Annual Symposium
Vegetable Grafting

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

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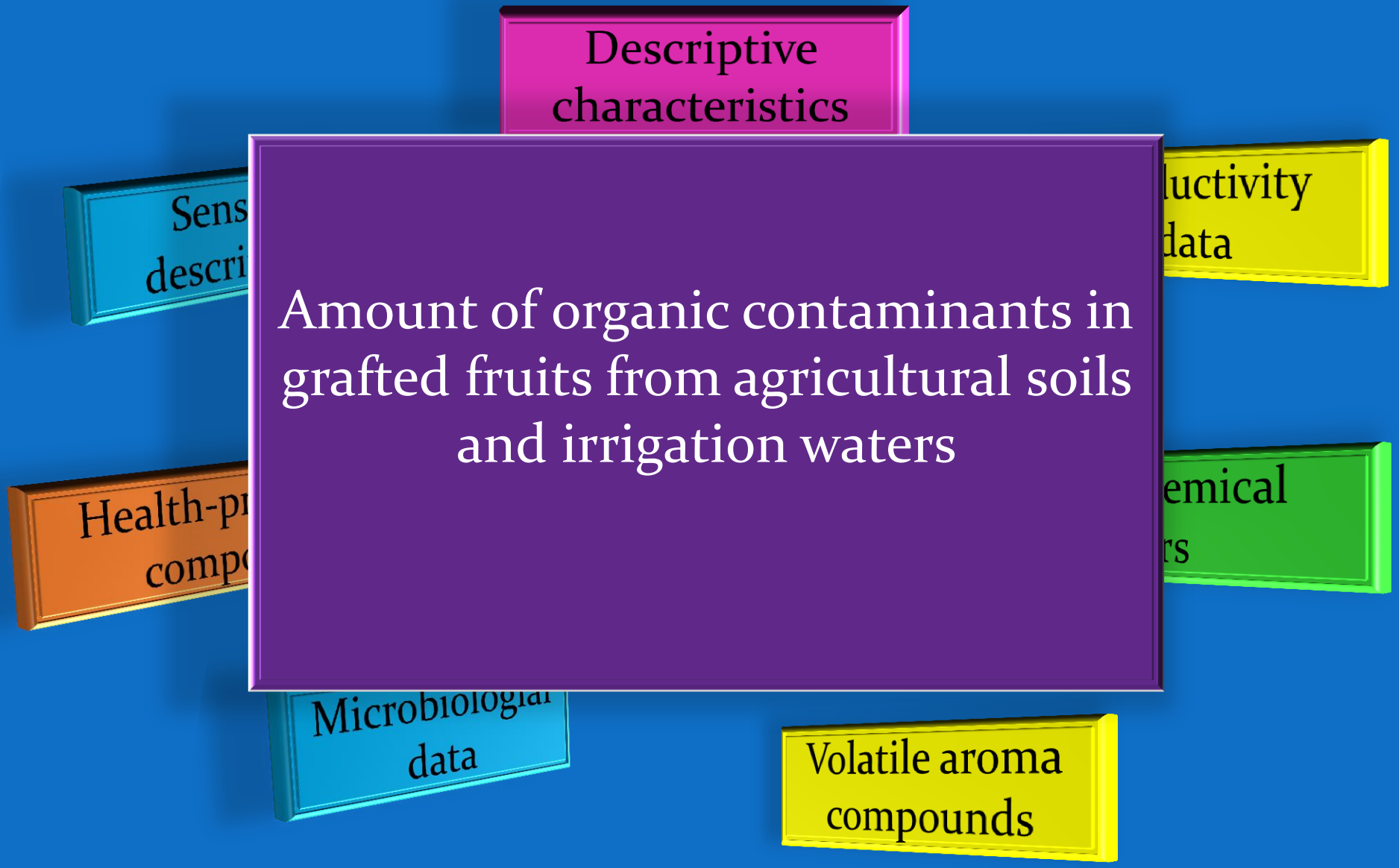
Our global approach for rootstock selection



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.

- personal care products from soils to pepper, tomato, lettuce and radish. Carter, et al. *Journal of agricultural and food chemistry* 62.4 (2014): 816-825.

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)

Savvas,D., Colla G., Rouphael Y., Schwarz D. (2010). *Scientia horticulturae*, *127*(2), 156-161.

✓Dieldrin concentration

✓OtaniTakashi, Nobuyasu Seike. *J. of Pesticide Sci.* 31.3 (2006): 316-321.

✓Persistent Organic Pollutants (POPs) including dioxins.

✓Mattina, MaryJane Incorvia, William A. Berger, Brian D. Eitzer. *Plant and soil* 291.1-2 (2007): 143-154.

