

## ORIGINAL PAPER

# Effects of homeopathic arsenic on tobacco plant resistance to tobacco mosaic virus. Theoretical suggestions about system variability, based on a large experimental data set

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**Context:** This research aimed at verifying the efficacy of homeopathic treatments by plant-based bioassays, which may be suitable for basic research, because they lack placebo effects and provide large datasets for statistical analyses.

**Objective:** To evaluate the effects of homeopathic treatments of arsenic trioxide (As<sub>2</sub>O<sub>3</sub>) on tobacco plants subjected to tobacco mosaic virus (TMV) inoculation as biotic stress.

**Design:** Blind, randomized experiment using tobacco leaf disks.

**Materials and methods:** Tobacco plants (*Nicotiana tabacum* L. cultivar Samsun) carrying the TMV resistance gene N. TMV inoculated leaf disks were floated for 3 days in the following:

- Distilled water (control)
- H<sub>2</sub>O 5 and 45 decimal and centesimal potencies
- As<sub>2</sub>O<sub>3</sub> 5 and 45 decimal and centesimal potencies

The main outcome measures is the number of hypersensitive lesions observed in a leaf disk.

**Results:** Homeopathic treatments of arsenic induce two effects on the plant: (i) increased resistance to TMV; (ii) decrease variability between experiments (system variability).

**Conclusions:** In this experimental model two actions of homeopathic treatment were detected: decrease in system variability and enhancement of the natural tendency of the system towards an 'equilibrium point'. *Homeopathy* (2003) 92, 195–202.

**Keywords:** homeopathy; tobacco plants; virus infection; arsenic; resistance; system variability

## Introduction

The efficacy of homeopathy, despite many years of experiment, is still controversial.<sup>1,2</sup> Most criticism concerns

whether treatments, in particular ultra-high dilutions (where the concentration of the original substance is beyond the Avogadro limit) have any real biological effect. Moreover, there is no complete theory to provide a plausible explanation for the action of homeopathic potencies,<sup>3</sup> so it is very difficult to understand the irreproducibility of the results often observed.<sup>4–6</sup> This lack of reproducibility represents a crucial difficulty in testing homeopathy and has stimulated explanations of homeopathic treatment effects using complexity theory.<sup>7</sup>

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Apart from efforts to find an interpretation of such 'unpredictable' effects of homeopathic treatment more experimental evidence should be obtained, by means of very strict scientific approaches to avoid objections concerning methodology. Basic research could provide a suitable tool because it uses relatively simple model systems, which enable a more direct effect/treatment relationship. Basic research also provides the large data samples needed for structured statistical analyses. In particular plant-based bioassays appear suitable for this kind of experimentation, as they overcome the main disadvantages of clinical trials (such as placebo effect, ethical difficulties, consumption of time, low number of replications, high costs). They rely on an appropriate, very cheap and nearly inexhaustible source of biological material.<sup>8</sup> Many papers on this topic have been published, are sometimes weak in methodology, accuracy and statistics.<sup>9</sup> Most experimental models were based on artificially diseased plants in order to better evaluate the effect of homeopathic treatments: the preliminary stress could be either abiotic, eg heavy metals, or biotic, eg fungal and viral pathogens.<sup>10–15</sup>

The present work is aimed at evaluating the effects of homeopathic arsenic trioxide ( $\text{As}_2\text{O}_3$ ) treatment on a well-known 'sensor' system: tobacco plants subjected to tobacco mosaic virus (TMV) inoculation as biotic stress. The 'remedy' was selected, according to the well-known *Principle of similarity*, on the basis of the hypersensitive-like reaction (necrotic spots) induced by  $\text{As}_2\text{O}_3$  in phytotoxic concentration on tobacco leaves (Betti *et al.*, unpublished data): these lesions resemble those provoked by TMV during a hypersensitive response (HR). HR is a typical plant defence mechanism, it occurs in resistant plants in response to different stresses. It is defined as a rapid localised necrosis of cells at the infection site and is associated with restricted pathogen multiplication and spread.<sup>16</sup> The higher the host resistance the quicker the defence response, expressed by fewer and smaller necrotic lesions.<sup>17</sup> Several lines of evidence suggest that HR cell death is a form of programmed cell death (PCD) in plants and recently striking homologies have been detected between plant and animal PCD.<sup>18,19</sup> The defence responses of pathogen-invaded plants occur within a few minutes and are followed by rapid and transient changes in the ion fluxes across the plasma membrane, accumulation of reactive oxygen intermediates (ROI), referred to as oxidative burst, and phosphorylation of various proteins associated with intracellular signal transduction mechanisms.<sup>20,21</sup> A rapid generation of ROI has been demonstrated in response to certain chemical treatments which induce resistance<sup>22–24</sup> and to inorganic arsenic species.<sup>25,26</sup>

