Rwanda Antestia-Potato Taste Research Group

WHAT DO THE VOLATILE COMPOUNDS IN GREEN COFFEE REVEAL ABOUT POTATO TASTE?

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Rwanda Collaboration Colloquium Sponsored by:

Outline

- Background about potato taste defect
- Our research questions
- Surface volatile organic compounds as a means to study manifestation of PTD in coffee
- Experiment: SVOCs by SPME GC-MS
- Results: a profile for PTD
- Conclusions
- Antestia bug SVOCs: relationship to PTD profile
- Acknowledgements

Background

- In 2001, producing specialty coffee became part of a strategy to develop the Rwandan economy
- Washing stations were implemented to support specialty coffee: specialty production rose from 1% in 2002 to 27% in 2012
- Potato Taste Defect (PTD) threatens this plan.[1]

[1]Government of Rwanda, *Strategic Plan for Agricultural Transformation in Rwanda* (Ministry of Agriculture and Animal Resources, Kigali, 2004).

What is PTD?

- **Potato Taste Defect (PTD)** leads to roasted coffee that smells and tastes like **potato skins**
- **PTD is not detectable** in green coffee beans by smell or appearance
- **PTD is detected in the cup** of coffee
- **PTD is detected in roasting coffee** near the first crack
- **PTD affects:**
  - 400,000 smallholder farmers, 86% of whom are women[1]
  - 25% of Rwandan coffee crops in 2008[2]
  - 51% of Cup of Excellence entries affected by PTD in 2013

[1]World Bank 2011

[2]Ngabitsinze, J. C., A. Mukashema, M. Ikirezi and F. Niyitanga. (October 2011). Planning and costing adaptation of perennial crop systems to climate change: Coffee and banana in Rwanda. Case study report. http://pubs.iied.org/pdfs/G03174.pdf#page18 .

TO AVOID PTD COFFEE: ALL DAMAGED BEANS ARE SORTED OUT

Unfortunately, sorting is not 100% effective

- Sometimes PTD is detected during coffee cupping, or tasting, before coffee buyer makes a purchase
- Affected beans may also manifest the defect during export away from Rwanda and be noticed upon arrival
  - Buyers decline to purchase lots with PTD

BUYERS SELECT COFFEES TO PURCHASE BY ROASTING AND TASTE-TESTING

One PTD cup in 40 or fewer cups will result in the coffee being rejected.

Need research to detect and eradicate PTD

- The **Global Knowledge Initiative** organized an international effort called LINK: Learning and Innovation Network for Knowledge and Solutions
- Dr. Daniel Rukazambuga and colleagues at the **National University of Rwanda** won the first LINK grant for international team approach toward a solution for PTD
- **We were invited** to join the GKI team as chemists, to analyze coffee for PTD in order to characterize the difference between PTD and non-PTD coffee

ROGERS FAMILY COMPANY

- Partnered with teams at SU and UC Riverside
- Ed Whitman characterized coffee samples
- Mario Serracin in the field in Rwanda
- Research support by RFC acknowledged

Previous Research on PTD

- Studies of PTD occurred mostly in 1980's and 1990's
- **Most common hypothesis is that PTD originates from feeding damage by the antestia bug followed by infection by a bacterium that produces a malodorous metabolite adhering to the beans**

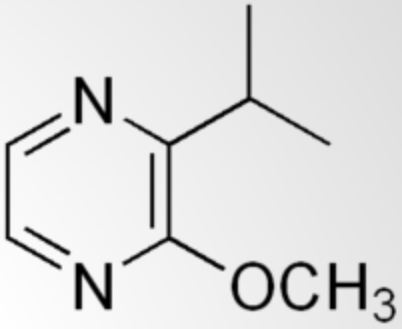
Antestia bug on coffee cherry

Coffee cherries that survived attack by the antestia bug.

Decaying coffee cherry after antestia bug attack

3-isopropyl -2 methoxypyrazine

- Intensely odiferous potato skin smelling molecule
- Detectable by human nose as low as 2 parts per trillion
- Associated with PTD in 1980s[3]
  - Sourced from ground green beans by solvent extraction and chromatography
  - Found by GC/MS in both good and PTD coffees; 30 times higher in PTD coffee
  - Not detected by nose in whole green coffee, but is sensed in ground green coffee and roasted coffee
  - No search has been made for other molecules that might serve as indicators for PTD
- Associated with several types of bacteria



IMP

[3]Becker, R., Döhla, B., Nitz, S.,Vitzthum, O. G. (1987). Identification of the peasy off-flavour note on central african coffees. In *12th International Conference on Coffee*, Montreux, pp 203-215.

OUR RESEARCH QUESTIONS: HOW MIGHT WE…

I. Understand the manifestation of PTD in coffee

II. Determine the difference between PTD and non-PTD coffee and what it means

III. Answer the question of whether PTD is distributed evenly or resident in only a “few bad beans”

IV. Use what we learn to propose a means of detecting PTD, or a means to sort out PTD, or a means to correct a sample to remove PTD

Objective to understand the manifestation of PTD in coffee

- Study the **surface volatile organic compounds** (**SVOC**s) in green coffee that define the chemical nature of PTD on the surface of beans
  - Surface volatiles should concentrate compounds deposited through antestia feeding activity and/or bacterial growth
- Study the **interior volatile organic compounds** (**IVOC**s) inside the green coffee beans
  - Interior volatiles should reflect compounds produced by the coffee bean itself in response to stress of antestia feeding activity and/or bacterial growth

OBJECTIVE TO FIND THE DIFFERENCE BETWEEN PTD AND NON-PTD COFFEE

- **Study a number of well characterized PTD and non-PTD samples for identification of a consistent pattern of compounds associated with PTD**
  - Analyze for SVOCs
- **Samples were supplied by Rogers Family Company through Dr. Mario Serracin and Mr. Ed Whitman from the harvests of 2012 and 2013**
  - Cupped to characterize for PTD

