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Determining mehlich-3 and DTPA extractable soil zinc optimum economic threshold for maize

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Summary

Maize (*Zea mays L.*) is one of the most susceptible crops to zinc (Zn) deficiency. However, in much of the world, soil Zn tests are poorly calibrated, and thus determining a critical soil test level for Zn is challenging. The objectives of this study were to: i) produce a field calibration of the Zn Mehlich-3 (M3-Zn) method for predicting maize grain yield response to Zn fertilizer application, ii) compare the capacity of DTPA extractable Zn (DTPA-Zn) with M3-Zn for predicting the response of maize yield to Zn fertilizer, iii) determine whether inclusion of soil pH, organic matter (SOM) and extractable phosphorus in a M3-Zn model improves its predictive capacity, and iv) evaluate an economic approach for determining soil Zn thresholds. We conducted 55 field experiments covering a wide range of edaphic and climatic conditions. Maize responded to Zn fertilizer in 29% of the trials. The capacity of M3-Zn and DTPA-Zn to predict relative yield of maize was similar. Inclusion of other soil variables (pH, extractable phosphorus, and SOM) did not or only slightly improve the prediction of M3-Zn. Based on the relationship between M3-Zn and DTPA-Zn (R^2 = 0.89), one test can be predicted from the other without affecting the calibration. The M3-Zn "economic threshold" ranged from 0.98 to 2.79 mg kg^{-1}, while for DTPA-Zn it varied from 0.41 to 1.61 mg kg^{-1}. The broad range of economic thresholds shows that differences in maize productivity and grain price between regions and seasons make establishing a single Zn threshold for all economic-productive situations inappropriate.

Keywords: availability, corn, diagnosis, field calibration, micronutrient, threshold

Introduction

Zinc (Zn) deficiency is the most widespread micronutrient deficiency in crops, and it affects more than 160 million ha of arable land worldwide (Alloway, 2009). Maize (*Zea mays L.*) is one of the most susceptible crops to this nutrient deficiency (Brown *et al.*, 1993). Zinc deficiency can be avoided by the application of Zn fertilizers. However, to achieve rational Zn fertilizer management, the development, correlation, calibration, and interpretation of soil test methods to diagnose plant-available soil Zn are necessary (Dahnke & Olson, 1990). The use of an adequate diagnostic method would avert under- or over-application of Zn, thereby reducing economic losses.

One of the methods used for extracting available Zn, is known as Mehlich-3 (M3-Zn) and was developed for acidic-neutral soils. Ethylenediaminetetraacetic acid (EDTA) is used as the chelating agent (Mehlich, 1984). Previous greenhouse correlation studies determined a significant relationship between M3-Zn and uptake of Zn by maize (Takrattanasaran *et al.*, 2010; Farshadirad *et al.*, 2017). However, there are no studies that provide a field-based calibration relating soil M3-Zn with the response of maize grain yield to Zn fertilizer application. Such M3-Zn field calibrations are important because, without them the determination of M3-Zn has limited meaning (Evans, 1987).

Another soil test method used for available Zn involves the extractant diethylenetriamine-pentaacetic acid (DTPA-Zn) and was developed for near-neutral and calcareous soils by Lindsay & Norvell (1978). The buffered pH and the presence of soluble Ca^{+2} prevent the dissolution of $CaCO_3$, thereby avoiding desorption of unavailable micronutrients occluded in this solid phase. This extractant has been used to calibrate the probability of maize yield response to Zn fertilizer application (Barbieri *et al.*, 2017).

The predictive capacity of a micronutrient soil test may be improved using multiple regression analysis, including particular edaphic properties that affect availability of micronutrients (Sims & Johnson, 1991). For example, Junus & Cox (1987) reported that the correlation between maize Zn uptake and M3-Zn was improved when pH was included into the model. The rationale for including pH is that it influences the replenishment of soil-solution Zn from solid phases (Gupta *et al.*, 2016) and the capacity of M3 to extract Zn fractions that are not readily available for plants in alkaline soils (Srivastava & Srivastava, 2008). Additionally, Zn deficiencies in crops are often observed in soils with high phosphorus (P) concentration (Zhang *et al.*, 2017). Therefore, the inclusion of an indicator of P availability in multiple regression studies, together with extractable Zn, could potentially improve crop response models for Zn. Another soil property that can affect Zn availability is soil organic matter (SOM) (Catlett *et al.*, 2002). However, Barbieri *et al.* (2017) demonstrated that the capacity of the model to predict the relative yield of maize from DTPA-Zn was slightly improved by the inclusion of SOM but not by the inclusion of pH or extractable P. Nevertheless, the inclusion of SOM, pH, or extractable P has not been studied in models, based on M3-Zn, for predicting the response of grain yield to Zn in maize.