## Material and methods

### Plants and pathogen

*Nicotiana tabacum* L. cultivar Samsun, carrying the TMV resistance gene N,<sup>27</sup> was used for all the experiments.

Plants were grown in a glasshouse under controlled conditions (16 h/8 h day/night cycle at  $25 \pm 1^\circ\text{C}$ ,  $1.87 \text{ W m}^{-2}$  intensity light with fluorescent tubes Osram luminux 58 W/ col. 84 and 75–80% RH) up to the vegetative stage when approximately nine leaves were present (c. 2 months old).

The highly purified TMV-type strain suspension used for virus inoculation was obtained, as previously described, from systemically infected leaves of *N. tabacum* cultivar Samsun nn susceptible to TMV.<sup>28,29</sup>

### Homeopathic treatments

The treatments used during the experiments were as follows:

- Merck distilled water (C, control)
- $\text{H}_2\text{O}$  DH 5 and DH 45 (W 5x, W 45x)
- $\text{H}_2\text{O}$  CH 5 and CH 45 (W 5c, W 45c)
- $\text{As}_2\text{O}_3$  DH 5 and DH 45 (As 5x, As 45x)
- $\text{As}_2\text{O}_3$  CH 5 and CH 45 (As 5c, As 45c)

Decimal and centesimal potencies were obtained by serial dilutions with distilled water (Merck) and succussions, starting from a mother tincture of 1 mM  $\text{As}_2\text{O}_3$  (Aldrich). The dynamization was made with a specially designed succussion machine which vertically shakes 100 ml volumes (in polyethylene bottles filled to 90%) at 70 times  $\text{min}^{-1}$  with an oscillation amplitude of 24 cm. Each potency was succussed for 1 min. The potentized water was prepared by exactly the same method of serial dilution and succussion, the only difference being the absence of arsenic in the starting solution. All these preparations were made at the same time and stored in dark at  $4^\circ\text{C}$ .

### Experimental set-up

The third and fourth mature leaves (counting from the top) of each plant were inoculated with 200  $\mu\text{l}$  per leaf of TMV suspension (20  $\mu\text{g ml}^{-1}$  in 10 mM phosphate buffer pH 7.0), using carborundum 400 mesh size (0.1  $\text{mg ml}^{-1}$ ) as an abrasive. Ten disks (20 mm in diameter) were cut from each inoculated leaf with a cork borer and were floated, according to a randomized scheme, in Petri dishes, each containing 15 ml of treatment: either distilled water (control), or decimal/centesimal potencies of  $\text{As}_2\text{O}_3$  or  $\text{H}_2\text{O}$ . In each experiment the randomized scheme was implemented as follows: the 10 disks punched out from one of the two leaves/plant were arranged one by one in 10 Petri dishes containing the same treatment; the same procedure was repeated for a total of nine leaves (by turns the third or the fourth from the top) from nine different plants. In this way 90 disks per treatment were obtained. The working variable was the number of hypersensitive lesions per disk evaluated, through a semi-automated counting method, 3 days after virus inoculation. All experiments in the present study were performed using a blind protocol and were repeated at least three times.

### Semi-automated counting method

The digital images of leaf disks were recorded by scanning the inoculated side of the disks using a flatbed scanner (Hewlett-Packard Scanjet 5470c) at a resolution of 600 dots per inch (dpi); the images were saved for subsequent counting as bitmap files. Suitable software developed for the purpose allowed easy recognition of the lesions and counting. Preliminary trials were conducted to evaluate the accuracy of the counting method, results showed no significant differences between different operators. The counting of hypersensitive lesions was performed blind for all the experiments.

