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Hormesis: Biphasic Dose-Responses to Fungicides in Plant Pathogens and Their Potential Threat to Agriculture

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1. Introduction

Hormesis is a toxicological concept characterized by low-dose stimulation and high-dose inhibition (1, 2, 3, 4, 5). Extensive examinations of scientific literature by Calabrese and his collaborators reported that hormetic dose-responses are common across biological systems and stressors (4, 6, 7, 8). Scientific literature provides evidence that hormesis can be caused by multiple stimuli (9), such as chemicals (4, 10, 11), radiation (12; 29), heat (13), stress (14), and even exercise (15). Dose-response curves displaying hormesis are characterized by a biphasic behavior (Fig.1). The hormetic zone includes a range of subinhibitory doses that are stimulant, with a peak at the maximum stimulation dose (MSD), and ends at the no observable adverse level (NOAEL), that typically precedes the inhibitory doses (Fig. 1). Our interest in hormesis pertains to the effects of fungicides at subinhibitory doses on fungal and oomycete growth and pathogenicity. Thereafter, for the purposes of this book, we will focus on chemical hormesis alone. Some of the most familiar examples of biphasic dose-responses include vitamins, alcohol, essential minerals, and many drugs (16, 17, 18, 19, 20). Hormesis has been measured using diverse endpoints in multiple biological systems (8). One of the most common endpoints used in hormesis research is growth, but several others have been studied including CO_2 production (1; 21), longevity (14), other metabolic processes, and cellular functions (22). Southam and Ehrlich (2) coined the term hormesis to describe the biphasic dose-response because its Greek etymological root *horm-* means "to exite" (18). Several reports of subinhibitory stimulation of fungi and oomycetes due to exposure to fungicides are available in the mycological and phytopathological literature, but little attention has been paid to fungicide hormesis in spite of its potential detrimental effects to crop productivity.
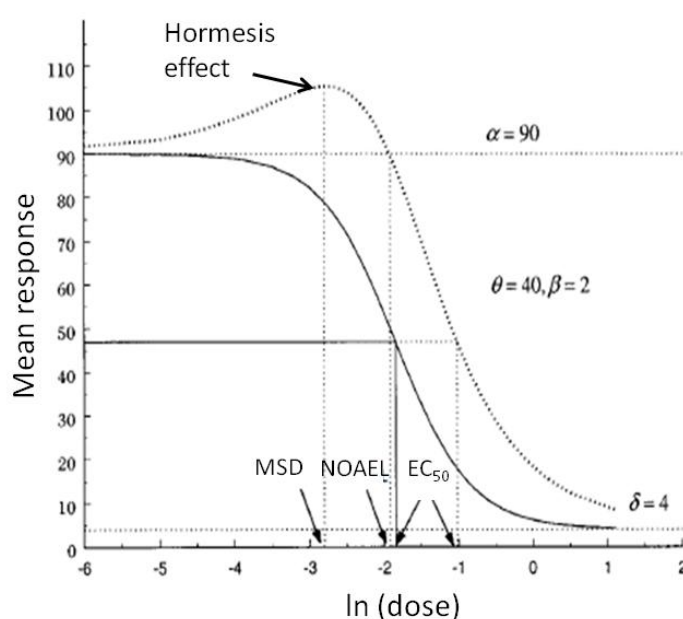
Figure 1. Dose-response curves comparing the traditional threshold model (solid) and the Brain-Cousens model (dotted) that includes hormesis in the dose-response concept. The Brain-Cousens model permits to estimate the no-observable adverse effect level (NOAEL), the maximum stimulation dose (MSD), and the half maximal effective concentration (EC_{50}). Modified from Shabenberg et al. (23).

2. Historic background

The first references to dose-responses have been attributed to Paracelsus, who allegedly said "all things are poison and nothing is without poison, only the dose permits something not to be poisonous" (24). However, the first scientific reports of chemical stimulation at low doses and inhibition at high concentrations go back to the 1800s. In 1854, Virchow (22) reported that low concentrations of sodium hydroxide increased the frequency and intensity of beating of ciliae of human tracheal ciliated epithelia; while at higher concentrations or longer exposures the same compound paralyzed ciliae and caused cell death. In 1865, Reveil (25) reported that sodium hypochlorite stimulated seed germination at low concentrations (0.1% solution) but it was phytotoxic at high concentrations. According to Calabrese and Baldwin, in their review of the historical foundations of chemical hormesis as a biological hypothesis (5), two researchers are considered as the founders of modern hormesis, Rudolf Arndt and Hugo Schulz. In 1887, Schulz (1) observed experimentally that several chemicals caused low-dose stimulation and high-dose stimulation on yeast fermentation. Because Schulz's views supported those of Arndt, a homeopathic physician, soon they were merged in to what was known as the "Arndt-Schulz law". The law stated that "for every substance, small doses stimulate, moderate doses inhibit and large doses kill" and that their findings could be generalized to all organisms and all toxic agents. Mainly because of the difficulty at the time to demonstrate the universality of this law without offering explanation of its biological causes, and probably also because at the time it was conceptually associated to homeopathy, over time it fell out of use. However, a few years later Ferdinand Hueppe

(1896), a distinguished bacteriologist, made similar observations in bacteria and described the phenomenon in his books and scientific publications (26). Hueppe recognized the validity of Schulz's scientific research but stated certain limitations and exceptions to the Arndt-Schulz law. Soon the notion that "substances which inhibit biological processes at sublethal doses may be expected to stimulate them at lower levels" became known as the "Hueppe rule" and was broadly adopted in international literature (3, 5).

Years later, in 1929, Branham (21) confirmed Schulz's observations using a series of different chemicals and an improved apparatus to detect CO_2 production, and demonstrated that very small doses of inhibitor compounds had an apparent stimulatory effect on carbon dioxide production by yeasts. One of her experiments assessed the effect of adding crystals of 1,2,5,6-dibenzanthracene to yeast suspensions, finding that at a concentration of 9×10^{-4} molar yeast proliferation increased. The term hormesis was coined by Southam and Ehrlich in their 1943 report of growth stimulation of a wood-decaying fungus (*Fomes officinalis*) in culture by extracts of western red-cedar heartwood at low doses, while higher doses were inhibitory (2).

Research focused on chemical subinhibitory dose-responses continued through the early decades of the 1900s, but scientific attention faded away as the science of Toxicology became established and few hormesis studies were published until the 1980s (3, 27, 28). Toxicology is the study of the adverse effects of chemical, physical, or biological agents on biological systems, their prevention and amelioration. Hence, by definition, Toxicology deals with the negative effects of such agents at doses above the no observed adverse effect level (NOAEL), and essentially ignores the effects of subinhibitory doses. Stebbing (3) reexamined the hormesis concept and provided the first update on the validity of this concept based on the abundance of scientific reports of data with biphasic distributions. Stebbing reported his conclusions after searching the scientific literature for an explanation to his own observations of growth stimulation of the colonial hydroid *Campanularia flexuosa* (3) by exposure to sublethal concentrations of various metals and organometallic compounds; and raised the question whether growth hormesis by diverse toxic substances in multiple biological systems had a common explanation. Although examples of stimulation by subinhibitory levels of chemicals and radiation are numerous in the toxicological literature, interest on hormesis has slowly increased again in the last few decades. Several studies on radiation hormesis followed Stebbing's paper (29, 30, 31, 32), with limited initial impact initially, but the interest on the subject gathered momentum by the end of the decade (33, 34). The few chemical hormesis reports that followed Stebbing's paper had much better acceptance (35, 36, 37). Hormesis once again, although still viewed as a polemic concept by some toxicologists, attracted the attention of the scientific community on the basis of its relevance to risk assessment and optimization of research resources (38). In the late 80s Dr. Edward Calabrese, currently the Director of the Department of Public Health at University of Massachusetts School of Public Health and Health Sciences, became deeply interested in hormesis and began a campaign to create awareness of the biological significance of this phenomenon.

