Emerging organic contaminants in fruits of *Cucumis melo* spp. grafted on different rootstocks. Preliminary data.

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Our global approach for rootstock selection

Amount of organic contaminants in grafted fruits from agricultural soils and irrigation waters
The term *emerging contaminants* refers to compounds previously not considered which are present in the environment on a global scale.

They are anthropogenic organic compounds and their transformation products.

They emerge as result of:
- Changes in use of manufactured chemicals
- Advances in analytical techniques
- Better monitoring

Many emerging contaminants remain unregulated, but the number of regulated contaminants will continue to grow slowly over the next several decades.
They include as ex.

- **Pesticides** – parent compounds (e.g. metaldehyde), metabolites
- **Pharmaceuticals** – human, veterinary, illicit
- **Life style** – nicotine, caffeine
- **Personal care** – DEET, parabens, triclosan, musks, UV filters
- **Industrial additives and by-products** – dioxanes, bisphenols, MTBE.
- **Water and wastewater treatment by-products** – NDMA, THM
- **Food additives** – BHA, BHT
- **Flame/fire retardants** – PBDE, alkyl phosphates, triazoles
- **Surfactants** – alkyl ethoxylates, PFOS & PFOA
- **Hormones and sterols** – estradiol, cholesterol
• The widespread occurrence of *emerging contaminants* in the irrigation water and agricultural soil is a major problem.

• They can easily enter the *food chain* and could be absorbed by humans.

• The consequences *on our health are not so well understood.*
From soils to human intake

Recent researches suggest that a group of emerging contaminants could be transferred into plants, as ex.

• some antibiotics from manured soils to a variety of plants including Brassicaceae, Redshaw et al., Phytochemistry 69.13 (2008): 2510-2516.

Plants grafted onto different rootstocks may exhibit dissimilar abilities to take up contaminants. 

The root structure and the uptake efficiency of the root cells are determined by the rootstock.

In case of *Cucurbitaceae* and *Solanaceae*

- Heavy metals (e.g. Cd, Ni, Cr) and micronutrients (e.g. Cu, B and Mn)


- Dieldrin concentration


- Persistent Organic Pollutants (POPs) including dioxins.

Aim of the research

Evaluate the ability of different rootstocks, selected for their resistance to diseases, to restrict contaminant concentrations in grafted melon fruits.

The following emerging contaminants were investigated:

- Glycol ether compounds
- Phatalates
- BTXE
- BHT, BHA
Fruit samples analysed.

*Cucumis melo* L. var. *Inodorus cv Incas*

*Cucumis melo* L. var. *Reticolatus cv Proteo*
<table>
<thead>
<tr>
<th></th>
<th>Rootstock</th>
<th>Suppliers</th>
<th>Pathogen resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Energia (<em>Cucumis melo</em>)</td>
<td>SEMINIS Vegetable Seeds Italia Srl (Pr)</td>
<td>FOM 1,2</td>
</tr>
<tr>
<td>2</td>
<td>Sting (<em>Cucumis melo</em>)</td>
<td>Nunhems Sementi srl, S. Agata (Bo)</td>
<td>FOM 0-1; 1,2; 2</td>
</tr>
<tr>
<td>3</td>
<td>Polifemo (<em>C. maxima x C. moschata</em>)</td>
<td>Esasem Spa, Casaleone (Vr)</td>
<td>FOM 1,2, <em>D. bryoniae</em></td>
</tr>
<tr>
<td>4</td>
<td>AS 10 (<em>C. maxima x C. moschata</em>)</td>
<td>SEMINIS Vegetable Seeds Italia Srl (Pr)</td>
<td>FOM 0-1; 1,2; 2</td>
</tr>
<tr>
<td>5</td>
<td>RS841 (<em>C. maxima x C. moschata</em>)</td>
<td>SEMINIS Vegetable Seeds Italia Srl (Pr)</td>
<td>FOM 1,2, <em>D. bryoniae</em></td>
</tr>
<tr>
<td>6</td>
<td>P 360 (<em>C. maxima x C. moschata</em>)</td>
<td>SAIS Sementi S.P.A. (FC)</td>
<td>FOM 1,2, <em>D. bryoniae</em></td>
</tr>
<tr>
<td>7</td>
<td>Elsi (<em>C. maxima x C. moschata</em>)</td>
<td>Vilmorin Italia srl, Funo (Bo)</td>
<td>FOM 1,2, <em>D. bryoniae</em></td>
</tr>
</tbody>
</table>
Production Location

The experiment were conducted in a experimental field located in Pachino, Siracusa, an area that has exceptional soil and climatic conditions.

- The Mediterranean red soils
- The high salinity of irrigation water determined by proximity to the sea
- The typical Mediterranean climate with mild winters and hot summers and dry with little rainfall well distributed throughout the year
- The light and solar radiations that are among the highest in Europe.
The experiment was conducted in a 1000-m² unheated polyethylene greenhouse.

The soil was a sandy loam with a pH of 7.5.

Plants were grown under natural light conditions.

The daily \( T_{\text{max}} \) and \( T_{\text{min}} \) varied from 19.0 to 36.4 °C and from 14.4 to 24.9°C.

The tongue approach was used for grafting the scion onto the rootstocks.

