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Wheat and ultra high diluted gibberellic acid – further experiments and re-analysis of data

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Background: **Following studies (a) on wheat seedlings and ultra high diluted silver nitrate, and (b) on amphibians and an ultra high diluted hormone, (c) a bio-assay on wheat and extremely diluted gibberellic acid was standardized. This assay was intended to combine the easy-to-handle aspect of (a) and biologically interesting aspects of (b). The purpose of the data analysis presented here was to investigate the influence of an extreme dilution of gibberellic acid on wheat stalk length and to determine the influence of external factors on the experimental outcome.**

Methods: **Grains of winter wheat (*Triticum aestivum*, Capo variety) were observed under the influence of extremely diluted gibberellic acid (10^{-30}) prepared by stepwise dilution and agitation according to a protocol derived from homeopathy ('G30x'). Analogously prepared water was used for control ('W30x'). 16 experiments including 8000 + 8000 grains were performed by 9 researchers.**

Results: **Experiments that were performed between January and April showed inconsistent results, whereas most of the experiments performed between September and December showed *shorter* stalks in the G30x group. This was confirmed by correlation analysis ($p < 0.01$). Thus winter/spring experiments and autumn experiments were analysed separately. When all 10 autumn experiments were pooled, mean stalk lengths (mm) were 48.3 ± 21.4 for the verum group and 52.1 ± 20.4 for control (mean ± SD) at grain level (N = 5000 per group) and ±5.3 and ±5.1 respectively at dish level. In other words, verum stalk length (92.67%) was 7.33% *smaller* than control stalk length (100%). The effect size is small when calculation is done on the basis of grains (d = 0.18) but, due to the smaller SD at dish level, medium when done on the basis of dishes (d = 0.73). The inhibiting effect was observed by 6 of the 6 researchers who performed the autumn experiments.**

Conclusion: **The model may be useful for further research as there exists a theoretical justification due to previous studies with wheat and extremely diluted silver nitrate, as well as to previous studies with amphibians and diluted hormones, and its methods are well standardized. Data confirm the hypothesis that information can be stored in the test liquid, even at a dilution of the original substance beyond Avogadro's value; and that the wheat bio-assay is sensitive to such information.** *Homeopathy* (2015) **104**, 257–262.

Keywords: Wheat; Gibberellic acid; Ultra high dilution; Homeopathy

Introduction

Bio-assays on wheat stalk growth have been used in studies on homeopathy since the 1920s, originally with homeopathically prepared metal salts.[1] Following the authors' studies (a) on wheat seedlings and ultra high diluted silver nitrate,[1] and (b) on amphibians and an ultra high diluted hormone,[2] (c) a bio-assay on wheat and extremely diluted gibberellic acid was standardized. This

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assay was intended to combine the easy-to-handle aspect of (a) and biologically interesting aspects of (b).

However, plant studies may cause special challenges with regard to the interpretation of their results.[3] Betti *et al.*[4] and Brizzi *et al.*[5] reported a *stimulation* of wheat growth through treatment of the seeds with high potencies of arsenic. On replicating the experiment however, Binder *et al.*[6] found a significant *decrease* in longitudinal growth. It is interesting to note that in these cases, data were usually found to be homogeneous within groups.[7] Homeopathically prepared gibberellic acid was first tested on barley stalk length, with different results according to seedlings' vigour levels.[8]

Thus, apart from avoiding false positive conclusions on the basis of mere random outcomes, careful research into the determinants of contradictory effects is needed. Furthermore, the idea was raised that calculation on the basis of absolute differences between means of verum and control group may be a useful statistical tool complementing calculation of means alone.[3]

For the author's project, the use of ultra high diluted gibberellic acid has been inspired by botanical studies of Baumgartner *et al.*[9–11] and an inter-researcher think-tank.

The purpose of the data analysis presented here was to investigate the influence of an extreme dilution of gibberellic acid (10^{-30}, '30×') on wheat stalk length and to determine the influence of external factors on the experimental outcome.