Calabrese first report related to hormesis described stimulation of *Mentha piperita* grown, in soils and *in vitro,* by the growth retardant phosfon (2,4 dichlororobenzyl tributyl phosphonium chloride) at concentrations of 1.26×10^{-5} M to 7.77×10^{-4} M and 6.30×10^{-7} M to 3.78×10^{-5} M respectively, while higher concentrations inhibited plant growth (39); nonetheless, no reference to hormesis was made, but wondered about the nature of the stimulation. Eleven years later, Calabrese and collaborators examined the occurrence of chemically induced hormesis in biological and toxicological systems by looking for evidence of low-dose stimulation in literature (37). They found multiple historical and contemporary publications reporting biphasic dose-responses to chemical stressors in plants, fungi, and animals. During the decade that followed Calabrese and Baldwin published four articles reviewing possible examples of chemical hormesis in previously published studies, and discussing the importance of expanding the reference dose concept to incorporate the effect of subinhibitory doses (4, 40, 41, 42). Products of their work were the description of the first quantitatively-based methodology to evaluate chemical hormesis and the development of a chemical hormesis database. These publications served as catalysts for what Calabrese and Baldwin referred to as "the dose-response revolution". The prolific research inspired by these papers has produced hundreds of publications, providing well supported evidence of hormetic effects of a broad spectrum of stressors, including radiation, heat, caloric intake, and even exercise, on plants, fungi, bacteria, protozoa, and animals, including humans (6, 10, 12, 43, 44, 45, 46, 47).

3. Hormesis as a general phenomenon

Based on their extensive literature review, Calabrese and Baldwin stated that hormetic responses often, but not always, display the following characteristics: i) Stimulation zone of the dose-response could be found within a 10-fold range; ii) Stimulatory responses were 30-60% greater than the controls; and iii) the NOAEL three to six-fold greater than the MSD (4). Using these criteria they identified hundreds of toxicological studies that potentially displayed hormetic responses (48). In recent years researchers from many different disciplines have been inspired by Dr. Calabrese's work and have studied hormesis in their biological systems of interest. There are numerous recent reports of biphasic dose-responses in plants, animals, as well as eukaryotic and prokaryotic microorganisms in the scientific literature. A few examples of studies reporting chemical hormesis are presented below.

Velini *et al.* (49) examined the effect of the herbicide Glyphosate on target and non-target plants. The growth of glyphosate sensitive soybean was inhibited when applied at concentrations between 72–720 g AE ha^{-1}; however, low doses (1.8 – 18 g AE ha^{-1}) of herbicide induced significant increases in shoot and total dry weight (up to 28% and 22%, respectively). Similar growth enhancements were observed in maize, *Eucalyptus grandis* Hill ex Maiden, *Pinus caribea* L. and *Commelina benghalensis* L. by 1.8 – 36 g AE ha^{-1}. Barceló and Poschenrieder (10) reported their observations of rapid root growth in corn plants sensitive to soil aluminum (Al). Hormesis was observed in plants exposed to subinhibitory Al levels or as a transient effect after brief exposure to potentially toxic concentrations. A study by Migliore *et al.* (50) of the effects on plants of fluoroquinolone antibiotics administered to

cattle and excreted in their feces, which are later used as field manure, demonstrated that enrofloxacin was toxic to *Cucumis sativurs* L., *Lactuca sativa* L., *Phaseolus vulgaris* L., and *Raphanus sativus* L. seedlings at concentrations equal or above 5000 µg l^{-1}, while growth hormesis was often observed at concentrations 50-100 µg l^{-1} in the four plant species.

The better documented example of hormesis in animals is lifespan increase as a result of restricted caloric intake in diet (24). While high calorie diets have been associated with increased risk of several age-related diseases in animal systems (cardiovascular disease, type 2 diabetes, stroke, among others), dietary energy restriction (i.e. controlled caloric restriction or intermittent fasting) has been reported to have anti-oxidative effects, increasing the cells' tolerance to several types of stresses. For example, restricted calorie diets protected rodents against several types of cancers (51); furthermore, alternate day calorie restricted diet in humans seems to improve inflammatory symptoms in asthmatic patients (52). A now classic example of chemical hormesis are vitamins in human diet, since small amounts of them are necessary and beneficial, but large amounts are toxic and can cause hypervitaminosis, tissue mineralization, and chemical imbalances (45). Other examples of hormesis in animal systems include inhibition of N-diethylnitrosamine (DEN)- initiated pre-neoplastic lesions by phenobarbital at low-doses, while higher doses promote activity (53), and survival and fertility enhancement in *Podisus distinctus* Stål (Heteroptera: Pentatomidae) due to exposure to sublethal doses of the pyrethroid insecticide permethrin (54), among others.

There are abundant examples of hormesis in prokaryotic and eukaryotic microbial systems. As related in the historical review, the first reports of biphasic dose-responses were on bacteria and fungi (1, 2, 26, 21) and several more studies have been published in more recent years. Hotchkiss (55) found that TiCl$_2$, MgCl$_2$ and, NaCl had hormetic effects on the growth of *Escherichia coli* in culture. Low doses of penicillin produced doubled the growth of *Staphylococcus* (No. 6571 N.C.T.C.) in culture compared to the non-treated control (56). Linares *et al.* (57) demonstrated that three antibiotics (tobramycin, tetracycline, and norfloxacin) trigger the expression of determinants that influence the virulence of *Pseudomonas aeruginosa* at subinhibitory concentrations. Wang *et al.* (58) and Gong *et al.* (59) reported increased production of mycrocystin by *Microcystis aeruginosa* (cyanobacteria, Cyanophyta) in non-linear responses to nonylphenol and arsenic pollution. Hong et al (60) reported growth stimulation in *Selenastrum capricornutum* (Chlorophyta, Selenastraceae) due to exposure to subinhibitory doses of the algicide ethyl 2-methyl acetoacetate at high initial algal densities. Many of the early studies on hormesis were done on the effects of multiple chemicals on yeast metabolism (2, 21). Yeasts continue to be used as models for the study of hormesis, particularly related to cancer (61), ageing (43, 62), and UV radiation hormesis research (63).

