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Special Report

Significant Influence of EC_{50} Estimation by Model Choice and EC_{50} Type

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Abstract

The effective control to 50% growth inhibition (EC_{50}) is a standard statistic for evaluating dose-response relationships. Many statistical software packages are available to estimate dose-response relationships but, recently, an open source package ("drc") in R has been utilized. This package is highly adaptable, having many models to describe dose-response relationships and flexibility to describe both hormetic relationships and absolute and relative EC_{50}. These models and definitions are generally left out of phytopathology literature. Here, we demonstrate that model choice and type of EC_{50} (relative versus absolute) can matter for EC_{50} estimation using data from *Pythium oopapillum* and *Fusarium virguliforme*. For some *P. oopapillum* isolates, the difference between absolute and relative EC_{50} was significant. Hormetic effects changed *F. virguliforme* EC_{50} distributions, leading to higher estimates than when using four- or three-parameter log-logistic models. Future studies should pay careful attention to model selection and interpretation in EC_{50} estimation and clearly indicate which model and EC_{50} measure (relative versus absolute) was used. We provide guidelines for model choice and interpretation for those wishing to set up experiments for accurate EC_{50} estimation.

Evaluating the effect of a fungicide in vitro is a standard bioassay to determine chemistries that influence growth of fungi and oomycete pathogens. According to the Fungicide Resistance Action Committee (FRAC), EC_{50} is defined as the fungicide dose that inhibits growth by 50% when compared with a nonamended control (http://www.frac.info/resistance-overview). This is estimated by analyzing dose-response curves. EC_{50} is not to be confused with ED_{50}, which describes the median effective dose, or the fungicide dose that gives a desired effect for 50% of a population, and should be used to describe in vivo dose-response relationships, not in vitro (Neubig et al. 2003). The two terms are used interchangeably in plant pathology literature but, for the purposes of consistency and simplicity, we will use the term EC_{50} as defined by FRAC. Typically, the EC_{50} is used to assess fungicide sensitivity shifts in pathogen populations over time as a means of assessing selection toward a less sensitive population. These monitored shifts in sensitivity are used to advise management decision for growers; thus, accuracy and clarity in describing these terms is important.

An amended medium assay, also called a poison plate assay, is a simple method used to estimate EC_{50} and can be applied to all culturable filamentous fungi and oomycetes. In this assay, fungicide is amended into agar medium at known concentrations, and the response (a measure of colony growth or spore germination) is recorded after a specified time interval. Despite the simplicity of the assay, estimating the EC_{50} may not be as simple because choice of analysis method can significantly impact data analysis and interpretation. Dose-response data are often analyzed by regressing the response against the log-transformed concentrations. These data result in a sigmoidal shape that can be described using nonlinear regressions. Statistical software such as Sigma-Plot (Systat Software, Inc., San Jose, CA), Prism (GraphPad Software, Inc., La Jolla, CA), and SAS (SAS Institute, Cary, NC) are used to make these nonlinear regressions easier to perform and interpret. These three software options are useful and robust to describe dose-response relationships but require commercial or academic licensure, which limits their use in some settings. Statistical packages in R have become popular within the academic community to assess EC_{50} due to the open source framework of the R language (R Core Team 2017) and workflow reproducibility when integrated with code repositories such as GitHub (https://github.com/) or Bitbucket (https://bitbucket.org/). The package "drc" (Ritz and Streibig 2005) was specifically written for estimating dose-response relationships across many disciplines, and many plant pathology labs use "drc" to estimate EC_{50} (Kunova et al. 2014; Saville et al. 2015; Stewart et al. 2014; Wang et al. 2017).

The "drc" package is robust in describing symmetric and asymmetric dose-response relationships, including those that describe hormetic effects (Brain and Cousens 1989; Cedergreen et al. 2005; Knezevic et al. 2007) (Fig. 1). Hormesis is observed in amended medium assays when isolates grown on low fungicide concentrations have a positive growth response, greater than a nonamended control (Fig. 1D). Symmetric dose-response curves describe relationships that can be modeled showing symmetry around an inflection point, whereas asymmetric dose-response curves do not have this property. Asymmetric models are flexible to estimate different response relationships at low and high doses. To estimate EC_{50}, dose-response models generally use the following parameters: the upper asymptote, lower asymptote, and slope (Table 1). The *f* parameter, in hormetic models, indicates the rate at which growth stimulation occurs (Belz and Piepho 2012). Other models that include a fifth parameter are beyond the scope of this study. In symmetrical dose-response curves, the relative EC_{50} is the inflection point, or when the second derivative equals zero (Kenakin 2009). More simply, this point is where the curve goes from concave-down to concave-up, and is the midpoint between the upper and lower asymptotes of the curve. The resulting concentration that corresponds to this point is the concentration modeled to be the point at which half maximal response occurs (Sebaugh 2011). This modeled concentration is not necessarily representative of the EC_{50} defined by FRAC. These two definitions describe two

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different meanings of EC_{50}: relative and absolute. Absolute EC_{50} is the concentration on a dose-response curve where the response equals 50% of the maximum response and is more consistent with the FRAC-defined EC_{50}, whereas relative EC_{50} is a model parameter on a dose-response curve. In other words, the relative EC_{50} is the inflection point on a dose-response curve, whereas the absolute EC_{50} is the concentration at which 50% maximal growth occurs. The two terms are identical in a four-parameter log-logistic model when the upper and lower asymptotes equal 100 and 0%, respectively (Fig. 1). The default settings of the "ED" function of "drc" estimate the relative EC_{50}.

