National Environmental Research Institute

Plant Biomarker Pattern

Apples grown with various availability of organic nitrogen and with or without the use of pesticides

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Introduction

In the recent years there has been an increasing focus on the quality and health value of organic plant products compared with conventional products.

The use of pesticides and concentrated fertilisers in conventional agriculture implies a risk of effects on plant composition, which may affect health of the consumer (Brandt & Mølgaard, 2001).

To determine if organically grown plant food could provide more or less benefits to health than conventional food, a first step is to investigate the differences in the composition and relative concentration of natural compounds in the plant products.

In this project apples were grown with two levels of nitrogen availability and with or without the use of pesticides. The apples were screened for changes in the phytochemical composition and concentration.

The work is affiliated to the project "Organic food and health" supported by the Danish Research Centre for Organic Farming (DARCOF).

Hypothesis

The composition and relative concentration of natural compounds differs (a biomarker pattern) if the plants are grown:

- with low or high N and with or without pesticides

Screening programme

Groups of phytochemical compounds detected in 46 Thin layer chromatography (TLC) systems (Christensen & Diedrichsen, 2002):

- Alcohols and phenolic compounds
- Carbohydrates
- N-containing compounds
- Organic acids and lipids
- P-containing compounds
- S-containing compounds
- Terpenoids
- Several of the above mentioned groups

Plant material

Apples, *Malus domesticus* Borkh. var. Otava were harvested primo October 2001. The trees and alleyway ground-cover treatments were established in 1995 in Aarslev and cultivated organically and unsprayed until 2001.

Parcel 1:
High N: The alleyways between the tree rows were sown with a mixture of Persian clover (*Trifolium resupinatum*) and Italian ryegrass (*Lolium multiflorum*) each year primo July. Mulched in April, followed by mechanical weed cleaning. No pesticides.

Parcel 2:
Low N: The alleyways were covered with a permanent grass mixture, consisting of red fescue (*Festuca rubra*) and meadowgrass (*Poa pratensis*). No pesticides.

Parcel 3:
High N: As in parcel 1. Pesticides (only in 2001): Kresoxim-methyl, Dithianon, Mancozeb, Triforin (Fungicides), Phosalon (Insecticide).

Parcel 4:
Low N: As in parcel 2.
Pesticides: As in parcel 3.

Screening process

Sample preparation
↓
Application on stationary phase (TLC-plate)
↓
Development in mobile phase
↓
Derivatization with chemical reagent
↓
Detection of biomarkers

Conclusion

Biomarkers and biomarker patterns were presented in plants cultivated with low and high N and with pesticides.

One biomarker was related to:
- the type of N with and without pesticides
- pesticides at high N and type of N without pesticides
- pesticides at low and high N

One biomarker pattern was related to:
- the type of N
- the type of N without pesticides
- pesticides at low N and type of N without pesticides
- pesticides at high N and type of N with pesticides

Sample preparation

Plant extracts used for screening are obtained using fresh, frozen or freeze-dried plant material. The plant material is either pressed or extracted with suitable solvents and centrifuged before screening.

Reference

Brandt, K. & Mølgaard, J.P. (2001): *Organic agriculture: does it enhance or reduce the nutritional value of plant foods?* J. Sci. Food Agric. 81, 924-931.
Christensen, T.F. & Diedrichsen, B. (2002): *A new phytochemical screening programme used for organic and conventional crops.* Master Thesis, The Royal Danish School of Pharmacy and The National Environmental Research Institute.

Results

Biomarkers

Cultivated with or without:	Pesticides		No pesticides		
Cultivated with Type of N:	**Low N**	**High N**	**Low N**	**High N**	**Causal relation**
Organic acid/lipids	n.d	x	n.d	x	Type of N
Carbohydrates	x	n.d	x	n.d	Type of N
S-containing compounds	n.d	n.d	x	n.d	Type of N without pesticides
S-containing compounds	n.d	n.d	x	n.d	Type of N without pesticides
Organic acid/lipids	n.d	x	x	n.d	Type of N with and without pesticides
Organic acid/lipids	x	x	n.d	x	Pesticides at low N and type of N without pesticides
Organic acid/lipids	x	x	n.d	x	Pesticides at low N and type of N without pesticides
N-containing compounds (amino acid)	x	x	x	n.d	Pesticides at high N and type of N without pesticides
Phenolic compounds (flavonoid)	(x)	n.d	x	x	Pesticides at high N and type of N with pesticides
Phenolic compounds (flavonoid)	x	n.d	x	x	Pesticides at high N and type of N with pesticides
Phenolic compounds (flavonoid)	(x)	n.d	x	x	Pesticides at high N and type of N with pesticides
Terpenoids	n.d	n.d	x	x	Pesticides at low and high N

x: presence of biomarkers
n.d: not detected

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In 2000 an international PCT patent application: "An assay method and kit for testing biological material for exposure to stress using biomarkers" (WO 01/92879 A1), PCT /DK01/00377) was filed at the European Patent Office with the purpose of international patent protection.