4. Evidence of chemical hormesis in phytopathological literature

Our review of mycological and phytopathological literature found several studies of fungicide effects on fungi and oomycetes with results that reflect potential hormetic responses. We present some interesting examples below, while an exhaustive literature

review will be reported elsewhere. Southam and Ehrlich (2) observed that extracts of western red-cedar heartwood were stimulatory at low doses on the growth of a wood-decaying fungus (*Fomes officinalis*) in culture, while higher doses were inhibitory. This was the first scientific study that demonstrated stimulation by a mycotoxic compound on a plant pathogenic fungus, and the authors coined the term hormesis to describe their observations. Later studies of hormesis in plant pathogens demonstrated a positive effect of trichothecin, a compound produced by *Trichothecium roseum*, on the growth of *Fusarium oxysporum* (64, 65) while assessing the production of trichothecin in different soil types. Although not further references to hormesis were made in the phytopathological literature until recently (66), several reports of stimulation by exposure to fungicide are available. Baraldi *et al.* (67) reported higher percentages of germination in seven out of 41 thiabendazole (TBZ) resistant isolates of *Penicillium expansum* from pear when grown TBZ-amended media than without the fungicide. The authors suggested that germination stimulation and fitness advantage in certain TBZ-resistant *P. expansum* isolates could be due to the ability of this isolates to metabolize the fungicide as a nutrient compound, but further study was recommended to understand the nature of the stimulation. Audenaert *et al.* (68) observed increased production of the mycotoxin deoxynivalenol (DON) by *Fusarium graminearum*, *in vitro* and *in planta*, when exposed to sub-lethal doses of the triazole fungicide prothioconazole, and proposed that mycotoxin production was stimulated by H_2O_2 production triggered by the fungicide at low concentrations. While studying the effects of different fungicides on the infection of *Sphagnum* by fungi, Landry *et al.* (69) observed significantly increased radial growth of *Lyophyllum palustre* (Peck) *in vitro* when media was amended with the fungicide propamocarb compared to the non-amended control.

Similar references can be found on oomycetes literature. Fenn and Coffey (70) observed that 69 µg/ml of phosphorous acid (H_3PO_3) was stimulatory on the growth of *Pythium ultimum in vitro* and that 138 µg /ml was also stimulatory on the growth of *Pythium myriotylum*. In a study comparing the sensitivities of various oomycetes to the fungicides mefenoxam (a metalaxyl enantiomer, FRAC code: 4) and hymexazole (FRAC code: 32), Kato *et al.* (71) observed a range of responses to these fungicides among the different groups that could be used to classify them by taxa as reflected by DNA analysis. Significant findings included the differential fungicide sensitivity of the plant pathogens *Pythium* and *Phytophthora*. Although fungicide sensitivity responses varied within the two genera, general trends suggested that *Pythium* species were more sensitive to hymexazol than to mefenoxam, while the opposite was true for *Phytophthora* species, with a few exceptions. The reported sensitivity response curve for *Phytopthora undulata* reflected slight stimulation at the lowest hymexazol doses. Radial growth stimulation was observed in three out of four metalaxyl-resistant *Phytophthora infestans* isolates when grown on cleared lima bean agar medium amended with 20µl/ml (72). Since one of the isolates grew more in the presence of metalaxyl only when nutrients were limited, it was hypothesized that under certain circumstances metalaxyl could be beneficial to metalaxyl-resistant *P. infestans* strains. Moorman and Kim (73) reported for the first time strains with dual resistance to mefenoxam and propamocarb in *Pythium aphanidermatum*, *P. irregulare,* and *P. ultimum* and described radial growth

stimulation in some strains of the three species by propamocarb at a concentration of 1µg/ml, and of *P. aphanidermatum* by 1,000 µg/ml propamocarb.

5. Fungicides hormesis and its impact on fungal plant pathogens

Recent research on chemical hormesis on fungal pathogens has focused on the effects of subinhibitory doses of fungicides on radial growth and pathogenicity of fungi and oomycetes (66, 74). Garzon *et al.* (66) examined the effects of subinhibitory doses of mefenoxam on the radial growth and pathogenicity of a mefenoxam and propamocarb-resistant isolate of *P. aphanidermatum*. In this study we found modest radial growth stimulation, with an average of 10% increase over the control, and very significant increase of pathogenicity, with an increase of 61% severity of damping-off of seedlings in geranium. Flores and Garzon (74) reported standardized laboratory and statistical protocols for detection of chemical hormesis using radial growth *in vitro* as endpoint. Using the reported methods hormetic responses were detected in *Pythium aphanidermatum*, *Rhizoctonia solani* and *R. zeae* exposed to ethanol (Fig. 2), and on *P. aphanidermatum* exposed to subinhibitory doses of the fungicides propamocarb and cyazofamid (Fig. 3).
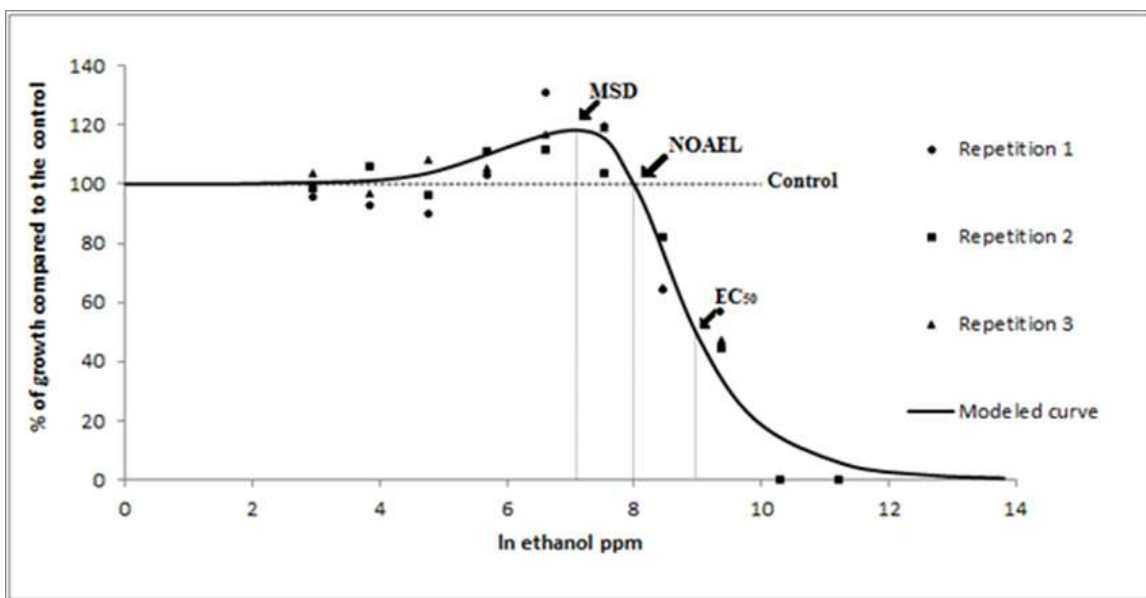
Figure 2. Modeled curve of the radial growth *in vitro* of *P. aphanidermatum* in response to subinhibitory doses of ethanol (Flores and Garzon *in press*). Radial growth is expressed as percentages relative to a non-amended control, and concentrations as natural logarithm of ppm. Figure reproduced with permission of Dose-Response Journal.
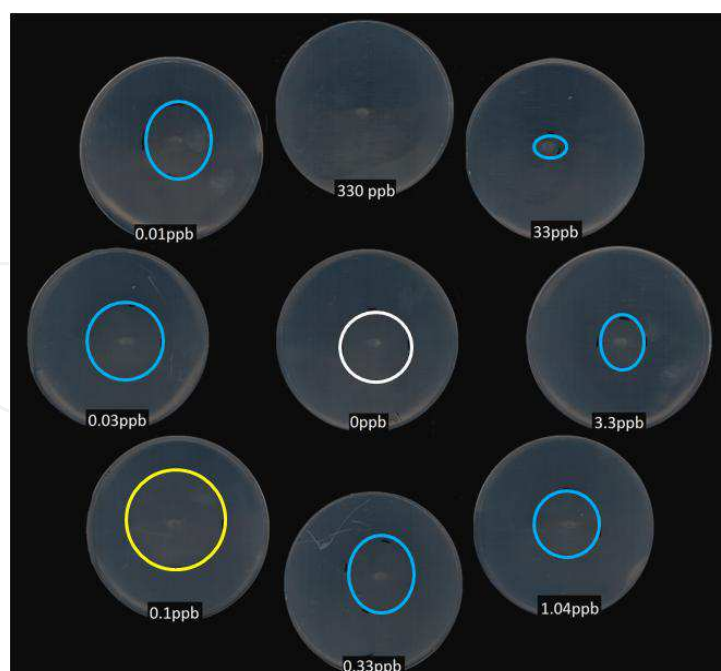
Figure 3. Radial growth of *Pythium aphanidermatum* on corn meal agar amended with cyazofamid. Significant hormetic stimulation was observed at 0.1 ppb cyazofamid, with 17% radial growth increase (yellow) over the control (white).