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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

Scion	*Cucumis melo* L. var. *inodorus* cv. Incas
	Cucumis melo L. var. *reticulatus* cv. Proteo

	Rootstock	Suppliers	Pathogen resistance
1	Energia (*Cucumis melo*)	SEMINIS Vegetable Seeds Italia Srl (Pr)	FOM 1,2
2	Sting (*Cucumis melo*)	Nunhems Sementi srl, S. Agata (Bo)	FOM 0-1; 1,2; 2
3	Polifemo (*C. maxima* x *C. moschata*)	Esasem Spa, Casaleone (Vr)	FOM 1,2, *D. bryoniae*
4	AS 10 (*C. maxima* x *C. moschata*)	SEMINIS Vegetable Seeds Italia Srl (Pr)	FOM 0-1; 1,2; 2
5	RS841 (*C. maxima* x *C. moschata*)	SEMINIS Vegetable Seeds Italia Srl (Pr)	FOM 1,2; *D. bryoniae*
6	P 360 (*C. maxima* x *C. moschata*)	SAIS Sementi S.P.A. (FC)	FOM 1,2; *D. bryoniae*
7	Elsi *(C. maxima x C. moschata)*	Vilmorin Italia srl, Funo (Bo)	FOM 1,2; D. bryoniae

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.

Greenhouse Conditions

- 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_{max} and T_{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
- Aquisition range: 40-200 *m/z*

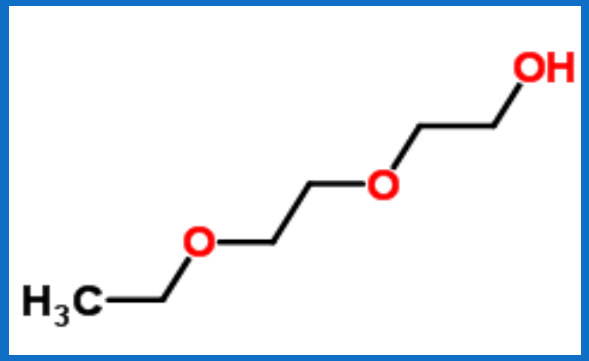
Results

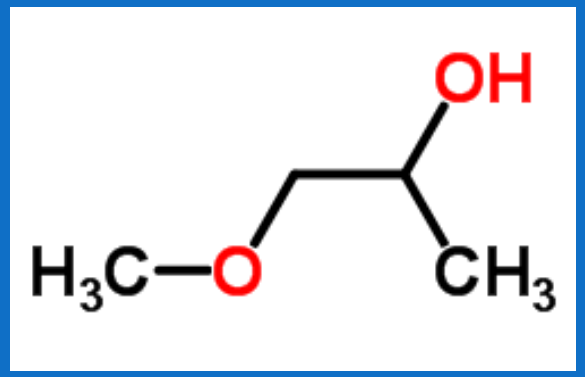
Emerging *contaminants identified*

Glycol ether compounds	✓
Phatalates	-
BTXE	✓
BHT, BHA	✓

Glycol Ether Products identified in our samples

Carbitol (Diethylene Glycol Monoethyl Ether)