Determining a method

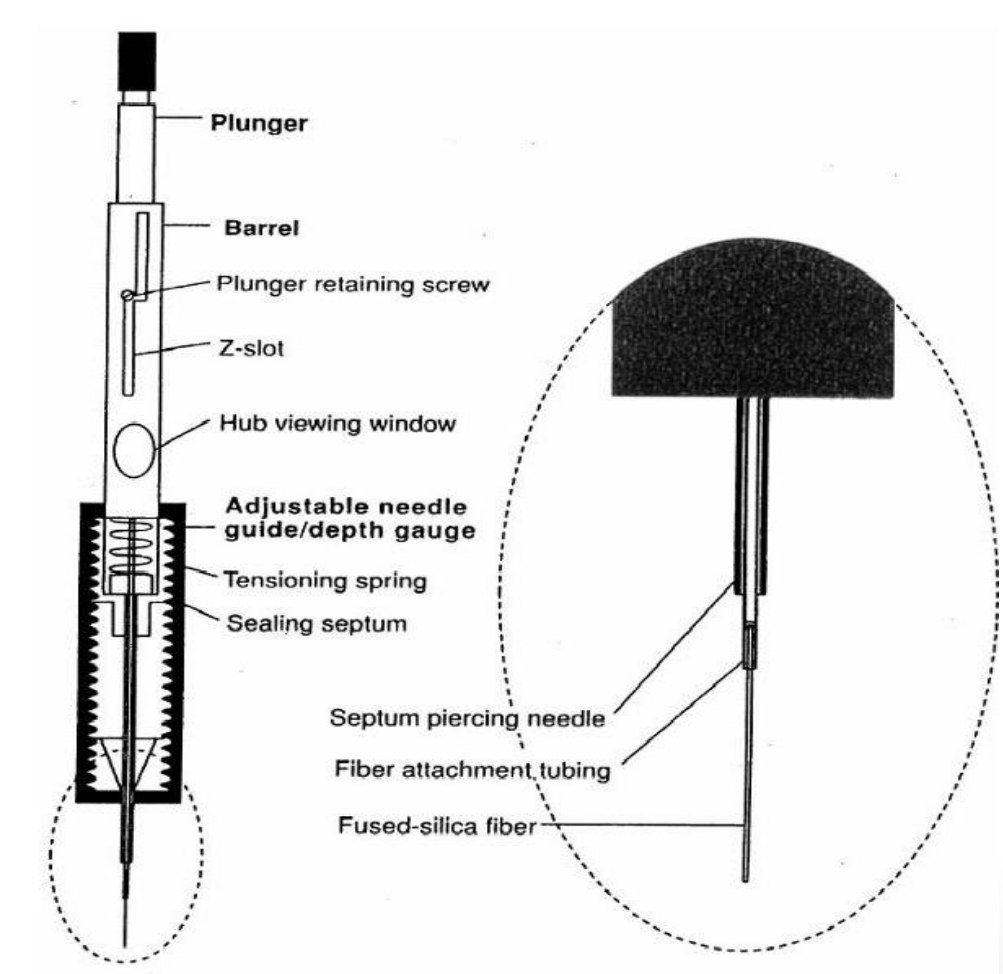
Starting point: a method previously used successfully for roasted ground coffee : heated coffee is sampled by solid phase micro-extraction (SPME)[5]

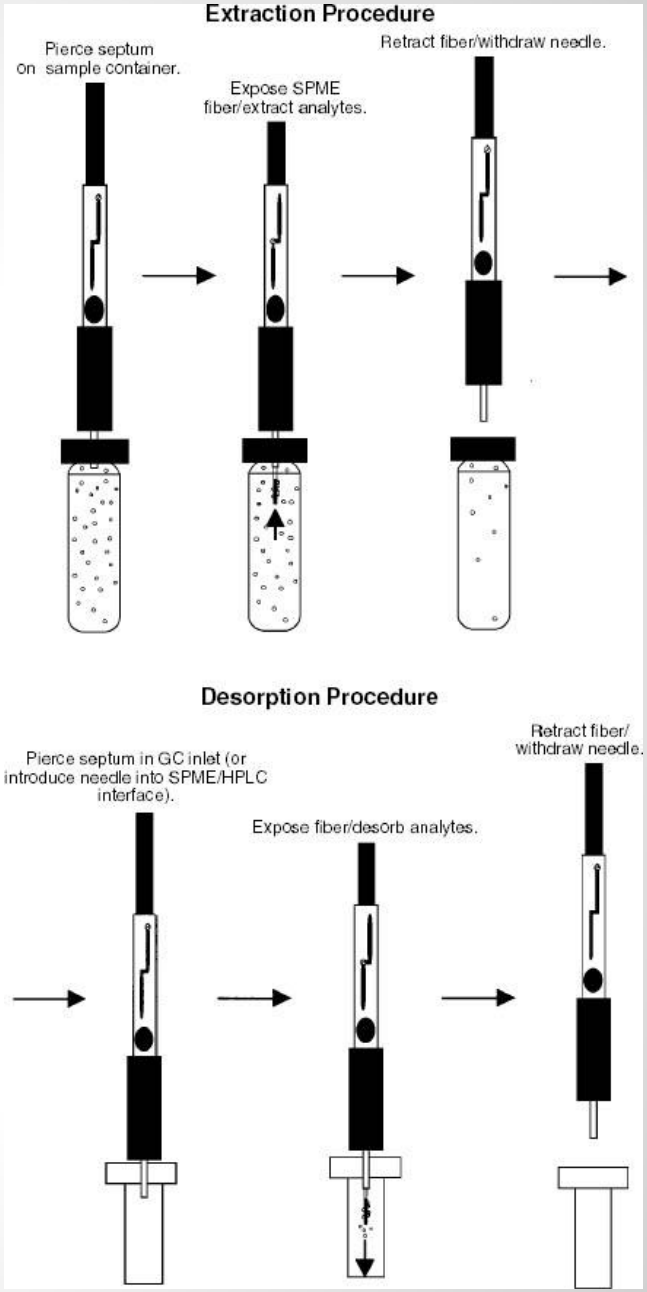
Need to apply to PTD analysis:

- **Green coffee aroma is not as intense as roasted coffee**
  - Need longer sampling time
- **Anecdotes propose 1 in 100 (or more) beans may be affected by PTD pyrazine deposited on surface**
  - Need to use more beans and whole beans for surface analysis
- **Green coffee has fewer compounds in it than roasted coffee**
  - Optimized conditions of chromatography method
- **SPME has been successfully applied to volatiles, including IMP down to nanogram level[6]**

[5]Mondello, L., et al, (2005) Reliable characterization of coffee bean aroma profiles by headspace solid phase microextraction-gas chromatography-mass specrometry… J. Separation Science 2005, 28, 1101 – 1109.
[6]Sala, C, Mestres, M., Marti, M.P., Busto, O., and Guasch, J. (2002) Headspace solid phase micro-extraction analysis of 3-alkyl-2-methoxypyrazines in wines. Journal of Chromatography A, 953, 1-6.

Solid Phase Micro-Extraction (SPME)

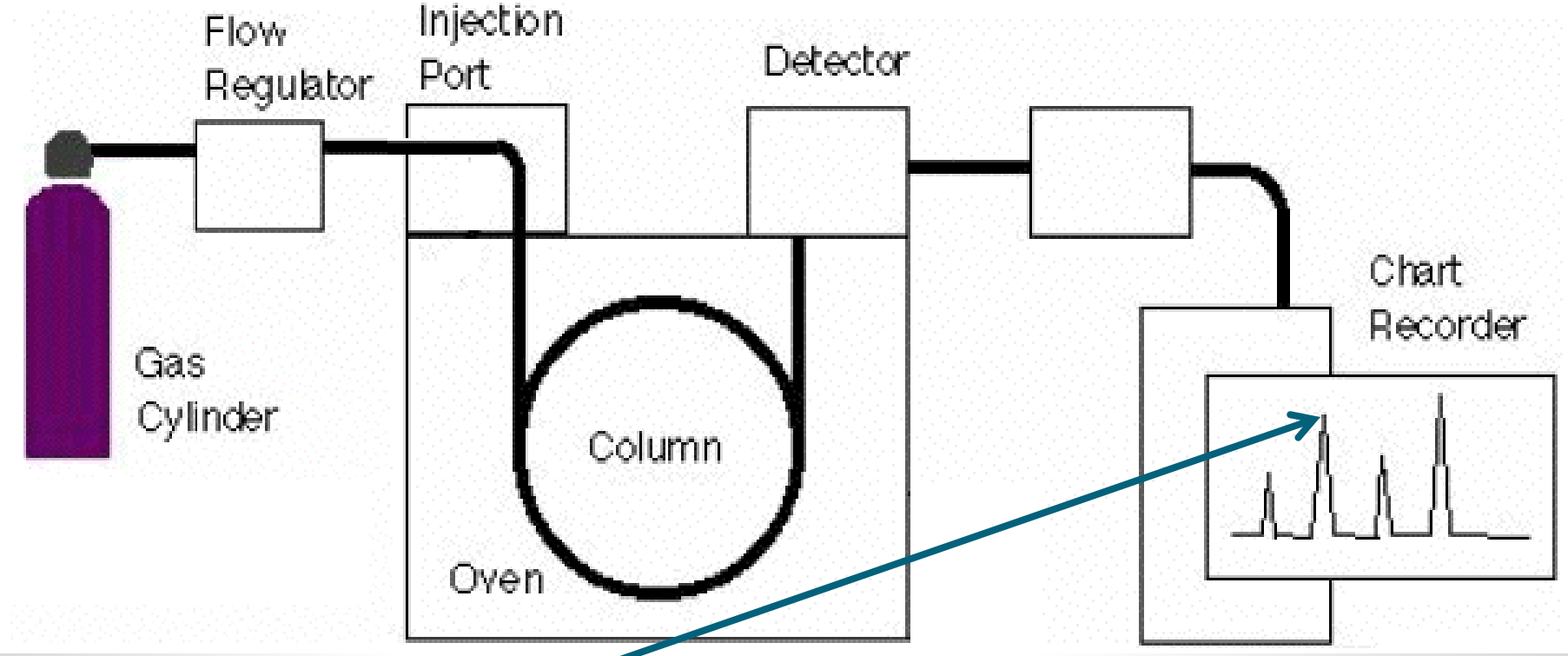




Method for volatile compounds

- Prepare 70g of whole coffee beans (about 400 beans) in headspace vial
- Heat at 60^{o}C in a water bath
- Volatiles are collected on SPME fiber for one hour
- Transfer SPME to gas chromatograph injection port for desorption at 250^{o}C
- Run gas chromatography with mass spectrometric detection

Gas Chromatography-Mass Spectrometry (GC-MS)