The most popular and widely accepted models to describe dose-response relationships are the four- and three-parameter log-logistic models (Ritz et al. 2015). The four-parameter log-logistic model becomes a three-parameter model when the lower asymptote is fixed at 0%. When the upper and lower asymptote are fixed at 100 and 0%,

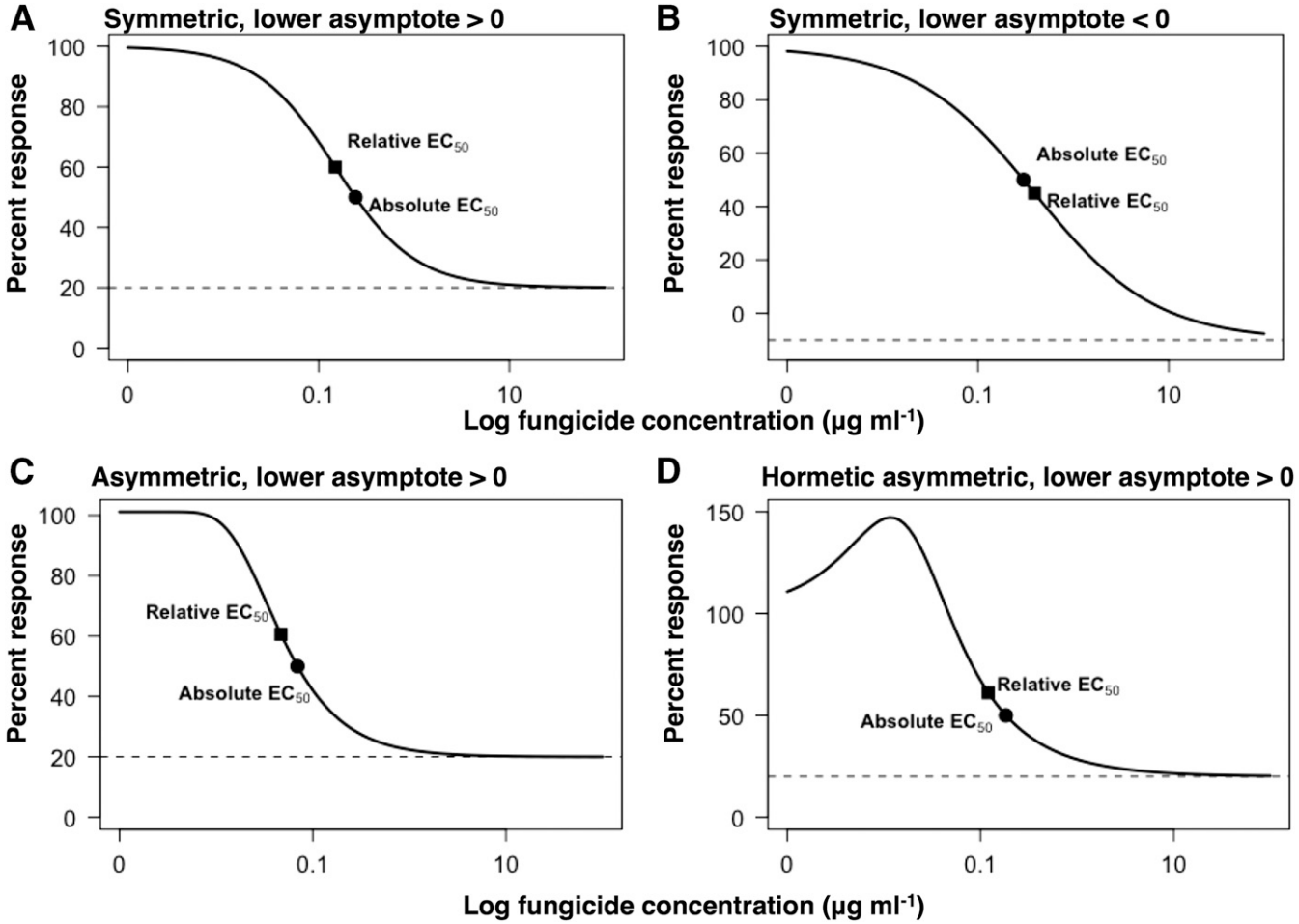

Fig. 1. Properties of symmetric, asymmetric, and hormetic dose-response curves in relation to absolute and relative effective control to 50% growth inhibition (EC_{50}). The relative EC_{50} is the inflection point on a dose-response curve whereas the absolute EC_{50} is the concentration at which 50% maximal growth occurs. **A** and **B,** Symmetric dose-response curves, like log-logistic curves, show symmetry about an inflection point (i.e., the relative EC_{50}). **C,** Asymmetric dose-response curves, like Weibull curves, do not show symmetry about an inflection point. **D,** Asymmetric hormetic curves show a growth stimulation at low doses. When the lower asymptote (dotted line) is modeled >0 (A, C, and D), the relative EC_{50} is less than the absolute EC_{50}. The opposite is true when the lower asymptote is modeled as <0 (B). When the lower and upper asymptotes are fixed at 100 and 0, respectively, the relative and absolute EC_{50} are equal.

Table 1. Models and description of parameters used

Model, "drc"[a]	Model name	Model type[b]	Equation[c]
LL.4	Four-parameter log-logistic	Symmetric	$f(x)=c+\frac{d-c}{1+\exp((b(\log(x)-\log(e)))}$
LL.3	Three-parameter log-logistic	Symmetric	$f(x)=\frac{d}{1+\exp((b(\log(x)-\log(e)))}$
LL.2	Two-parameter log-logistic	Symmetric	$f(x)=\frac{1}{1+\exp((b(\log(x)-\log(e)))}$
BC.4	Brain and Cousens	Asymmetric hormetic	$f(x)=c+\frac{d-c+fx}{1+\exp((b(\log(x)-\log(e)))}$
CRS.4c	Four-parameter Cedergreen-Ritz-Streibig	Asymmetric hormetic	$f(x)=c+\frac{d-c+fexp(-1/x^{0.25})}{1+\exp((b(\log(x)-\log(e)))}$
W2.4	Four-parameter Weibull2	Asymmetric	$f(x)=c+(d-c)(1-exp(-exp(b(log(x)-log(e)))))$