### Statistical analysis

Each treatment group involved 10 Petri dishes, each containing nine leaf disks; the disk is the elementary statistical unit. The working variable  $Y$  is the number of hypersensitive lesions observed in a leaf disk. First of all, some exploratory statistical indices were calculated: the mean  $M(Y)$ , the standard error  $SE(Y)$ , the median  $Me(Y)$ , the mean absolute deviation about the median  $MAD(Y)$ , the Pearson's index of skewness  $\gamma$ , as well as the tail average of the ten largest observations. Since the data are evidently skewed, we used a non-parametric approach for testing significance. The data of each experiment were compared using the Wilcoxon rank sum test, in which the null hypothesis is the equivalence of two median values. The test statistic involves all the sample observations; therefore, it may show significant results even if the sample medians are not obviously different (or vice versa). Observations of the two groups were paired using a criterion based on co-graduation. The results were then corrected by Bonferroni adjustment, to reduce the probability of false significance in repeated tests.<sup>30</sup> We then compared the overall samples (including all the experiments) of each treatment with control; since these samples were large enough to justify a parametric approach, the comparisons were performed by Student's  $t$ -test.

The variability of  $Y$ , computed for each treatment group, was split into two components: the variability *within* experiment and *between* experiments; the relative weight  $\eta$  of the latter component is an index of dependence on experiments. If we denote the number of experiments  $k$ , the  $j$ th observation of the  $i$ th experiment  $Y_{ij}$ , the number of data and the mean of  $Y$  the  $i$ th experiment  $n_i$  and  $M(Y_i)$ , respectively; the index  $\eta$  is defined as follows:

$$\eta = \sqrt{\frac{\sum_{i=1}^k [M(Y_i) - M(Y)]^2 n_i}{\sum_{i=1}^k \sum_{j=1}^{n_i} [Y_{ij} - M(Y)]^2}}$$

All the results were compared with an 'overall control mean' ( $\tilde{M}$ ), calculated from the whole set of 3900 data points collected in control groups in several analogous experiments (including those here presented) performed to test the experimental model reliability. We

denote  $\bar{x}_{ik}$  the average number of lesions corresponding to the  $i$ th treatment in the  $k$ th experiment, and with  $n_i$  the number of the experiments performed with the  $i$ th treatment. We then focused our attention on the differences

$$d_{ik} = |\bar{x}_{ik} - \tilde{M}|$$

calculating for each treatment group the mean difference from the overall control mean

$$M(d_i) = \frac{\sum_{k=1}^{n_i} d_{ik}}{n_i}$$

and the standard deviation

$$S(d_i) = \sqrt{\frac{\sum_{k=1}^{n_i} [d_{ik} - M(d_i)]^2}{n_i}}$$

Finally, the mean differences were compared by a one-sided Student's  $t$ -test and the set of values  $\bar{x}_{ik}$  has been plotted graphically.

## Results

Preliminary studies (data not shown) demonstrated that HR is dependent on the age and size of the TMV inoculated leaf: the upper (ie younger) leaves show a lower number of hypersensitive lesions (up to 50% less), that is a higher resistance level. The randomized scheme applied in our tests made it possible to average the intrinsic physiological differences between the plants and the leaves: the results obtained in several experiments, each consisting of control groups only, showed no statistically significant differences (within each experiment) in the number of hypersensitive lesions. The model we adopted therefore proved to be reliable: within an experiment the working variable can be considered as being mainly related to the treatment effect.

The results obtained in successive experiments with decimal and centesimal potencies of  $H_2O$  and  $As_2O_3$ , are reported in Tables 1 and 2, respectively. It can be seen that all the treatments gave significant differences *vs* control in the distribution of hypersensitive lesions, except W 45c (Table 2) not significant in experiment VI. The test results show different directions (increase or decrease of lesion number) depending on the experiment. A trend towards a decrease was observed for decimal potencies (greater number of minus than plus ones signs). It can also be seen that the test statistic for  $H_2O$  and  $As_2O_3$  treatments within each experiment always showed the same sign *vs* control (except for 45x potencies in experiment VI), both for decimal and centesimal potencies. Moreover, if we compare the variability within each experiment (in terms of MAD) of  $H_2O$  and  $As_2O_3$  treatment groups *vs* control, we observe that it is rather homogenous for decimal potencies, whereas it increases for centesimal ones (see the values of MAD in each row of Tables 1 and 2).