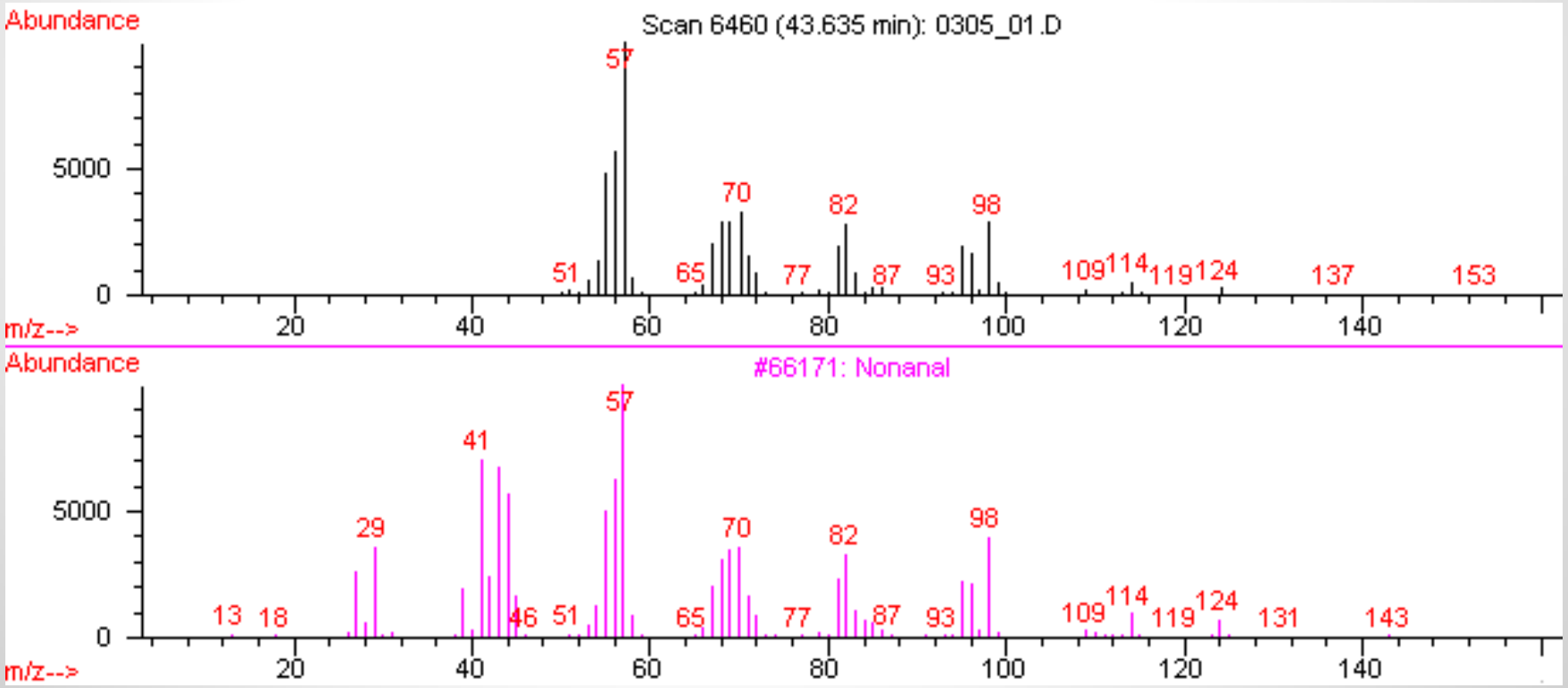


Chromatogram with peaks and mass spectrum for each compound
Compounds identified by 1)comparison of mass spectrum with NIST05 and FFNSC2 database s(>75% match) and 2) retention time matches with candidate compound.

Identifying compounds from GCMS data

With a good MS match (>75% quality), to ensure correct MS compound identification:

- **Check retention times** to make sure they correlate for compound and our GC settings
- **Run standards** to verify compound identification for key SVOCs



SAMPLES WERE CHARACTERIZED AND SUPPLIED BY THE ROGERS FAMILY COMPANY

54 non-PT samples

12 PT samples

8 samples with other defect (baggy, chemical, fertilizer)

In all, over 200 chromatograms were obtained. When possible, samples were run in triplicate.

Results: SVOCs for whole bean coffee

Figure 1: Chromatogram of a typical **non-PTD** coffee

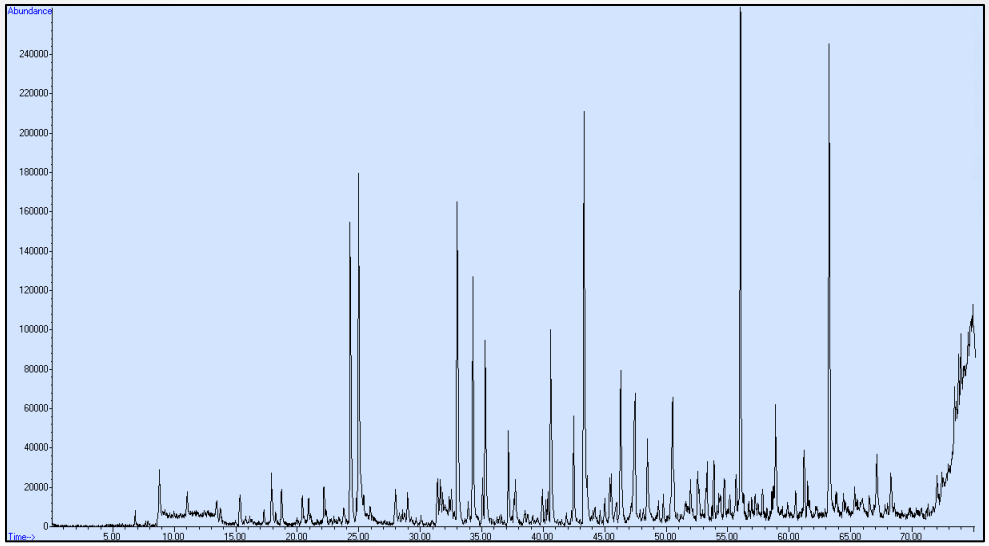
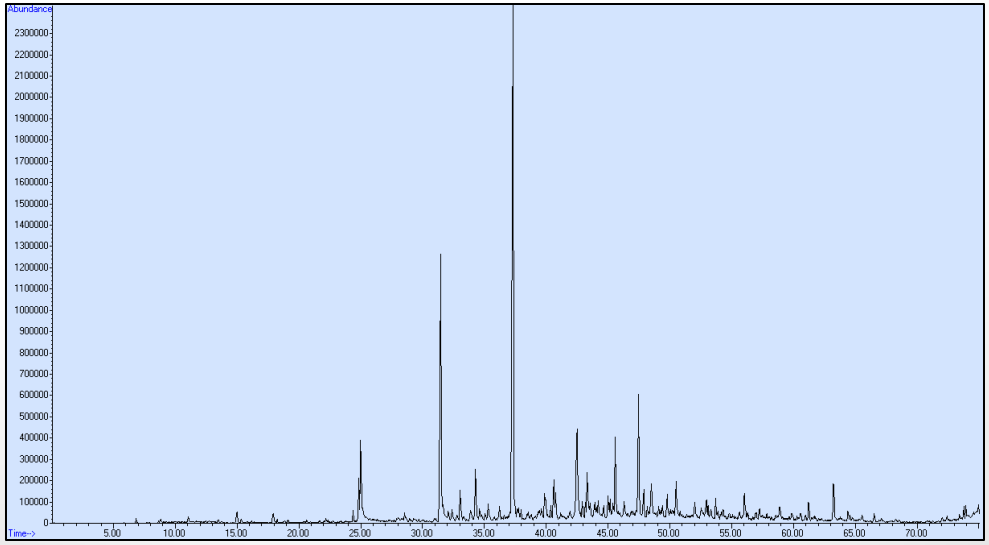