[a] Open source package "drc" in R.
[b] Symmetric and asymmetric indicate that the curve is symmetric or asymmetric about the upper and lower asymptotes, respectively. Hormetic describes growth stimulation at low doses.
[c] Definitions: d = lower asymptote, c = upper asymptote, b = slope, e = effective control to 50% growth inhibition (EC_{50}), and x = fungicide dosage.

respectively, the four-parameter model is reduced to a two-parameter model. A two-parameter model is not useful to describe continuous dose-response data, and is usually reserved for binary data (dead or alive, germinated or not germinated, and so on) (Ritz et al. 2015). Hormetic models such as the Brain and Cousens model and the Cedergreen-Ritz-Streibig model are derivations of the log-logistic models that include the ability to describe growth simulation at low doses (Brain and Cousens 1989; Cedergreen et al. 2005). Hormesis can be practically important because sublethal applications of mefenoxam can increase the growth and virulence of mefenoxam-resistant *Pythium aphanidermatum* (Garzón et al. 2011). The models and descriptions presented here are not comprehensive but, rather, attempt to illustrate some of their properties in relation to plant pathology. Garzón and Flores (2013) describe the hormetic effect in the context of plant pathology in more detail. For more descriptions of the models addressed, use of "drc", and general discussion on dose-response statistics we recommend Ritz et al. (2015), Ritz and Streibig (2005), Knezevic et al. (2007), and Sebaugh (2011).

The objectives of the following research are to (i) investigate whether relative or absolute EC_{50} can change the practical interpretation of fungicide sensitivity in a pathogen population, (ii) understand whether model choice and hormetic effects may change the interpretation of a population, and (iii) provide practical guidelines for plant pathologists performing fungicide sensitivity studies.

Materials and Methods

Oomycete and fungal isolates. *P. oopapillum* isolates were collected in 2011 and 2012 from symptomatic soybean roots in fields with a history of stand establishment issues across 11 states in the Midwest by collaborators of the National Institute of Food and Agriculture–Oomycete Soybean Cooperative Agricultural Project (Rojas et al. 2017). From this collection, 70 isolates of *P. oopapillum* from nine states were used in this study. *Fusarium virguliforme* isolates (n = 27) were collected from commercially grown soybean fields from Michigan (n = 20), Arkansas (n = 2), Kansas (n = 2), Indiana (n = 1), Illinois (n = 1), and Iowa (n = 1) (Wang et al. 2017).

Amended media assays. Formulated-grade mefenoxam (Apron XL; Syngenta Crop Protection Inc., Greensboro, NC) was dissolved in distilled H_2O and passed through a 0.22-μm filter to prepare stock solutions. Formulated-grade fluopyram (Luna Privilege; Bayer CropScience LP, Research Triangle Park, NC) was used to prepare stock solutions. Stock solutions of active ingredients (a.i.) were added to molten medium after autoclaving and the medium had cooled to 50 to 55°C.

P. oopapillum isolates were evaluated with mefenoxam at 0.01, 0.1, 0.5, and 1 μg a.i. ml^{-1} in half-strength V8 medium (82 ml of V8 juice filtered through eight layers of cheesecloth, 0.5 g of $CaCO_3$, 918 ml of distilled water, and 17 g of agar), and nonamended half-strength V8 medium was used as the control. Mycelial plugs (3.7 mm in diameter) from the edge of a 2- to 3-day-old *P. oopapillum* colony, grown on corn meal agar amended with pentachloronitrobenzene (50 mg/liter), ampicillin (250 mg/liter), rifampicin (10 mg/liter), pimaricin (5 mg/liter), and benomyl (10 mg/liter), were placed mycelium side down onto the center of medium in 100-mm Petri plates for each fungicide concentration evaluated. Three replicate plates were used per fungicide concentration. The colony diameter was measured in two perpendicular directions using digital calipers (Mitutoyo, Aurora, IL) after incubating at 24°C for 48 h in the dark. The size of the plug was subtracted from the colony diameter before calculating percent relative response or estimating EC_{50}.

F. virguliforme isolates were evaluated with fluopyram at 1, 5, 10, 25, 50, and 100 μg ml^{-1} in half-strength potato dextrose agar (PDA) (Acumedia, Lansing, MI). Nonamended half-strength PDA was used as the control. Three replicate plates were used per fungicide concentration. Mycelia plugs (2 mm^3) from the edge of 10-day-old fungal colonies were placed mycelium side down on the center of each plate. Plates were incubated at 24°C for 10 days in the dark, and the colony of each isolate was scanned 3 and 10 days after inoculation at 300-dpi image quality with a blue background to provide contrast between the scanned colony and the background. American Phytopathological Society Assess v.2.0 was used to calculate the colony area at each fungicide concentration. A 1-cm^2 reference scale was included in all images to calibrate the ratio between pixel and distance. Growth rate of colony areas was used as the response variable because *F. virguliforme* colonies are irregularly shaped, making colony diameter measurements inaccurate (Wang et al. 2017). Growth rate (square centimeters per day) was calculated by subtracting the colony area after 3 days from the colony area after 10 days divided by seven.

Percent relative response was calculated by dividing the response—colony diameter (*P. oopapillum*) or growth rate (*F. virguliforme*)—by the mean response on the nonamended plate, multiplied by 100. The relative EC_{50} was estimated by fitting the response against the log concentration using a four-, three-, and two-parameter log-logistic model and specifying type = "relative" within the "ED" function of "drc" (Ritz and Streibig 2005). This option solves for the concentration on a dose-response curve at which the inflection point occurs. The absolute EC_{50} was estimated by fitting percent relative growth against the log concentration using a four-, three-, and two-parameter log-logistic model but specifying type = "absolute" within the "ED" function of "drc" (Ritz and Streibig 2005). This option solves for the concentration on the dose-response curve where 50% inhibition takes place. A t test of the estimated EC_{50} was performed using the "comped" function of "drc" to determine whether the difference between the relative and absolute EC_{50} was different than zero.

