Homeopathy (2015) 104, 257–262

http://dx.doi.org/10.1016/j.homp.2015.09.007, available online at http://www.sciencedirect.com

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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Received 24 February 2015; revised 18 August 2015; accepted 25 September 2015

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