HORMESIS: IS IT AN IMPORTANT FACTOR IN HERBICIDE USE AND ALLELOPATHY?

Stephen O. Duke[1], Nina Cedergreen[2], Edivaldo D. Velini[3], and Regina G. Belz[4] describe the stimulatory effects of low doses of herbicides that are inhibitory or severely phytotoxic at normal use rates and consider the potential of this phenomenon

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Introduction

Paracelsus (Fig. 1), regarded by many as the father of toxicology, is often paraphrased to have said that the poison is in the dose [“*Alle Ding sind Gifft und nichts ohn Gifft. Allein die Dosis macht das ein Ding kein Gifft ist.*” (All things are poison and are not poison; only the dose makes a thing not a poison)]. With this statement, Paracelsus considered the apparent safety of toxicants at low doses. Moreover, some substances, although toxic at higher doses, can be stimulatory or even beneficial at low doses. This is the case with compounds such as pharmaceuticals that are used for their beneficial effects, as well as with compounds such as pesticides that are normally used as toxicants. This stimulatory effect of a low dose of a toxicant is called hormesis. Although this phenomenon was recognized earlier, the term hormesis was first used by Southam and Erlich (1943) to describe the effect of an oak bark compound that promoted fungal growth at low doses, but strongly inhibited it at higher doses. They coined this term using the Greek word “hormo” (to excite), the same root used in the word hormone.

Figure 1. Aureolus Philippus Theophrastus Bombastus von Hohenheim (1493-1541), known as Paracelsus, practiced alchemy, surgery, and medicine in Germany, Italy, and Switzerland.

The concept of hormesis has highly controversial implications in the areas of environmental and medical toxicology (*e.g.*, Calabrese, 2005; Thayer *et al.*, 2005). Renewed interest in this phenomenon has led to the recent founding of the International Hormesis Society (www.HormesisSociety.org), whose main effort is to document and understand hormesis with different toxicants and organisms and to encourage the assessment of the implications of hormesis in all fields of science. Hormesis has been found within all groups of organisms, from bacteria and fungi to higher plants and animals (Calabrese, 2005). Most focus has been on animals and mammalian test-systems, as there is a large interest in hormetic effects within the pharmaceutical and toxicological sciences. Less documentation exists on hormesis in plants, and there is practically no information concerning the mechanisms underlying the observed hormetic effects.

In this short review, we present examples of hormesis in plants exposed to herbicides and other phytotoxins, which by definition are toxic to plants at certain doses. Possible mechanisms that underlie the observed stimulatory responses are discussed, together with the possible positive or negative implications of hormesis for both crops and non-target plants. One should note that the definition of hormesis does not state whether the hormetic effect is beneficial or harmful to the organism, only that it is stimulatory to the parameter being measured (Calabrese & Baldwin, 2002a).

Proving hormesis

To prove the existence of hormesis, dose-response curves that include several doses below the adverse effect concentrations must be generated. Since most research concerning herbicides and phytotoxins focuses on the toxic effect of these compounds, experiments containing several doses below the adverse effect concentrations are rare. But, when they are tested, stimulatory effects on one or several traits are often observed. The statistical significance of the stimulation is frequently assessed by simple comparative tests, such as

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t-tests, that compare controls with treated plants. A stronger test for hormesis is obtained by regression analysis, comparing non-linear regression models with and without hormetic growth stimulation, and evaluating which model describes the data best (Cedergreen *et al.*, 2005). This approach not only gives a more reliable test, since data from the entire dose response curve is used, but recent regression models also allow the estimation of the maximal hormetic effect, the concentration giving that effect, and the concentration range that produces a stimulatory response (Cedergreen *et al.*, 2005). Hence, using these tools, research in hormesis can move from detecting its existence towards quantifying the magnitude of hormesis and the concentration range where it takes place.

A survey of hormesis caused by herbicides in crop and aquatic plants demonstrated that hormesis can range from a few percentages up to a 100% increase in the measured parameter, but with an average of 20-30% stimulation compared to the control (Cedergreen *et al.*, 2005). In this survey of more than 30 cases of hormesis, the average concentration eliciting the maximal hormetic response was approximately 20% of the concentration causing 50% growth reduction.