F. virguliforme isolates with >100% relative growth rate at 1 μg ml^{-1} were defined as isolates with a potential hormetic dose-response relationship (i.e., increased growth rate at low fungicide dosage). Nonlinear regressions of the growth rate against the log-transformed fungicide concentrations using two nonhormetic models and two hormetic models were performed to determine whether hormetic models had a better fit to these data and whether there was an effect on the resulting EC_{50}. The nonhormetic models used were the symmetric four-parameter log-logistic and asymmetric four-parameter Weibull2, specified by setting the "fct" option equal to "LL.4()" and "W2.4()", respectively, within the "drm" function of "drc". The hormetic models used were the four-parameter Brain and Cousens and four-parameter Cedergreen-Ritz-Streibig models, specified by setting the "fct" option equal to "BC.4()" and "CRS.4c()", respectively, within the "drm" function of "drc" (Brain and Cousens 1989; Cedergreen et al. 2005; Ritz and Streibig 2005). The best-fit model was evaluated based on the log-likelihood, Akaike Information Criteria (AIC), and residual sum of squares using the "mselect" function of "drc". The workflow, data, and analysis performed in this study can be found and downloaded at https://github.com/noelzach/fungalEC.

Results

To examine the relationship between the relative and absolute EC_{50}, we analyzed dose-response data of 70 *P. oopapillum* isolates challenged with mefenoxam using a four-, three-, and two-parameter log-logistic model. The difference between the relative and absolute EC_{50} was significantly different than zero for 27% of the isolates tested using a four-parameter log-logistic model. The difference between the absolute and relative EC_{50} could not be calculated for one isolate (isolate 2) because the absolute EC_{50} was outside the concentration range tested. Isolate 43 had the largest difference between relative and absolute EC_{50}, where the relative EC_{50} was 0.27 μg ml^{-1} less than the absolute EC_{50} (Fig. 2). When the absolute EC_{50} was greater than the relative EC_{50}, the lower asymptote of the four-parameter log-logistic model was greater than zero. When the relative EC_{50} was greater than the absolute EC_{50}, the lower parameter of a four-parameter log-logistic model was negative. When the lower asymptote of a four-parameter log-logistic model was fixed at 0% (i.e., a three-parameter log-logistic), the difference between the relative and absolute EC_{50} was not significantly different than zero for any isolate, and there were no examples where the difference was undefined. When the lower and upper limits were fixed at 0 and 100% (i.e., a two-parameter log-logistic), the difference between the relative and absolute EC_{50} was 0 for all isolates (Fig. 2).

P. oopapillum isolates 2 and 5 were chosen for a closer analysis (Fig. 3, Table 2). Isolate 2 represents a scenario where the absolute