**Table 1** Effect of H<sub>2</sub>O (W) and As<sub>2</sub>O<sub>3</sub> (As) decimal potencies on the distribution of hypersensitive lesions and Wilcoxon test results

*	Control		W 5x			As 5x		
	Me(Y) [M(Y)]	MAD(Y)	Me(Y) [M(Y)]	P-value W vs C	MAD(Y)	Me(Y) [M(Y)]	P-value As vs C	MAD(Y)
I	64.5 [71.7]	43.8	75.5 [78.8]	<0.001(+)	44.5	77.5 [87.1]	<0.001(+)	53.9
III	117.0 [116.7]	49.9	106.5 [114.4]	<0.050(-)	47.5	75.0 [87.5]	<0.001(-)	41.1
V <sup>†</sup>	82.0 [81.1]	21.0	96.0 [91.6]	<0.001(+)	21.8	83.0 [84.3]	<0.050(+)	25.3
VII	72.0 [93.0]	49.6	63.0 [81.4]	<0.001(-)	50.3	54.0 [69.7]	<0.001(-)	44.5
VIII	98.0 [105.8]	47.7	66.5 [80.2]	<0.001(-)	44.1	80.0 [83.7]	<0.001(-)	40.7
*	Control		W 45x			As 45x		
	Me(Y) [M(Y)]	MAD(Y)	Me(Y) [M(Y)]	P-value W vs C	MAD(Y)	Me(Y) [M(Y)]	P-value As vs C	MAD(Y)
II	77.0 [76.6]	32.7	63.0 [68.7]	<0.001(-)	37.5	55.0 [58.6]	<0.001(-)	38.0
IV	110.5 [118.2]	47.1	57.5 [64.4]	<0.001(-)	35.1	70.5 [81.4]	<0.001(-)	42.3
VI	92.0 [93.3]	52.0	102.5 [108.8]	<0.001(+)	62.4	84.0 [85.1]	<0.001(-)	42.0

\* Roman numerals indicate the experiments in order of time; n=90 data per treatment and experiment, except <sup>†</sup> = 60 data; Me(Y) = median; M(Y) = mean; MAD(Y) = mean absolute deviation about the median; (+), (-) = direction of test statistic.

**Table 2** Effect of H<sub>2</sub>O (W) and As<sub>2</sub>O<sub>3</sub> (As) centesimal potencies on the distribution of hypersensitive lesions and Wilcoxon test results

*	Control		W 5c			As 5c		
	Me(Y) [M(Y)]	MAD(Y)	Me(Y) [M(Y)]	P-value W vs C	MAD(Y)	Me(Y) [M(Y)]	P-value As vs C	MAD(Y)
I	78.0 [98.2]	54.0	109.0 [107.6]	<0.001(+)	68.2	80.0 [106.3]	<0.020(+)	73.3
III	53.0 [61.8]	41.2	84.0 [90.8]	<0.001(+)	49.3	80.0 [83.6]	<0.001(+)	43.3
V	113.5 [118.0]	52.1	106.0 [112.7]	<0.020(-)	73.1	109.5 [109.8]	<0.020(-)	71.5
*	Control		W 45c			As 45c		
	Me(Y) [M(Y)]	MAD(Y)	Me(Y) [M(Y)]	P-value W vs C	MAD(Y)	Me(Y) [M(Y)]	P-value As vs C	MAD(Y)
II	70.0 [87.4]	54.6	104.0 [122.0]	<0.001(+)	83.9	96.0 [116.4]	<0.001(+)	72.7
IV	89.0 [95.2]	49.9	65.0 [76.2]	<0.001(-)	48.2	66.0 [87.2]	<0.001(-)	52.0
VI	125.0 [126.9]	55.0	120.5 [123.9]	n.s.	79.6	99.0 [100.2]	<0.001(-)	62.4

\* Roman numerals indicate the experiments in order of time; n=90 data per treatment and experiment; Me(Y) = median; M(Y) = mean; MAD(Y) = mean absolute deviation about the median; (+), (-) = direction of test statistic; n.s. = not significant.