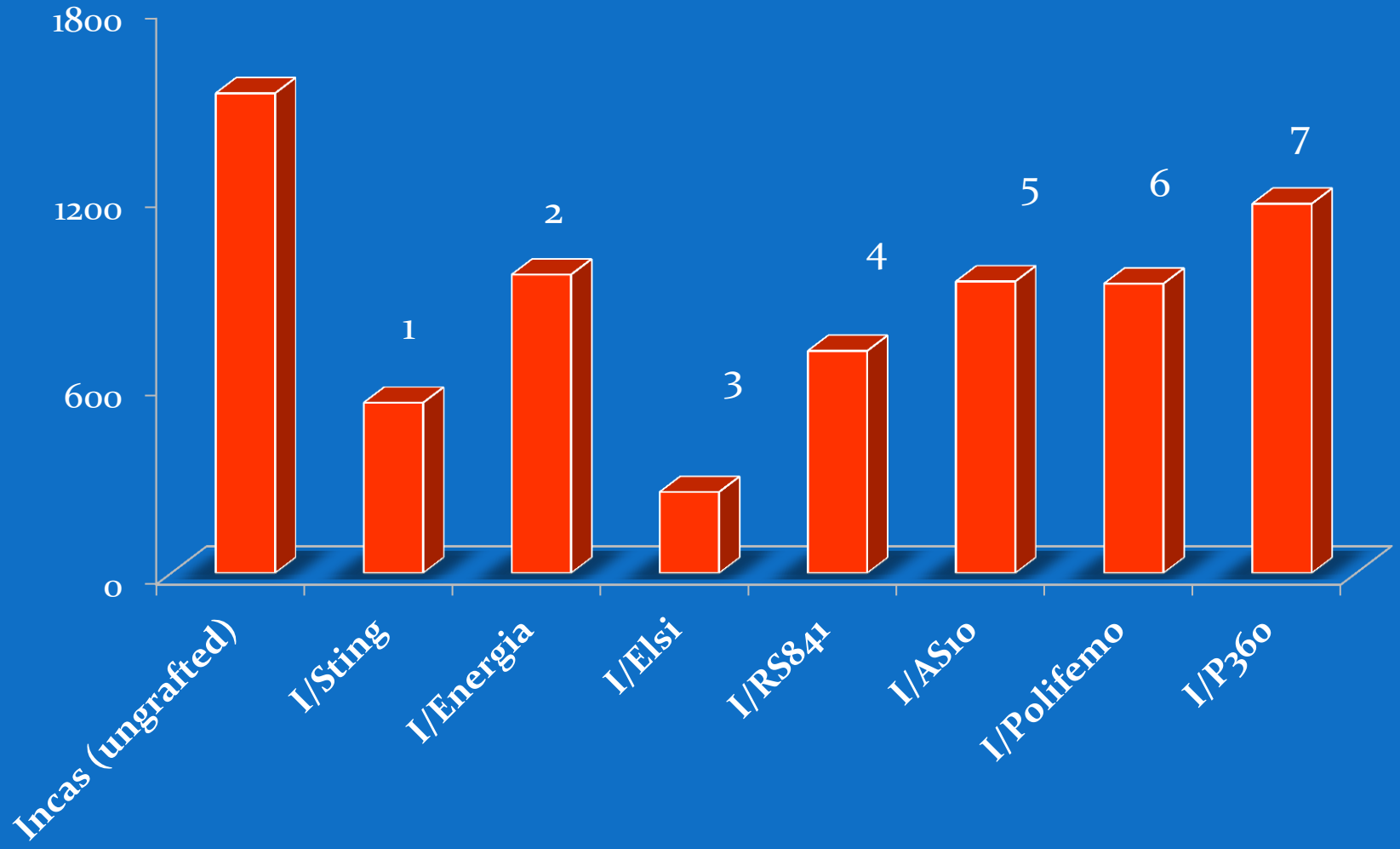


1-Methoxy-2-propanol (Propylene glycol methyl ether)

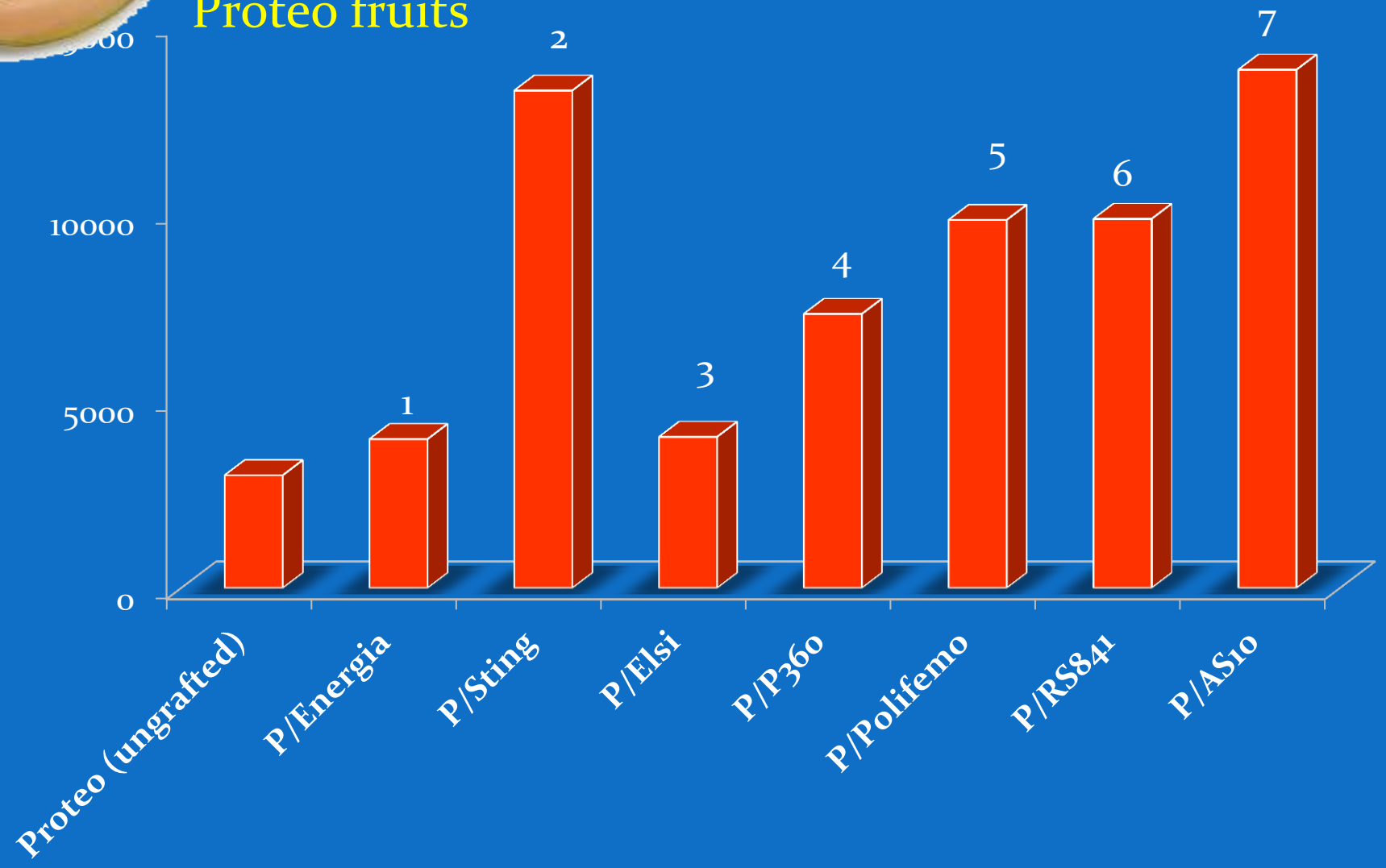
- In human studies, exposure to glycol ethers has been associated with low sperm mobility (Cherry et al. 2008), hematological effects (Starek et al. 2008), and asthma and allergies (Choi et al. 2010).
-