Plants were grown in single rows (2 m x 1.2 m) at a plant density of 0.42 plants/m².
Sampling

- The fruits were collected in two different years.
- The ripe fruits were harvested in July (cv Incas and cv Proteo), transported under low temperatures to the laboratory and immediately analyzed.
- The samples were organized in eight theses, one for the control (cv. Incas or cv. Proteo) and seven for each rootstock.
- Three slices were taken from different orientations of each fruit and used for one sample.
- All the analyses were carried out in duplicate.
Contaminants were analysed by the static headspace solid phase microextraction method (HS-SPME) coupled with gas chromatograph-mass spectrometer (GC-MS)

- Gas chromatograph Varian 3800 interfaced with a Varian 2000 ion trap mass spectrometer
- Injection mode: splitless
- Column: CP-Wax 52 CB 60 m, 0.25mm i.d., 0.25 μm film thickness
- Oven temperature: 45°C for 5 min to 80°C at a rate of 10°C/min, to 240 at 2°C/min.
- Carrier gas: helium at 10 psi
- Transfer line temperature: 250°C
- Ionization mode: EI 70 eV
- Acquisition range: 40-200 m/z
Results

Emerging contaminants identified

- Glycol ether compounds
- Phatalates
- BTXE
- BHT, BHA
Glycol Ether Products identified in our samples

Carbitol (Diethylene Glycol Monoethyl Ether)

1-Methoxy-2-propanol (Propylene glycol methyl ether)

• In human studies, exposure to glycol ethers has been associated with low sperm mobility (Cherry et al. 2008), hematological effects (Starek et al. 2008), and asthma and allergies (Choi et al. 2010).
**Glycol ethers**, a chemical class with > 80 compounds, used in a broad array of cleaning applications. They are often used in paints, varnishes, cosmetics and have been detected in a variety of household products. Monitoring methods are currently being developed, so large-scale studies are limited.
Amount of Glycol ether products in Inodorus cv. Incas fruits
Amount of Glycol ether products in Reticulatus cv. Proteo fruits

<table>
<thead>
<tr>
<th>Variety</th>
<th>Amount (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteo (ungrafted)</td>
<td>1</td>
</tr>
<tr>
<td>P/Energia</td>
<td>1</td>
</tr>
<tr>
<td>P/Sting</td>
<td>2</td>
</tr>
<tr>
<td>P/Elsi</td>
<td>3</td>
</tr>
<tr>
<td>P/P360</td>
<td>4</td>
</tr>
<tr>
<td>P/Polifemo</td>
<td>5</td>
</tr>
<tr>
<td>P/RS841</td>
<td>6</td>
</tr>
<tr>
<td>P/AS10</td>
<td>7</td>
</tr>
</tbody>
</table>
BHT
Butylated hydroxytoluene

BHA
Butylated hydroxyanisole
**BHA** (butylated hydroxyanisole), **BHT** (butylated hydroxytoluene) are synthetic compounds used as antioxidants in foods, such as butter, meats, chewing gum, snack foods, dehydrated potatoes, and even beer. They are also used in a wide range of ‘non food’ products including cosmetics, toiletries and medicines.
Foods that may contain BHA and BHT.

<table>
<thead>
<tr>
<th>Active dry yeast</th>
<th>Flavourings other than essential oils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cake mixes</td>
<td>Frying oil and frying fat excluding olive pomace oil</td>
</tr>
<tr>
<td>Cereal based snack foods</td>
<td>Glazed fruit</td>
</tr>
<tr>
<td>Chewing gum</td>
<td>Lard, fish oil, beef, poultry and sheep fat</td>
</tr>
<tr>
<td>Defoaming agents for beet sugar and yeast</td>
<td>Milk powder for vending machines</td>
</tr>
<tr>
<td>Dehydrated meat</td>
<td>Pre cooked cereals</td>
</tr>
<tr>
<td>Dehydrated potatoes: potato flakes, mashed potato</td>
<td>Processed nuts</td>
</tr>
<tr>
<td>Dehydrated soups and broths</td>
<td>Ready Meals</td>
</tr>
<tr>
<td>Dry mixes for beverages and deserts</td>
<td>Sauces</td>
</tr>
<tr>
<td>Emulsion stabilisers for shortenings</td>
<td>Sausage, poultry and meat products</td>
</tr>
<tr>
<td>Essential oils</td>
<td>Seasonings and condiments</td>
</tr>
<tr>
<td>Fats and oils for the professional manufacture of heat treated foodstuffs</td>
<td>Snack Foods</td>
</tr>
</tbody>
</table>
Amount of BHT, BHA in Inodorus cv. Incas fruits

- Incas (ungrafted)
- I/Sting
- I/Energia
- I/Elisi
- I/RS841
- I/AS10
- I/Polifemo
- I/P360
Amount of BHT, BHA in Reticulatus cv. Proteo fruits
**BTEX**, are aromatic compounds such as toluene, ethylbenzene, styrene, etc. Gasoline leaking from underground storage tanks, distribution facilities, and various industrial operations represents a prime source of soil and aquifer contamination. BTEX are classified as priority pollutants because of their high mobility and toxicity.
BTEX identified in our samples

- Toluene
- o-Xylene
- m-Xylene
- Ethylbenzene
- Styrene
Amount of BTEX in Inodorus cv. Incas fruits
Amount of BTEX in Reticulatus cv. Proteo fruits
The results here reported demonstrated that

the rootstock influenced the uptake of contaminants such as:

- Glycol ether compounds
- BTXE
- BHT, BHA

both in Cucumis melo L. var. inodorus and reticulatus.
In fact,
a different amount was observed in the grafted fruits in relation to the rootstock for the two varieties. *Inodorus* melon fruits grafted on Elsi rootstock showed the lowest amount of Glycol ether compounds, BTXE and BHT, BHA. *Reticulatus* melon fruits grafted - on Elsi showed the lowest amount of BTXE - on Energia and Elsi showed similar amount of Glycol ether compounds and of BHT, BHA to the ungrafted.
In conclusion,

Our results must be considered preliminary and much more needs to be done, but we agree that:

Selecting low-uptake rootstock cultivars is a promising practical technique to reduce in fruits contaminant concentration

Since the responses depend on the rootstock/scion combination each grafting combination must be checked.
Thanks for your attention!