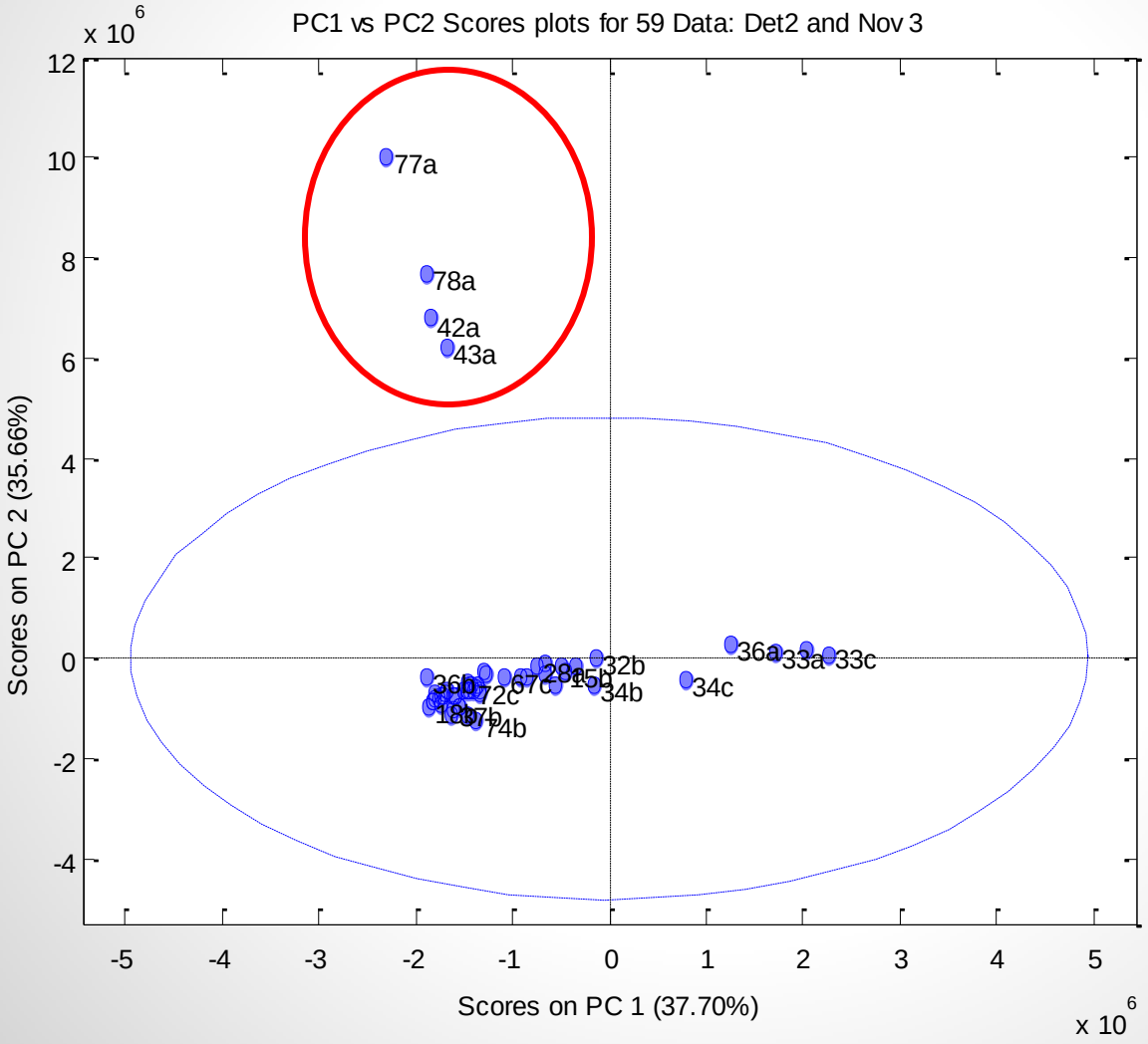
Figure 2: Chromatogram of a typical **PTD** coffee



Note:
10X Different Scales For Y axis

PTD coffee appears to have large peaks in addition to typical non-PTD peaks.

