



Effects of cultivation conditions for apples on growth rates of fruit fly larvae and contents of phenolics

Drosophila melanogaster (2 mm)

Introduction

Apples of the variety Otava were grown with 3 different alleyway ground-cover management strategies, providing a wide range of nutrient availabilities to the trees, spanning the ranges normally found in both conventional and organic orchards. These treatments had significant effects on both yields and incidence of fungal diseases. However, the differences in yields and disease incidence cancelled each other out, resulting in identical average yields of marketable fruit, as reported earlier (Lindhard Petersen & Bertelsen 2002).



High N
(Annual clover/grass)

Medium N
(Perennial clover/grass)

Low N
(Perennial grass)

The nutrient status of apple orchards is known to affect the content of phenolic compounds in leaves (Mihalek et al. 1996), which are involved in protection against diseases (Buchter-Weisbrodt 1996), so we expected the apples to show corresponding variation of phenolic compounds.

Several plant secondary metabolites have been implicated in the effects of fruits and vegetables on human health, including phenolic compounds such as flavonoids. Many investigations have focused on their anti-oxidant properties, but in the present study we wanted to test the hypothesis by Brandt & Mølgaard (2001), that plant secondary metabolites could increase longevity through anti-nutrient effects, by mimicking the effects of caloric restriction (reviewed by Gerhard 2001). We would thus expect a slower development of larvae in apple material with high content of relevant secondary metabolites.

Results

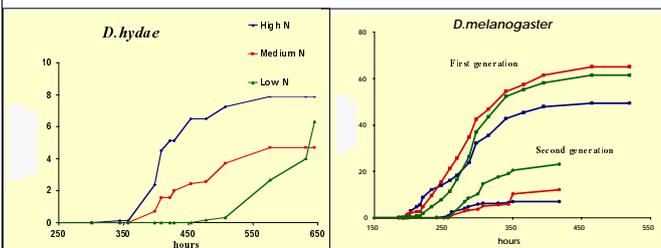
In all 3 tests, the average time for development was longer for flies developing on low N apples than the 2 other treatments. This is particularly clear for *D. hydrae*, which has a longer lifecycle than *D. melanogaster*. (Figure 1)

In *D. melanogaster* the second generation developed slower than the first generation. (Figure 1)

The total number of flies emerging differed very much between containers and treatments, and no clear trend was observed. Each of the 3 cultivation treatments resulted the highest number of flies in one of the 3 tests. (Figure 2)

Figure 2

Emergence of adult fruit flies in the 3 tests



There are no systematic effects on the number of flies emerging, indicating that nutrient supply is adequate in all treatments.

References:

- 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.
- Buchter-Weisbrodt, H. (1996) Phenole gegen schorf. *Obstbau* 2: 62-64.
- Gerhard, GS. (2001) Caloric restriction in nonmammalian models. *Journal of Anti-aging Medicine* 4: 205-213
- Mikalek, S., Treutter, D., Mayr, U., Lux-Endrich, A., Gutmann, M. & Feucht, W. (1996) Role of flavan-3-ols in resistance of apple trees to *Venturia inaequalis*. *Polyphenols Communications* O26.
- Pedersen H.L. and Bertelsen M. (2002) Alleyway groundcover management and scab resistant apple varieties. ECO-FRU-VIT. 10th International Conference on Cultivation technique and phytopathological problems in Organic Fruit-Growing and Viticulture, 19-21. www.infodienst-mlr.bwl.de/la/lwvo/ecofru/vit/alleywayground3.pdf

Drosophila hydrae (4 mm)

Methods

Two fruit fly species were used: *Drosophila melanogaster* and *D. hydrae*.