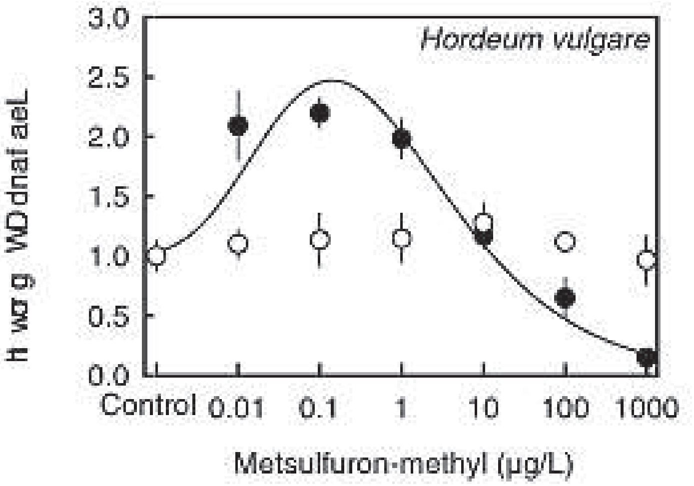

Figure 2. Growth of barley exposed to the herbicide metsulfuron-methyl measured as increase in leaf length (filled symbols) and dry weight-specific relative growth rates (open symbols). Both growth parameters are normalized to the controls and given as means ± 1SE. The data on leaf length increase are described with a logistic dose-response curve, including a function for hormesis (Cedergreen *et al.*, 2005).

Examples of hormesis

Published examples of hormesis with herbicides are provided in Table 1. In most of these cases, there was statistical proof of hormesis based either on *t*-test or regression analyses. The stimulatory response is measured on different parameters varying from growth based on weight, height or leaf area to measured changes in physiological parameters such as protein content (Table 1). A stimulatory response in one trait does not necessarily correlate with a stimulatory response in other traits. For example, some herbicides (*e.g.*, bromacil, bromoxynil, chloramben, propachlor, terbacil, EPTA, and MSMA), can stimulate root growth at low doses, but have no stimulatory effect on shoot growth at any dose (Weidman & Appleby, 1972). Similarly, a 100% increase in barley leaf length occurred when plants were exposed to metsulfuron-methyl, while the same plants did not increase in total dry weight (Figure 2).

One of us (E. D. Velini) has found that glyphosate induces hormesis in crops and plant species as different as sorghum, soybean, coffee, eucalyptus, *Arabidopsis thaliana*, maize, and *Pinus* spp. In general, the hormetic response was more pronounced in woody genera such as *Eucalyptus* spp. (Figure 3). Others have observed hormesis with glyphosate in maize and barnyard grass (Schabenberger *et al.*, 1999, Wagner *et al.*, 2003) (Table 1).

Allellochemicals, the phytotoxins released from plants, are known to induce hormesis as well. An *et al.* (1993) even hypothesized that biphasic dose-response relationships are a universal biological property of allelochemicals. However, reports that prove hormesis of allelochemicals or natural mixtures of allelochemicals (*i.e.*, extracts or exudates of

Table 1. Some examples of herbicide-related hormesis.

Herbicide (dose)	Plant	Increased parameter	Reference
Simazine (0.05-0.8 μM)	Rye & pea	protein content	Reis *et al.*, 1967
	Barley	protein content	Pulver & Reis, 1973
Oxyfluorfen (17.5 g ai/ha)	Soybean	resistance to plant pathogens	Nelson *et al.*, 2002
MSMA (5 μg/pot)		growth	Wiedman & Appleby, 1972
Dalapon (0.05 μg/pot)	Wheat	growth	Wiedman & Appley, 1972
Bromoxynil (0.01 mg/pot)	Wheat	growth	Wiedman & Appleby, 1972
Terbacil (70 g/ha)	Wheat	protein content	Strbac *et al.*, 1974
(0.01 mg/pot)	Wheat	growth	Wiedman & Appleby, 1972
Glyphosate (53-105 g ai/ha)	Barnyard grass	growth	Schabenberger *et al.*, 1999
(<0.6 μg taken up/plant)	Maize	whole plant fresh weight	Wagner *et al.*, 2003
Terbuthylazine (5-20 μg L^{-1})	Seven aquatic macrophyte species	dry weight biomass	Cedergreen *et al.*, 2004
Sulfosulfuron (0.1 μg L^{-1})	*Glyceria maxima*, *Myriophyllum spicatum*	Shoot height	Davies *et al.*, 2003

