unless otherwise indicated. Regression analysis between rates of MBM application and corn growth, yield, and soil water NO<sub>3</sub>-N

parameters was conducted using JMP<sup>®</sup> Version 13.1 (SAS Institute, Inc., 2013). Linear regression analysis was also used to evaluate accuracy of NO<sub>3</sub>-N analyses between the ion-selective electrode and standard methods of the ADSC laboratory at the University of Hawaii.

## RESULTS

### *Precipitation and temperature data*

The mean precipitation during the study period at Waimanalo was 3.7 mm d<sup>-1</sup>, ranging from 0 to 87 mm d<sup>-1</sup> with several heavy rainfall events related to a tropical storm occurring during the last 14 days of the study (Figure 1). This was six times the amount of rainfall as compared to multiyear averages. The temperature ranged from 21°C to 28°C with a mean temperature of 25.9°C during the study period at Waimanalo (Figure 2); this is 0.87°C warmer than the multiyear average temperature during these months. Poamoho received less precipitation than Waimanalo with a mean of 2 mm d<sup>-1</sup> and a range of 0 to 38 mm d<sup>-1</sup> during the study period. At Poamoho, the mean daily temperature ranged from 21°C to 26°C and the mean temperature was 24°C during the study period. The temperature at Poamoho was generally similar to multiyear averages and precipitation was about double the multiyear average due to two heavy rainfall events at the end of May 2016. Crop ET (ET<sub>c</sub>) was calculated using the Hargreaves ET model with 7-day moving average of daily readings was between 4 and 5 mm day<sup>-1</sup> in Waimanalo and 3 and 7 mm day<sup>-1</sup> in Poamoho during the study periods. Using the water balance approach to check soil moisture we found that inputs of rainfall and irrigation (544.49



mm) exceeded outputs ( $ET_c = 233$  mm) at Waimanalo and inputs (431.9 mm) exceeded outputs ( $ET_c = 316$  mm) at Poamoho.

### *Yield, growth, and quality*

At Waimanalo, MBM rates had a highly significant effect on sweet corn yield, shoot fresh and dry weights, root fresh and dry weights, and leaf area ( $P \leq 0.01$ ), and plant height ( $P \leq 0.05$ ) significantly (Table 3 & 4). Shoot and root biomass increased with increasing MBM rate. Although the highest yield of marketable sweet corn (213.88 g plant<sup>-1</sup>) was achieved with the highest application rate of 448 kg N ha<sup>-1</sup>, yields from the MBM rate of 224 kg N ha<sup>-1</sup> were not significantly different than those fertilized with 336 or 448 kg N ha<sup>-1</sup>. Corn yield regressed with MBM rate quadratically ( $P = 0.011$ ,  $R^2 = 0.38$ , Fig. 3). However, application timing did not have an effect for any of the parameters measured. Plant tissue analysis at the V8 growth stage showed N deficiencies in the control and split 112 kg N ha<sup>-1</sup> treatments and low levels of Ca, Mg, Mn, and B (Table 5) as compared to nutrient sufficiency ranges (Maynard & Hochmuth, 2007). Kernel quality based on SCC averaged 13.3% ( $\pm 1.15$ ) and was not different among the treatments.

At Poamoho, MBM rates had a highly significant effect on sweet corn yield, ear fresh or dry weights, shoot fresh and dry weights, root fresh and dry weights, plant height, and leaf area ( $P \leq 0.01$ ), and shoot dry weights significantly ( $P \leq 0.05$ ; Table 6 & 7). The highest sweet corn yield (288.08 g plant<sup>-1</sup>) was achieved with the highest MBM application rate (672 kg N ha<sup>-1</sup>), but yield in all other MBM rates were not different from 672 kg N ha<sup>-1</sup> except for lower yield in the 112 kg N ha<sup>-1</sup> rate (Table 6). Corn yield

regressed with MBM rates quadratically ( $P \leq 0.01$ ,  $R^2 = 0.27$ , Fig. 3). Unlike Waimanalo, application timing of MBM was significant ( $P \leq 0.05$ ), where yield decreased from 255.52 to 220.71 g plant<sup>-1</sup> for preplant compared to split application. From the plant tissue analysis, results showed N deficiencies in the unfertilized control and Mg levels were marginal in all treatments. Kernel quality based on SCC averaged 12.71% ( $\pm 1.16$ ) and was not significantly different among the treatments. Regression analyses between growth and yield parameters and MBM application rates are shown in Figures 4 -10.