The common approach used for interpreting results of field calibration studies for nutrients with little soil mobility, such as Zn, is based on the "sufficiency level" (Cate & Nelson, 1971; Ware *et al.*, 1982). This methodology uses an arbitrarily defined relative yield level to determine a fixed critical threshold (Tang *et al.*, 2009), which is the soil test value at which crop yield reaches a 90-98% relative yield (Jordan-Meille *et al.*, 2012). Adoption of this 90-98% relative yield goal is based on the assumption that a grain yield reduction of 2-10% does not represent a significant economic loss. However, there is no agronomic reason justifying use of a fixed relative yield goal. Alternatively, a soil test threshold could be based on optimizing economic profit instead of maximizing yield (Mallarino, 2004), allowing a Zn soil threshold prediction based on both economic and productive criteria (economic threshold).

Although Zn availability thresholds for maize have been developed under greenhouse conditions, M3-Zn thresholds have not been evaluated under field conditions nor interpreted based on economic criteria. Thus, the objectives of this study were to: i) calibrate the M3-Zn method for predicting response of grain yield in maize to Zn fertilizer application in the field, ii) compare the capacity of DTPA-Zn with M3-Zn for predicting the response of maize yield to Zn fertilizer, iii) determine whether the inclusion of soil pH, SOM, and extractable P in a M3-Zn model improves its predictive capacity, and iv) evaluate an economic approach for determining soil Zn thresholds, across several Argentinian soils.

Materials and methods

Field experiments and soil and plant analysis

A total of fifty-five field trials, evaluating maize response to Zn fertilizer were conducted during 2009 (8 sites), 2010 (6 sites), 2011 (4 sites), 2013 (12 sites), 2014 (4 sites), 2015 (1 site), 2016 (13 sites), and 2017 (7 sites) over an extensive area of mainland Argentina (Fig. 1). The dataset is representative of soils where row-crop production predominates (fifteen soil survey subgroups; USDA Soil Taxonomy, 2014; Table S1, Supporting Information) allowing to perform a robust field calibration (Dahnke & Olson, 1990).

At each trial, a composite soil sample per block (15 cores, 0 to 20 cm depth) was collected before maize sowing using a stainless-steel probe. Soil samples were air-dried at 30°C, ground, and passed through a 2-mm nylon screen. Soil pH (1:2.5 soil to water ratio), SOM (Schulte & Hopkins, 1996), M3-P (Mehlich, 1984), DTPA-Zn (Lindsay & Norvell, 1978), and M3-Zn (Mehlich, 1984) were determined. For each sample, the presented result is the average of three laboratory replications. For the DTPA extraction, soil samples were shaken at room temperature (20° C ± 1) for 2 h on a horizontal shaker (120 cycles min^{-1}) with an extracting solution composed by DTPA 0.005 mol L^{-1}, $CaCl_2$ 0.01 mol L^{-1}, and TEA 0.1 mol L^{-1} buffered at pH 7.30 (soil-to-solution ratio 1:2 w/v). For the Mehlich-3 extraction, soil samples were shaken for 5 min at room temperature (20° C ± 1) on a horizontal shaker (200 cycles min^{-1}) with an extractant composed by CH_3COOH 0.2 mol L^{-1}, NH_4NO_3 0.25 mol L^{-1}, NH_4F 0.015 mol L^{-1}, HNO_3 0,013 mol L^{-1}, and EDTA 0.001 mol L^{-1} buffered at pH 2.5 (soil-to-solution ratio 1:10 w/v). The resulting suspensions were filtered through Whatman No. 42 filter paper and analyzed for Zn concentration using atomic absorption spectrophotometry (AAS) (Model AA-6200, Shimadzu Co., Kyoto, Japan) equipped with air-acetylene flame. The detection limit and the sensitivity of the spectrophotometer were 0.010 mg L^{-1} and 0.012 mg L^{-1}, respectively. All the used reagents were of analytical grade and solutions were prepared using double-distilled deionized water. Zinc standard solutions for AAS were prepared using the appropriate background electrolyte, to overcome matrix and background interferences.