6. The biological basis of hormesis

Hormesis can result from overcompensation after a disruption of homeostasis by stressors, by direct stimulation, or as a response to an adapting dose followed by a larger dose (3, 75, 76). The research by Branham (21) on the effect of 16 chemicals on CO_2 production by Baker's yeast, provided clear evidence, in 12 of the chemicals, of an initial mild inhibition followed by significant stimulation. These results supported the hypothesis of stimulation due to overcompensation, being most evident for formaldehyde, phenol, iodine, and metaphen. Other examples of over-compensatory responses include ethanol stimulation of locomotion in mice (77), increased serotonin levels in rat neurons after treatment with below toxic doses of 5,6-dihydroxytryptamine (78); and growth stimulation in peppermint following an initial decrease after treatment with phosfon, a plant growth regulator (39), among others. Stebbing (79) provided an "improved" explanation for hormesis due to overcompensation by describing a model using two overlapping curves, an *effect* curve and a *response* curve, relative to a particular endpoint and the compensatory mechanisms involved, respectively. Under this model, below a threshold an stressor may be undetectable by an organism, and after reaching that threshold compensatory mechanisms would be triggered by a range of doses (represented by the *response* curve), however no effects would be visible, due to neutralization by compensation responses; effects would be evident only after the capacity to neutralize inhibition is exceeded and may follow a linear pattern. In systems where compensation responses completely neutralize inhibition, the alfa curve is present (threshold dose-response) with no evident low-dose effects, while in those where compensation responses exceed inhibition the beta curve is observed (hormetic curve).

Overcorrection of inhibition may have an adaptation role with impact on fitness. This hypothesis was supported by Stebbing's observations that hydroid colonies pre-exposed to a subinhibitory dose of copper (10 μg.l^{-1}) had increase tolerance across the range (0 – 50 μg.l^{-1}), when compared with non-pre-exposed colonies. Calabrese suggested direct stimulatory response as another possible cause of hormesis (8).

The underlying mechanisms that generate hormetic responses have not yet been fully understood. Conolly and Lutz (80) hypothesized that hormetic responses may occur due the superimposition of two monotonic dose-responses, one that takes effect at low doses and other that overtakes at higher doses undermining the first one. They demonstrated by computational modeling that four different cellular models could generate biphasic dose-responses: i) Membrane receptor subtypes with opposite downstream effect; ii) Androgen receptor mediated gene expression; iii) Induction of DNA repair and "co-repair" of background DNA damage; and, iv) Modulation of the cell cycle and effect on rate of mutation (80). Subsequent studies have found empirical evidence of hormesis attributable to the presence of antagonistic membrane receptors (81) or to the induction of DNA repair (82). Bae *et al.* (83) suggested that hormesis may arise because of the heterogenic susceptibilities of different tissues to the same stimulus; such difference can result in the expression of a U shaped dose-response curve. The observations that drove this conclusion were made from the response of different cell types normally present in human blood vessels to the presence of small doses of arsenic and a reactive oxygen species generator (menadione). Allender (84) and Allender *et al.* (85) provided indirect evidence of the influence of calcium influx to the cell on hormetic responses related to plant growth. The diversity of the models that may show a hormetic response suggests that the mechanisms acting may not be the same for different systems. Experimental evidence suggests that multiple metabolic processes may be involved in hormetic responses, some acting during the stimulation phase and others, probably different, acting during the inhibition phase of the beta curve.

7. Studying hormesis

Detection of hormesis is often challenging due to the multiple factors that can affect metabolic responses of the target organisms. For example, when studying fungicide hormesis in oomycetes using radial growth as endpoint it is fundamental to standardize every experimental factor involved; in addition to growing media type and concentration, fungicide treatments, and incubation temperature, other factors are also relevant, including light, growing media depth, inoculum age and developmental stage, mixing time when preparing fungicide dilutions, using fungicide stock solutions prepared on the same day of the experiments, etc. Variation in any of these parameters can influence mycelial growth significantly, hence introducing experimental variation that could affect the reproducibility of results (66).

When trying to prove the existence of hormesis there are some requirements that the experimental design should fulfill: i) The NOAEL should be determined; ii) doses below the NOAEL need to be tested with five equally spaced doses providing enough data to detect

hormesis; and iii) the separation between doses should generally be smaller than one order of magnitude since the hormetic zone is usually within a ten-fold range (42). To test for hormesis researchers must compare the effect of small doses with the response of the non-treated control. Therefore, there should always be background incidence in the control, without background incidence there is no way to detect a stimulus (80). Evaluation of data is very important when proving hormesis. Crump suggests the criteria for evaluating hormesis as follows: strength of evidence, soundness of data, consistency and biological plausibility (86). Statistical analyses should be performed in order to differentiate a small stimulus from background occurrence.

Different methods have been used throughout the years for the detection and estimation of hormesis including parametric, non-parametric, and model-based approaches. The hormetic zone of a dose-response curve follows a non-monotonic relationship between two variables, similar to what is known as umbrella alternatives. Umbrella alternatives are important in many fields of science; a classical example is the ability of learning as a function of age in humans (87). As we grow older our ability to learn new things reaches a peak and later declines. Tests for umbrella alternatives can be used to detect if a dose-response curve follows a non-monotonic trend compared to a monotonic one where hormesis would not be present. The firsts to describe a test for umbrella alternatives were Mack and Wolfe (87) who used a non-parametric method where the distribution of the data is not assumed a-priori. In the Mack and Wolfe test (87) the maximum stimulation detected experimentally is compared to the response at all the other doses using Mann-Whitney counts, a test statistic is calculated and compared with simulated critical values to determine if the dose-response is biphasic. Buning and Kossler (88) demonstrated that the Mack and Wolfe (87) test with Mann-Whitney counts is appropriate for testing data with symmetric and medium-up to long tailed distributions but they suggested the use of different two-sample statistics, i.e. Hogg *et al.* (89) and Gastwirth (90), for asymmetric and short-tailed distributions respectively.