Methods

In preparing the documentation of the experiments, the recommendations for good fundamental research documentation in homeopathy were observed, which were elaborated by the K and V Carstens Foundation, Essen.[12]

Plants

Experiments were performed on wheat (*Triticum aestivum*, Capo variety) grain grown without herbicides or pesticides. As a rule, a new batch of grains was harvested in August of each year and used for the experiments (Table 1). Around 10% of the grains were ruptured and around 10% were distorted, and these were all removed prior to the experiment.

Researchers, seasons and sites (inter-researcher control)

Experiments were performed between 2007 and 2012 by different researchers, at different locations and at different times of the year (Table 1, for further details see[13–15]).

Laboratory workers received thorough training in the methods and procedures to be used by WS-P.[1] They had no contact with each other while experiments were in progress.

Laboratory conditions

All glass bottles and fastenings were disposable products; dishes, covering glass vessels and glass pipettes for administration of the probes were heat sterilized and were (additionally) rinsed twice with double distilled water prior to treatment. Plastic pipettes used for the dilution process were disposable products. Seedling development took place in complete darkness at a temperature of 21.5 ± 1°C regulated by central heating, depending on the laboratory. Temperatures were homogeneous for all dishes in one and the same experiment. The experimental setup was explicitly meant to be 'low threshold' to maintain its easy-to-handle aspect.

Table 1 Experimental details. Experiment = experiment number referring to the sequence between January and December; researcher = Thomas Reischl, Karin Thieves, Andrea Pfleger, Wolfgang Matzer, Maria Hartmann, Waltraud Scherer-Pongratz, Sonja Hribar, Jürgen Hofäcker, Christian Reich; site = location of the experimental site, Weiz (southern Austria), Geilenkirchen (Germany), St. Johann (northern Austria); year = 2007–2011; month = Jan–Dec; age = age of the grains (years); acetone = use of acetone for preparation of the stock solution

Experiment	*Researcher*	*Site*	*Year*	*Month*	*Age*	*Acetone*
1	Reischl	Weiz	2009	1	1.5	Yes
2	Thieves	Gels	2009	1	0.5	Yes
3	Thieves	Gels	2009	1	0.5	Yes
4	Pfleger	Jo	2009	2	0.5	No
5	Matzer	Weiz	2010	2	0.5	No
6	Pfleger	Jo	2008	4	0.5	Yes
7	Hartmann	Weiz	2009	9	0	No
8	Pfleger	Weiz	2007	10	0	Yes
9	Pfleger	Weiz	2007	10	0	Yes
10	Scherer	Weiz	2009	10	0	No
11	Hribar	Weiz	2011	10	0	Yes
12	Hofäcker	Weiz	2007	11	0	Yes
13	Hofäcker	Weiz	2007	12	0	Yes
14	Reich	Weiz	2008	12	0	Yes
15	Scherer	Weiz	2009	12	0	No
16	Scherer	Weiz	2009	12	0	No

Preparation of test solutions

The test substance and control were prepared inspired by Baumgartner[9] according to the method of stepwise dilution and succussion as derived from homeopathy. The degree of dilution was set to 10^{-30} in order to exceed Avogadro's limit of theoretical 0-molarity (10^{-24}). Botanic hormone 10^{-30} (30×) was chosen with regard to previous experiments with a zoological hormone 30×.[2] Grains were observed under the influence of gibberellic acid 30×, or of analogously prepared water control (30×), respectively. Different sets of test substance and control, respectively, were prepared by different researchers (see Table 1).

For preparation of the test dilutions, 0.017 g of gibberellic acid (Sigma–Aldrich company, art. nr. 36575) were added to 9 ml of either of two possible solvents of gibberellic acid: acetone or double distilled water (see Table 1) and the liquid was gently swung (not 'agitated') for one minute (='mother substance, 1×'). Then, using a disposable pipette (Brand company, Transferpette), 1 ml of the mother substance was added to 9 ml of double distilled water in a 20 ml brown glass bottle (Heiland company, art. nr. 380020) and the product was agitated vigorously according to a standardized protocol: the vial was manually banged

30 times against an elastic surface at intervals of approximately 0.5 s to create mechanical shocks (=gibberellic acid 2× or 'G2x'). In a total of 30 steps of dilution 1:10 and 29 steps of agitation (as agitation was omitted at the first dilution step), the test substance 'G30x' was thus prepared. Preparation details differed from the homeopathic pharmacopoeia due to laboratory convenience. Starting from the 28th step, quantities larger than 1 ml were added to the tenfold amount of double distilled water in order to prepare a sufficient quantity of test substance. Larger brown glass bottles (source: different pharmacies depending on the researcher) (each of which was filled ½ with the liquid) were used for these last steps (29×: 250 ml, 30×: 500 ml). A new glass bottle was used at each step of dilution.

