INFORME TÉCNICO

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Ten steps for reports of new cases of herbicide resistance of weeds in Brazil

Introduction

To establish criteria for new reports of cases of herbicide resistance in weeds, this technical briefing includes procedures prepared by the Brazilian Society of Weed Science (SBCPD, Portuguese acronym) in conjunction with the Committee for Action on Herbicide Resistance in Brazil. (HRAC-BR).

The definition of herbicide resistance in weeds considered in this document is "the inherited ability of a plant to survive and reproduce after exposure to a dose of normally lethal herbicide, i.e., the maximum dose indicated in the package insert for the natural population. In a plant, herbicide resistance can occur naturally or be induced by techniques such as genetic engineering or the selection of mutants produced by tissue culture or mutagenesis".

Currently, a case of weed resistance is considered new worldwide when a report is made "online" in the international database on the website www.weedscience.org. Researchers who intend to publish new cases of weed resistance herbicide on the SBCPD website (sbcpd.org), HRAC-BR (hrac-br.org) website, and the International Herbicide-Resistant Weed Database (weedscience.org) should follow the steps described below:

First step – Field identification of a resistant plant

Under field conditions, a series of adverse effects, such as unfavorable climatic conditions, water stress, plants at the incorrect stage at the time of application, sub-doses of commercial products, inadequate adjuvants, and inadequate application technology, can often

be factors that mistakenly lead to the assumption that surviving plants are "resistant to a particular herbicide".

After eliminating the possibilities mentioned above and identifying a weed that survived herbicide application at the stage and dose recommended in the package insert, plants and/or seeds of the species with herbicide resistance should be collected. For georeferencing, it is necessary to collect the geographic coordinates as well as the municipality and state. Additional information, such as the date, weed stage at the time of application, application data (spray volume, tip type, product(s), dose(s), adjuvants, environmental conditions), and other relevant data on collection, are also important.

Second step - Dose-response curve studies (confirmation of resistance)

Seeds collected from plants with suspected field resistance should be used in greenhouse studies of doseresponse curves.

Screening using the plants originating from the seeds collected in the field should be performed, usually by applying the dose recommended in the package insert and by doubling this dose to ensure the control failure is strictly caused by the herbicide.

First generation (F1). After ascertaining survival in the triage, the F1 generation should be obtained, and the dose-response curve should be generated in a greenhouse. Doses in multiples of two should be applied to weeds with medium susceptibility to herbicides, as suggested: 0 D, 1/8 D, 1/4 D, 1/2 D, D, 2 D, 4 D, and 8 D, where D is the recommended herbicide dose in the package insert (Gazziero et al., 2008). During the experiment, general information, such as the date and location (municipality and state as well as GPS information), weed stage at the time of application, application data (spray volume, tip type, product(s), dose(s), adjuvants, environmental conditions), greenhouse or open environment conditions, and other relevant information, should be recorded.

Second generation (F2). A second experiment using the treatments and details used for the study of the F1 generation should be performed with plants originating <u>from seeds collected from the plants surviving the first experiment</u>, i.e., of the F1 generation, using the recommended dose or a higher dose of the treatment. Both experiments should be conducted using a susceptible biotype of the same species and from the same region to determine the comparative effect while respecting the requirements of statistical analysis during data processing. More than one susceptible biotype can be included in the studies to increase the consistency of the related analyses.
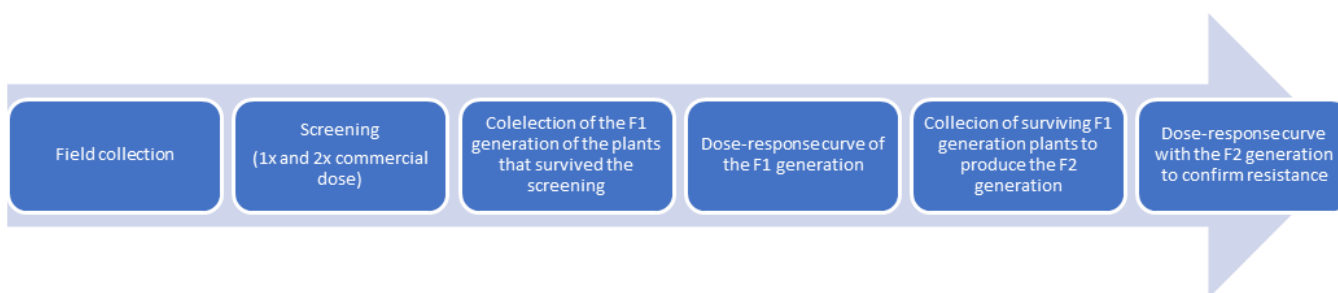
Organizational chart for confirmation of resistance:
Third step - Regression models to demonstrate resistance