### *SPAD*

At both study locations, relative leaf chlorophyll content was different among MBM application rates by week ( $P \leq 0.01$ ), but was not different between application timing (Table 9 & 10). At Poamoho, a significant interaction of week×rate×timing ( $P \leq 0.01$ ) was observed. The range of SPAD readings between both locations was 25.3 - 65.3 and over the growing season, the average was 46.70 and 41.71 for Waimanalo and Poamoho, respectively. SPAD measurements were converted to a N sufficiency index (S.I.) to express in relative terms to evaluate sufficiency of leaf N in corn as compared to a well-fertilized reference using the equation (Varvel et al., 1997):

$$S.I. \% = \frac{\text{average reading}}{\text{average reference}} \times 100$$

In both locations, S.I. was calculated by dividing average readings from each plot by the average reference using averages of plots with the N rate of 448 kg ha<sup>-1</sup>. The S.I. corresponding N status was then interpreted according to the guidelines of Brouler (2003). At Waimanalo, the mean S.I. over 7 weeks of readings showed rates of 336 and 448 kg N ha<sup>-1</sup> were above 95% and therefore considered sufficient (Table 11).

Conversely, the 224 kg N ha<sup>-1</sup>, 112 kg N ha<sup>-1</sup>, and unfertilized control plots were 93, 89, and 85% of S.I., respectively. When S.I. is below 95%, it is recommended to apply more N to achieve maximum agronomic yields. The same results were found at Poamoho over 8 weeks, where the rate of 336 kg N ha<sup>-1</sup> was considered sufficient at 96% while N rates of 224 kg ha<sup>-1</sup>, 112 kg ha<sup>-1</sup>, and control plots indicated a S.I. of 89, 84, and 79%, respectively, when compared to the well fertilized (672 kg N ha<sup>-1</sup>) reference plots.

#### *Nitrate concentrations*

At Waimanalo, for NO<sub>3</sub>-N within the root zone, rates and week effects were significant, and significant interactions of rate×timing and week×rate×timing were observed ( $P \leq 0.10$ ; Table 12). Split application of MBM reduced NO<sub>3</sub>-N load within the corn root zone during the first few weeks after application compared to preplant application. For preplant application, high N rates of 336 and 448 kg ha<sup>-1</sup> resulted in 56.93 and 71 mg/L NO<sub>3</sub>-N, respectively. Whereas when MBM was applied in split applications, the NO<sub>3</sub>-N levels within the root zone at the two highest N rates of 336 and 448 kg ha<sup>-1</sup> were 32.60 and 33.58, respectively.

For NO<sub>3</sub>-N below the root zone, rates and week effects, as well as the interactions between rate×timing and week×rate×timing were significant ( $P \leq 0.05$ ; Table 13). A quadratic yield regression between MBM rate and NO<sub>3</sub>-N within and below the root zone was observed ( $P \leq 0.01$ , Fig. 11). Timing of application did not affect on nitrate concentrations within or below the root zone at Waimanalo. Nitrate-N concentrations were highest during weeks 2 – 4 within the root zone (30 cm depth) and highest in weeks 2 - 5 below the root zone (60 cm depth). The highest measurement of NO<sub>3</sub>-N within the

root zone ( $162.8 \text{ mg L}^{-1}$ ) was recorded on week 4 from a preplant  $448 \text{ kg N ha}^{-1}$  plot and the highest measurement below the root zone ( $110.8 \text{ mg L}^{-1}$ ) was from a preplant  $336 \text{ kg N ha}^{-1}$  plot (Fig. 12). The mean  $\text{NO}_3\text{-N}$  concentrations during the study period at Waimanalo were  $25.6$  and  $20.7 \text{ mg L}^{-1}$  for within and below the root zone, respectively. Mean separation according to the Waller-Duncan  $k$ -ratio ( $k=100$ )  $t$ -test of rates at Waimanalo showed N rates of  $448 \text{ kg ha}^{-1}$  to be in the same group with  $336$ ,  $224$  and  $112 \text{ kg ha}^{-1}$  designated with the letter A while the B letter group was shared with  $336$ ,  $224$ ,  $112$  and  $0 \text{ kg ha}^{-1}$ .