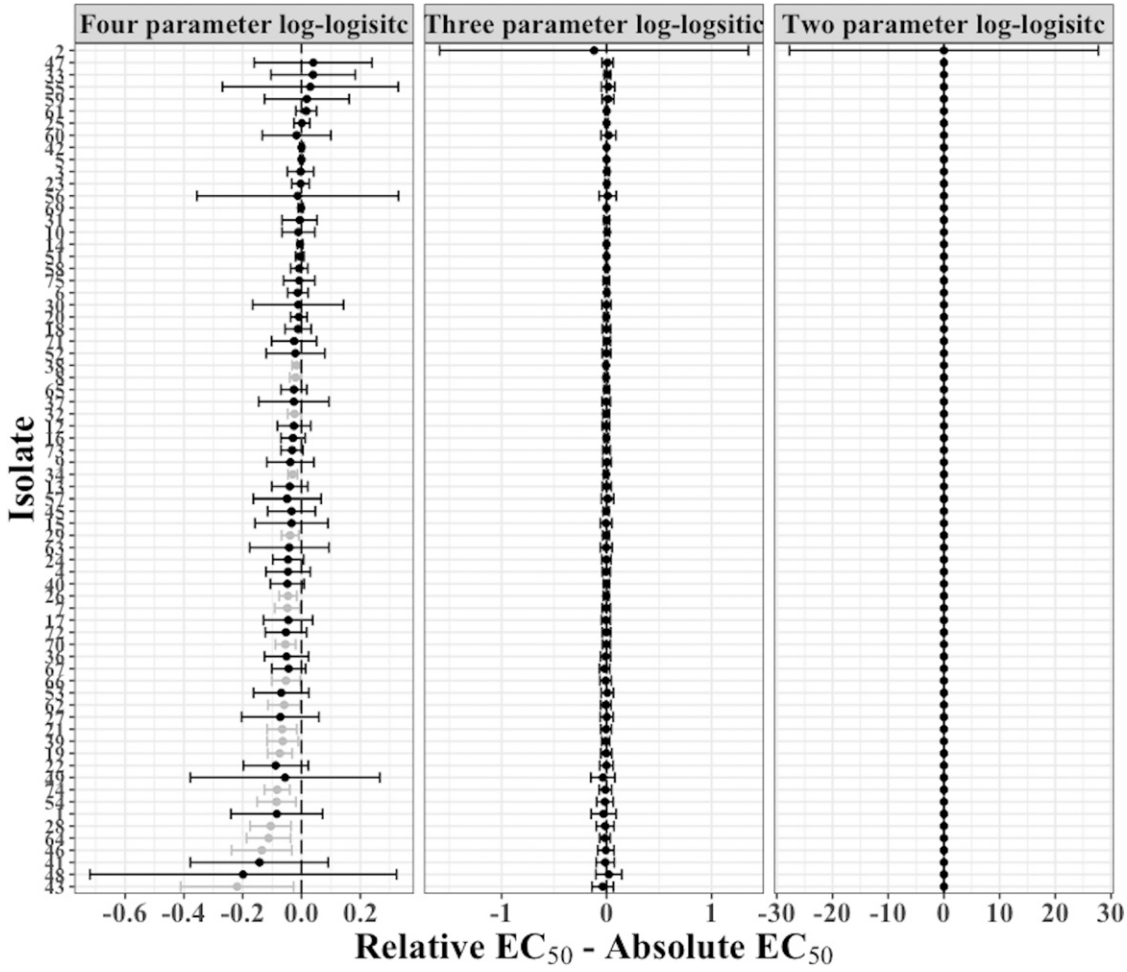

Fig. 2. Difference between relative and absolute effective control to 50% growth inhibition (EC_{50}) of 70 *Pythium oopapillum* isolates challenged against mefenoxam using a four-, three-, and two-parameter log-logistic model and compared using a *t* test. Gray points with 95% confidence intervals that do not overlap zero are significantly different from zero. The difference for isolates without an absolute EC_{50} (isolate 2) cannot be calculated using the four-parameter log-logistic model.

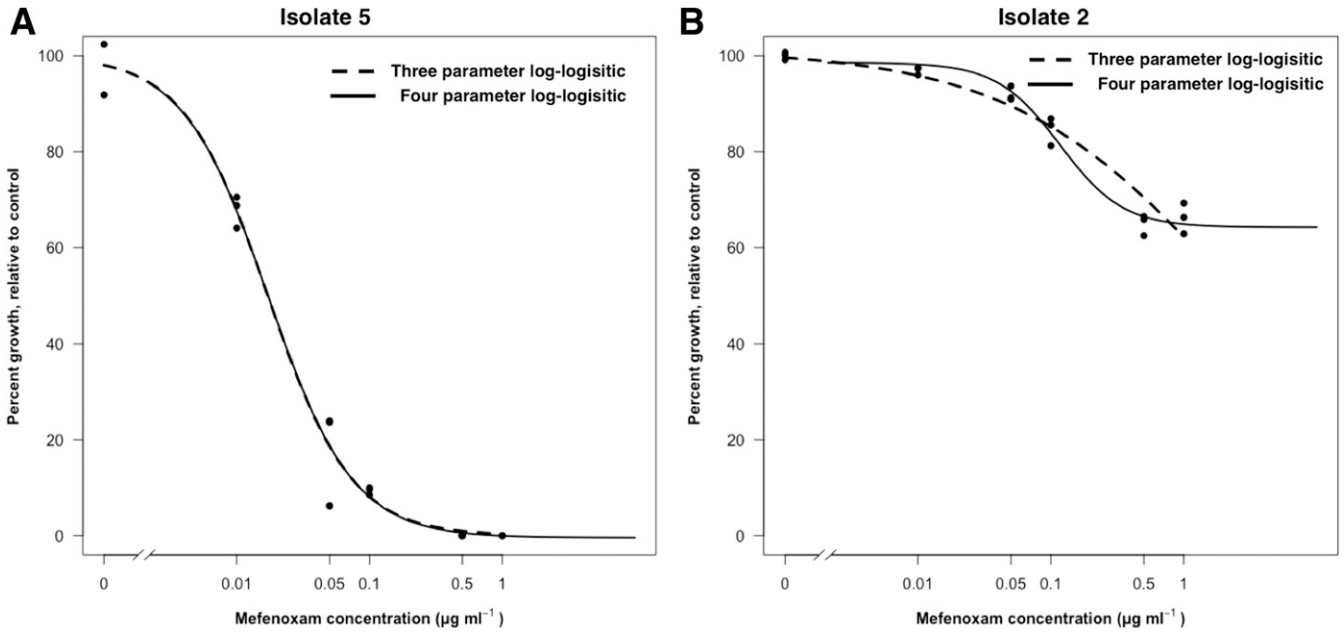

Fig. 3. Four- and three-parameter dose-response curves for *Pythium oopapillum* isolate 5 and 2 challenged against mefenoxam. **A**, Isolate 5 represents a situation where the dose-response model can be adequately described by a three-parameter model. **B**, Isolate 2 represents a situation where it is statistically more appropriate to describe the dose-response relationship using a four-parameter dose-response model but the absolute effective control to 50% growth inhibition does not exist.

EC_{50} was undefined but the relative EC_{50} was defined using a four-parameter log-logistic model. Isolate 5 (Fig. 3A) represents a situation where the difference in absolute and relative EC_{50} was not significantly different than zero. For isolate 2 (Fig. 3B), the estimated relative EC_{50} for the four-parameter log-logistic model was 0.117 ± 0.139 μg ml^{-1} mefenoxam but the absolute EC_{50} was not defined (Table 2). The estimated relative EC_{50} for the three-parameter log-logistic model was 2.545 ± 0.548 μg ml^{-1} mefenoxam and the absolute EC_{50} was 2.425 ± 0.513 μg ml^{-1} mefenoxam. The log-likelihood and AIC for the four-parameter log-logistic model was −38.890 and 87.780, respectively. The log-likelihood and AIC for a three-parameter log-logistic model was −48.060 and 104.110, respectively. A significant likelihood ratio test ($P = 0.0002$), or analysis of variance, between the two models, indicated that the four-parameter model should not be reduced to a three-parameter log-logistic model. A nonsignificant lack-of-fit test revealed no indication of lack of fit for a four-parameter log-logistic model. For isolate 5 (Fig. 3A), the relative and absolute EC_{50} was 0.017 ± 0.002 (Table 2). The log-likelihood and AIC for the four-parameter log-logistic model was −52.100 and 114.190, respectively. The log-likelihood and AIC for a three-parameter log-logistic model was −52.120 and 112.240, respectively. A nonsignificant likelihood ratio test ($P = 0.8487$) between the two models indicated that the four-parameter model could be reduced to a three-parameter log-logistic model and there was no lack of fit for either model, indicating that these data can be adequately described by a three- or four-parameter log-logistic model.

