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Method Article

A method for assessing the frequency of hormetic trade-offs in plants

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A B S T R A C T

Hormesis is a biphasic response to stress, involving low-dose stimulation and high-dose inhibition. Currently, attempts are being made to use the hormetic stimulation of plant parameters to increase resilience to severe stress. However, due to hormetic trade-offs, low-dose stress does not always cause synchronous improvement of different plant parameters. Some parameters do not change and even some even worsen compared to the control. Therefore, there is a need to evaluate the frequency of hormetic trade-offs. In this study, a method for estimating the probabilities of two types of hormetic trade-off in plants was developed. This method is intended solely to improve the scientific understanding of plant hormesis and a first step towards a more extensive evaluation of hormetic trade-offs in plants.

- The proposed method estimates the probabilities of hormetic trade-offs 1 and 2 in plants using simple calculations.
- This method can be applied to estimate the probability of hormetic trade-offs 1 and 2 both for a set of independent experiments and in a single experiment.
- This method could be used in the future to identify doses and factors that stimulate the greatest number of traits without worsening others.

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Name and reference of original method	*Not applicable*
Resource availability	*Not applicable*

Background

An evolutionary trade-off is a phenomenon when an evolutionary adaptation is accompanied by deterioration in some parameters that are presumably of less or no importance in the new environment [1]. Trade-off is revealed also for biological plasticity, including hormesis, and represents the stimulation of parameters/functions against the background of the suppression of others that are considered non-essential to prepare for coming threats [2]. Various authors have noted that low doses of stress can cause unsynchronized stimulation of plant parameters [3,4].

Previously, we identified 2 types of hormetic trade-off in plants [5]:

(1) At a particular dose of a factor, the parameter is statistically significantly worse than in the control. However, the same dose hormetically stimulates other plant parameters (hormetic trade-off 1).

(2) At a particular dose of a factor, the parameter does not differ significantly from the control. However, the same dose hormetically stimulates other plant parameters (hormetic trade-off 2). We suggested considering this case as a hormetic trade-off, since due to limited resources, the plant cannot hormetically stimulate all parameters/functions that may be relevant to increase resistance to a strong stressor. The plant must 'choose' the parameters to stimulate, which depends on features of the low-dose stressor and the plant's resources [5].

It should be noted that the same dose can cause trade-off 1 for one plant parameter and trade-off 2 for another parameter (Table 1). This does not contradict the definitions of trade-offs, because potentially possible stimulation is not realized for these parameters or even goes into inhibition.

We found that trade-off 2 is much more common for growth, photosynthesis and antioxidant defense indicators than the first variant. Apparently, this is due to the fact that trade-off 2 does not disrupt plant homeostasis, that is, it does not worsen the parameters/functions in plants [5].

When choosing plant parameters used to calculate the frequency of trade-offs, the following points should be taken into account because the physiological and biochemical responses are highly time-dose-dependent:

(1) Plant parameters should be sensitive to this type/dose of impact/impacts. For example, we evaluated the frequency of hormetic trade-offs for growth, photosynthesis and antioxidant defense indicators with different stresses (various pollutants and abiotic factors). These plant indicators respond non-specifically to any stress, as shown by the analysis of numerous published data. This allowed the combining of data on the effect of various stresses on growth into one sample, with a similar procedure being used for photosynthesis and antioxidant defense [5].

(2) Growth is an integral response of plants to stress and is closely related to plant productivity; therefore, it is necessary to evaluate trade-offs for growth indicators along with other plant parameters.

(3) The duration of exposure to the stress factor should be sufficient for hormetic responses in the studied plant parameters. If the study has several time points, then the time point with the greatest manifestation of hormesis in the studied indicators should be selected.

(4) The dose range used to analyze trade-offs should include both stimulating and inhibitory doses, since trade-offs often occur in the dose range at the border between stimulation and inhibition (Table 1) when resources for resistance to this stressor are no longer sufficient.