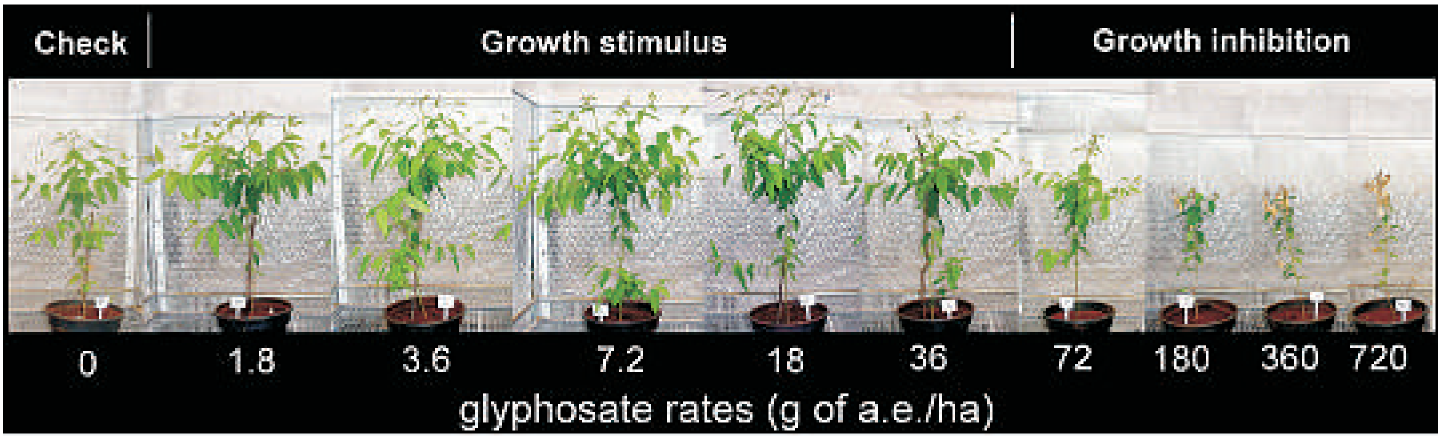

Figure 3. The effect of different doses of glyphosate on grown *Eucalyptus grandis* 60 days after spraying (unpublished work of E. D. Velini).

allelopathic plants) are still rare, due to dose-response designs that rarely include the concentrations expected to elicit hormesis.

An example from our own work (R. G. Belz) involves the invasive weed *Parthenium hysterophorus* whose allelopathic properties may contribute to its invasiveness. *P. hysterophorus* biosynthesizes several secondary metabolites (Kanchan & Jayachandra, 1980) that, once released from plant material, may act jointly as allelochemicals in mixtures that vary in composition over the plant's life cycle. Our studies showed that the developmental stage of *P. hysterophorus* was important in eliciting a hormetic response with leaf extracts. No hormetic dose response appeared if leaf extracts of an early developmental stage were assayed, while hormesis was common for leaf extracts at the beginning of flower bud development (Figure 4). The extracts differed significantly in the level of the allelochemical parthenin, which dominated the spectrum of compounds in the leaf extracts only at bud development. Pure parthenin caused an up to 70% hormetic response on root growth of the test species, and is therefore likely to be the chemical causing the hormetic response of the plant extracts. This example illustrates that hormesis can be pronounced with allelochemicals or mixtures of allelochemicals. However, qualitative and quantitative differences in the chemical composition of allelopathic extracts or exudates may complicate and constrain the testing of hormesis, especially if the mixtures are not completely characterized.

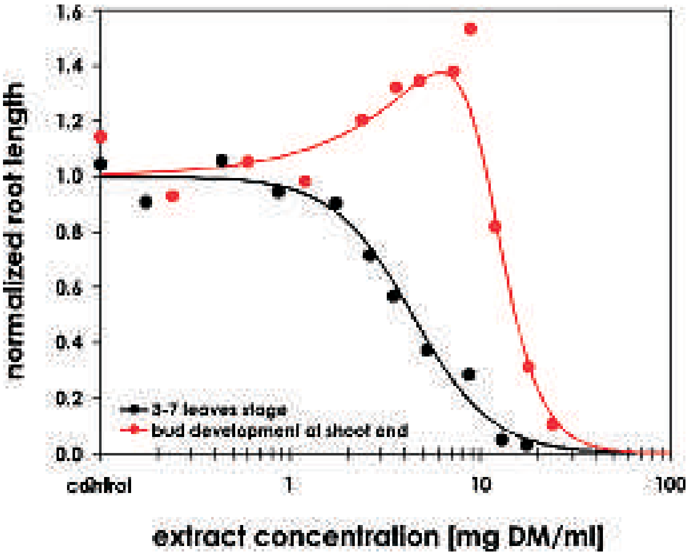

Figure 4. Effects of aqueous leaf extracts of *Parthenium hysterophorus* on *Lactuca sativa* L. as influenced by *Parthenium* plant age. Responses are normalized to the control and described by a logistic dose-response curve, including a function for hormesis. DM = dry mass (unpublished data, R. G. Belz).