STATISTICAL ANALYSIS OF SIXTY SAMPLES BY PRINCIPAL COMPONENT ANALYSIS



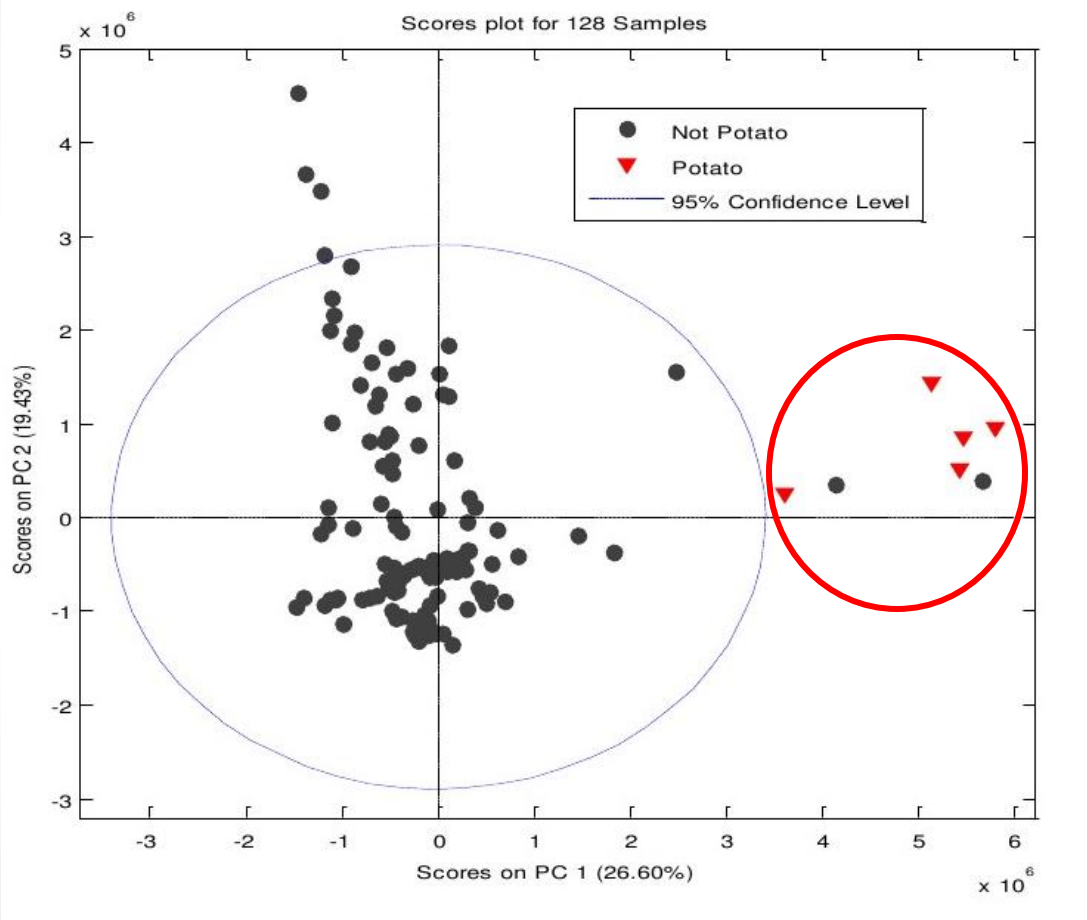
Key:

Red group is PTD coffee

Blue group is Non-PTD coffee

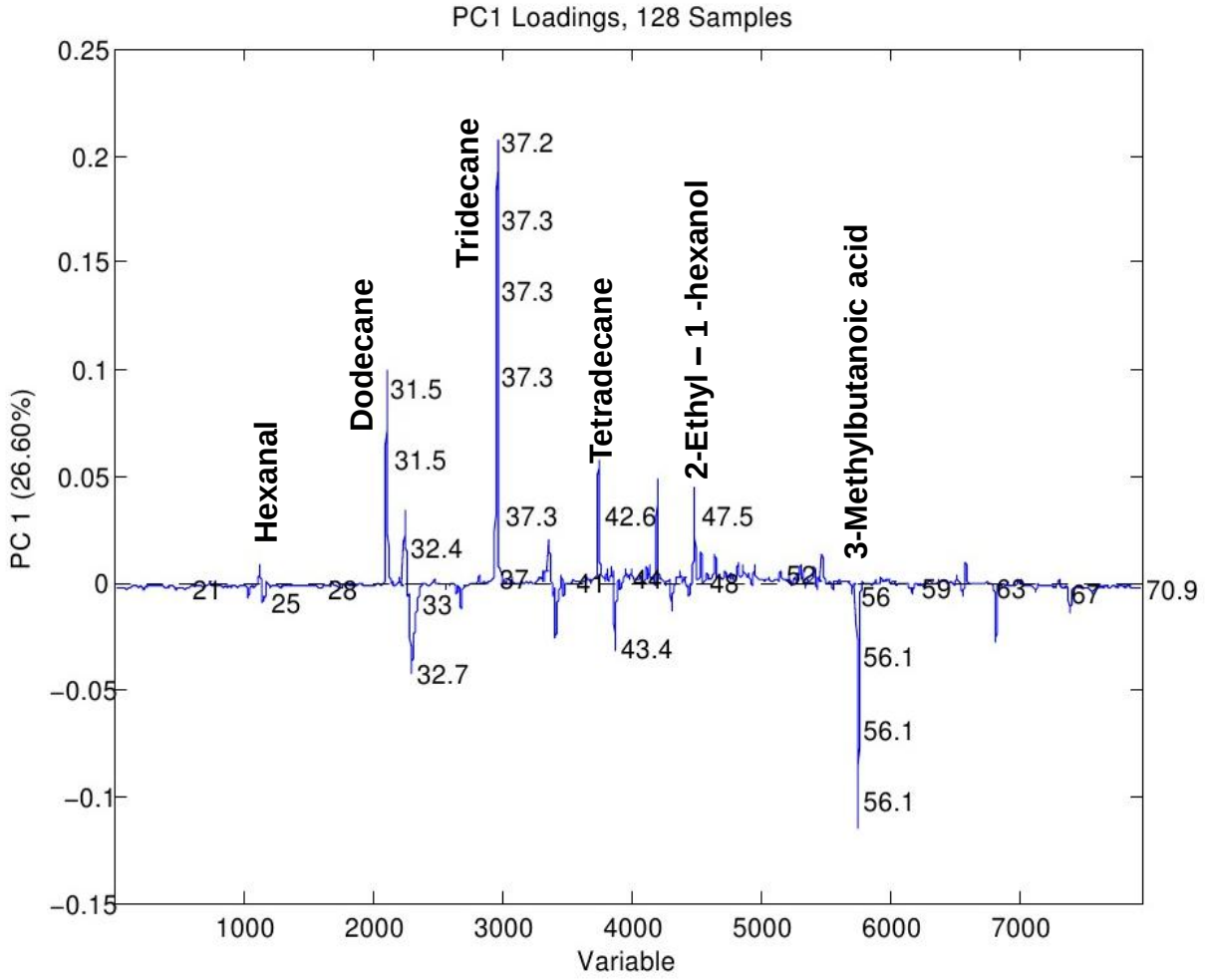
PC1 has one compound, 1-dodecene, that separates samples according to age.

