Further experiments involving GDV technique in agronomy

{Technical report on experiments performed at FiBL Institute in October 2002}

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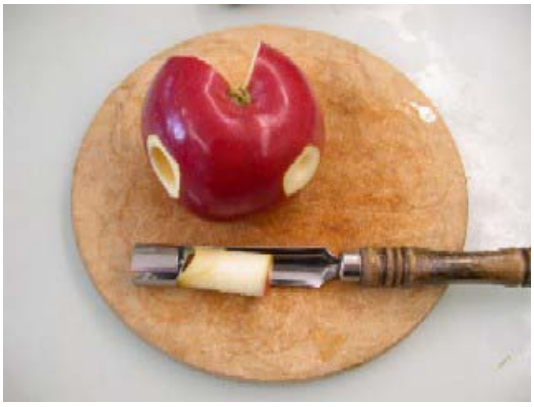
Introduction

This report sums up newest experiments involving GDV technique in agronomy performed at FiBL Institute, Frick, Switzerland [8]. The work presented here is the continuation of experiments done at the same institute in the previous two years. Most of our previous work is described in [1, 2, 3, 4].

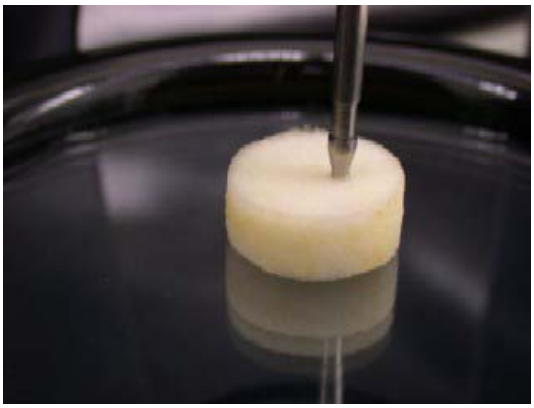
The focus of this year's research was twofold. We tried to distinguish between organically and conventionally grown apples, based on corona images of ripe apples. We performed two experiments to this end. Secondly, we tried to detect the influence fertilization method has on growing apples, also based on corona images of ripe apples. One experiment was performed to this end. A side effect of these experiments was the development of new, standardized way of recording bioelectromagnetic fields (coronas) of ripe apples.

Recording methodology

During last year's experiment we already developed a standardized method of recording ripe apples. But one problem with it was the skin of the apple. Agronomists felt that it may be too easily influenced by means beyond our control. This was the reason that this year we decided to record the apple tissue alone, cutting off the skin. Cutting is a very intrusive method and we would like to avoid it, but the fact that ripe apples are too big to be recorded as a whole (because of the electrode size) meant that we could not avoid cutting them anyway.



Our methodology for recording bioelectromagnetic fields of ripe apples with the Kirlian camera [5] is as follows. First we wash the apple with water and dry it with a towel. Then we pinpoint the sun and shadow side of the apple. These two points are diametrically opposed to each other. Sun side is the side of the apple that was exposed to the sun the most when the apple was growing and is usually brighter or of a warmer colour. The point from where in the apple we extract the tissue is located exactly in the middle between the sun and shadow



sides. This somewhat eliminates the effect of positioning the apple has while growing with respect to the sun (see next chapter for discussion on this effect). At the point of extraction we cut off the skin and take out a cylindrical part of the apple tissue with a special knife (Figure 1). It is important to first cut off the skin, because then we can extract the tissue with less pressure on the knife, which in turn damages the tissue to a lesser extent. From this cylinder of apple tissue we extract a smaller cylinder by cutting the original one centimeter under the apple surface and one and a half centimeter under the surface. This gives us a standard piece of apple tissue, which is cylindrically shaped, its height is half a centimeter and diameter is slightly more that one centimeter (Figure 2). Sample is then positioned on the electrode, grounded and recorded (Figures 3 and 4).

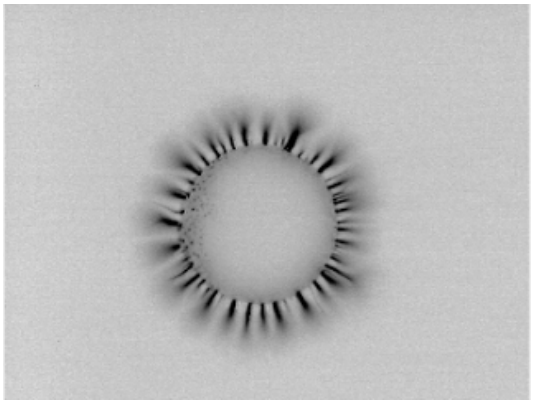
Apart from standardizing the way of obtaining a recording sample another benefit of this method is that the sample's cylindrical shape means we get a roughly circular corona (Figure 5). Circular coronas have the advantage that they are similar (in shape) to coronas of human fingers for which there was the most scientific interest [5] and hence the most methods of describing them with numerical parameters. This is also true for our own analytical program GDV Assistant [6], which we used for analysis during our experiments.