Several nonlinear regression models are available in the literature to compare various biotypes (Streibig, 1988; Brain & Cousens 1989; Seefeldt et al., 1995). These models are necessary to determine the GR50 or GR90 (dose required to reduce dry mass; GR = growth reduction) and the LD50 and LD90 (control; LD = lethal dose) of the suspected biotype with resistance compared to the susceptible biotype.

$$y = \frac{a}{\left[1 + \left(\frac{x}{b}\right)^{c}\right]}$$

One of the most widely used nonlinear regression models is that of Streibig (1988) shown below:

where
y is the response variable (percentage of control or shoot dry mass);
x is the herbicide dose (g ha-1); and
a, b, and **c** are estimated parameters of the equation;

a is the amplitude between the maximum and minimum points of the variable;
b is the dose that provides 50% variable response; and
c is the slope of the curve near b.

The nonlinear logistic model provides an estimate of the parameter C50 (50% of control) or GR50 (50% growth reduction).

Based on the C50 and GR50 values, the resistance factor is calculated (RF = C50 or GR50 of the resistant population/ C50 or GR50 of the susceptible population). The RF expresses the number of times higher that the dose required to control 50% of the resistant population is than the dose that controls 50% of the susceptible population (Burgos et al., 2013, Ritz et al., 2015).

Fourth step - Identification of the species

The "key of botanical identification" (dichotomous key) and, if necessary, molecular techniques should be used to conclusively identify the species, including both the resistant biotype(s) and the susceptible biotype(s).

Fifth step - Certification of previous criteria

The answers to the questionnaire below should be "yes" in all fields to be considered:

5.1. Does the case meet the definition of weed resistance in relation to surviving the maximum recorded herbicide dose for the species evaluated?

5.2. Did the confirmation occur through results obtained from scientifically based protocols?

5.3. Was the heredity of herbicide resistance in weeds confirmed?

5.4. Is there a potential practical agronomic impact of the reported resistance?

5.5. Was the involved species conclusively botanically identified and is not the result of deliberate or artificial selection?

Sixth step - Documentation

Resistant biotypes that meet the previous steps will be considered, with appropriate scientific evidence.

Seventh step - Case submission

According to the proposed form (www.hrac-br.org), the PDF document, together with the other statistical analysis documents (PDF) containing the dose-response curve results and tables with the GR, LD, and RF values obtained in the two experiments conducted in the greenhouse and general information, such as the following, should be sent to the Weed Resistance Committee of the SBCPD (sbcpd@sbcpd.org), which will send the results to the HRAC-BR for joint analysis of the case:

7.1 date and location of collection (municipality),

Rapporteur → SBCPD → HRAC-BR

7.2 weed stage at the time of application,

7.3 application data (spray volume, tip type, product(s), dose(s), adjuvants, environmental conditions), and

7.4 other pertinent information.

Eighth step - Analysis

After analyzing the data from the SBCPD Resistance Committee with the help of the HRAC-BR in accordance with the criteria for official statistical reports of herbicide-resistant weed biotypes of the SBCPD, as well as needs covered by weedscience.org, the report will be uploaded to the SBCPD website and forwarded to the HRAC-BR and to the website www.weedscience.org by the SBCPD Resistance Committee.

SBCPD → Rapporteurs/ HRAC-BR → Agriculture Sector

Ninth step - Communication

A formal statement from the SBCPD will be sent to the rapporteurs of the case and widely disseminated to those involved with the agricultural sector.

Tenth step - Control strategies

The rapporteurs of the case, with the support of the SBCPD and HRAC-BR, should develop strategies for management of the areas and disseminate them widely, especially in the region where the case was identified.

Additional information on the criteria for reporting new cases of weed resistance can be found on the SBCPD website (sbcpd.org), HRAC-BR website (hrac-br.org), and International Herbicide-Resistant Weed Database website (weedscience.org).

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