THE 1-DODECENE PEAK WAS REMOVED AND THE VARIATION LOOKED LIKE THIS:



Note: two samples that had not been classified as PTD showed up in our group. These samples were from farms near where PTD was identified.

PC1 DISTINGUISHES PTD FROM NON-PTD COFFEES



Key:

Numbers are GC retention times for peaks that distinguish PTD from non-PTD coffee.

Positive means more of this compound in PTD coffee

Negative means less of this compound in PTD coffee

Results – PTD associated Compounds in SVOCs

Table 1: SVOCs elevated in PTD coffee

Name	Retention Time	Odor Description
Dodecane	31.5	Alkane
Tridecane	37.3	Alkane
Tetradecane	42.6	Alkane
2-Ethyl-1-hexanol	47.5	Citrus, fresh, floral, oily, sweet
3-Methylbutanoic acid	56.1	Peasy, cheesy, smelly feet

IMP was <u>not detected</u> in SVOC of any PTD or non-PTD coffee

Conclusions from study of RFC samples:

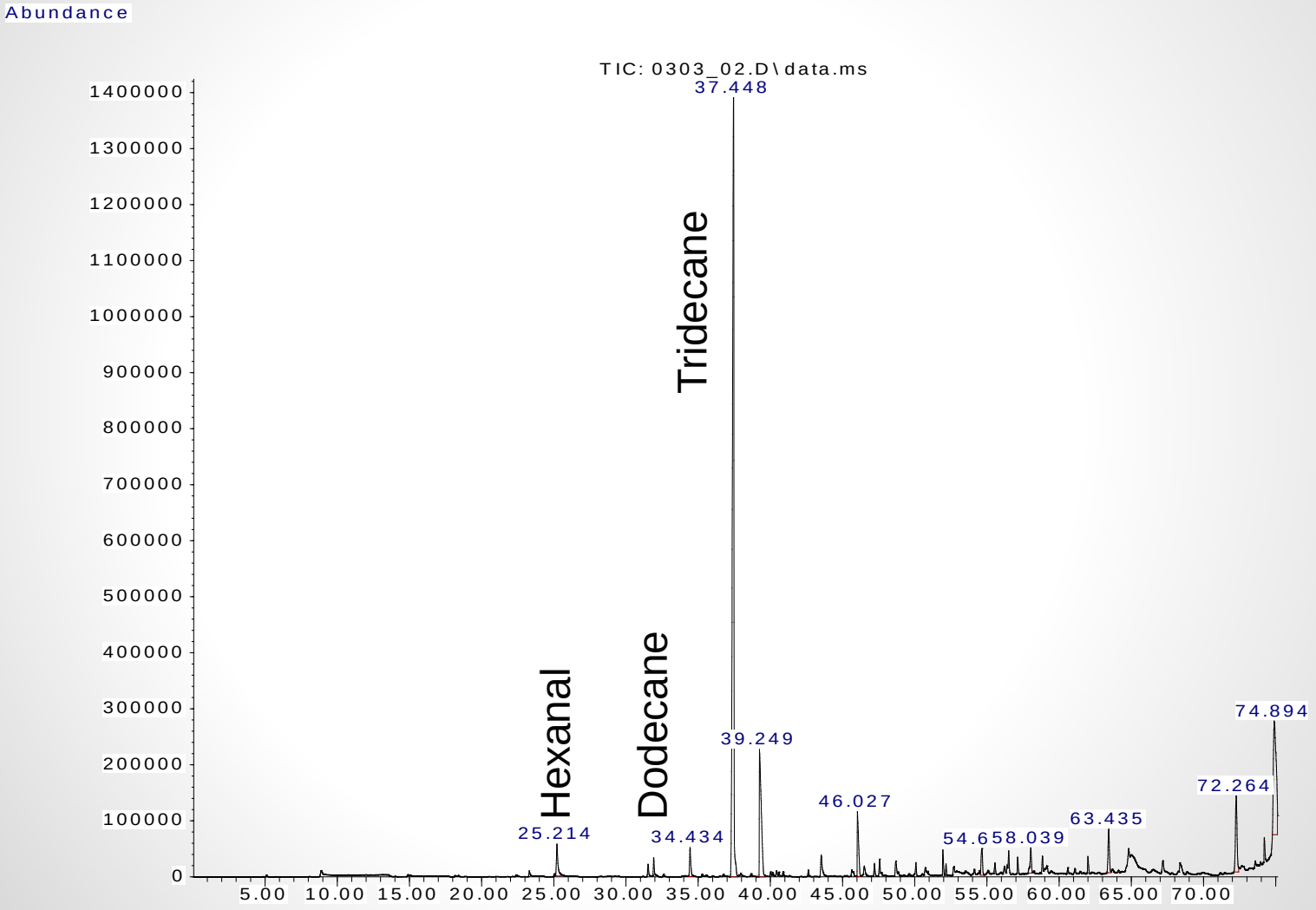
- We have developed a new method capable of analyzing green coffee SVOCs
- We have observed replicable pattern distinctions between PTD and non-PTD green coffees
- We have created a general PTD SVOC profile
- PTD volatile profile compounds are distributed throughout PTD coffee samples (not concentrated in a few beans)
- PTD SVOC profile is dominated by alkanes (tridecane, dodecane and tetradecane)
- PTD volatile profile compounds do not produce odor but could be detected by other means (for example, electronic nose)
- In contrast to expectations, IMP is not found on the surface of the beans in either the PTD or the non-PTD samples

NEXT QUESTION: WHAT IS THE MEANING OF ALKANE VOLATILES ASSOCIATED WITH PTD?