Analogously prepared solvent was used for control (water 30×) to ensure that possibly solute contents of the glass wall were equally present both in verum 30× and control 30× and thus their possible effect was ruled out, and that the content of solute oxygen was alike. If a difference in growth occurred between seedlings treated with verum and control, it should then be due to the presence or absence of gibberellic acid in the mother substance.

System performance controls

Experiments have shown that differential treatment with water 30× or with water that has not undergone any preparation process at all (W0, negative control) produces no differences in stalk length measured after one week (water 30×: 49.7 ± 21.6 mm; W0: 49.9 ± 21.2 mm). In 4 experiments performed by WS-P, total N of grains per group was 2000, and temperature was 21.5 ± 1°C.

By way of a positive system control it has been observed that after one week stalk lengths are greater under treatment with gibberellic acid at molecular doses (10^{-4}: 53.8 ± 22.1 mm; 10^{-6}: 46.9 ± 22.5 mm) than in water control (44.8 ± 22.6 mm) (WS-P, N of grains per group = 200, temperature 20 ± 1°C).

Analyses of water control in analogous experiments in the past[13,14] with the same spatial arrangement of dishes and plants have shown a high degree of homogeneity within dishes of one and the same group ($p > 0.05$, i.e. there are no significant differences *within* the group). Homogeneity is also investigated in the present study.

Independent probe coding

Control and verum were encoded by further independent persons. All probes were applied blindly, codes were broken only after the data had been calculated.

Data base

Sixteen experiments were carried out including 500 + 500 grains each. Depending on the researcher, 20 or 25 grains (see[13–15]) were put into one dish, i.e. a total of 8000 grains were observed per treatment group G30x and 8000 per treatment group W30x.

Placement of grains

The grains were put into glass dishes (diameter 11 cm) (i.e. lids of preserving jars), each containing 2 layers of filter paper (Whatman, cellulose, 90 mm, sort 2), with the germination furrow facing down (Figure 1).

Exposition to probes

Five millilitre of the verum or control probe were added to each dish with the help of a disposable 5 ml pipette and pipetting ball (VWR company, art. no. 612-1328 and 612-1947). Dishes were then covered with 1000 ml glass beakers (i.e. the preserving jars) and dishes and covers were wrapped in aluminium foil (Figure 2).

They were placed in alternating rows according to the procedure of stratified randomization. Grains had not been soaked prior to treatment.

Observed development (endpoints)

Stalk length (Figure 3) was observed after 7 days according to standard protocol.[1] Stalks were cut off and their length was measured on a mm scale. The person performing the measurements knew neither whether the stalks measured were verum or control treated (see blinding procedure above) nor what their blind code (A or B) was. Any possibility of an assignment bias was thus ruled out. Subsets were harvested in the same sequence as they had been planted. Measurement of endpoints was done blindly.

Data evaluation

For description of stalk length, at the level of the 16 individual experiments, always referring to the number of seedlings that have actually germinated ('living seedlings'), the statistical mean was used, and lengths were compared by one way analysis of variance. SD of the mean was calculated. Mean and SD were also calculated by dish, i.e. for each cohort of 20 or 25 grains. In order to avoid false negative results, analysis of variance was not calculated at dish level, and to avoid false positive results, it was also not calculated for the pooled experiments.

Figure 1 Example for placement of grains. From Ref. 12.

Figure 2 Example for placement of beakers. From Ref. 12.

Figure 3 Example of stalk growth. From Ref. 11.

For the pooled experiments, however, the effect size (Cohen's d, standardized difference of means = absolute difference between means of verum and control group, divided by SD) was calculated. An effect size >0.2 is regarded as small, >0.5 as medium and >0.8 as large.

Homogeneities of stalk lengths within the verum group and within the control group, respectively, were investigated by one way analyses of variance with *post-hoc* pairwise comparisons by means of Tukey HSD test.