For the detection of hormesis there are also parametric analyses which assume a normal distribution of the data and can have more statistical power than non-parametric analyses if such assumptions are correct. Among the parametrical tests we can highlight the one proposed by Buning and Kossler (88), a modification for umbrella alternatives of the test by Barlow *et al.* (91) for monotonic alternatives. And the method by Bailer and Oris (92) that employs generalized linear models for the detection of hormesis. There are also parametric models that can be used to detect the hormetic response. A parametric model is an equation with a finite number of parameters that describe the relationship between two variables, in the case of hormesis it describes a byphasic relationship between dose and response. If the data fits the model within a confidence limit, usually of 95%, it is assumed that hormesis is present. There are some models that can be modified to better fit the hormetic response, including the quadratic function (93), Gompertz function (94, 95) and logistic function (96). Deng *et al.* (97) summarized some of these model-based approaches and developed a method to estimate the magnitude of the hormetic response. When testing for chemical hormesis the Brain and Cousens model (96), based on the logistic function, is the most

commonly used. Further modifications of the Brain and Cousens model have been made by Van Ewijk and Hoekstra (98), and Schabenberger *et al.* (23) in order to allow estimation of different parameters that are relevant for toxicology such as EC_{50}, NOAEL and maximum stimulation dose (MSD). According to Cedergreen *et al.* (99), a deficiency of the Brain and Cousens model is that if the slope at the EC_{50} (β) is smaller than 1 then the model will not yield a curve; therefore they proposed a model that circumvents this drawback. Cedergreen *et al.* (99) provide a robust detection method but it does not estimate the EC_{50}, NOAEL or MSD. In general, a combination of methods may be used to detect hormesis, determine its magnitude and estimate the EC_{50}, NOAEL and MSD, depending on the type of data that is being analyzed and on the behavior of the dose-response curve.

When testing for the stimulation at low doses of chemicals on fungal plant pathogens, Flores and Garzon (74) used a model-based approach. The Brain and Cousens model was appropriate for this case where most of the dose-response datasets analyzed yielded a β higher than 1. Because of the relevance of EC_{50}, NOAEL, and MSD for disease management, the modified model by Schabenberger *et al.* (23) was used. Flores and Garzon (74) tested 9 to 11 doses of different chemicals including pesticides and disinfectants on *Pythium aphanidermatum* and *Rhizoctonia zeae* with at least five doses below the NOAEL. Datasets were analyzed using a non-linear modeling procedure (PROC-NLIN, SAS 9.2, SAS Institute, Cary, NC) and EC_{50}, NOAEL, and MSD were estimated as described by Schabenberger *et al.* (23). The procedure yielded dose-response curves that properly described the behavior of the data and showed their biphasic nature (Fig 4).
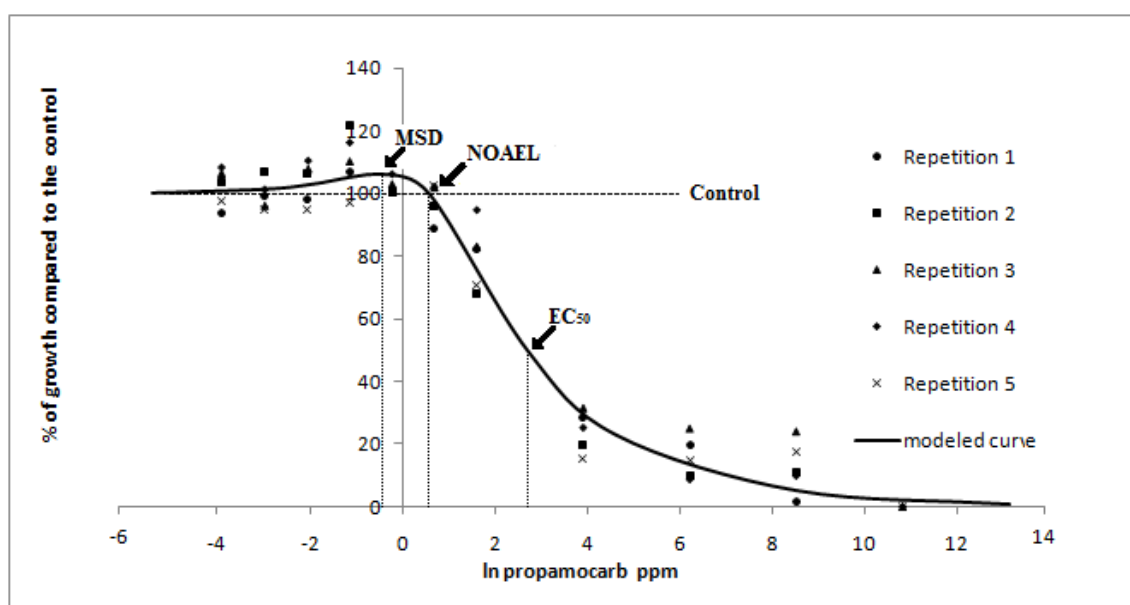
Figure 4. Observed values and modeled curve of the radial growth *in vitro* of *P. aphanidermatum* in response to low doses of propamocarb. Radial growth is expressed as percentages relative to a non-amended control, and concentrations as natural logarithm of ppm. Each data point in the figure represents the mean value of five replicates. Arrows indicate the natural logarithm of the EC_{50} (14.91 ppm), NOAEL (1.77 ppm) and MSD (0.64 ppm). The slope of the curve at the EC_{50} (β) was 1.37 and the rate of increase at low doses (γ) was 35.17. Low-dose stimulation was observed (Flores and Garzon, [74]). Figure reproduction authorized by Dose-Response.

8. Why is chemical hormesis relevant for crop management?

Multiple chemicals with distinct modes of action are available for management of fungal and oomycete diseases. Although integrated disease management is practiced extensively, the productivity of many agricultural systems relies strongly on chemical control. The limited access to registered products for certain agricultural environments, such as greenhouses, as well as inappropriate use has led to the emergence of fungicide resistant strains in multiple species (100, 101, 102, 103, 104, 105, 106). Currently, the effects of subinhibitory doses of fungicides on fungal plant pathogens are unknown. The evidence gathered from literature indicates that stimulation of fungi and oomycetes by sub-inhibitory doses of fungicides has been observed in ascomycetes (64, 65, 67, 68), basidiomycetes (2, 69, 74), as well as in oomycetes (66, 70, 71, 72, 73, 74). Several fitness factors could be affected for the benefit of pathogens, including mycelial growth, spore germination, toxin production and pathogenicity (66, 67, 68, 74). Exposure to subinhibitory doses can occur accidentally in agricultural fields, orchards, nurseries and greenhouses, under diverse circumstances, such as inappropriate fungicide application, low-dose applications to reduce costs, presence of fungicide resistant strains, etc. Thereafter, the possibility of fungal pathogen stimulation due to fungicide hormesis in actual agricultural scenarios is real. The potential effects of fungicide hormesis are highly detrimental, since it could result in larger crop losses, reduced seed and crop quality, higher mycotoxin levels in grain, and wasteful use of fungicides.