To examine the effect of model selection on the 27 *F. virguliforme* isolates showing hormesis, two hormetic and two nonhormetic models were used to estimate the EC_{50} distributions. The four-parameter Brain and Cousens (BC.4) hormetic model had a log-likelihood of −198.493, AIC of 630.986, and residual variance of 0.142 (Table 3). The Cedergreen-Ritz-Streibig model (CRS.4c) model had a log-likelihood of −237.946, AIC of 709.892, and residual variance of 0.163. The two nonhormetic models—four-parameter log-logistic (LL.4) and Weibull four-parameter (W2.4)—had higher AIC values, lower log-likelihood values, and larger residual variance, indicating that the two hormetic models were better to describe the dose response for these isolates. The fluopyram EC_{50} distributions generated with the BC.4 and CRS.4c hormetic models had similar shapes, with means of 4.74 and 4.94, medians of 3.50 and 3.82, and ranges of 1.11 to 16.76 and 1.21 to 16.89 μg ml^{-1}, respectively. The EC_{50} distributions for the LL.4 and W2.4 models had means of 3.07 and 2.74, medians of 3.20 and 2.78, and ranges of 0.86 to 4.48 and 0.92 to 4.62 μg ml^{-1}, respectively (Table 3). The EC_{50} distributions for hormetic models had longer right-hand tails, indicating higher EC_{50} estimates for some isolates (Fig. 4).

Table 2. Three and four-parameter log-logistic estimated effective control to 50% growth inhibition (EC_{50}) values and model evaluation for two *Pythium oopapillum* isolates (isolates 2 and 5), challenged against mefenoxam[a]

ID	Model[b]	AIC[c]	Log-likelihood	Relative EC_{50} ± SE	Absolute EC_{50} ± SE	Lack-of-fit *P* value	ANOVA *P* value[d]
2	LL.3	104.110	−48.060	2.545 ± 0.548	2.425 ± 0.513	0.0006	0.0002
	LL.4	87.780	−38.890	0.117 ± 0.139	NA	0.0998	…
5	LL.3	112.240	−52.120	0.017 ± 0.002	0.017 ± 0.002	0.9516	0.8487
	LL.4	114.190	−52.100	0.017 ± 0.002	0.017 ± 0.002	0.8614	…

[a] SE = standard error and NA = not applicable.
[b] LL.3 = three-parameter log-logistic and LL.4 = four-parameter log-logistic.
[c] AIC = Akaike Information Criterion.
[d] ANOVA = analysis of variance.

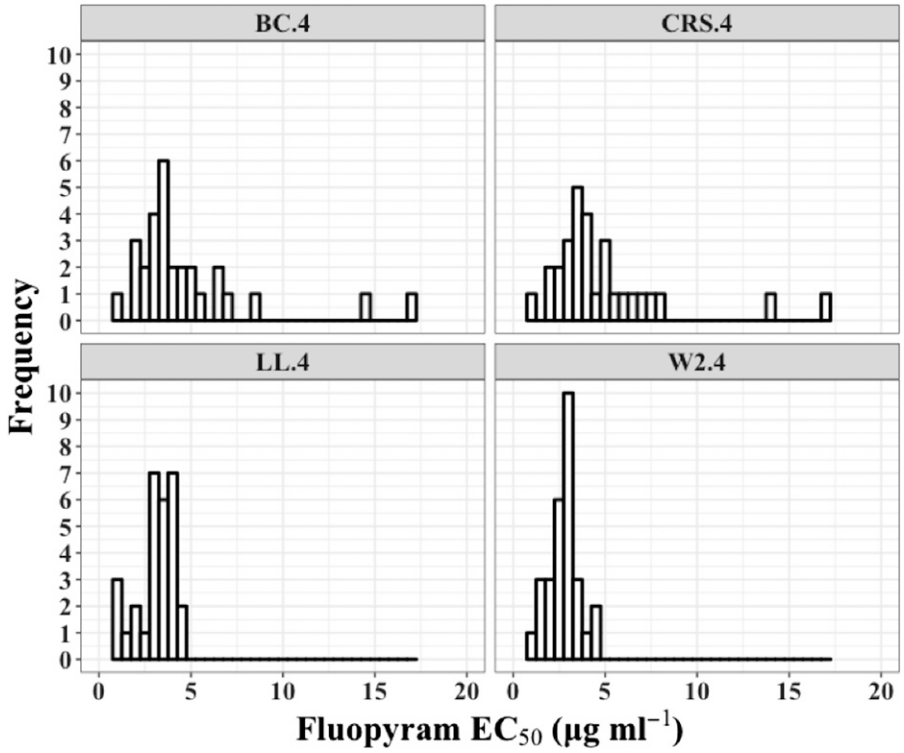

Fig. 4. EC_{50} distributions of 27 *Fusarium virguliforme* isolates challenged with fluopyram fit with two hormetic models (BC.4 and CRS.4c) and two nonhormetic models (LL.4 and W2.4).

Discussion

In this study, we have demonstrated the use of the open source R package "drc" in estimating EC_{50} of fungal and oomycete pathogens' response to fungicide. We examined *P. oopapillum* isolates to illustrate the difference between relative and absolute EC_{50}. The relative EC_{50} is the inflection point on a dose-response curve whereas the absolute EC_{50} is the concentration at which 50% maximal growth occurs. The FRAC definition of EC_{50} is more consistent with absolute EC_{50} and not the same as the default relative EC_{50} output within the "ED" function of "drc". The relative EC_{50} in a four-parameter log-logistic function is determined when the second derivative is equal to 0 and is represented as the inflection point on the curve. As the top and bottom parameters of a four-parameter log-logistic approach 100 and 0%, the relative and absolute EC_{50} become equal (Fig. 1).