Table 1
An example of calculating the frequency of hormetic trade-offs in plants

Experiments	Photosynthetic pigments			Growth indicators			
1. Cd mg L^{-1} [6]	Ch a	Ch b		Shoot dry weight	Root dry weight		
1.0	**Stimulation**	**Stimulation**		No effect	**Stimulation**		
7.5	No effect	No effect		No effect	**Stimulation**		
15.0	**Inhibition**	**Inhibition**		**Inhibition**	**Inhibition**		
30.0	**Inhibition**	**Inhibition**		**Inhibition**	**Inhibition**		
Dose proportion causing trade-off 1	**0**	**0**		0	0		
Dose proportion causing trade-off 2	1/4 = 0.25	1/4 = 0.25		2/4 = 0.50	0		
Average proportion of trade-off 1	**0**			**0**			
Average proportion of trade-off 2	(0.25+0.25)/2 = **0.250**			(0.50+0)/2 = **0.250**			
2. W mg L^{-1} [7]	**Ch a**	**Ch b**	**Car**	**Total fresh weight**	**Total dry weight**	**Shoot length**	**Root length**
1	**Stimulation**	**Stimulation**	**Stimulation**	**Stimulation**	**Stimulation**	**Stimulation**	**Stimulation**
5	**Stimulation**	**Stimulation**	**Stimulation**	**Stimulation**	**Stimulation**	**Stimulation**	**Stimulation**
10	**Stimulation**	**Stimulation**	**Stimulation**	**Stimulation**	**Stimulation**	**Stimulation**	**Stimulation**
50	No effect	**Inhibition**	**Stimulation**	No effect	No effect	**Stimulation**	No effect
100	**Inhibition**	**Inhibition**	**Inhibition**	**Inhibition**	**Inhibition**	**Inhibition**	**Inhibition**
Proportion of doses causing trade-off 1	**0**	1/5=**0.20**	**0**	**0**	**0**	**0**	**0**
Proportion of doses causing trade-off 2	1/5 = **0.20**	**0**	**0**	1/5 = **0.20**	1/5 = **0.20**	**0**	1/5 = **0.20**
Average proportion doses causing trade-off 1	(0+0.20+0)/3 = **0.067**			**0**			
Average proportion doses causing trade-off 2	(0.20+0+0)/3 = **0.067**			(0.20+0.20+0+0.20)/4 = **0.150**			

Note: Stimulation is an improvement of the parameter relative to the control; inhibition is a deterioration of the parameter compared to the control. Grey indicates trade-off 1, and yellow indicates trade-off 2. The table shows the calculations only for two experiments [6,7]. Earlier, we performed this analysis using data extracted from 18 independent experiments [5].

Method details

This method can be used to estimate the frequencies of hormetic trade-offs in two cases:

(1) Data from several independent experiments.
(2) Data from a single experiment.

Estimation of the frequency of hormetic trade-offs using data from several independent experiments

Here, the calculation of the proportions of trade-offs 1 and 2 using as an example data on growth indicators and the content of photosynthetic pigments is presented step by step. These data were obtained by different authors for hormesis caused by various stress factors in plants (Table 1).

(1) Construct a table showing statistically significant changes (stimulation, inhibition or no effect) in growth and photosynthesis indicators compared to the control at all doses of the factor for each experiment (Table 1).
(2) Calculate the proportion of doses that cause trade-offs 1 and 2 for each plant parameter in all experiments (Table 1). The use of proportions instead of absolute values enables the different number of factor doses in different experiments to be taken into account. As trade-off 1, consider the situation when a given dose of the factor significantly worsens a parameter relative to the control and stimulates any of the other parameters. For example, tungsten (W) at a dose of 50 mg L^{-1} reduced the level of chlorophyll b compared to the control and stimulated shoot growth and carotenoid level, that is, this dose caused trade-off 1 (Table 1). One of the five doses caused this type of trade-off for the level of chlorophyll b; therefore, the proportion of doses with this effect for chlorophyll b is 1/5 or 0.20 (Table 1).

If this dose does not affect the parameter and significantly stimulates any of the other parameters, then consider this as trade-off 2. For example, cadmium (Cd) at dose of 7.5 mg L^{-1} did not affect the level of chlorophyll a and increased the root dry weight relative to the control (Table 1). This is a manifestation of trade-off 2. For example, one of the four doses caused this type of trade-off for the level of chlorophyll a; therefore, the proportion of doses with this effect for chlorophyll a is 1/4 or 0.25 (Table 1).

In the first example of Table 1, cadmium did not induce trade-off 1 (grey indicates trade-off 1, and yellow indicates trade-off 2 in Table 1); therefore, the proportion of such doses was zero for pigments and growth indicators. At the same time, the proportion of cadmium doses causing trade-off 2 was 1/4 and 2/4 for chlorophylls and shoot dry weight, respectively. That is, 1 of 4 doses or 2 of 4 doses caused trade-off 2 (Table 1).

In the second example of Table 1, only tungsten (W) at a dose of 50 mg L^{-1} induced trade-off 1 for chlorophyll b (marked in gray in Table 1). Hence, the proportion of doses causing trade-off 1 was 1/5 (one dose of 5). For other indicators, this proportion was zero (Table 1). At the same time, the proportion of tungsten doses causing trade-off 2 was 1/5 (one dose of 5) for chlorophyll a, root length, total fresh and dry weight and was zero for the remaining indicators.

(3) To perform statistical analysis, combine the proportions of trade-off 1 for all pigments into one sample, and the proportions of trade-off 2 for all pigments into another sample. Perform the same procedure for growth.