In spite of the potential detrimental effects of fungicide hormesis on fungal plant pathogens to agricultural productivity, a complete lack of awareness has meant the exclusion of this important concept from the design of disease chemical management strategies. In some fungicide-pathogen systems, the value of the EC_{50} can be different when hormesis is included in the analysis, hence it is important to consider this concept to avoid bias in EC_{50} calculations. Awareness of the risk taken by growers by the inappropriate use of reduced-dose fungicides (reduced-dose fungicides can be used in combination with two or more other formulations, with different active ingredients [107]) and careless chemical application will help to promote the use of best-management practices and responsible use of fungicides.

More research is needed to understand the processes involved in fungicide hormesis, the prevalence of hormesis in fungi and oomycete species and populations, fungicide class risks, whether mixtures can prevent stimulation, etc. Hormesis is not a new concept but its use in plant pathology is recent, and its application to disease management may open new opportunities to improve plan health and crop productivity.

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9. References

[1] Schulz H 1888. Ueber mefegifte. Plügers Archiv fur die gesamte Physiologic 42:517.

[2] Southam C.M. and Erhlich J. 1943. Effects of extracts of western red-cedar heartwood on certain wood-decaying fungi in culture. Phytopathology 33:517–524.

[3] Stebbing A. R. 1982. Hormesis the stimulation of growth by low levels of inhibitors. The Science of the Total Environment 3:213-234.

[4] Calabrese E.J. and Baldwin L.A. 1997b. The dose determines the stimulation (and poison): Development of a chemical hormesis database. International Journal of Toxicology 16: 545-559.

[5] Calabrese E. J. and Baldwin L. A. 1999. Chemical hormesis: its historical foundations as a biological hypothesis. Toxicologic Pathology 27:195-216.

[6] Calabrese E.J. and Baldwin L.A. 2001a. Radiation hormesis: its historical foundations as a biological hypothesis. Human and Experimental Toxicology 19:41-75.

[7] Calabrese E.J. and Baldwin L.A. 2001b U-shaped dose-responses in Biology, Toxicology, and Public Health. Annual Review of Public Health 22:15-33.

[8] Calabrese E.J. and Baldwin L.A. 2001c. Hormesis: a generalizable and unifying hypothesis. Critical Reviews in Toxicology 31:353-424.

[9] Calabrese E.J. and Baldwin L.A. 2003a. Hormesis: the dose-response revolution. Annual Review of Pharmacology and Toxicology 43: 175-197.

[10] Barcelo J. and Poschenrieder C. 2002. Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review. Environmental and Experimental Botany 48: 75–92.

[11] Calabrese E. J. and Baldwin L. A. 2000. Chemical hormesis: its historical foundations as a biological hypothesis. Human and Experimental Toxicology 1:2-31.

[12] Feinendegen L.E. 2005. Evidence for beneficial low level radiation effects and radiation hormesis. The British Journal of Radiology 78: 3-7.

[13] Rea S.L., Wu D., Cypser J.R., Vaupel J.W. and Johnson T.E. 2005. A stress-sensitive reporter predicts longevity in isogenic populations of *Caenorhabditis elegans*. Nature Genetics 37:894-898.

[14] Cypser J.R. and Johnson T.E. 2002. Multiple stressors in *Caenorhabditis elegans* induce stress hormesis and extended longevity. Journal of Gerontology: Biological Sciences 57A: B109-B114.

[15] Gomez-Cabrera M.C., Domenec E. and Vina J. 2008. Moderate exercise is an antioxidant: Upregulation of antioxidant genes by training. Free Radical Biology and Medicine 44:126-131.

[16] Calabrese E.J. and Baldwin L.A. 2003. Ethanol and hormesis. Critical Reviews in Toxicology 33:407-424.

[17] Calabrese E.J. and Baldwin L.A. 2003. Toxicology rethinks its central belief. Nature 421: 691-692.

[18] Hayes A.W. 2008. Principles and Methods of Toxicology. Informa Healthcare U.S.A. Inc. New York.

[19] Yu X.K., Zhao W.M., Ma J.F., Fu X.Q. and Zhao Z.Z.J. 2011. Beneficial and harmful effects of alcohol exposure on *Caenorhabditis elegans* worms. Biochemical and Biophysical Research Communications 412: 757-762.

[20] Sridhary K.R. and Bärlocher F. 2011. Reproduction of aquatic hyphomycetes at low concentrations of Ca2, Zn2, Cu2, and Cd. Environmental Toxicology and Chemistry 30: 2868–2873.

[21] Branham, S. E. 1929. The effects of certain chemical compounds upon the course of gas production by baker's yeast. Journal of Bacteriology 4:247-264.

[22] Virchow R. 1854. Uber die Erregbarkeit der Flimmerzellen. Virch Arch 6:133-134. In: Henschler D. (2006). The origin of hormesis: historical background and driving forces. Human and Experimental Toxicology 25: 347-351.

[23] Schabenberger O., Tharp B. E., Kells J. J., and Penner D. 1999. Statistical tests for hormesis and effective dosages in herbicide dose-response. Agronomy Journal 91:713-721.

[24] Mattson M.T. and Calabrese E.J. 2010. Hormesis: A Revolution in Biology, Toxicology and Medicine. Humana Press, Springer New York.

[25] Reveil P.O.1865. Recherches de physiologie vegetale. In: De l'action des poisons sur les plantes. Paris.

[26] Hueppe F. 1896. Principles of Bacteriology. Open Court Publishing Company, Chicago. In: Calabrese EJ and Baldwin LA (1999). Chemical Hormesis: Its Historical Foundations as a Biological Hypothesis. Toxicologic Pathology 27:195-216.

[27] Randall W.A., Price C.W., and Welch H. 1947. Demonstration of hormesis (increase in fatality rate) by penicillin. American Journal of Public Health 374:421-425.

[28] Campbell, C. C., and S. Saslaw. 1949. Enhancement of growth of certain fungi by streptomycin. Proceedings Of The Society For Experimental Biology And Medicine 3:562-562.

[29] Prichard H. M. 1984. Air ionization and radiation hormesis. Health Physics 46: 1139-1140.

[30] Hickey, R. J., Bowers E. J., and Clelland R. C. 1983. Radiation hormesis, public health, and public policy: a commentary. Health Physics 44:207-219

[31] Roessler G.S. 1987. Radiation Hormesis. Health Physics 52: 517.

[32] Cohen J.J. 1987. Conference on radiation hormesis- Overview. Health Physics 52:519.

[33] Shu Z. L., Liu W. H., Sun J. B. 1987. Radiation hormesis- Its expression in the immune system. Health Physics 52: 579-583.

[34] Miller M. W. 1987. Radiation hormesis in plants. Health Physics 52: 607-616.

[35] Weis P. and Weis J. S. 1986. Cadmium acclimation and hormesis in *Fundulus heteroclitus* during fin regeneration. Environmental Research 39: 356-363.

[36] Furst A. 1987. Hormetic Effects in pharmacology – Pharmacological inversions as prototypes for hormesis. Health Physics 52: 527-530.

[37] Calabrese E. J., McCarthy M. E. and Kenyon E. 1987. The occurrence of chemically induced hormesis. Health Physics 52: 531-541.

[38] Sagan L. A. 1989. On radiation, paradigms, and hormesis. Science 245: 574.

[39] Calabrese E. J. and Howe K. J. 1976. Stimulation of growth of peppermint (*Mentha piperita*) by phosfon, a growth retardant. Physiologia Plantarum 37: 163-165.