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

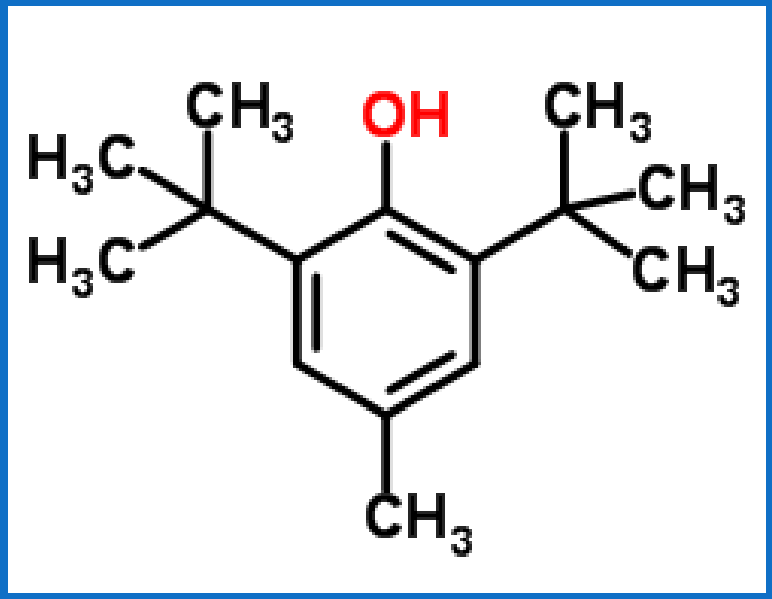


Amount of Glycol ether products in Reticulatus cv. Proteo fruits



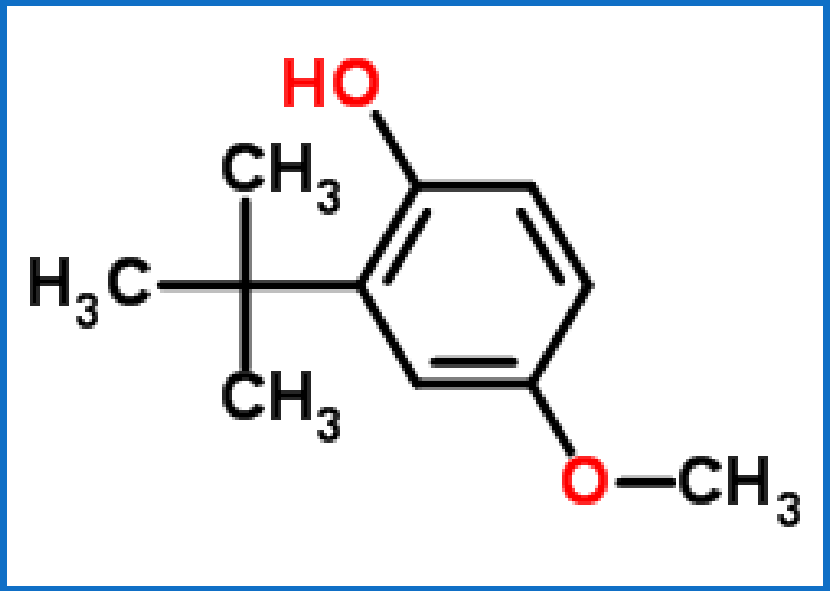
BHT

Butylated hydroxytoluene



BHA

Butylated hydroxyanisole

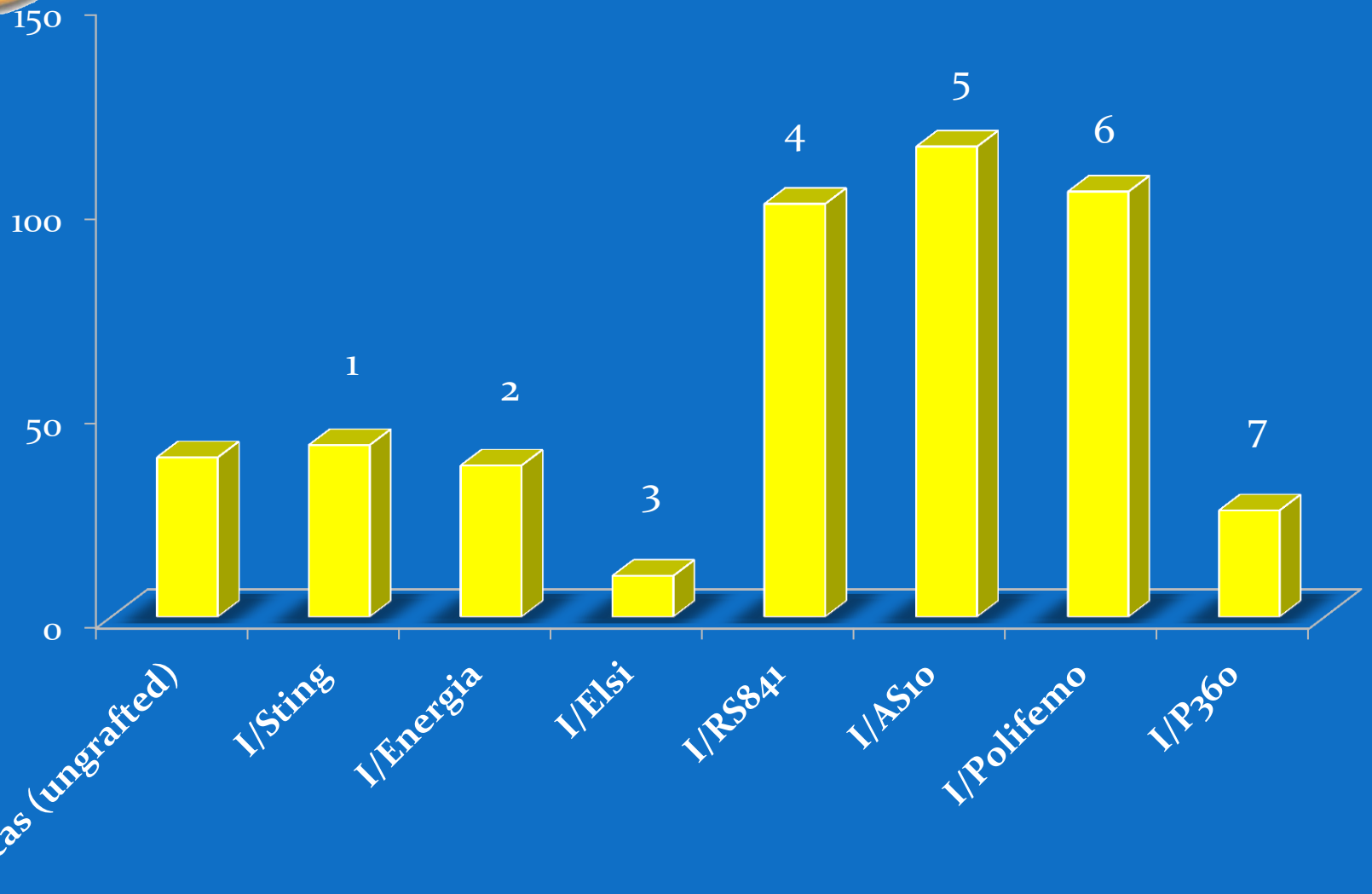


BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene) are synthetic compounds used as antioxidants in foods, such as butter, meats, chewing gum, snack foods, dehydrated potatoes, and even beer. They are also used in a wide range of 'non food' products including cosmetics, toiletries and medicines.

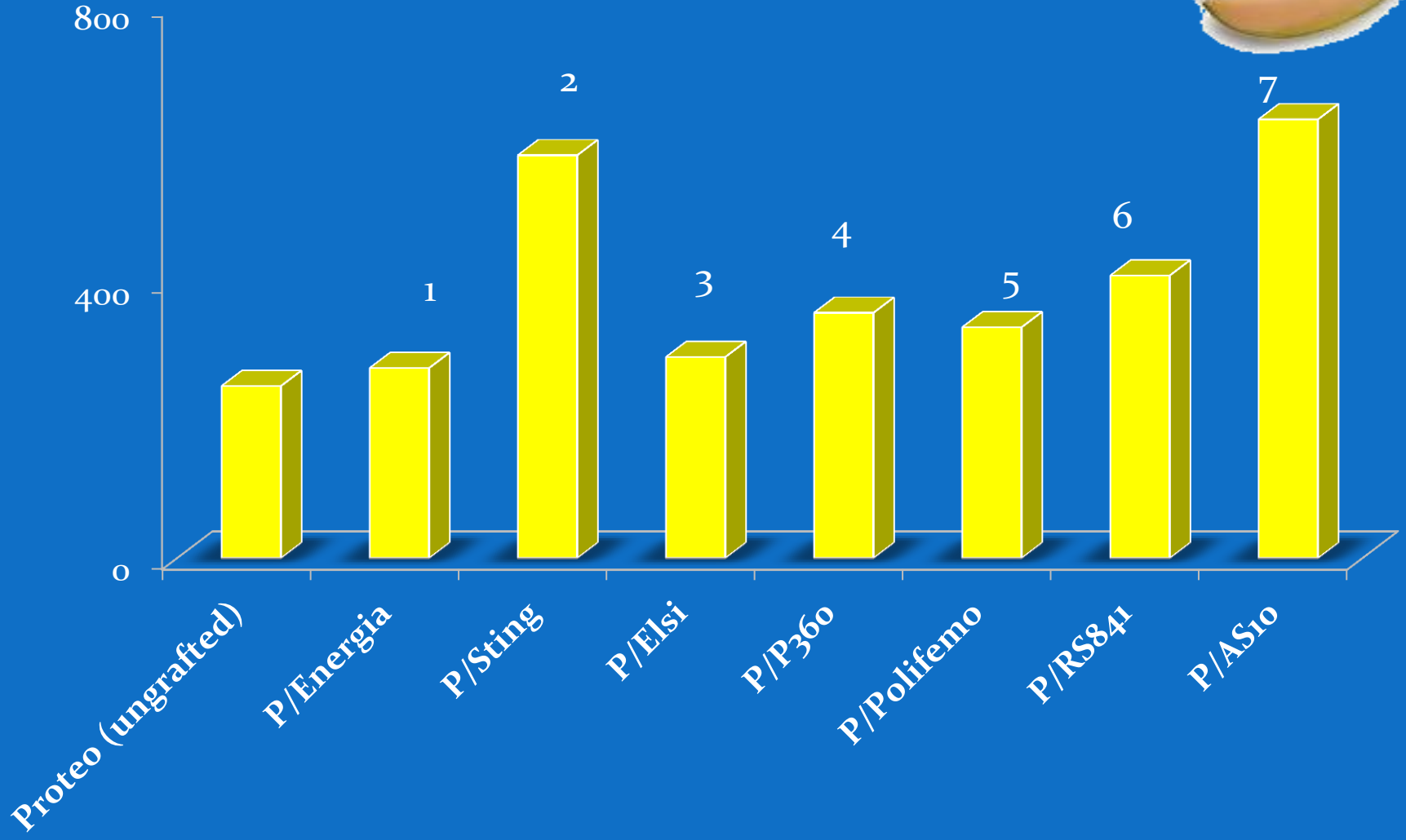
Foods that may contain BHA and BHT.