It is also possible to use the average proportions of trade-offs 1 and 2 for pigments and growth for statistical analysis as shown in Table 1. For example, the average proportion of trade-off 2 induced by cadmium for pigments was (0.25+0.25)/2=0.250 (Table 1). We found the sum of the dose proportions causing trade-off 2 for chlorophylls a and b, and divided this sum by the number of these dose proportions. However, some authors use only one growth indicator (for example, total dry weight of plants) and the total content of chlorophylls, so averaging for this group of indicators is not always possible.

(4) Statistically compare the proportions of trade-offs 1 and 2 for each group of plant parameters (for pigments and for growth) and between these groups (between pigments and growth.). Since, in this case, the trade-off proportions calculated for a set of experiments are quantitative indicators and sample distributions are usually not normal use nonparametric tests for statistical analysis. Compare proportions of trade-offs 1 and 2 for pigments using the Wilcoxon signed-rank test (i.e., two dependent samples) and perform a similar analysis for growth indicators. Apply, for example, Dunn's test to compare the proportions of hormetic trade-offs 1 and 2 between pigments and growth (i.e., two independent samples).

Estimation of the frequency of hormetic trade-offs for a single experiment

The method described above can also be used for statistical comparison of trade-off proportions for different plant parameters studied in the same experiment:

(1) Construct a table showing statistically significant changes (stimulation, inhibition or no effect) in two compared parameters relative to the control at all doses of the factor as shown above (Table 1).
(2) Compare of the proportions of trade-off 1 for two studied plant parameters in the same experiment using McNemar's test. Similarly, compare the proportion of trade-off 2 for these parameters. McNemar's test is used for dichotomous data, that is, categorical (qualitative) observations that have only 2 values. For example, there is a trade-off or no trade-off for this plant parameter at the factor dose. The test is suitable for small dependent samples, when plant parameters of the same set of objects were studied. However, for sufficient statistical power of this test (i.e., the probability that the test correctly rejects the null hypothesis), the number of doses of the factor in the experiment should be about 15–20. This creates certain difficulties, because the authors usually do not use more than 3–6 doses of the stress factor.

Conclusions and future prospects

This is the first attempt to quantify the probability of hormetic trade-offs in plants, which undoubtedly requires further development and improvement, especially in terms of developing a statistical apparatus suitable for analysing data from small samples obtained in a single experiment. In the case of analysing a set of independent experiments, it is possible to perform the trade-off analysis for each individual plant parameter, different stress factors or plant species if the researcher has a sufficiently large set of experiments with the same plant indicators. This method is intended solely to improve the scientific understanding of plant hormesis and a first step towards a more extensive evaluation of hormetic trade-offs in plants.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Elena A. Erofeeva: Conceptualization, Investigation, Writing – original draft, Visualization.

References

[1] A.F. Bennett, R.E. Lenski, An experimental test of evolutionary trade-offs during temperature adaptation, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 8649–8654, doi:10.1073/pnas.0702117104.
[2] E. Agathokleous, E.J. Calabres, A global environmental health perspective and optimisation of stress, Sci. Total Environ. 704 (2020) 135263, doi:10.1016/j.scitotenv.2019.135263.
[3] E.A. Erofeeva, Hormesis and paradoxical effects of wheat seedling (*Triticum aestivum* L.) parameters upon exposure to different pollutants in a wide range of doses, Dose Response 12 (1) (2014) 121–135 Erofeeva, doi:10.2203/dose-response.13-017.

[4] E. Agathokleous, R.G. Belz, V. Calatayud, A. De Marco, Y. Hoshika, M. Kitao, C.J. Saitanis, P. Sicard, E. Paoletti, E.J. Calabrese, Predicting the effect of ozone on vegetation via linear non-threshold (LNT), threshold and hormetic dose-response models, Sci. Total Environ. 649 (2019) 61–74, doi:10.1016/j.scitotenv.2018.08.264.
[5] E.A. Erofeeva, Environmental hormesis of non-specific and specific adaptive mechanisms in plants, Sci. Total Environ. 804 (2022) 150059, doi:10.1016/j.scitotenv.2021.150059.
[6] N. Aibibu, Y. Liu, G. Zeng, X. Wang, B. Chen, H. Song, L. Xu, Cadmium accumulation in vetiveria zizanioides and its effects on growth, physiological and biochemical characters, Bioresour. Technol. 101 (16) (2010) 6297–6303, doi:10.1016/j.biortech.2010.03.028.
[7] M.F.A. Dawood, M.M. Azooz, Concentration-dependent effects of tungstate on germination, growth, lignification-related enzymes, antioxidants, and reactive oxygen species in broccoli (*Brassica oleracea* var. italica L.), Environ. Sci. Pollut. Res. 26 (2019) 36441–36457, doi:10.1007/s11356-019-06603-y.