[40] Calabrese E. J. and Baldwin L. A. 1993. Possible examples of chemical hormesis in a previously published study. Journal of Applied Toxicology 13: 169-172.

[41] Calabrese E. J. and Baldwin L. A. 1996. Expanding the RfD concept to incorporate and optimize beneficial effects while preventing toxic responses from nonessential toxicants. Ecotoxicology and Environmental Safety 34: 94-101.

[42] Calabrese E. J. and Baldwin L. A. 1997a. A quantitatively-based methodology for the evaluation of chemical hormesis. Human and Ecological Risk Assessment 4:545-554.

[43] Masoro E. J. 2005. Overview of caloric restriction and ageing. Mechanisms of Ageing and Development 126: 913-922.

[44] Ji L. L., Gomez-Cabrera M. C., Vina J. 2006. Exercise and Hormesis Activation of Cellular Antioxidant Signaling Pathway. In: Understanding and modulating aging. Annals of the New York Academy of Sciences Vol. 1067, p. 425-435.

[45] Hayes D. P. 2007. Nutritional hormesis. European Journal of Clinical Nutrition 61: 147-159.

[46] Ristow M., Zarse K., Oberbach A., Kloting N., Birringer M., Kiehntopf M., Stumvoll M., Kahn C. R. and Bluher M. 2009. Antioxidants prevent health-promoting effects of physical exercise in humans. Proceedings of the National Academy of Sciences of the United States of America 106: 8665-8670.

[47] Tlili, A., Montuelle, B., Berard, A., and Bouchez, A. 2011. Impact of chronic and acute pesticide exposures on periphyton communities. Science of the Total Environment 409: 2102-2113.

[48] Calabrese, E. J., and Blain R. B. 2009. Hormesis and plant biology. Environmental Pollution 1:42-48.

[49] Velini E. D., Alves E., Godoy M. C., Meschede D. K., Souza R. T., Duke S. O. 2008. Glyphosate applied at low doses can stimulate plant growth. Pest Management Science 64: 489-496.

[50] Migliore L., Cozzolino S., Fiori M. 2003. Phytotoxicity to and uptake of enrofloxacin in crop plants. Chemosphere 52: 1233-1244.

[51] Roebuck BD, Yager Jr. JD, Longnecker DS. 1981. Dietary modulation of azaserine-induced pancreatic carcinogenesis in the rat. Cancer Reserach 41: 888–893.

[52] Johnson, J.B., Summer, W., Cutler, R.G., Martin, B., Hyun, D.H., Dixit, V.D., Pearson, M., Nassar, M., Tellejohan, R., Maudsley, S., Carlson, O., John S, Laub DR, Mattson MP. 2007. Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. Free Radical Biology Medicine 42: 665–674.

[53] Kinoshita N., Sueki K., Sasa K., Kitagawa J., Ikarashi S., Nishimura T., Wong Y. S., Satou Y., Handa K., Takahashi T., Sato M. and Yamagata T. 2011. Assessment of individual radionuclide distributions from the Fukushima nuclear accident covering central-east Japan. Proceedings of the National Academy of Sciences of the United States of America 108:19526–19529.

[54] Guedes R. N. C., Magalhaes L. C., and Cosme L. V. 2009. Stimulatory sublethal response of a generalist predator to permethrin: hormesis, hormoligosis, or homeostatic regulation? Journal of Economic Entomology 102: 170-176.

[55] Hotchkiss M. 1923. Studies on salt action. Journal of Bacteriology. 8:141-162. In Stebbing, A. R. (1982). Hormesis - The stimulation of growth by low levels of inhibitors. The Science of the Total Environment 3:213-234.

[56] Miller, W. S., C. A. Green, and H. Kitchen. 1945. Biphasic action of Penicillin and other sulphonamide similarity. Nature 1:210-211.

[57] Linares J. F., Gustafsson I., Baquero F., Martinez J. L. 2006. Antibiotics as intermicrobial signaling agents instead of weapons. Proceedings of the National Academy of Sciences of the United States of America 103: 19484-19489.

[58] Wang J. X., Xie P., Guo N. 2007. Effects of nonylphenol on the growth and microcystin production of Microcystis strains. Environmental Research 103: 70-78.

[59] Gong Y., Song L. R., Wu X. Q., Xiao B. D., Fang T., Liu J. T. 2009. Effects of arsenate on microcystin content and leakage of Microcystis Strain PCC7806 under various phosphate regimes. Environmental Toxicology 24: 87-94.

[60] Hong Y., Hong-Ying H., Li F. M. 2008. Growth and physiological responses of freshwater green alga Selenastrum capricornutum to allelochemical ethyl 2-methyl acetoacetate (EMA) under different initial algal densities. Pesticide Biochemistry and Physiology 90: 203-212.

[61] Calabrese E. J., Staudenmayer J. W., Stanek E. J. 2006. Hormesis outperforms threshold model in National Cancer Institute antitumor drug screening database. Toxicological Sciences 94: 368-378.

[62] Mesquita A., Weinberger M., Silva A., Sampaio-Marques B., Almeida B., Leao C., Costa V., Rodrigues F., Burhans W. C., Ludovico P. 2010. Caloric restriction or catalase inactivation extends yeast chronological lifespan by inducing H2O2 and superoxide dismutase activity. Proceedings of the National Academy of Sciences of the United States of America 107: 15123-15128.

[63] Stevens C., Khan V. A., Lu J. Y., Wilson C. L., Pusey P. L., Kabwe M. K., Igwegbe E. C. K., Chalutz E., and Droby S.1998. The germicidal and hermetic effects of UV-C light on reducing brown rot disease and yeast microflora of peaches. Crop Protection 17: 75-84.

[64] Hessayon D. G. 1951. Double-action of trichothecin and its production in soil. Nature 4284:998-999.

[65] Hessayon, D. G. 1953. Fungitoxins in the soil: II Trichothecin, its production and inactivation in unsterilized soils. Soil Science 5:395-404.

[66] Moorman, G. W. 2011. Sublethal doses of mefenoxam enhance Pythium damping-off of geranium. Plant Disease 95:1233-1238.

[67] Baraldi E., Mari M., Chierici E., Pondrelli M., Bertolini P. and Pratella G. C. 2003. Studies on thiabendazole resistance of *Penicillium expansum* of pears: pathogenic fitness and genetic characterization. Plant Pathology 52: 362–370.

[68] Audenaert K., Callewaert E., Höfte M., De Saeger S., and Haesaert G. 2010. Hydrogen peroxide induced by the fungicide prothioconazole triggers deoxynivalenol (DON) production by *Fusarium graminearum.* BMC Microbiology 10:112.

[69] Landry J., Martinez C. and Rochefort L. 2011. The use of fungicide Nova to mitigate infection of *Sphagnum* by parasitic fungi in the greenhouse. Botany 89: 655-661.

[70] Fenn M. E. and Coffey M. D. 1984. Studies on the *in vitro* and *in vivo* antifungal activity of fosetyl-Al and phosphorous acid. Phytopathology 74:606-611.

[71] Kato S., Coe R., New L., and Dick M. 1990. Sensitivities of various Oomycetes to hymexazol and metalaxyl. Journal of General Microbiology 136: 2127-2134.