Study of SVOCs in whole desiccated antestia bugs gives a clue. Thanks to Mario Serracin of Rogers Family Company for harvesting and sending antestia bugs.

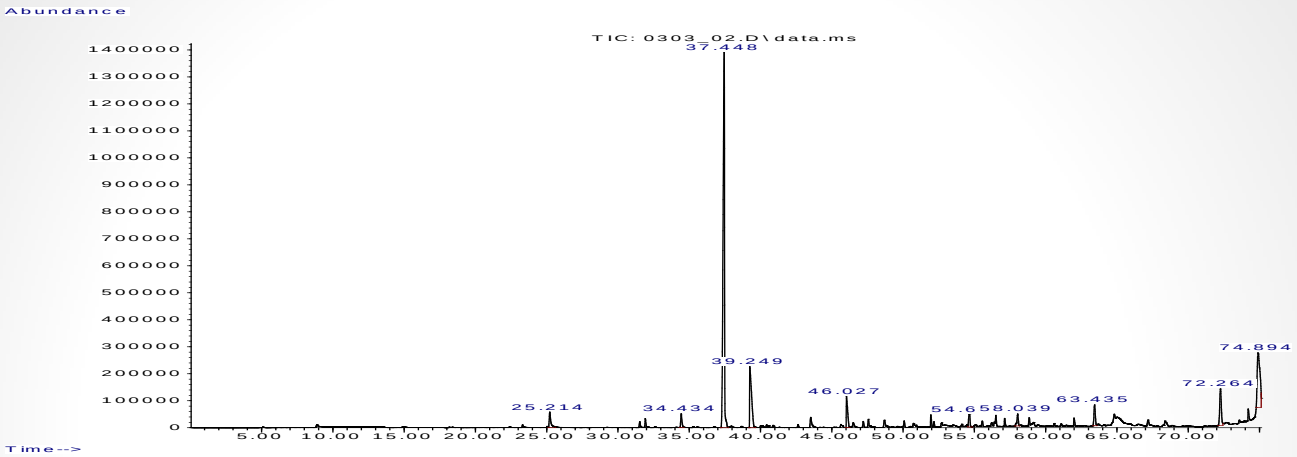
Method: a single whole frozen antestia bug is warmed to 60 ^{o}C and volatiles are sampled with SPME. Analysis is by gas chromatography separation and mass spectrometry.

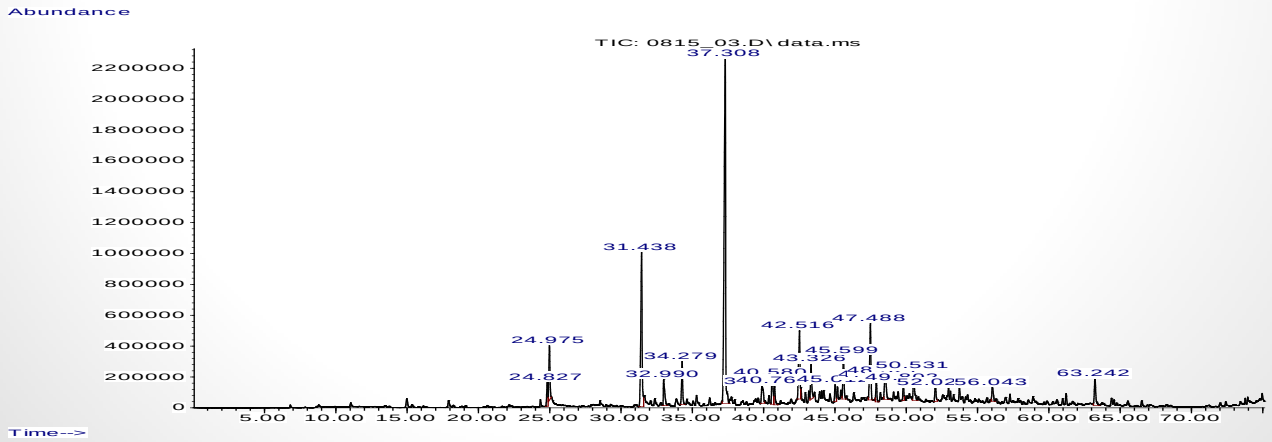
RESULTS: CHROMATOGRAMS FOR MALES AND FEMALES ARE IDENTICAL



Note: No IMP was found in antestia volatiles.

ANTESTIA (TOP)
PTD COFFEE (BOTTOM)





TRIDECANE IS A POTENTIAL MARKER FOR PTD COFFEE

Tridecane is a known pheromone associated with stink bugs (but not yet studied for antestia)

Borges, M; Jepson, PC; Howse, PE: Long-range mate location and close-range courtship behaviour of the Green Stink bug, Nezara viridula and its mediation by sex pheromones, Entomol. Exp. Appl. (1987) 44: 205-212.

The mix of tridecane, dodecane and three other compounds elicited long-range mate location behavior.

TENTATIVELY,

- Tridecane, dodecane and hexanal in the surface profile of PTD coffee may be associated with antestia activity in the coffee.
- Antestia may have left its “scent” on the PTD coffee!
- Tridecane may serve as a marker for PTD coffee.

Future (presentation tomorrow)

- Continue to analyze more PTD and non PTD coffee , now concentrating on **volatiles inside the beans**
- Analyze samples of **sorted coffee** to see if the PTD indicators are concentrated in any category of defect: insect damaged, broken, etc.
- Initiate the **search for the "bad bean"** that has high IMP

Acknowledgements

Rwanda Collaboration Colloquium Sponsored by:

COUNTER CULTURE COFFEE

WeatherSafe

Rwanda Antestia-Potato Taste Research Group

- Dr. Mario Serracin and Mr. Ed Whitman, Rogers Family Company
- Mr. Steve Miller, Seattle University Chemistry Department
- The Rogers Family Company
- The SU Center for Environmental Justice and Sustainability
- Murdock Undergraduate Research Program of Seattle University

Questions?