At Poamoho, soil water  $\text{NO}_3\text{-N}$  concentrations within the corn root zone were affected by MBM application timing ( $P \leq 0.05$ ) and week ( $P \leq 0.01$ ) and significant interaction between rate $\times$ timing ( $P \leq 0.01$ ) was observed (Table 14 & 15). Similarly,  $\text{NO}_3\text{-N}$  below the root zone was affected by timing of MBM application but only at marginal level ( $P \leq 0.10$ ) and the effect varied by week. Nitrate-N concentrations were highest in the first 5 weeks of the study (Fig. 13). In week 4, significant differences showed between levels of  $\text{NO}_3\text{-N}$  within the root zone by timing with concentration of  $101.65$  and  $34.78 \text{ mg L}^{-1}$  for preplant and split application, respectively. Overall ANOVA tables for both locations for  $\text{NO}_3\text{-N}$  within and below the root zone are shown in Table 16.

In comparison of the nitrate ion-selective electrode to the ADSC method 353.2 of colorimetric analysis,  $R^2 = 0.99$  and  $R^2 = 0.68$  at Waimanalo and Poamoho, respectively (Figure 14). Statistical analyses of  $\text{NH}_4^+\text{-N}$ , pH, and EC follow similar trends to  $\text{NO}_3\text{-N}$  in both locations where week is often highly significant ( $P \leq 0.01$ ), and rate and timing are sometimes significant or marginally significant (Tables 17-19).

## DISCUSSION

This study evaluated response of MBM application rates (0 - 672 kg N ha<sup>-1</sup>) and fertilization timing (preplant, split) on sweet corn growth, yield, and quality and soil water nitrate concentrations within and below the root zone in two locations in Oahu, Hawaii. Overall, yield was comparable to other tropical sweet corn hybrids (J.L. Brewbaker, 2008). At Waimanalo, yields from rates of 224 kg N ha<sup>-1</sup> were not different than N rates of 336 or 448 kg ha<sup>-1</sup> suggesting that 224 kg N ha<sup>-1</sup> is enough to meet crop uptake needs. The tissue testing at the V8 growth stage showed the unfertilized plots were below N sufficiency levels. In addition, the split 112 kg N ha<sup>-1</sup> at Waimanalo was below N sufficiency levels. The relative chlorophyll content from our SPAD measurements was the same for both locations and showed the rates of 224 kg N ha<sup>-1</sup> and below were not reaching their full yield potential as compared to the high rates. This may have been due to deficient concentrations of NO<sub>3</sub>-N (lower than 20 mg/L) that were observed in the root zone from N rates 224 kg ha<sup>-1</sup> and below during plant growth.

In concurrence with Chen (2011), we found increased along with increasing rate of MBM. However, others reported yield increased with increasing rate of MBM, but no further yield increase beyond MBM rates of 1.5 t ha<sup>-1</sup> on cereal crops in Poland (Nogalska et al., 2014) and 120 kg N ha<sup>-1</sup> with jicama in Hawaii (Valenzuela et al., 2000). Maresma et al. (2016) reported increase in yield beyond 240 kg N ha<sup>-1</sup> for maize grown in Spain from various treatments of organic and synthetic fertilizers. High rates of fertilization are also used in sweet corn crops to achieve increased yield goals and corn, like many grasses, is known to be able to consume large amounts of nitrogen.

Lazicki et al. (2016) reported that organic fertilizers with high N and rapid mineralization are good candidates for sidedress N. In our attempt to use MBM for sidedress N, we found split application reduced potential for NO<sub>3</sub>-N leaching while it did not have much of an effect on yield. At Waimanalo the N rates of 224 kg ha<sup>-1</sup> preplant and 336 kg ha<sup>-1</sup> split provided the target range of nitrate-N (20-30 mg/L) to the rhizosphere of the corn plants during the growing season, although higher rates of N have been documented to increase corn yield closer to maximum yield. The benefits of split application are also seen in week 8 at the end of the corn's growth cycle when lower nitrogen levels are needed, the NO<sub>3</sub>-N levels for preplant rates of 336 and 448 were 23.40 and 29.28 mg/L respectively, whereas split application NO<sub>3</sub>-N levels were both below 20 mg/L, at 5.63 and 17.33, respectively. At both locations the N rates of 448 kg ha<sup>-1</sup> and above provided beyond the sufficiency range for nitrate-N in the root zone and subsequently had considerable leaching as evidenced by the high nitrate-N concentrations below the root zone. Considering economical costs and environmental impacts of MBM, we suggest the application rate of 224 kg N ha<sup>-1</sup> is sufficient at preplant, while higher rates of 336 kg N ha<sup>-1</sup>, when applied in split, can provide fertility without increasing risks to the environment.