[72] Zhang S., Panaccione S. G., Gallegly M. E. 1997. Metalaxyl stimulation of growth of isolates of *Phytophthora infestans*. Mycologia 89: 289-292.

[73] Moorman, G. W., and Kim, S. H. 2004. Species of *Pythium* from greenhouses in Pennsylvania exhibit resistance to propamocarb and mefenoxam. Plant Disease 88:630-632.

[74] Flores F. and Garzon C. D. 2012. Detection and assessment of chemical hormesis on the radial growth in vitro of oomycetes and fungal plant pathogens. Dose-Response *In press.*

[75] Calabrese E.J. 2008. Hormesis and Medicine. British Journal of Clinical Pharmacology 66: 594-617.

[76] Davies J. M., Lowry C. V., Davies K. J. 1995. Transcient adaptation to oxidative stressing yeast. Archives of Biochemestry and Biophysics 317: 1–6.

[77] Matchett J. A. and Erickson C.K. 1977. Alteration of ethanolinduced changes in locomotor activity by adrenergic blockers in mice. Psychopharmacology 52:201–206.

[78] Baumgarten H. G., Lachenmayer A., Bjorklund A., Nobin A., and Rosengren E. 1973. Long-term recovery of serotonin concentrations in the rat CNS following 5,6-dihydroxytryptamine. Life Sciences 12:357–364.

[79] Stebbing A. R. 2009. Interpreting 'Dose-Response' curves using homeodynamic data: with an improved explanation for hormesis. Dose-Response 7: 221-233.

[80] Conolly, R. B., and Lutz W. K. 2004. Nonmonotonic dose-response relationships: Mechanistic basis, kinetic modeling, and implications for risk assessment. Toxicological Sciences 1:151-157.

[81] Gómez-Icazbalceta G., Huerta L., Soto-Ramirez L. E., and Larralde C. 2007. Extracellular HIV-1 Nef protein modulates lytic activity and proliferation of human CD8+ T lymphocytes. Cellular Immunology 1-2:85-90.

[82] Kushida M., Sukata T., Uwagawa S., Ozaki K., Kinoshita A., Wanibuchi H., Morimura K., Okuno Y., and Fukushima S. 2005. Low dose DDT inhibition of hepatocarcinogenesis initiated by diethylnitrosamine in male rats: Possible mechanisms. Toxicology and Applied Pharmacology 3:285-294.

[83] Bae O. N., Lim K. M., Han J. Y., Jung B. I., Lee J. Y., Noh J. Y., Chung S. M., Lee M. Y. and Chung J. H.. 2008. U-shaped dose-response in vasomotor tone: A mixed result of heterogenic response of multiple cells to xenobiotics. Toxicological Sciences 1:181-190.

[84] Allender W. J. 1997. Effect of trifluoperazine and verapamil on herbicide stimulated growth of cotton. Journal of Plant Nutrition 1:69-80.

[85] Allender W. J., Cresswell G. C., Kaldor J. and Kennedy I. R. 1997. Effect of lithium and lanthanum on herbicide induced hormesis in hydroponically-grown cotton and corn. Journal of Plant Nutrition 1:81-95.

[86] Crump K. 2001. Evaluating the evidence for hormesis: A statistical perspective. Human and Ecological Risk Assessment 4:781.

[87] Mack, G. A., and D. A. Wolfe. 1981. K-Sample Rank Tests for Umbrella Alternatives. Journal of the American Statistical Association 76:175-181

[88] Buning, H., and W. Kossler. 1997. Power of some tests for umbrella alternatives in the multi-sample location problem. Biometrical Journal 39:481-494

[89] Hogg, R. V., D. M. Fisher, and R. H. Randles. 1975. A Two-Sample Adaptive Distribution-Free Test. Journal of the American Statistical Association 70:656-661

[90] Gastwirth, J. L. 1965. Percentile Modifications of Two Sample Rank Tests. Journal of the American Statistical Association 60(312):1127-1141

[91] Barlow, R. E. 1972. Statistical inference under order restrictions: the theory and application of isotonic regression. J. Wiley, 388p.

[92] Bailer, A. J., and J. T. Oris. 1997. Estimating inhibition concentrations for different response scales using generalized linear models. Environmental Toxicology and Chemistry 16:1554-1559

[93] Hickey, R. J., E. J. Bowers, and R. C. Clelland. 1983. Radiation hormesis, public health, and public policy: a commentary. Health Phys 44(3):207-19

[94] Boxenbaum, H., P. J. Neafsey, and D. J. Fournier. 1988. Hormesis, Gompertz functions, and risk assessment. Drug Metab Rev 19:195-229

[95] Neafsey, P. J., H. Boxenbaum, D. A. Ciraulo, and D. J. Fournier. 1988. A Gompertz age-specific mortality rate model of aging, hormesis, and toxicity: fixed-dose studies. Drug Metab Rev 19:369-401

[96] Brain, P., and R. Cousens. 1989. An equation to describe dose-responses where there is stimulation of growth at low doses. Weed Research 29:93-96

[97] Deng, C. Q., R. Graham, and R. Shukla. 2001. Detecting and estimating hormesis using a model-based approach. Human and Ecological Risk Assessment 7(4):849-866

[98] Van Ewijk, P. H., and J. A. Hoekstra. 1993. Calculation of the EC50 and its confidence interval when subtoxic stimulus is present. Ecotoxicology and Environmental Safety 25:25-32

[99] Cedergreen, N., C. Ritz, and J. C. Streibig. 2005. Improved empirical models describing hormesis. Environmental Toxicology and Chemistry 24(12):3166-3172

[100] Albourie, J. M., Tourvieille, J., and de Labrouhe, D. T. 1998. Resistance to metalaxyl in isolates of the sunflower pathogen Plasmopara halstedii. European Journal of Plant Pathology, 104: 235-242.

[101] Lamour, K. H. and Hausbeck, M. K. 2003. Susceptibility of mefenoxam-treated cucurbits to isolates of *Phytophthora capsici* sensitive and insensitive to mefenoxam. Plant Disease, 87: 920-922.

[102] Moorman, G. W., Kang, S., Geiser, D. M., and Kim, S. H. 2002. Identification and characterization of *Pythium* species associated with greenhouse floral crops in Pennsylvania. Plant Disease, 86: 1227-1231

[103] Taylor, R. J., Salas, B., Secor, G. A., Rivera, V., and Gudmestad, N. C. 2002. Sensitivity of north American isolates of Phytophthora erythroseptica and Pythium ultimum to mefenoxam (metalaxyl). Plant Disease, 86: 797-802.

[104] Grunwald, N. J., Sturbaum, A. K., Montes, G. R., Serrano, E. G., Lozoya-Saldana, H., and Fry, W. E.2006. Selection for fungicide resistance within a growing season in field populations of *Phytophthora infestans* at the center of origin. Phytopathology, 96: 1397-1403.

[105] Gisi, U. and Sierotzki, H. 2008. Fungicide modes of action and resistance in downy mildews. European Journal of Plant Pathology, 122: 157-167.

[106] FRAC 2011.FRAC list of plant pathogenic organisms resistant to disease control agents. Fungicide Resistance Action Committee, 71p.

[107] Moorman, G. W., and Lease, R. J. 1992. Benzimidazole- and dicarboximide-resistant Botrytis cinerea from Pennsylvania greenhouses. Plant Dis. 76:477-480.