This relationship can be visualized by *P. oopapillum* isolate 5 (Fig. 3A, Table 2), where the inflection point accurately estimates 50% reduction in response and the relative and absolute EC_{50} both correspond to the definition proposed by FRAC. However, in the case of isolate 2 (Fig. 3B, Table 2), the lower asymptote of the four-parameter log-logistic curve is above 50% relative response. The inflection point on the curve (relative EC_{50}) does not equal the absolute EC_{50}, and an incorrect estimation of the FRAC-defined EC_{50} is made, even though a four-parameter log-logistic model was the better model to describe these data. In this case, if the relative (2.545) or absolute (2.425) EC_{50} produced by the three-parameter log-logistic model was taken as true, it may be a more accurate numeric EC_{50} estimate but is extrapolated beyond the concentration range tested. Instead, the EC_{50} should not be expressed as an exact number but as greater than the highest concentration tested or, in this example, >1 $\mu g\ ml^{-1}$. If the dosage of fungicide was increased to include response values at 10 or 100 $\mu g\ ml^{-1}$ in these examples, the lower asymptote might be closer to 0% and the difference between relative and absolute EC_{50} might have been closer to 0, regardless of model choice. In plant pathology literature, which EC_{50} measures are reported is not often specified. Therefore, it is important to make this distinction so that future research is more reproducible and clear.

Isolate 43 had the largest defined difference in relative and absolute EC_{50} values (Fig. 2). This amount of error may not contribute much practical significance but it illustrates the importance of choosing concentrations that will fully inhibit growth and bring the lower asymptote of the dose-response curve to 0%, or at least below 50%. Preliminary experiments may be needed to determine this concentration. In future research, isolates like this could be retested at higher concentrations to test whether these isolates' absolute EC_{50} were defined or whether these isolates were potentially resistant to the chemistry. However, in most situations, it would be acceptable to use relative EC_{50} estimation, especially if the highest dose completely inhibits growth, such as for isolate 5. In the case of isolate 5, the model could be reduced to a three-parameter model by fixing the lower asymptote at 0%. This avoids the issue of four-parameter models having a negative lower asymptote (i.e., modeling negative growth). It makes biological sense to use a three-parameter log-logistic model because, as the concentration of a fungicide approaches infinity, the growth of the colony approaches zero, not negative growth. However, the objectives of fungicide sensitivity studies often include characterizing resistant or less sensitive isolates. In these situations, the fungicide concentrations tested do not allow the lower asymptote of the dose-response curve to be close to zero. Therefore, the relative EC_{50} estimate may not be correct, regardless of how well the data fit a four-parameter model, and describing these data with a three-parameter model would result in extrapolating the EC_{50} estimate. Sebaugh (2011) discussed that, in order for accurate usage of absolute EC_{50}, there should be at least two responses above and below 50% relative growth inhibition, whereas the accurate usage of relative EC_{50} requires two responses above and below the bend points of a dose-response curve. We did not specifically address this but it could be looked at in future studies.

The model choice was also an important factor to consider when interpreting results because model choice can change the overall EC_{50} distribution. This was demonstrated with 27 *F. virguliforme* isolates showing a possible hormetic effect (Fig. 4). In this study, the hormetic models described these data better than the nonhormetic models, and the resulting hormetic distributions had a bimodal shape and higher EC_{50} estimates than the nonhormetic models, which showed a unimodal shape. This may change the interpretation of the population from a population that is sensitive to one that is shifting toward a more resistant phenotype, particularly for pathogens with high dispersal and polycyclic disease cycles, where only a few isolates with a resistant phenotype can spread quickly.

Based on the results of this study, we have generated a decision system to aid the process used to obtain and interpret useful EC_{50} values (Fig. 5). Our first suggestion after data entry is to check whether 50% inhibition has occurred within the concentration range tested for

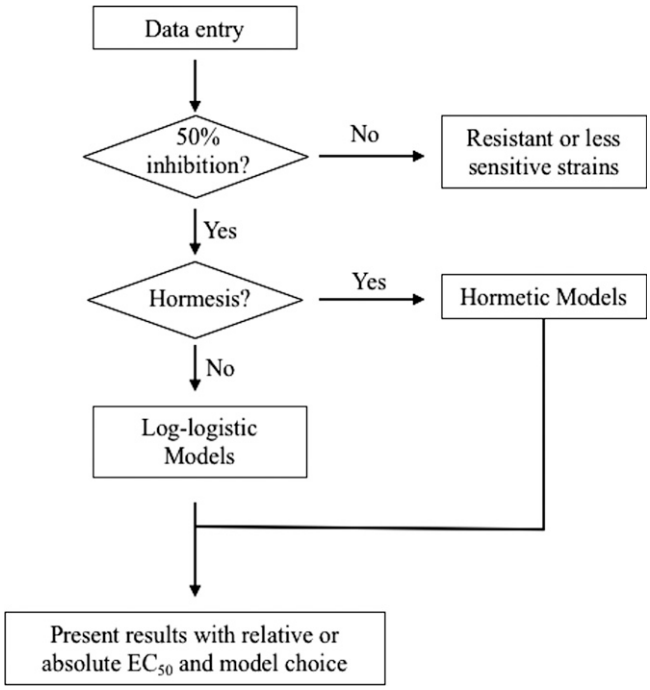

Fig. 5. Decision tree for fitting a dose-response curve for effective control to 50% growth inhibition (EC_{50}) estimation.