Interaction between 'month' and 'result' was calculated by correlation analysis.

Results at the level of single experiments were represented graphically by zeroing the results of the W30x control groups and plotting the difference to the G30x groups on the ordinate.

Results for G30x were also expressed as *percent* of W30x normalized to 100%.

Results

Figure 4 shows the differences between the mean stalk length of G30x and W30x seedlings (each of 16 experiments comprising 500 + 500 grains).

As can be seen, experiments 1−6, which were performed between January and April, showed inconsistent results, whereas most of the experiments 7−16, performed between September and December, showed shorter stalks in the G30x group. This was confirmed by correlation analysis ($p < 0.01$) (Figure 5).

Thus winter/spring experiments and autumn experiments were analysed separately.

When all winter/spring experiments (1−6) were pooled, mean stalk lengths (mm) were 54.6 ± 16.4 for the verum group and 52.7 ± 14.4 for control at grain level ($N = 3000$ germinated seedlings per group) and ± 4.9 and ± 3.6 respectively at dish level, i.e. overall verum stalk length (103.64%) was 3.64% *greater* than control stalk length (100%). The effect size is small both when calculation is done on the basis of grains ($d = 0.13$) and on the basis of dishes ($d = 0.45$).

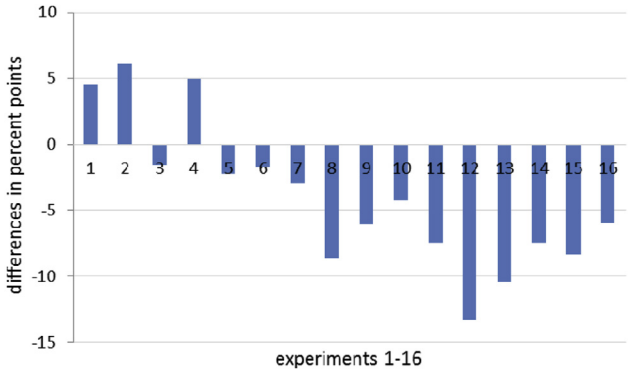

Figure 4 Differences in stalk length between W30x groups (zeroed) and G30x groups (ordinate). Abscissa: experiments 1−16.

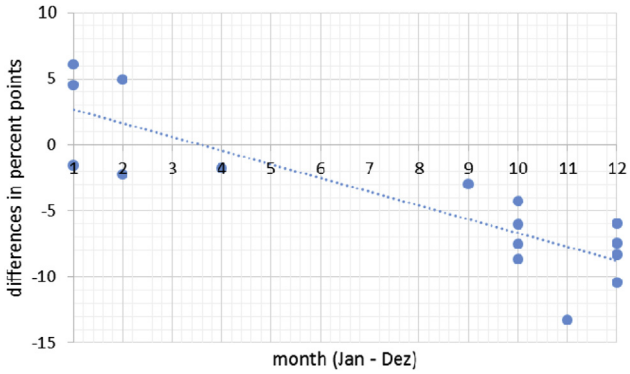

Figure 5 Differences in stalk length between W30x groups (zeroed) and G30x groups (ordinate). Abscissa: time (Jan−Dec).

When all the autumn experiments (7−16) were pooled, mean stalk lengths (mm) were 48.3 ± 21.4 for the verum group and 52.1 ± 20.4 for control (mean ± SD) at grain level (N = 5000 per group) and ±5.3 and ±5.1 respectively at dish level. In other words, verum stalk length (92.67%) was 7.33% *smaller* than control stalk length (100%). The effect size is small when calculation is done on the basis of grains (d = 0.18) but, due to the smaller SD at dish level, medium when done on the basis of dishes (d = 0.73). The stimulating effect was observed by 6 of the 6 researchers: with $p < 0.01$ by Pfleger, Hribar, Hofäcker and Scherer and as a trend ($p > 0.05$) by Hartmann.

These results suggest that in the experiments performed in autumn, there was a growth inhibiting influence of gibberellic acid 30×. In contrast, no clear effect was found in experiments performed in winter/spring. This pointed to autumn as a promising season for obtaining significant effects in 2007−2010[13,14] an assumption which was confirmed 2012[15] and by the overall analysis.