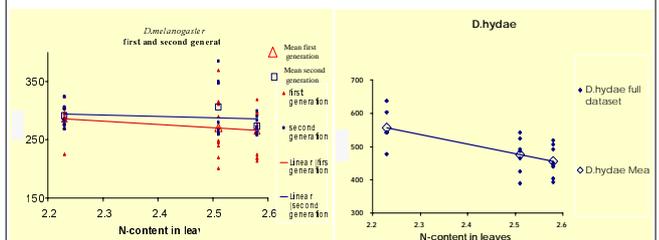
First grade Otava apples from year 2000 were washed, the center with kernels removed, and the flesh with skin was grated and frozen. Identical tests were done for the two species of fruit flies and the 3 different cultivation treatments of apples.

Each fly species were tested for one generation, with *D. melanogaster* also a second generation test was done. For each apple cultivation treatment, 10 fruit fly containers were prepared, with 2 cm of newly thawed grated apple placed in the bottom (5 ml). 20 adult fruit flies were added to each container (only 10 for the second generation test). After 12 hours the flies were removed and each container were observed for developing of larva and flies. The containers were placed at 21°C and a 12 hour photoperiod.

Newly emerged adult flies were removed and counted at least every other day.

Figure 1

Time of 50% emergence of flies, as function of N concentration in leaves



| | <i>D. melanogaster</i> | | <i>D. hydrae</i> |
|------------------------------|------------------------|-------------------|------------------|
| | First generation | Second generation | |
| R ² Mean: | 0.9973 | 0.0527 | 0.9998 |
| R ² Full dataset: | 0.0506 | 0.0121 | 0.4411 |

Despite large variation within each test, all three tests show trends of slower rate of development for lower N supply to the apple trees.

Measurements of phenolic compounds

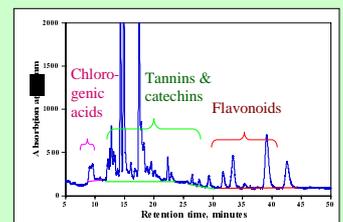
Experimental

Extraction-0.5 g freeze-dried material was extracted in 12 ml 30% CH₂Cl₂-0.5% TFA (trifluoroacetic acid) for 1 hr at room temperature.

Flavonoid and phenolic acid. HPLC analysis-The filtrated extracts and standards of chlorogenic acid, rutin, quercetin and epicatechin were analyzed by HPLC using a LiChrospher 100 RP-18 (5 μm) column at 35 °C, detection at 280 nm, flow rate 1.0 ml min⁻¹.

The gradient solvent system consisted of solvent A: (HCO₂H-H₂O (1:19)) and solvent B (MeOH-H₂O-HOAc (10:3:7)) as follows: 0 min 0%B, 5 min 15%B, 8 min 15%B, 20 min 30%B, 35 min 30%B, 45 min 40%B, 55 min 40%B, 75 min 100%B, 85 min 0%B, 95 min.

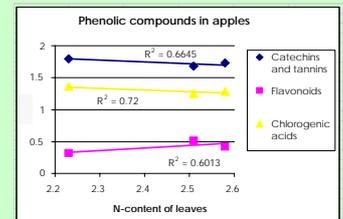
Eluted peaks were distinguished by their ultraviolet-visible absorption profiles as either chlorogenic acids, flavonoids, tannins or catechins, and expressed in μg/g dry weight of plant material as equivalents of chlorogenic acid, rutin, quercetin and epicatechin, respectively.



Results

The contents of phenolic compounds in apple differed among the cultivation treatments.

Tannins and chlorogenic acids, assumed to be defence related compounds, decreased with increasing N-supply to the trees. While the flavonoids, which are most likely more important in UV protection, increased.



Conclusion:

The different cultivation treatments significantly and systematically affected both rate of development of fruit fly larvae and contents of phenolic compounds, but not the total number of flies produced. This is in accordance with the hypothesis that cultivation methods can give large enough changes in composition of plant foods to affect physiological aspects important for health of consumers. It indicates that further studies should be made of the links between plant cultivation, plant composition and health, for example regarding the question of the nutritional value of organic versus conventional food.