The comparison between As<sub>2</sub>O<sub>3</sub> and H<sub>2</sub>O treatment groups, evaluated by Wilcoxon test, is reported in Table 3: the results are always significant with only one exception (5c in experiment V), demonstrating a greater effect of As<sub>2</sub>O<sub>3</sub> than H<sub>2</sub>O ones potencies.

The overall mean number of hypersensitive lesions for each treatment is reported in Table 4: H<sub>2</sub>O and As<sub>2</sub>O<sub>3</sub> decimal potencies induce a significant lesion number decrease vs the control (ie increased resistance), more evident for As<sub>2</sub>O<sub>3</sub> potencies (see

**Table 3** Comparison between As<sub>2</sub>O<sub>3</sub> (As) and H<sub>2</sub>O (W) treatment groups (decimal and centesimal potencies); statistical significance evaluated by Wilcoxon test

	*	M(Y)		Me(Y)		P-value As vs W
		W	As	W	As	
Decimal potencies						
5x	I	78.8	87.1	75.5	77.5	<0.001(+)
	III	114.4	87.5	106.5	75.0	<0.001(-)
	V <sup>†</sup>	91.6	84.3	96.0	83.0	<0.001(-)
	VII	81.4	69.7	63.0	54.0	<0.001(-)
	VIII	80.2	83.7	66.5	80.0	<0.001(+)
45x	II	68.7	58.6	63.0	55.0	<0.001(-)
	IV	64.4	81.4	57.5	70.5	<0.001(+)
	VI	108.8	85.1	102.5	84.0	<0.001(-)
Centesimal potencies						
5c	I	107.6	106.3	109.0	80.0	<0.050(-)
	III	90.8	83.6	84.0	80.0	<0.001(-)
	V	112.7	109.8	106.0	109.5	n.s.
45c	II	122.0	116.4	104.0	96.0	<0.010(-)
	IV	76.2	87.2	65.0	66.0	<0.001(+)
	VI	123.9	100.2	120.5	99.0	<0.001(-)

\*Roman numerals indicate the experiments in order of time; n=90 data per treatment and experiment, except <sup>†</sup>=60 data; M(Y)=mean; Me(Y)=median; (+), (-)=direction of test statistic; n.s.=not significant.

**Table 4** Overall mean of hypersensitive lesions for H<sub>2</sub>O (W) and As<sub>2</sub>O<sub>3</sub> (As) treatment groups (decimal and centesimal potencies), standard error and Student's t-test

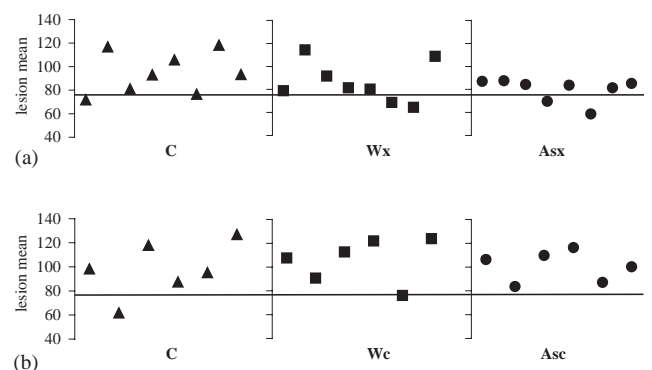
	Treatment group	n	M(Y)	SE(Y)	t-Statistic	P-value
Decimal potencies						
	Control	690	95.1	2.3	—	—
	W 5x	420	89.2	2.7	1.643	0.050
	W 45x	270	80.7	3.7	3.367	<0.001
	As 5x	420	82.4	2.6	3.575	<0.001
	As 45x	270	74.9	3.2	4.855	<0.001
Centesimal potencies						
	Control	540	97.9	3.0	—	—
	W 5c	270	103.7	4.7	-1.074	n.s.
	W 45c	270	107.4	5.4	-1.665	0.048
	As 5c	270	99.9	4.8	-0.369	n.s.
	As 45c	270	101.3	4.8	-0.621	n.s.

n=number of data per treatment; M(Y)=overall mean; SE(Y)=standard error; n.s.=not significant.

t-statistic); whereas centesimal potencies did not show significant results.