As a rule, data were found to be homogeneous *within* the control groups of the single experiments ($p > 0.05$) as well as *within* the verum groups ($p > 0.05$). In other words, there are significant differences between the average stalk lengths *between* the groups (verum or control, see above), but no significant differences *within* the groups. This holds true both for the experiments performed in autumn and in winter/spring.

Discussion

First results from experiments performed in autumn 2007 had suggested an *inhibition* of stalk growth by gibberellic acid 30×.[13] Further experiments then led to the idea that gibberellic acid 30× causes *inhibition* of growth *in autumn season* only, whereas in winter it causes a small stimulation of growth.[14] Again in 2012, further experiments confirmed inhibition in autumn.[15] To investigate the hypothesis of seasonal dependency, all data (2007−2012) were analysed.

All of the 10 *autumn* experiments showed *less* stalk growth in the G30x group (statistically significant with $p < 0.01$ in 7 cases, with $p < 0.05$ in 1 case, trend in 2 cases).

In contrast, *more* stalk growth or no clear effect was found in experiments performed in winter/spring (more growth with $p < 0.01$ in 3 cases, less growth with $p < 0.01$ in one case, sub significant trend in 2 cases).

The working hypothesis, derived from,[3,13] was that the time of season (autumn versus winter/spring) is a crucial factor in predicting the effect of homeopathically prepared gibberellic acid (G30x). This hypothesis, reflected in the arrangement of Table 1, appears to have been confirmed by the present analysis. Other parameters such as the researcher and the person preparing the dilutions involved,[14] the year the experiment was carried out, the presence or absence of acetone in the mother substance 1× did not seem to play a role with regard to the outcome. However, the following factors may also play a key role: The age of the grains (a few weeks in the autumn experiments, as opposed to 0.5−1.5 years in the winter/spring experiments, with differences possibly attributable to growth inhibition by G30x in fresh seeds), as well as the laboratory (Weiz in southern Austria versus Sankt Johann in northern Austria and Geilenkirchen in Germany). These factors, as well as a possible influence of slight temperature differences between the experiments, require further investigation.

Generally, results seem to be in line with[4−7,9] in as far as (small) changes in the setup can lead to significant changes in the experimental result, even in outcomes pointing to opposite directions.

The model may be useful for further research as there exists a theoretical justification due to previous studies with wheat,[1] as well as with potentized plant hormones,[8,9,16] its methods are well standardized. A weakness is that homeopathic studies on plants sometimes yield contradictory results, e.g. stimulation of growth in one and inhibition of growth in another laboratory, both findings being homogeneous and statistically significant within themselves.[17] One of the tasks of fundamental homeopathy research − and thus further repetition of this study − must be to better define the conditions (methodological, seasonal, geographic) which produce such consistent, yet contradictory results.

It may here be referred to a project on amphibian metamorphosis under the influence of dilutions of thyroxin.[2] When in experiments special highland amphibians were used, effects of extremely diluted agitated probes added to the basin water (30×, decrease of metamorphosis speed) were found. In contrast, animals from lowland biotopes obviously did not react to thyroxin 30×. The project helped to highlight pitfalls and challenges in high dilution research.

Data further confirm the hypothesis that information can be stored in the test liquid,[18] even at a dilution of the original substance beyond Avogadro's value; and that the wheat bio-assay is sensitive to such information. It also was established that outcomes to this effect are best obtained in the autumn season, i.e. that experiments should be performed in autumn season. Further research work to explore time-related dependencies could include other seasons of the year.

In order to optimize the experimental setup and facilitate its manageability, further experiments on wheat and gibberellic acid were performed, namely on one week stalk growth of seedlings that have previously been treated by molecular doses of silver nitrate[15] and on one day germination.[19,20] Results, however, suggest to follow the main line of experiments as described in[13,14] and in this paper.

References

1 Pongratz W, Endler P. Reappraisal of a classical botanical experiment in ultra high dilution research. In: Endler PC, Schulte J (eds). *Ultra High Dilution: Physiology and Physics*. Dordrecht: Kluwer Academic Publishers, 1994, pp 121−128.

2 Endler PC, Scherer-Pongratz W, Harrer B, Lingg G, Lothaller H. Amphibians and ultra high diluted thyroxine − further experiments and re-analysis of data. *Homeopathy* 2015; **104**: 250−256.

