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Cranberry Chemistry for Plant Disease Resistance and Human Health

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Director, UMass Cranberry Health Research Center
A bridge between two goals

• The chemistry of plants is related to both plant health and human health
• Cranberries contain unique and diverse compounds produced in defense against UV light, fungi, predators and other stresses
• Many of these “phenolic” defense compounds are antioxidants and antimicrobials that may also improve human health and nutrition
Collaboration between UMD and Cranberry Experiment Station

Goal (2006-present): Study relationship between pest/disease resistance and plant chemistry

– Insect pests
– Pathogenic fungi
– Use data for sustainability
– Funded by USDA Special Grant and UMD Cranberry
– Justine Vanden Heuvel, Frank Caruso, Anne Averill, Carolyn Demoranville, Cathy Neto
# Greenhouse Feeding Trial

## Feeding Damage on New Growth of Howes and Early Black Cranberry Uprights by Three Insect Pests

<table>
<thead>
<tr>
<th>Insect</th>
<th>Cultivar</th>
<th>Time</th>
<th>No. of Uprights Exhibiting:</th>
<th>Avg. Score</th>
<th>p-value (cultivar)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No feeding</td>
<td>Light feeding</td>
<td>Medium feeding</td>
</tr>
<tr>
<td>Gypsy Moth</td>
<td>Howes</td>
<td>24 h</td>
<td>9</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Early Black</td>
<td></td>
<td>13</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Weevil</td>
<td>Howes</td>
<td>72 h</td>
<td>2</td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Early Black</td>
<td></td>
<td>6</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Flea Beetle</td>
<td>Howes</td>
<td>5 d</td>
<td>1</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Early Black</td>
<td></td>
<td>6</td>
<td>14</td>
<td>7</td>
</tr>
</tbody>
</table>

Uprights were scored as having no feeding (0), light feeding (1), medium feeding (2), or heavy feeding (3).

(Vanden Heuvel, Averill and Sylvia, 2005)
Early finding: Quercetin and other phenolics may deter insects from feeding

- Feeding study showed two cranberry pests feed more heavily on Howes cultivar vs. Early Black foliage
- HPLC analysis identified several phenolic compounds present at higher levels in Early Black leaves
- May play a role in resistance

Objectives 2008-present

- Collect berry and leaf samples from pest and disease resistant and susceptible cranberry cultivars
- Prepare and analyze extracts from different bogs, seasons and growing regions
- Compare their chemical compositions
- Identify compounds that may play a role in resistance or serve as markers
- Develop new analysis methods
Casting a wider net

- 2008-09 samples collected in MA (F. Caruso)
  - Howes, Early Black, Ben Lear, Bugle
- Broader range of samples collected 2010-11
- Includes newer cultivars:
  - Crimson Queen, Demoranville, Mullica Queen
- MA, NJ, Wisconsin, British Columbia (N. Vorsa)
- Analysis for phenolic composition and proanthocyanidin (PAC) content
1. How do “pest-resistant” phenolics vary with cultivar, bog, stress?

- Leaves were collected in June 2009 & 2010 from 14 sites in MA (F. Caruso)
- Healthy and stressed plants (*Phytophthera* root rot, tipworm)
- Extracts were prepared and analyzed by HPLC for content of quercetin and coumaric acid derivatives (Eleni Yiantsidis, Anthony Dovell)
Production of quercetin compounds in Early Black varies between bogs

State Bog was highest
Production of several metabolites was decreased in stressed Ben Lear plants.

Stressed plants had lower coumaroyl content.
2. Resistance to fungal pathogens

- Cultivars vary in resistance to fruit rot and other diseases caused by fungi
- Bugle > Early Black > Ben Lear
- Which compounds play a role? How do they affect fungi? (F. Caruso, M. Nunnelley)

- Fruit and leaf samples collected, extracted and analyzed by HPLC in 2008-09
- Tested for effect on growth of several fungi using disc-diffusion assays
Cranberry plant pathogens of concern in Southeastern MA

**Fruit rot:**
- *Fusicoccum putrificiens*
- *Coleophoma emptetri*
- *Phomopsis vaccinii*
- *Physalospora vaccinii*
- *Colletotrichum acutatum*

**Leaf spot (young beds):**
- *Colletotrichum acutatum*
- *Phyllosticta vaccinii*

**Root rot**
*Phytophthora spp.*

General trend in resistance by cranberry cultivar:

Bugle > Early Black > Ben Lear > HyRed
Fungi treated with cranberry extracts showed a stress response

• Extracts of berries and leaves containing PACs (proanthocyanidins) caused melanin production

• PACs may play a defense role against fungi
3. How does PAC content vary between cultivars, seasons, regions?