In Figure 1 the results obtained in successive experiments (reported in Tables 1 and 2) are plotted with respect to the overall control mean ( $\bar{M}$ , 76.08 (horizontal line)), calculated from about 3900 data points taken from control groups. It can be seen that the values obtained with As decimal potencies are closer to  $\bar{M}$  than in other groups: the oscillation is less marked than elsewhere. The statistics shown in Table 5 give numerical support to this evidence: in the As decimal potencies the average difference from  $\bar{M}$  shows the lowest value ( $P<0.001$  vs control) and the standard deviation is significantly reduced.

In Table 6 some exploratory statistics, referring to the overall samples of each treatment group, are reported. All the experimental samples are positively skewed, and the  $\gamma$  index of skewness has similar values, except for the As 45c sample, whose data are more symmetric than elsewhere. Some differences emerge



**Figure 1** Variation of results of different experiments about the overall control mean  $\bar{M}$  (horizontal line): a—control (C), H<sub>2</sub>O decimal potencies (W x), As<sub>2</sub>O<sub>3</sub> decimal potencies (As x); b—control (C), H<sub>2</sub>O centesimal potencies (W c), As<sub>2</sub>O<sub>3</sub> centesimal potencies (As c).

when analysing the ‘tail’: indeed the ‘tail average’ is considerably lower for all the decimal treatment groups, and particularly for As 45x, whereas no

**Table 5** Mean and standard deviation of the differences of each treatment group from the overall control mean  $\tilde{M}$ , compared by Student's *t*-test

	Treatment group (i)	$n_i$	$M(d_i)$	$S(d_i)$	t-Statistic	P-value
Decimal potencies	C (1)	8	19.6	15.3	—	—
	W (2)	8	14.7	12.7	-1.009	n.s.
	As (3)	8	9.6	3.6	-7.404	<0.001
Centesimal potencies	C (4)	6	26.6	29.0	—	—
	W (5)	6	29.5	31.4	0.203	n.s.
	As (6)	6	24.5	24.4	-0.192	n.s.

$n_i$ = number of experiments related to the *i*th treatment;  $M(d_i)$ = mean of the differences from  $\tilde{M}$ ;  $S(d_i)$ = standard deviation of the differences; n.s.=not significant.

**Table 6** Overall exploratory statistical analysis: skewness ( $\gamma$ ), tail average and dependence on experiments ( $\eta$ )

	Treatment group	$\gamma$	Tail average [control=100]	$\eta$ (%)
Decimal potencies	Control	0.826	281.9 [100]	28.1
	W 5x	0.610	244.8 [87]	41.1
	W 45x	0.864	240.9 [86]	33.3
	As 5x	0.823	241.5 [86]	19.4
	As 45x	0.779	214.2 [76]	22.4
Centesimal potencies	Control	0.778	299.4 [100]	30.5
	W 5c	0.585	294.7 [98]	11.8
	W 45c	0.687	321.2 [107]	25.1
	As 5c	0.654	284.0 [95]	14.8
	As 45c	0.325	300.4 [100]	15.2

relevant differences were observed in the centesimal treatment groups. Finally, the  $\eta$  index shows that the variability of *Y* between experiments is reduced in all treatment groups except in H<sub>2</sub>O decimal potencies.

## Discussion

Our experimental set-up, based on the leaf disk as elementary statistical unit, allows us to obtain a large data set and to average the physiological differences between individual plants, as verified in preliminary experiments consisting of control groups only. Therefore, we considered, within an experiment, the 90 leaf disks of each treatment group may represent a 'virtual' *standard* tobacco plant, whose hypersensitive response is mainly related to the treatment effect. Taking into account all the experiments for each treatment group, we define as *system* the *standard* plants as a whole. In the light of these assumptions, the data are analysed at two different levels.

