Endophytic microbiome of fruit stem-end and its relation to development of postharvest decay

Noam Alkan
Mango stem-end rot

- Israeli mango season of 2014 was accompanied with 30-40% fruit losses due to stem-end rots.
- Stem-end rot (SER) are the second most devastating mango disease after anthracnose.
- The stem-ends of healthy mango fruit are colonized with microorganisms.
- Mango stem-end-rots are caused by the pathogenic fungi: *Alternaria, Lasiodiplodia, Neofusicoccum, Phomopsis* and *Colletotrichum*.
- During fruit ripening those fungi becomes pathogenic.

(Galsurker et al., 2018)
Lasiodiplodia theobromae

Lasiodiplodia is a wide host pathogen that inoculate mango among other 500 different plant species.

Lasiodiplodia live as an endophyte without causing visible damage.

In response to abiotic stress or ripening the Lasiodiplodia will switch to Necrotroph.

Lasiodiplodia cause canker, dieback, and stem-end rot.
Model for Botryosphaeria colonization cycle
Model for stem-end rot development

(Galsurker et al., 2018)
Fruit-fungal interaction is a complex system

Could we modify this interaction to create a healthy microbiome?
Research topics: microbiome dynamics in response to...

1. Fungicide treatments at flowering

2. Fruit exposure to light (color of the peel)

3. Storage and ripening

4. Harvesting with/without stem
Mango flowering around the Galilee Sea
Effect of four fungicide treatments during flowering on fruit microbiome and SER

Shelly

Keitt

Microbial community

SER (%)
Spraying effect on stem dieback and yield

**Stem and Inflorescence Dieback / Tree**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean</th>
<th>SD</th>
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<tbody>
<tr>
<td>Control</td>
<td>25</td>
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<tr>
<td>PMt</td>
<td>28</td>
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<tr>
<td>Luna</td>
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<td>Tranquility</td>
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<td>Serenade</td>
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**Fruit/tree (#)**

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<tr>
<th>Treatment</th>
<th>Mean</th>
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<tr>
<td>Control</td>
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<td>Luna tr</td>
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<td>Combination</td>
<td>220</td>
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**Photos:**
- Control: Stem and inflorescence dieback
- Treatment: Spraying effect on stem dieback and yield
Spraying effect on postharvest decay

Stem-end rot (%)

<table>
<thead>
<tr>
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<th>SL 7d</th>
<th>SL 14d</th>
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Side decay (%)

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Control

Luna tr
Summary: flowering spray

Inhibition of Botryosphaeria penetration and changing the endophytic microbiome

- Reduced postharvest loss
- Reduced fruit loss
- Reduced die back
- More yield

(Feygenberg et al., Under review)
Research topics: microbiome dynamics in response to...

1. Fungicide treatments at flowering

2. Fruit exposure to light (color of the peel)

3. Storage and ripening

4. Harvesting with/without stem
Red mango fruit are more resistant

- Anthocyanin and red color
- Antifungal compounds
- Antioxidant compounds

Induced resistance

Biotic stress ‘anthracnose’

Abiotic stress ‘Chilling’

‘Chilling’ lipid peroxidation

(Sivankalyani et al., 2015; Diskin et al., 2017; Sudheeran et al., 2018; Sudheeran et al., 2019)
Postharvest decay in red and green mango

- **Side decay (%)**
  - After cold: 2%
  - After SL: 10%

- **Stem decay (%)**
  - After cold: 0%
  - After SL: 10%

Postharvest decay in red and green mango.
Flavonoids and anthocyanin characterization in mango fruit peel

5 - quercetin 3-O-diglycoside
6 - quercetin 3-O-galactoside
7 - quercetin 3-O-glucoside
8 - quercetin 3-O-xiloside
9 - quercetin 3-O-arabinopyranoside
10 - quercetin 3-O-arabinofuranoside
11 - quercetin 3-O-rhamnoside
12 - kaempferol 3-O-glucoside
13 - kaempferol 3-O-galactoside
14 - quercetin (aglycone)

Flavonoids Peak area / gFW (x10^6)

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Anthocyanins (Peak area / gFW (x10^6))

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Un-hydrolyzed samples (353 nm)

Un-hydrolyzed samples (513 nm)

7-O-methylcyanidin 3-O-β-D-galactopyranoside

Cyanidin 3-O-galactoside

Control

Red extract

Green extract

Red extract

Green extract
The effect of peel color on stem-end microbiome

Fungal community

Bacterial community

(Diskin et al., phytobiomes, 2017)
The effect of peel color on stem-end microbiota

**Fungal community**

- Sclerotiniaceae
- Debaryomycesaceae
- Hypocreaceae
- Sporidiobolaceae
- Metschnikowiaaceae
- Trichosporonaceae
- Botryosphaeriaceae
- Trichocomaceae
- Dothioraceae
- Davidiellaceae
- Pleosporaceae

**Bacterial community**

- Micrococcaceae
- Bacillaceae
- Moraxellaceae
- Sphingomonadaceae
- Corynebacteriaceae
- Staphylococcaceae
- Bradyrhizobiaceae
- Pseudomonadaceae
- Comamonadaceae
- Chitinophagaceae
- Enterobacteriaceae

(Diskin et al., phytobiomes, 2017)
Research topics: microbiome dynamics in response to...

1. Fungicide treatments at flowering

2. Fruit exposure to light (color of the peel)

3. Storage and ripening

4. Harvesting with/without stem
Stem end microbiome using NGS

Harvest → Storage at 12°C → Self-life at 20°C

DNA → Fungal ITS → NGS (Illumina MiSeq) → Bioinformatic analysis

Fungal ITS → DNA

Bacterial 16S → DNA
The effect of long storage on stem-end microbiota

Fungal community

Bacterial community

(Diskin et al., phytobiomes, 2017)
The effect of long storage on stem-end microbiota

Fungal community

Bacterial community

(Diskin et al., phytobiomes, 2017)
Research topics: microbiome dynamics in response to...