- PACs may be vital both to human health (UTI protection) and plant health
- Scant data on PAC content by cultivar
- Fruit samples collected in 2009 and 2010 (F. Caruso, N. Vorsa)
- Fruit extracts were analyzed for PAC content using DMAC assay (J. Carpenter)
Average PAC content in 2010 MA fruit samples by cultivar (5 cultivars, 1-3 bogs)

- Howes variety was significantly higher in PACs than all other cultivars (except Early Black)
- Ben Lear was significantly lower in PACs than the others (p < 0.001)
PAC content in 2010 samples by growing region

- **2010 New Jersey**

  - PAC Concentration (mg PAC A2/100g dry powder)
  - 0, 2000, 4000, 6000, 8000, 10000

- **2010 Wisconsin**

  - Cranberry Sample: S-WI, BL-WI, CQ-WI, Dem-WI
  - PAC Concentration (mg PAC A2/100g dry powder)
  - 0, 1000, 2000, 3000, 4000, 5000, 6000

- **2010 British Columbia**

  - PAC Concentration (mg PAC A2/100g dry powder)
  - 0, 2000, 4000, 6000, 8000, 10000

- **Observations**

  - PAC content in Ben Lear, Stevens, Crimson Queen did not differ significantly by region in 2010
  - Mullica Queen did: BC > MA ≈ NJ
Seasonal difference in PACs: 2009 vs. 2010

<table>
<thead>
<tr>
<th>Cranberry Samples</th>
<th>2009 Samples</th>
<th>2010 Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of PACs (mg A2/100g dried fruit)</td>
<td>0</td>
<td>2000</td>
</tr>
</tbody>
</table>

2010 was a harsher growing season with a high incidence of fruit rot. PAC content in EB, Ben Lear and Bugle fruit was significantly lower in 2010 (p < 0.05).

2011 samples TBA.
NMR analysis of plant extracts
“metabolomics”

• Fast, efficient
• Gives a snapshot of chemical composition
• Can be used to verify botanical species, origin, where a plant is grown
• Can detect samples that differ in composition, identify and quantify key compounds
• Bruker and UMD are developing it as a tool for cranberry sample analysis
NMR data shows differences between foliage of *Vaccinium* species that can be used to validate samples. Separation of samples in clusters indicates differences in chemical composition.
Variation in cranberry leaf samples

Healthy vs. diseased plants

*(Phytophthera)*

Clustering of data points shows that cultivars differ in composition

- **SHAWC-16 Ben Lear Healthy**
- **SHAWC-16 Ben Lear with root rot**
- **Ben Lear**
- **Bugle**
- **Early Black**
- **Howes**
- **Wild Vines**
Determination of PACs by NMR

- Bruker – UMD collaboration (K. Colson, B. Pereira)
- $^1$H NMR analysis of cranberry extracts can detect A-type PACs
- Signal intensity is proportional to content
- Developing NMR method to quantify A-type PACs in cranberry samples
Summary

- Quercetin, phenolics and PACs play both plant defense and human health roles
- Composition varies in response to stress, location, season
- Analysis of 2011 samples will better define regional, cultivar, and seasonal differences
- Further characterization of key compounds
- Development of NMR method for PACs
- How can we use this information to help cranberry industry?
UMass Cranberry Health Research Center
http://www.umassd.edu/chrc/

Funded by a $110 K grant from the UMass President’s Science and Technology Fund
Directors: Cathy Neto and Maolin Guo (UMD)
Cranberries and Human Health