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

Amount of BHT, BHA in Inodorus cv. Incas fruits

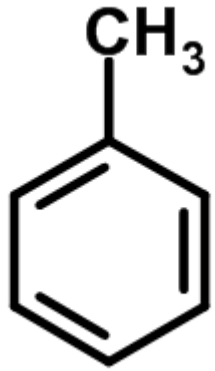


Amount of BHT, BHA in Reticulatus cv. Proteo fruits

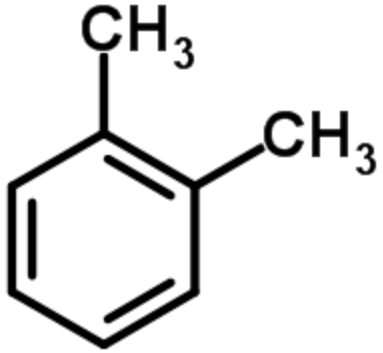


BTEX, are aromatic compounds such as toluene, ethylbenzene , styrene, etc. Gasoline leaking from underground storage tanks, distribution facilities, and various industrial operations represents a prime source of soil and aquifer contamination. BTEX are classified as priority pollutants because of their high mobility and toxicity.

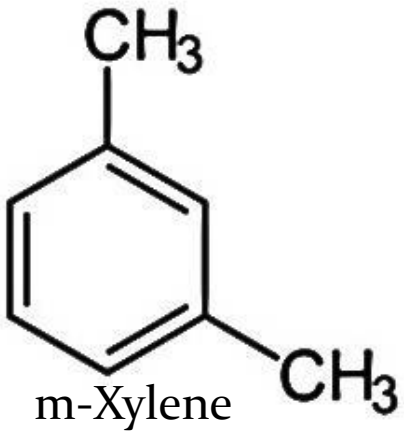
BTEX identified in our samples



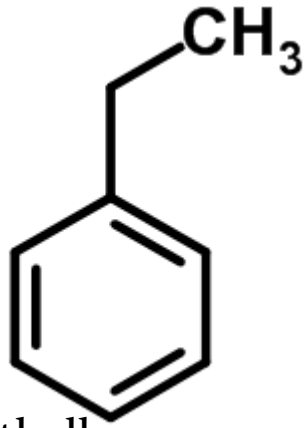
Toluene



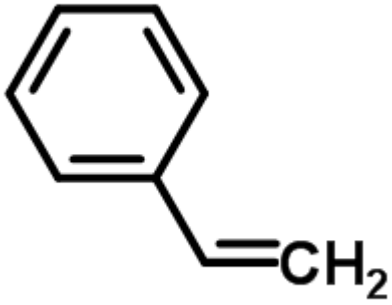
o-Xylene



m-Xylene

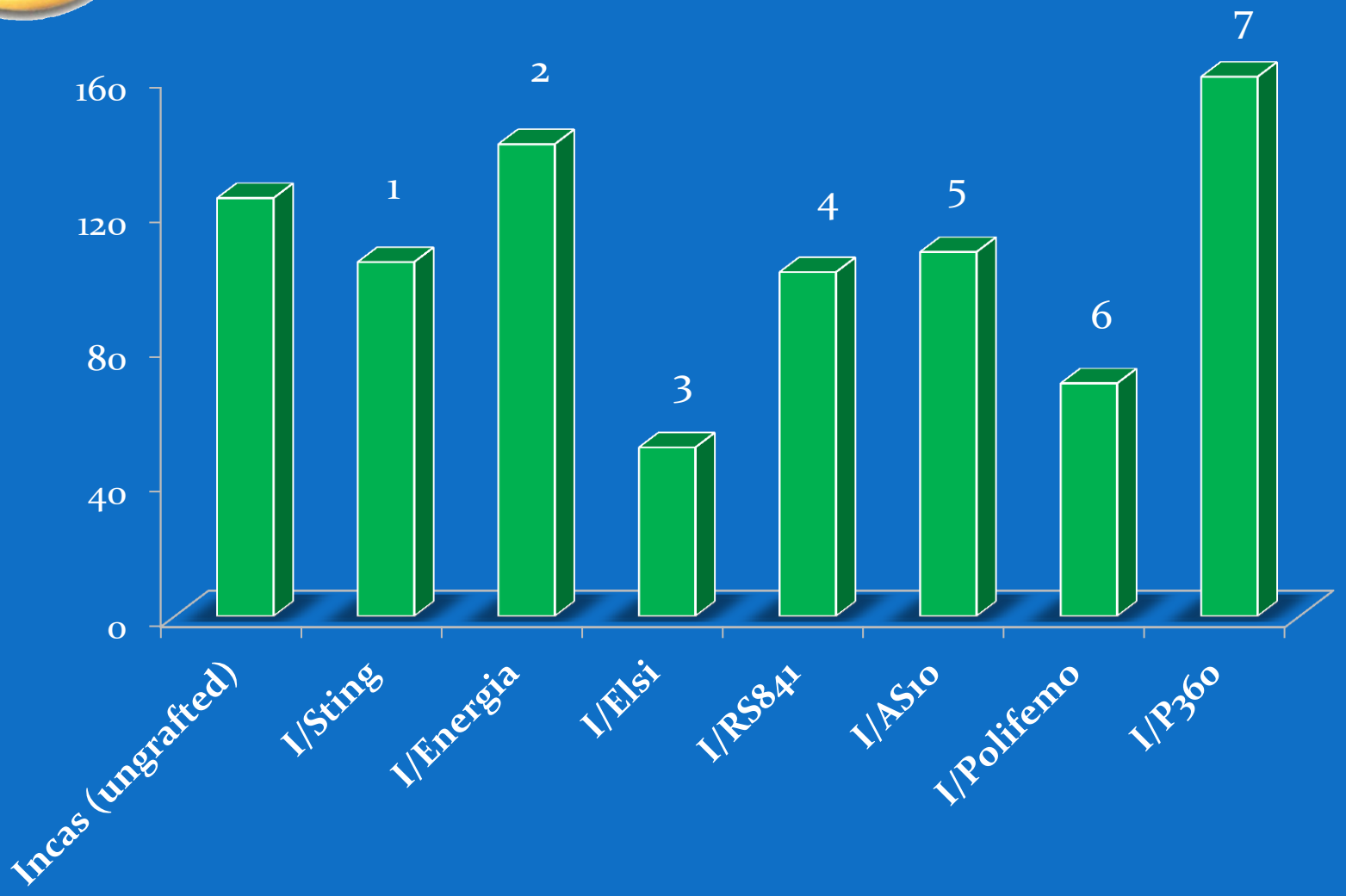


Ethylbenzene



Styrene

Amount of BTEX in Inodorus cv. Incas fruits



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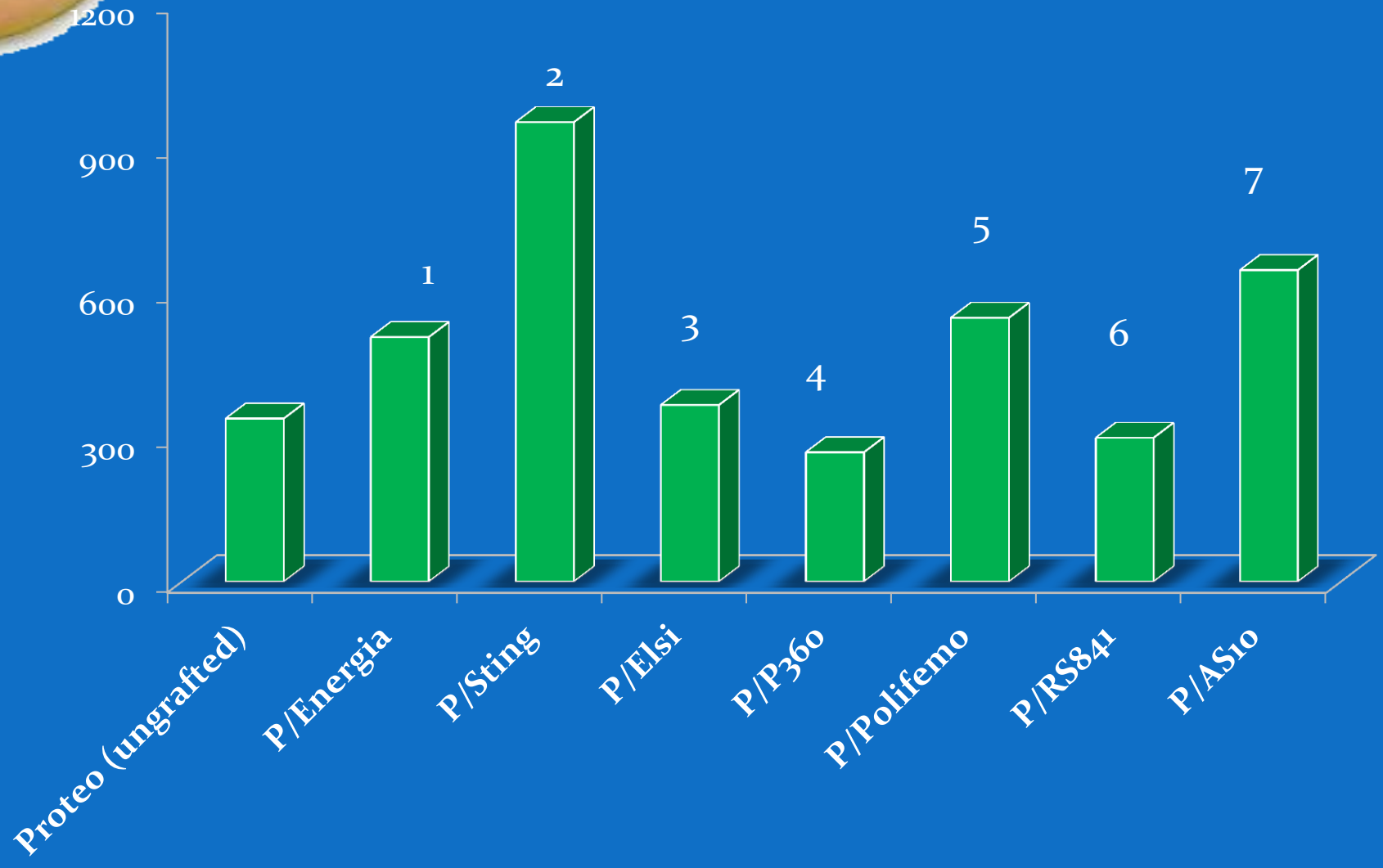
Amount of BTEX in Reticulatus cv. Proteo fruits