In comparing the two locations, the increase in yield at Poamoho may be attributed to the climate of the higher elevation including cooler temperatures and increased solar radiation, which may have allowed more time for grain fill, as compared to Waimanalo. The weather was warmer than the multiyear averages at Waimanalo and that may have promoted quicker physiological development that resulted in a shorter period of grain fill. Similar to Ahmad et al. (2009), we found that soil water nitrate

concentrations were higher in Poamoho than in Waimanalo for depths, 30 and 60 cm. The increased leaching at Poamoho could be due to the differences in soil physical and mineral properties between the two locations (Filho et al., 2017). At both locations, N losses were numerically lower when MBM was applied as split compared to preplant.

In conclusion, these findings suggest that MBM is an effective source of N for sweet corn production in the tropics and split application of MBM may reduce potential groundwater pollution. To the best of our knowledge this is the first report of the independent and interactive effects of rate and split application of MBM in sweet corn. Meat and bone meal contains very little K and farming systems may benefit from supplemental fertilization with invasive algae to provide K and replace imported inputs (Gangaiah et al., 2017). In-season monitoring of nutrients using tools such as SPAD and tracking soil water  $\text{NO}_3\text{-N}$  concentrations is very important in organic production systems where N release can be variable as affected by soil temperature, moisture and microbial activity. This research can help develop crop specific best management practices for applying MBM as an organic fertilizer. Meat and bone meal should also be considered a viable fertilizer in organic seedling production, landscaping industry and for native plant growth and establishment.

## CHAPTER 3

### SUMMARY AND CONCLUSION

Application rate and timing of meat and bone meal (MBM) was evaluated in field trials at two University of Hawaii Agricultural Research Stations in 2015-2016. Meat and bone meal is a promising locally produced and available organic fertilizer because of its high N content. Use of MBM can reduce reliance on imported fertilizers and increase self-sufficiency of our agricultural inputs in Hawaii. With its rapid mineralization rate for an organic fertilizer, MBM should be managed appropriately. Fertilizer best management practices should be followed to provide sufficient plant nutrition by applying nutrients at the correct rate, at the right time and placement in order to minimize harm to the environment. This study built upon a foundation of work that included nutrient analyses and mineralization studies. To the best of our knowledge, this study was the first to examine independent and interactive effects of rate and timing of MBM application.

#### *Extending knowledge and understanding of MBM*

While several researchers have looked at the effect of MBM application rate on vegetable crops, this research is the first to examine application timing of MBM as a treatment and the interactive effects of rate and timing. This study verifies the work of Ahmad et al. (2016) from incubation with two Hawaiian soils on the mineralization rate and release pattern of meat and bone meal confirming that MBM rapidly mineralizes in two common agricultural soils. We found that the rapid mineralization may result in an asynchrony of nutrient release and transformation into plant available forms, mainly nitrate-N. It has been recommended that this type of organic fertilizer is a good candidate for being



applied in split doses, however our results were varied. At Waimanalo, no difference was found whereas at Poamoho a significant difference was observed where yields decreased with split application. Ideally, split application would result in better fertilizer use efficiency resulting in same yields with less fertilizer or increased yields with the same amount of fertilizer.

*Key themes that have emerged from the project*

Our findings indicated that split application of MBM reduced nitrate leaching below the root zone by 20% and 40% in Waimanalo and Poamoho, respectively. When the MBM was applied preplant we saw increased concentrations of  $\text{NO}_3\text{-N}$  levels within the rhizosphere well beyond the  $25 \text{ mg L}^{-1}$  recommended sufficiency level for plant growth. In addition, this early spike of  $\text{NO}_3\text{-N}$  occurred in weeks 2 and 3 in Waimanalo and weeks 1 and 2 in Poamoho at a time when the sweet corn seedlings were very small with limited root growth. This mismatch between nutrient release and plant uptake resulted in high amounts of  $\text{NO}_3\text{-N}$  leached below the root zone, especially at Poamoho, in the early growth period. When split applied, we saw a small bump in nitrate-N concentrations in week 6 and 7 in Poamoho that suggests the second application mineralized and was plant available, although  $\text{NO}_3\text{-N}$  levels in the soil water were below the  $25 \text{ mg L}^{-1}$  critical threshold level, which may have decreased yields. No such increase was detectable in the later part of the growing season in Waimanalo. This could be due to the heavy N demands from the sweet corn plant around the time of tasseling. It is possible that during this rapid growth stage the corn root system was well developed and the N was uptaken by the plant.