Mechanisms of hormesis

What physiological mechanisms can be hypothesized to cause the stimulatory responses on growth observed in morphological traits at subtoxic concentrations of a phytotoxin? There are probably several answers to that question, depending on the chemical being tested and/or the plant species exposed to the compound. Some mechanisms could represent physiological attempts to "escape" or compensate for chemical stress. This could explain the hormetic response in root growth observed by Weidman and Appleby (1972). Plants could also escape unfavorable growth conditions by producing more seeds, giving the next generation a larger opportunity to germinate under more favorable conditions. This type of response is often seen in animals exposed to low doses of chemicals, where the increased production of offspring is counterbalanced by lower off-spring survival (Fujiwara *et al.*, 2002; Zanuncio *et al.*, 2003).

The induction of different defense systems that can ameliorate the effect of chemicals in an organism has also been proposed to be a cause of hormetic responses (Parson, 2003). For example, Kovalchuck *et al.* (2003) showed that the induction of defense mechanisms induced by free radicals of oxygen can lead to increased growth in the presence of low doses of phytotoxic chemicals.

Some chemicals could affect plant hormones at low doses. For example, chemicals stimulating hormonal responses responsible for leaf or root elongation could initiate an increase in these traits at low doses, while they might have deleterious effects at higher doses due to the same or another mechanism of action. When viewed over a broad dose-range this would lead to a hormetic dose-response curve. The auxinic herbicides are well-known examples of chemicals that enhance growth at non-toxic concentrations by mimicking the growth hormone auxin, but which are lethal at higher doses (Allender, 1997).

Non-hormonal mechanisms may also be important in eliciting a hormetic response. For example, we propose a mechanism for the hormesis found with glyphosate that is related to the fact that glyphosate inhibits the shikimate pathway, the source of lignin precursors. It might preferentially inhibit lignin synthesis at low, non-herbicidal doses, making cells walls more elastic for a longer period during development. This should result in greater longitudinal growth. In support of this mechanism, one of us (E. D. Velini) has found that glyphosate-mediated hormesis occurs with non-transgenic soybean, but not with glyphosate-resistant soybean, in which the shikimate pathway is unaffected by glyphosate. Thus, the hormetic response is related to the effect of glyphosate on its molecular target site, rather than a secondary target site. There may be many different mechanisms of hormesis for phytotoxins with different modes of action.

Implications of hormesis

Evaluated from an energetic and evolutionary perspective, the stimulatory responses observed at low levels of chemical stress should seldom lead to an over-all improvement of the fitness of the organism (Parson, 2003; Forbes, 2000). In other words, there may be a cost of the hormetic response. This cost can be paid at the expense of the development of a trait other than the one showing the hormetic response. However, few studies have measured more than one trait in order to test whether such resource allocation takes place. The trade-off could also be paid over time, so that an initial increase in growth might be followed by a decrease, if the stress persists over time. This has been observed for hormesis in animal test-systems (Stebbing, 2002; Calabrese, 2005), but no studies have to our knowledge monitored hormesis over time in plants.

Hormesis is not necessarily beneficial or detrimental in agriculture. The judgment of desirability of hormesis depends on what outcome is desired and how hormesis enhances this. Treatment of a crop with a sublethal dose of a herbicide for a desirable phenotypic change could be valuable to a farmer. For example, subtoxic doses of glyphosate increase sucrose content in sugarcane (McDonald *et al.*, 2001). This hormetic effect is highly beneficial to farmers, and a low dose glyphosate treatment is used worldwide in sugarcane production. Low doses of other herbicides for desired agronomic effects have been proposed in papers and patents. For example, low doses of protoporphyrinogen oxidase-inhibiting herbicides provide crops protection from plant pathogens, apparently through elicitation of defenses against pathogens (Nelson *et al.*, 2002). However, to our knowledge, these and other reported beneficial hormetic effects of herbicides on crops are not used.

But just as stimulation of a certain trait in a crop can be of economic significance for a farmer, changes in resource allocation of a non-target plant exposed to a hormetic herbicide dose may reduce plant fitness over the long term. It could also increase fitness in cases such as the elicitation of resistance to pathogens. Whether fitness is increased or decreased by a hormetic effect, species-specific differences in responses to low doses of chemicals could change competition between species, thereby leading to changes in species composition within an ecosystem, as has been seen with low dose exposure of algal communities to different toxicants (Selck *et al.*, 2002). While little is known of hormesis in crops in response to commercial herbicides, virtually nothing is known of hormesis and its long-term effects on fitness in non-target plant species. If the mechanism of hormesis varies between species and between phytotoxins, understanding the potential benefits and risks of hormetic doses of these compounds on plants will require considerable research. However, we believe that obtaining this knowledge will be rewarding to agriculture and environmental toxicology.

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