The results here reported demontrated 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 rootstoock 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.

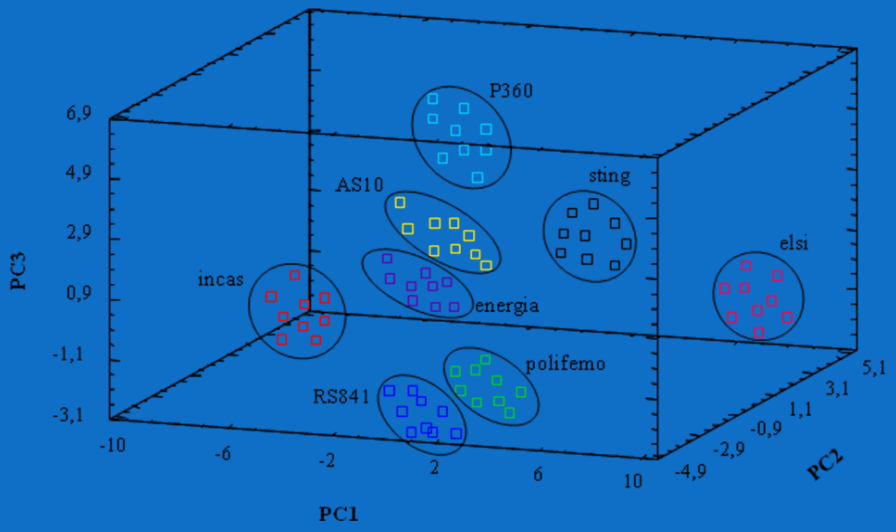
In conclusion,

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!


P360
AS10
sting
elsi
incas
energia
polifemo
RS841
6,9
4,9
2,9
0,9
-1,1
-3,1
PC3
-10
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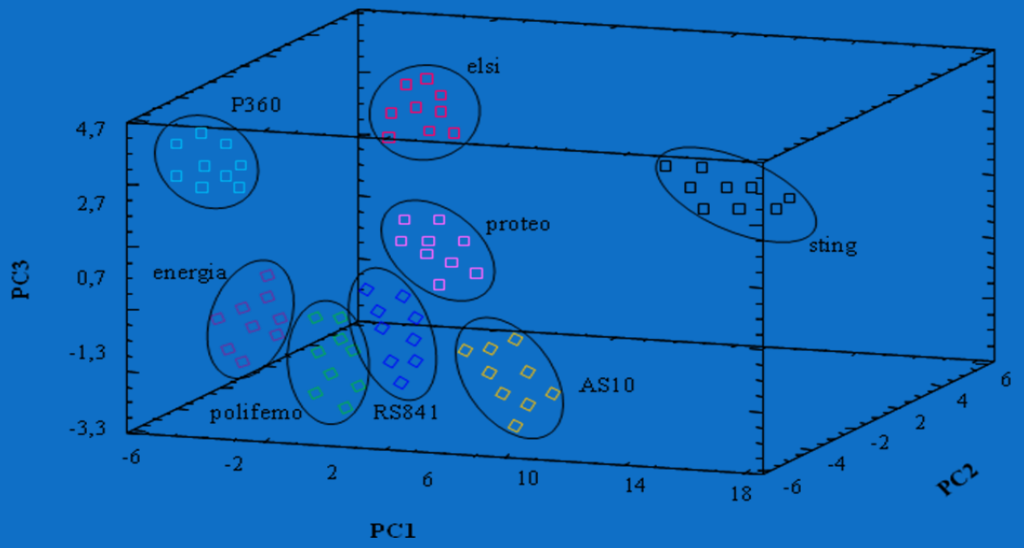
-2,9
-4,9
PC2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14
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PC1
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-4
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PC2