Two further issues regarding recording methodology

An extensive amount of time was devoted to two open questions affecting the recording methodology:

(a) What (if any) is the effect of positioning the apple has while growing with respect to the sun?
(b) Is the information apple contains stored in its skin or its tissue?

To find out the answers to these questions we performed an additional sub experiment of the organic vs. conventional experiment described in the next chapter. We took four (for some even eight) samples from each observed apple: one from the sun side, one from the shadow side and two from in between both sides (neutral) (Figure 6). Along with the recording of the tissue we also recorded the corona of the skin alone. Skin was approximately one millimeter thick.



The analysis, visual and numerical, did not give any clear answers. Different tissue samples from the same apple didn't differ much, while there were differences between tissue and skin samples.

However, with respect to the experiment itself, neither sample group yielded any information. It has to be noted that the main experiment's result was negative, therefore the answers to these two questions are still highly inconclusive, especially the issue regarding the skin vs. tissue.

Because of these inconclusive results we decided to stick with our agronomically founded philosophy regarding which side of the apple to use for further recordings. The side between sun and shadow sides is some sort of average and is therefore deemed most appropriate. Regarding the skin vs. tissue issue we decided in favour of the tissue because skin can be much easier influenced by external factors than tissue and this is highly undesirable. Until there is any evidence that some other methodology can give better results this will be our default recording methodology for ripe apples.

Experimental design

Three experiments were performed, two of them dealt with differentiating organically grown apples from conventionally grown ones while one experiment dealt with differentiating apples grown by using different fertilization methods. All were performed in a similar fashion. We first recorded the images of selected apples with the Kirlian camera (of type Korotkov 99 Clear Glass) using the previously discussed recording methodology. For the purposes of analysis and differentiation we had to describe the obtained images with numerical parameters. We have done that with the use of GDV Assistant program, developed at our laboratory. Each sample was described with a set of numerical parameters described in [4, 6]. Differentiation was then attempted with See5 software [7], used for constructing decision trees and rules. Analysis of the data was done with Microsoft Excel.

We used GDV Assistant, version 1.0.8a, August 2002, with the following settings:

- fragment size: 50;
- background: 65;
- GDV camera type: Korotkov GDV 99 Clear Glass.

See5 software was version 1.09a. Unless specified otherwise the results were obtained using default settings. Other settings were tried but did not give much improvement, if any. Testing method used was leave-one-out testing where number of samples was less than 100, otherwise 10-fold cross validation was used.

Organic vs. Conventional

This was a repeat of last year's experiment, which turned out negative. This year, however, we were able to obtain samples from controlled environment as opposed to last year where we picked the apples from the market. Idared apples were from a 6-years-old comparison trial on organic and integrated (IP or conventional) fruit production called ECOMAX from the Swiss Federal Research Station on Fruit Growing at Sion, VS. The organic block is certified organic and the integrated one certified IP. This made this study's results much more reliable. We recorded 80 apples, 40 from each class. Apples were of the same variety. In contradiction with our recording philosophy we recorded positional extremes (sun and shadow side) and averages (the usual in between sun and shadow sides)

and also the skin for all apples. That was done because we were developing our recording methodology in parallel with this experiment. So, we actually had additional data we could use for differentiation. For comparison we also recorded 3 standard agronomical parameters: sugar contents, firmness (Figure 7) and taste (this is subjective, done with two or more testers who each give a mark between 1 (worse) and 5 (best); marks are then averaged).

The results of differentiation turned out all negative. We tried differentiation with both positional extremes together and with both positional averages together, as well as with just the sun side and just the shadow side. Differentiation based on apple skin was also attempted. We did not have any success with any of them. Testing was done on all four camera's voltage ranges.

For comparison we tried differentiation using only standard agronomical parameters. Along with the measured three parameters we also used the calculated quality index, which is a parameter that agronomists very commonly use to describe the sample with a single all-inclusive parameter. The formula is:

$$QualityIndex = 2 \cdot Firmness + SugarContents + 4 \cdot Taste$$

We had available 54 reliable samples with these measurements, 27 of each class. See5 was able to differentiate between organically and conventionally grown apples with almost 80% accuracy. It used only one parameter, sugar contents.

Additionally, we observed whether there are any correlations between standard agronomical parameters and GDV parameters. No such correlations were found.

Since we were not satisfied with the negative result of our experiment, we decided to carry out yet another repeat of this experiment with even more reliable samples. Apples for this experiment were even more selectively picked. The design of this repeat experiment was the same as before. This time we recorded 60 apples, 30 of each class (organically and conventionally grown). We recorded both extremes (sun and shadow side) and three previously mentioned standard agronomical parameters. Again we tried differentiation based on both samples from one apple together and both version with single sample from one apple (sun and shadow). We tried all four camera voltage ranges. Results were negative for all tests.

This time the differentiation based on standard agronomical parameters also failed. This might indicate that these samples were even more reliable than the ones in the previous experiment.