### *Limitations to the research*

Limitations of this research include variability of soil conditions at both locations and difficulty obtaining regular soil water samples at Poamoho. At Poamoho there were times we were unable to get a soil solution sample from the lysimeters, possibly due to low soil moisture and inadequate vacuum suction. Also for this experiment, field areas were selected that have been fallow for several months under the assumption that nutrients were removed by previous crops or leached away. However, upon our first collection of our soil pore water samples at both locations we found nitrate-N levels high in some areas of the field. Soil tests were composited from entire field assuming homogeneity. With the inherent heterogeneity it would have been desirable to collect soil samples from each plot, although that process would have been time consuming and costly. Another way to improve the homogeneity of the study sites would have been to plant a nitrogen scavenging cover crop, such as ryegrass, and terminate the crop when it reaches reproductive stage and push crop residues to the side of the field outside of the designated experimental area. At Poamoho the field history included a biochar trial with sweet corn and napier grass where the biochar may have affected pH and nitrogen holding capacity. This study could have been further improved with larger plot sizes and more replications, which would have decreased the variation we saw related to non-homogeneous field conditions and small plot sizes.

### *Direction for future research*

Implementing a full nitrogen budget would have been good to get better resolution on fate of the nitrogen applied in the second split application. Because yield decreased at

Poamoho we can conclude the N from the split application did not fully reach the plants. This may be due to the lack of incorporation in the soil with our manual incorporation methods or sub-optimal conditions for mineralization of the MBM. From this project we know split application decreased nitrate leaching below the root zone. However, due to the unexpected heterogeneity in the field the differences between the treatments are not as clear as we would like. Further research should include more field trials in comparing rates, timing, and placement of MBM. Nutrient availability from split application may be improved with mechanical placement and incorporation as compared to manual placement as band applied and manual incorporation. Fertigation is another promising option to improving N synchronization with organic materials and would save labor costs from having to go through the field a second time to band apply fertilizer. Current challenges with fertigation of nutrients from organic sources include clogging from poor solubility and obtaining the proper equipment to inject the liquid organic fertilizers into the irrigation line. Many farmers are already using drip irrigation and could easily transition to fertigation with proper equipment and training. MBM is still one of the most promising sources of N from locally available fertilizers in Hawaii and more studies need to be conducted on crop response using different crops, in different locations over several seasons and years. Further investigation could be carried out for longer periods to determine the N mineralization beyond the cropping period. If 50 – 75% was available in the first 90 days, the remainder will continue to be available in the soil profile and it should be determined how to best be utilized by subsequent crops.

TABLES AND FIGURES

Table 1. Soil analyses from field trials at two locations in Oahu, Hawaii analyzed by the ADSC<sup>z</sup> at the University of Hawaii.

pH	P <sup>y</sup>	K	Ca	Mg	Mn	Fe	Cu	Zn	N	C
	-----mg/g-----				-----mg/dm <sup>3</sup> -----			----%----		
-----Waimanalo-----										
6.6	306	393	4510	1465	384	191	11	11	0.14	2.1
-----Poamoho-----										
5.4	288	623	887	179	695	41	28	26	0.07	1.2

<sup>z</sup>Agriculture Diagnostic Service Center

<sup>y</sup>The Modified Truog procedure was used for extractable P.

Table 2. Nutrient analyses from meat and bone meal fertilizer<sup>z</sup> used in field trials at two locations in Oahu, Hawaii analyzed by the ADSC<sup>y</sup> at the University of Hawaii.

N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B	C
-----%-----				-----ug/g-----				---%---		
-----Waimanalo-----										
8.9	2.8	0.67	5.5	0.15	992	12	79	3	12	46.7
-----Poamoho-----										
10.0	2.7	0.83	5.0	0.16	1526	17	87	3	5	47.1

<sup>z</sup>Meat and bone meal fertilizer obtained from Baker Commodities in Kapolei, HI.

<sup>y</sup>Agriculture Diagnostic Service Center