3 Endler P, Pfleger A, Thieves K, Reischl T, Reich C. Proposal for a comparison on relative differences in fundamental botanical homeopathy research (Abstract). *Eur J Integr Med* 2009; **1**: 246.
4 Betti L, Brizzi M, Nani D, Peruzzi M. Effect of high dilutions of Arsenicum album on wheat seedlings from seed poisoned with the same substance. *Br Homeopath J* 1997; **86**: 86–89.
5 Brizzi M, Nani D, Peruzzi M, Betti L. Statistical analysis of the effect of high dilutions of arsenic in a large dataset from a wheat germination model. *Br Homeopath J* 2000; **89**: 63–67.
6 Binder M, Baumgartner S, Thurneysen A. The effects of a 45x potency of arsenicum album on wheat seedling growth – a reproduction trial. *Res Complement Med – Forsch Komplementärmed* 2005; **12**: 284–291.
7 Nani D, Brizzi M, Lazzarato L, Betti L. The role of variability in evaluating ultra high dilution effects: considerations based on plant model experiments. *Res Complement Med – Forsch Komplementärmed* 2007; **14**(5): 301–305.
8 Hamman B, Koning G, Him Lok K. Homeopathically prepared gibberellic acid and barley seed germination. *Homeopathy* 2003; **92**: 140–144.
9 Baumgartner S, Thurneysen A, Heusser P. Growth stimulation of dwarf peas (*Pisum sativum* L.) through homeopathic potencies of plant growth substances. *Res Complement Med – Forsch Komplementärmed* 2004; **11**: 281–292.
10 Majewsky V, Scherr C, Arlt SP, *et al*. Reproducibility of effects of homeopathically potentised gibberellic acid on the growth of *Lemna gibba* L. in a randomized and blindes bioassay. *Homeopathy* 2014; **103**: 113–126.
11 Majewsky V, Arlt S, Shah D, *et al*. Use of homeopathic preparations in experimental studies with healthy plants. *Homeopathy* 2009; **98**: 228–243.
12 Stock-Schröer B, Albrecht H, Betti L, *et al*. Reporting experiments in homeopathic basic research (REHBaR) – a detailed guideline for authors. *Homeopathy* 2009; **98**: 287–298.
13 Pfleger A, Hofäcker J, Scherer-Pongratz W, Lothaller H, Reich C, Endler P. The effect of extremely diluted agitated gibberellic acid (10e-30) on wheat seedling development – a two researcher study. *Complement Ther Med* 2011; **19**: 164–169.
14 Endler P, Matzer W, Reich C, *et al*. Seasonal variation of the effect of extremely diluted agitated gibberellic acid (10e-30) on wheat stalk growth – a multi researcher study. *Sci World J* 2011; **11**: 1667–1678.
15 Hribar-Marko S, Graunke H, Scherer-Pongratz W, Lothaller H, Endler P. Prestimulation of wheat seedling development with gibberellic acid (10e-3, 10e-4 and 10e-5) followed by application of an agitated high dilution (10e-30) of the same hormone. *IJHDR* 2013; **12**(42): 26–39.
16 Jäger T, Scherr C, Shah D, *et al*. The use of plant-based bioassays in homeopathic basic research. *Homeopathy* 2015; **104**: 277–282.
17 Endler P, Thieves K, Reich R, *et al*. Repetitions of fundamental research models for homeopathically prepared dilutions beyond 10e-23: a bibliometric study. *Homeopathy* 2010; **2010**(99): 25–36.
18 Schulte J, Endler PC. Update on preliminary elements of a theory of ultra high dilutions. *Homeopathy* 2015; **104**: 337–342.
19 Hartung H, Schiestl S, Matzer W, Endler P. Wheat germination (20 hrs) and extremely diluted gibberellic acid (10e-30). Explorative experiments on a fundamental homoeopathy research model (Abstract). *Eur J Integr Med* 2010; **2**: 224–245.
20 Kiefer P, Matzer W, Schiestl S, *et al*. Wheat germination and extremely diluted agitated gibberellic acid (10e-30) – a multi researcher study. *Int J High Dilution Res* 2012; **11**(39): 45–59.