Effect of fertilization method

Second topic of our research this year was to investigate whether we can observe the effect of different fertilization methods by analyzing the corona images of apples grown using these methods. For this experiment we recorded 30 apples for each of five different fertilization methods, here denoted as v2, v3, v4, v5 and v10. This gave us a total of 150 samples. In this experiment we used recording methodology that was described earlier.

A few details about the origin of the samples for this experiment. Apples were taken from the KOB trial performed by FiBL's Franco Weibel and Andi Schmid at the Vogt organic farm in Remigen, AG. The apples are all of the same variety (Topaz), the only difference between them is the fertilization treatment they receive. Treatments taken under our observation were:

- v2: negative control, without compost, with PKCaMg addition;
- v3: fertilized with compost;
- v4: fertilized with compost of same raw material as v3, but made by a bio-dynamic recipe; no bio-dynamic preparations added during vegetation;
- v5: same as v4, except with bio-dynamic preparations added during vegetation 3 times per year on soil (bd 500) and on leaves (bd 501);
- v10: positive control, without compost, soil and leaf fertilizers applied, closest to conventional treatment.

Until now we tried only differentiating range 3 data for v4 method against the other fertilization methods. Method v4 was picked because agronomists felt that this method should be the most distinctive of them all. Results were not too encouraging though, because the best classification accuracy was 65% (for differentiating method v4 from method v2). Differentiation of all 5 methods at once failed, as did fail all the other v4 versus vX (X = 2,3,5,10) differentiations – classification accuracy for these was lower than 60%.

After receiving unsatisfactory results of differentiation attempts, we tried to separate whole groups instead of a single sample (apple). Here, the question was whether there is a difference in any of the GDV parameters between one fertilization method from the other. To find this out we performed statistical t-tests for all GDV parameters on all pairs of fertilization methods. Results were somewhat surprisingly positive and are shown in Table 1.

TTesting	pair	area	noise	br.dev	area/frag
	v2 vs v3	0.0000	0.0000	0.0009	0.4240
	v2 vs v4	0.0074	0.0000	0.6455	0.9759
	v2 vs v5	0.0531	0.0013	0.1898	0.7245
	v2 vs v10	0.0675	0.1216	0.4040	0.7510
	v3 vs v4	0.0105	0.0349	0.0056	0.0105
	v3 vs v5	0.0002	0.0000	0.0207	0.0002
	v3 vs v10	0.0009	0.0000	0.0001	0.0009
	v4 vs v5	0.2293	0.0002	0.4442	0.2293
	v4 vs v10	0.3435	0.0000	0.2150	0.3435
	v5 vs v10	0.9077	0.0442	0.0338	0.9077

Table 1 Results of t-tests for positive GDV parameters

Numbers in the table represent probabilities that the two groups of samples come from the same population according to the observed GDV parameter. For example, value 0.0531 in the fourth row of the first column means that there is 5.31% probability that groups v2 and v5 come from the same population. With red font we marked those probabilities that are less than 5% (a statistical standard). For these cases we can claim that observed GDV parameter(s) point out the differences between the groups and therefore show differences between fertilization methods. In the table we included only GDV parameters that showed such differences. Those not included failed to do so.

A little comment on the last GDV parameter included, area per fragment. It is correlated with area, and is equal to it for cases where number of fragments is 1. Since number of fragments as a parameter didn't provide any differentiation of groups, we can observe that area per fragment showed differences only when the former parameter was 1. As such it has no real use and is only included for completeness of the report.

For this experiment further statistical tests (especially principal component analysis) are ongoing at FiBL by dr. Franco Weibel and have preliminarily shown encouraging results.

Good vs. Bad apples

We performed an additional sub experiment, where we tried to see whether GDV parameters could distinguish between good and bad apples as defined by quality index, described before. Since quality index is highly influenced by taste, which is clearly a subjective measure, this whole test is a bit subjective. Nevertheless, it was attempted as a preliminary study, again only on range 3 data.

We used apples recorded for organic vs. conventional experiment I and II, separately of course. We have selected a group of good and a group of bad apples according to their quality index (selected by an agronomist so that we had three approximately even groups, good, medium and bad; medium group was discarded). Then we performed t-tests to see whether GDV parameters show us any differences for the two groups.

For samples acquired from organic vs. conventional experiment I, such differences were shown by two GDV parameters, area (1.5% – 2%) and noise (0.25% – 0.30%). However, standard agronomical parameters, firmness and sugar contents, performed such differentiation much better, in the range of less than one millionth of a percent for the probability that the two groups come from the same population.

For samples acquired from organic vs. conventional experiment II, no differences were shown by GDV parameters. The closest to showing anything was parameter brightness deviation at around 8.5%. Standard agronomical parameter firmness did not show any differences either, however, sugar contents did at around 3.7%. From these we can conclude that standard parameters work much better than GDV parameters for this type of problem.

We have analyzed whether there are any correlations between standard agronomical and GDV parameters. We did not discover any such correlations on these two sets of data.

Acknowledgements

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