- Cranberries are best known for protection against UTI
- Contain health-promoting **antioxidants** like those in red wine and blueberries
- We identified berry compounds that reduce cancer cell growth, oxidative stress, microbes
- Possible protection against colon cancer, heart disease, diabetes, Alzheimer’s, infections?
Goal: Establish the science behind health benefits

• How do these compounds work?
• Are they bioavailable in animals? Humans?
• What are the metabolites?
• How can we measure efficacy?
• What other diseases or conditions might cranberry protect against?
• Which varieties, products, formulations are the healthiest?
Goals of CHRC Project

- Establish a collaborative center for cranberry-health research across five UMass campuses
- Bring together researchers with common interests to work together
- Pursue federal grant funding (NIH, USDA, NSF), industry & state sources
- Focus areas year one:
  - Colon cancer
  - Oxidative stress
- Future: identify & develop other priority areas
Activities

• CHRC website on the UMD site
• Two $25 K seed grants to be awarded – RFP posted in January, proposals due in March
• Next: press release, publicize center to industry, seek new funding sources
• Collaborators meet for proposal development
• Cranberry Health Research Symposium (Fall 2012)
• Goals: NIH R01 proposals, identify additional funding to sustain center
Cranberry Health Research Center

The UMass Cranberry Health Research Center is a collaborative established through a 2011 Science and Technology Initiative grant from the UMass President's Office. The Center Directors are established cranberry health researchers Dr. Catherine Neto and Dr. Maolin Guo of the Department of Chemistry and Biochemistry at UMass Dartmouth.

The Cranberry Health Research Center encompasses researchers from all five UMass campuses, other academic institutions, hospitals and private laboratories throughout the U.S. and Canada. The Center’s collaborations combine the strengths of academia, medical institutions and industry to provide solid scientific evidence for cranberry’s role in health and nutrition.

News/Announcements:

- Click here for Current Programs and 2012 funding opportunities
- Click here for Upcoming Events
Neto Research Group

• >10 years’ expertise in cranberry phytochemical separation, analysis and health research, funded by state, federal, private grants
• Collaborators at UPEI, WPI & Cornell
• Our studies show cranberry compounds inhibit development of colon, prostate & breast cancer
• Antimicrobial research with oral bacteria, yeasts (Candida), E. coli, plant pathogens
• Anticancer and antioxidant studies, cellular protein and gene expression
• Teaming up with scientists using animal/clinical models is critical to furthering this research
Guo Research Group

- Biochemistry of iron and other metals, role of iron in biological processes and diseases, interactions with cranberry
- Molecular biology and protein biochemistry
- Live cell imaging methods, fluorescent tagging of molecules
- Sensor and probe development
- Bioassays – antibacterial, antioxidant, enzyme-based assays, spectroscopy
UMass CHRC helps support student research
Academic-Industry partnership

Advisory Board Members (industry):
• Cranberry Institute - Martin Starr
• Ocean Spray Inc. - Christina Khoo
• Decas Cranberries Inc. - Reza Ghaedian
• Cranberry Marketing Committee - TBA
• Brunswick Laboratories Inc. - Boxin Ou, Vice President
• Microbiotics Inc. - Terry Bowlin, President/CEO

Advisory Board Members (academic):
• UMass Medical School/CCTS - John Sullivan, Nate Hafer
• UMass Cranberry Station – Carolyn Demoranville
• UMass Amherst Food Science – Eric Decker
• Harvard Medical/MacLean Hospital – David Lee
Sustainability of CHRC?

• Federal grant funding levels are very low
• S & T grant provides only for Year 1
• Challenge: convince industry and state to invest in CHRC
• We need to provide continued support for students, new research projects
• We welcome your input and suggestions!
Acknowledgements

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• Frank Caruso, Carolyn Demoranville, Hilary Sandler, Anne Averill, Sunil Tewari (UMass)
• Justine Vanden Heuvel (Cornell)
• Nick Vorsa (Rutgers)
• Kim Colson, Josh Hicks, Sarah Luchsinger (Bruker-Biospin)
• UMD students: Christine Dao, Melissa Nunnelley, Eleni Yiantsidis, Anthony Dovell, Jessica Carpenter, Brian Pereira

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Cranberries:
Better health for the Commonwealth

http://heartcurrents.files.wordpress.com/2010