Table 3. Comparison of hormetic and nonhormetic models, describing 30 *Fusarium virguliforme* isolates challenged against fluopyram

Model[a]	Model type[b]	AIC[c]	Log-likelihood	Residual variance	Mean ($\mu g\ ml^{-1}$)	Median ($\mu g\ ml^{-1}$)	Range ($\mu g\ ml^{-1}$)
BC.4	Hormetic	630.986	−198.493	0.142	4.74	3.50	1.11–16.76
CRS.4c	Hormetic	709.892	−237.946	0.163	4.94	3.82	1.21–16.76
W2.4	Nonhormetic	718.069	−242.035	0.165	2.74	2.78	0.92–4.62
LL.4	Nonhormetic	744.942	−255.471	0.173	3.07	3.20	0.86–4.48

[a] BC.4 = four-parameter Brian-Cousens model, CRS.4c = four-parameter Cedargreen-Ritz-Streibig model, W2.4 = four-parameter Weibull model, and LL.4 = four-parameter log-logistic model.
[b] Hormetic models account for increased growth at low fungicide concentrations.
[c] AIC = Akaike Information Criterion.

all isolates. The easiest way to do this is to normalize the response values so that they are expressed as a percent relative growth to the control and check whether 50% occurs at any concentration. A four-parameter log-logistic model can be fit to these data, and absolute EC_{50} should be defined and the lower asymptote should be below 50%. If this does not occur, the EC_{50} for this isolate should be expressed as greater than the greatest concentration tested. Next, hormetic models should be considered for isolates displaying a possible hormetic affect. If hormetic models describe these data better than nonhormetic models, both the nonhormetic and hormetic EC_{50} estimate should be reported or, at the very least, hormesis may need to be acknowledged if presenting one model over the other for the sake of parsimony (Wang et al. 2017) (C. Ritz, personal communication). For isolates not displaying a hormetic affect, a four- or three-parameter log-logistic model should be considered. If most isolates screened are completely inhibited by the fungicide, a three-parameter model may be best to describe these data to avoid issues with negative lower parameter estimates. Finally, it should also be stated which EC_{50} estimate is reported (relative or absolute) along with the model choice. These guidelines will help improve the consistency and reproducibility of fungicide sensitivity studies in plant pathology.

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Literature Cited

Belz, R. G., and Piepho, H. P. 2012. Modeling effective dosages in hormetic dose-response studies. PLoS One 7:e33432.

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

Cedergreen, N., Ritz, C., and Streibig, J. C. 2005. Improved empirical models describing hormesis. Environ. Toxicol. Chem. 24:3166-3172.

Garzón, C. D., and Flores, F. J. 2013. Hormesis: Biphasic dose-responses to fungicides in plant pathogens and their potential threat to agriculture. Pages 311-328 in: Fungicides—Showcases of integrated plant disease management from around the world. Online publication. INTECH. http://cdn.intechopen.com/pdfs-wm/44734.pdf

Garzón, C. D., Molineros, J. E., Yanez, J. M., Flores, F. J., Jimenez-Gasco, M. M., and Moorman, G. W. 2011. Sublethal doses of mefenoxam enhance *Pythium* damping-off of geranium. Plant Dis. 95:1233-1238.

Kenakin, T. P. 2009. Statistics and Experimental Design. Pages 273-302 in: *A Pharmacology Primer*, 3rd ed. Academic Press, New York.

Knezevic, S. Z., Streibig, J. C., and Ritz, C. 2007. Utilizing R software package for dose-response studies: The concept and data analysis. Weed Technol. 21:840-848.

Kunova, A., Pizzatti, C., Bonaldi, M., and Cortesi, P. 2014. Sensitivity of nonexposed and exposed populations of *Magnaporthe oryzae* from rice to tricyclazole and azoxystrobin. Plant Dis. 98:512-518.

Neubig, R. R., Spedding, M., Kenakin, T., and Christopoulos, A. 2003. International union of pharmacology committee on receptor nomenclature and drug classification. XXXVIII. Update on terms and symbols in quantitative pharmacology. Pharmacol. Rev. 55:597-606.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ritz, C., Baty, F., Streibig, J. C., and Gerhard, D. 2015. Dose-response analysis using R. PLoS One. 10: e0146021.

Ritz, C., and Streibig, J. C. 2005. Bioassay analysis using R. J. Stat. Softw. 12. doi.org/10.18637/jss.v012.i05

Rojas, J. A., Jacobs, J. L., Napieralski, S., Karaj, B., Bradley, C. A., Chase, T., Esker, P. D., Giesler, L. J., Jardine, D. J., Malvick, D. K., Markell, S. G., Nelson, B. D., Robertson, A. E., Rupe, J. C., Smith, D. L., Sweets, L. E., Tenuta, A. U., Wise, K. A., and Chilvers, M. I. 2017. Oomycete species associated with soybean seedlings in North America—Part I: Identification and pathogenicity characterization. Phytopathology 107:280-292.

Saville, A., Graham, K., Grunwald, N. J., Myers, K., Fry, W. E., and Ristaino, J. B. 2015. Fungicide sensitivity of U.S. genotypes of *Phytophthora infestans* to six oomycete-targeted compounds. Plant Dis. 99:659-666.

Sebaugh, J. L. 2011. Guidelines for accurate EC50/IC50 estimation. Pharm. Stat. 10:128-134.

Stewart, J. E., Kroese, D., Tabima, J. F., Larsen, M. M., Fieland, V. J., Press, C. M., Zasada, I. A., and Grünwald, N. J. 2014. Pathogenicity, fungicide resistance, and genetic variability of *Phytophthora rubi* isolates from raspberry (*Rubus idaeus*) in the western United States. Plant Dis. 98:1702-1708.

Wang, J., Bradley, C. A., Stenzel, O., Pedersen, D. K., Reuter-Carlson, U., and Chilvers, M. I. 2017. Baseline sensitivity of *Fusarium virguliforme* to fluopyram fungicide. Plant Dis. 101:576-582.