As first level, we consider the action of the treatment on the hypersensitive response in terms of number of lesions, ie the increase or decrease of resistance vs control. All the treatments (both decimal and centesimal) induce on the *standard* plant a significant effect (either increase or decrease of resistance), confirming that both high (5x, 5c) and ultra-high (45x, 45c) potencies can provoke a biological action. The pattern of results, which at a first sight seems to be almost

random, could be explained as a trend of the *standard* plants towards an 'equilibrium point' (expressed by the overall control mean  $\tilde{M}$ , calculated on a very large set of data). This 'equilibrium point' would be due to an intrinsic constraint of the system Tobacco/TMV itself.<sup>31-34</sup> Decimal potencies seem to enhance the tendency towards this 'equilibrium point', significantly for arsenic treatments, which therefore seem to have an 'equilibrium-effect': when the control value is higher than the 'equilibrium point', the homeopathic treatment induces a decreased response, whereas, when the control value is lower than the 'equilibrium point', the treatment induces an increased response. The crucial problem of irreproducibility of homeopathic treatment effects, faced by many authors,<sup>4-6</sup> may be interpreted from this perspective. Considering the overall mean for each treatment, ie the effect on the *system*, only decimal potencies induce significantly increased resistance. These findings could indicate that decimal potencies are more suited to plants than centesimal potencies. Finally, the significant results obtained with potentized H<sub>2</sub>O treatments suggest, as previously reported,<sup>15</sup> that solvent potentization alone is able to induce effects similar (but weaker) than homeopathic arsenic. Potentization and dilution represent two essential sources of homeopathic activity: the original substance dissolved initially, despite being successively diluted beyond the Avogadro number, could give the particular quality of the homeopathic effect, as recently experimentally confirmed by Rey.<sup>35</sup>

As second level, we consider the action of the treatment on the variability of the hypersensitive response. Variability can be split into two parts: variability within experiment and between experiments. The variability within experiment for each treatment group (ie the variability in each *standard* plant) derives from various sources: different physiological state of individual plants and leaves, different micro-experimental conditions (such as inoculation pressure) and other factors unavoidably beyond experimental control. On the other hand, the variability between experiments for each treatment group (ie the variability of the *system*) includes not only the above-mentioned sources, but also the differences between the plant groups used in the successive experiments and seasonal differences. The treatments generally induce a decrease in *system* variability, as evidenced by the decrease of the index of dependence on experiments ( $\eta$ ). It is noteworthy that this decrease is induced also (and even more markedly) by centesimal potencies, which do not act at the first level (hypersensitive response in terms of lesion number). A similar decrease in system variability emerged from a 3000 data point collected in a parallel research performed on the same model and involving treatments with water conditioned by weak microwave radiations.<sup>36</sup> This finding could support the idea that electromagnetism and homeopathy are linked through a common natural mediator represented by water.

## Conclusions

The experimental model we developed seems to be particularly useful in evaluating the effects of homeopathic treatments, since it gives a large data set for statistical analysis without the disadvantages of clinical trials. Moreover, using a biotic stress (ie TMV infection) we are able to get close to a natural disease model, which could be suitable to represent a general response of living matter to homeopathic treatments (the main cell structures and functions being common to the majority of eukaryotes).

Summarizing the results, arsenic decimal potencies seem to act both on resistance and on its variability, whereas centesimal ones seem to affect only variability, which could be considered a higher level of complexity. Therefore, we suggest that decrease of system variability is one of the peculiar actions of homeopathy. This decrease in system variability (ie between different *standard* plants) does not exclude the possibility of an oscillatory effect, within an individual organism, due to the homeopathic treatment, as recently reported for clinical trials.<sup>7</sup> In fact, if we assume that the *standard* plant can be regarded as a virtual representation of an individual organism, the variability increase in the *standard* plant, observed in particular for centesimal potencies (which induce the

most marked decrease in *system* variability), is consistent with the increasing oscillatory effect reported by Hyland and Lewith.<sup>7</sup> Nevertheless, unlike Hyland and Lewith we took a 'snapshot' of the differences between treatment and control groups at a single time point, 3 days after inoculation.

Finally, we underline that these results are based on a large experimental data set and take into account not only the effect of the treatment on a well-determined parameter in a *standard* plant, but also the *system* behaviour: therefore they can support a phenomenological and non-reductionistic theory of homeopathy.

The polarity between the effects observed in a *standard* plant and in the *system*, regarded as a whole, recalls the polarity of water accepted in quantum physics as a free electric dipole laser,<sup>37</sup> where the apparent dualism particle/wave is solved by the fundamental equations of relativism.

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