1. Fungicide treatments at flowering

2. Fruit exposure to light (color of the peel)

3. Storage and ripening

4. Harvesting with/without stem
Harvest with and without the stem-end

![Image of mangoes with and without stems]

<table>
<thead>
<tr>
<th></th>
<th>After CS</th>
<th>After SL</th>
<th>Additional SL</th>
</tr>
</thead>
<tbody>
<tr>
<td>With stem</td>
<td></td>
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<td>*</td>
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<tr>
<td>No stem</td>
<td></td>
<td>*</td>
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</table>

SER (%)
Effect of stem-end extract on the inhibition of *Lasiodiplodia*

Graph 1: Hyphae diameter (mm) vs. Incubation time (h)
- Stem extract (red line)
- Control (purple line)

Graph 2: Absorbance (600 nm) vs. Incubation time (h)
- Upper part extract (no stem) - CS (green line)
- Stem extract - CS (black line)
- Control (purple line)

Images:
- Stem extract:
  - 24 h
  - 48 h
  - 132 h

- Control:
  - Images showing no significant difference
Stem end microbiome using NGS

Harvest without stem → Storage at 12°C → Self-life at 20°C

Harvest with stem → Storage at 12°C → Self-life at 20°C

DNA → Fungal ITS → NGS (Illumina MiSeq) → Bioinformatic analysis

Bacterial 16S
Effect of harvesting with stem on the endophytic microbiome

Fungal microbiome

- **PC1**
- **PC3**

Fungal community (%)

- **Harvest**
- **CS**
- **SL**

- Unassigned
- Tremellaceae
- Sclerotiniaceae
- Nectriaceae
- Montagnulaceae
- Lasiosphaeriaceae
- Metschikowiaaceae
- Botryosphaeriaceae
- Trichocomaceae
- Pleosporaceae
- Dothioraceae
Effect of harvesting with stem on the endophytic microbiome

Fungal microbiome
Effect of harvesting with stem on the endophytic microbiome
Co-occurrence of the stem-end endophytic-fungal microbiome
Effect of harvesting with stem on the endophytic microbiome

Relative abundance

Dothiorella
Aureobasidium
Alternaria
Aspergillus
Stemphyllum
Penicillium
Lasiodiplodia
Montagnulaceae sp.

Genus

SER (%)

Harvest
CS
SL

With stem
No stem

After CS
After SL
Additional SL
Conclusions

1. Pathogenic fungi colonize the phloem of the stem without causing any symptom until the fruit ripens.

2. Fungicide spray during flowering change the endophytic microbiome, reduced stem die-back, fruit dropping, increase yield and reduced postharvest SER.

3. Fruit that was exposed to sunlight and developed red color peel were more tolerant to postharvest pathogens and chilling.

4. Fruit that was harvested with stem had more antifungal compound, changed microbiome and reduced SER.

**Microbiome and SER:**

1. Pre and postharvest treatment affect microorganism diversity and SER.

2. Higher microbiome diversity was usually correlated with reduced SER.
Fruit-fungal interaction is a complex system
Conclusion

Fruit physiology (ripening, defense response)

Microbiome community

Fruit pathology ( Decay )

Environmental condition
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Mor International (Ekel Tevet…)
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Thank you for the attention
Research questions

1. Does the microbiome affect postharvest decay?

2. Is there a healthy or pathogenic microbiome?

3. Could different practice or treatments affect the microbiome?

4. Does microbiome variability correlate with resistance?

5. Is there a co-occurrence of pathogenic fungi or biocontrol agents?
Ethylene and ripening effect on stem-end rot (SER)

Ripened fruit are more susceptible to decay
Botryosphaeria

• Botryosphaeria is a genus of pathogenic fungi, which include 193 species.

• Botryosphaeria is a major fungal pathogenic that cause cankers, dieback and stem-end rots.

• Botryosphaeria complex of pathogens include: *Lasiodiplodia*, *Diplodia*, *Dothiorella*, *Neofusicoccum*...
Research topics: microbiome dynamics in response to...

1. Fungicide treatments postharvest

2. Fungicide treatments at flowering

3. Storage and ripening

4. Fruit exposure to light (color of the peel)

5. Harvesting with/without stem
European union revision

- **Imazalil**: potato, apple, plume, **citrus**, apricot, cantaloupe, banana, peach, persimmon.
  Regularly used in citrus against *Penicillium*.

- **Prochloraz**: **Avocado, mango**, eggplant, cucumber, apricot, tomato, pomegranate, plum, almond, strawberry.
  Regularly used in mango and avocado.

We need to find immediate alternatives!!!
Postharvest fungicide application on mango fruit

**Stem end rot (%)**
- Control
- 150ppm Prochloraz
- 300ppm Prochloraz
- 150ppm Fludioxonil
- 300ppm Fludioxonil
- Integrated (150ppm)

**Side rots (%)**
- Control
- 150ppm Prochloraz
- 300ppm Prochloraz
- 150ppm Fludioxonil
- 300ppm Fludioxonil
- Integrated (150ppm)

**Fungal community (%)**
- Cladosporium
- Yeast
- Lasiodiplodia
- Penicillium
- Bacteria
- Alternaria

(Diskin et al., Crop Protection, 2019)
Stem end microbiome using NGS

Green fruit harvest → Storage at 12°C → Self-life at 20°C

Red fruit harvest → Storage at 12°C → Self-life at 20°C

DNA → Fungal ITS → NGS (Illumina MiSeq) → Bioinformatic analysis

Bacterial 16S