At all trials, maize was cultivated under a no-tillage and no-irrigation system. Maize sowing rate, row spacing, and sowing date were selected to maximize maize yield, and varied among trials (Table S2, Supporting Information). At sowing, all plots were fertilized with nitrogen (N), P, and sulfur (S), as urea (46-0-0), triple superphosphate (0-46-0), and gypsum ($CaSO_4$), respectively. Fertilizer rates (70 - 200 kg N ha^{-1}, 20 - 30 kg P ha^{-1}, 10 - 20 kg S ha^{-1}) were established based on soil analysis and locally-calibrated models (Carciochi *et al.*, 2016; Orcellet *et al.*, 2017). The crops were not fertilized with potassium, since it was not a limiting nutrient at the studied area (soil available potassium was greater than 200 mg kg^{-1}; Sainz Rozas *et al.*, 2019).

Two treatments were included: Zn fertilizer applied (+Zn) and No Zn fertilizer (Control). These treatments were evaluated at each trial using a randomized complete block design with three or four replications. Plot size ranged from 30 to 50 m^2 depending on row spacing (8 to 10 rows per plot). Zinc fertilizer was foliar-applied to avoid fertilizer-soil interactions. Foliar applications were performed at V_6-V_8 stage (Salem & El-Gizawy, 2012). The Zn source was a Zn oxide (ZnO) - ethanediol flowable suspension applied at 0.7 kg Zn ha^{-1}. This Zn source has a solubility of 700 g Zn L^{-1} and was successfully used in other studies (White *et al.*, 2016; Mohammadi & Khezri, 2018). The applied rate exceeded maize Zn total uptake (0.44 - 0.56 kg ha^{-1}; Bender *et al.*, 2013). The fertilizer was mixed with water (150 L H_2O ha^{-1}) and applied with a backpack sprayer using a CV-IA 110–04 hollow cone spray nozzle (MagnoJet, Ibaiti, Brazil). No additives or surfactants were used.

Maize grain yield was determined at physiological maturity by hand-harvesting 10 m^2 from the two center rows of each plot. Grain yield was expressed at 155 g kg^{-1} moisture content. At 34 trials, a grain sample from each plot was dried, ground, and digested in a double acid mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) (1:1) (Benton, 1991). Zinc concentration in the digests was determined by AAS.

Data analysis

Maize yield response to Zn fertilizer was analyzed for each trial by analysis of variance (ANOVA). The effects were considered significant at $p < 0.1$, following the rationale described by Garland-Campbell (2018), who suggested selecting a p-value based on the goals of the study rather than using the usual 0.05 p-value. The field spatial variability of soil properties and Zn availability can mask economically significant yield responses to fertilizer, which may not be detected when using a 0.05 p-value. A linear regression analysis was performed using the stepwise selection method ($p < 0.1$) to determine the best variable combination to explain relative yield from DTPA-Zn or M3-Zn, pH, SOM, and M3-P. All statistics were performed using SAS (version 9.4, SAS Institute, Inc, Cary, NC, 2018).

The Arcsine Logarithm Calibration Curve (ALCC) (Dyson & Conyers, 2013; Brennan & Bell, 2013; Bell *et al.*, 2013; Speirs *et al.*, 2013; Correndo *et al.,* 2017) was used to describe the relationship between soil Zn availability and relative yield. The ALCC method uses the arcsine of the square root to transform and linearize the relative yield, and uses the natural logarithm to transform soil test values. The relative yield was calculated by dividing the yield of the unfertilized treatment at each trial by the yield of the Zn fertilized treatment, multiplied by 100 (Bell *et al.*, 2013).

A residual sum of squares analysis (Milliken & Debruin, 1978) was used to determine if the model to predict relative yield from DTPA-Zn or M3-Zn was different when Zn soil test values were measured or estimated from one another (estimating DTPA-Zn from M3-Zn and vice versa).

Economic threshold approach

We also developed a novel approach for the interpretation of results from tests to estimate the availability of nutrients with little soil mobility. This interpretation strategy, which takes into account economic and productive factors for crop production, would be especially useful for farmers when the expected profit margin is narrow or the farm business suffers from economic-financial restrictions. Throughout the paper we use the term “economic threshold” to refer to this approach, which was based on the criterion that the economic profit from the grain yield response to Zn fertilizer should be greater than the Zn fertilizer application cost, as follows:

$$Yr \times Pm > Cf \tag{1}$$

where Yr refers to grain yield response (Mg ha^{-1}); Pm refers to maize price ($ Mg); and Cf is the cost of Zn fertilizer application ($ ha^{-1}).

The break-even yield response (BYr) is a yield value (Mg ha^{-1}) at which the costs and benefits of fertilizer application are equal. In other words, yield response must be greater than or equal to the ratio of Zn fertilizer application cost to maize price. Rearranging the previous equation:

$$BYr \geq \frac{Cf}{Pm} \tag{2}$$

Then, BYr is accounted for the definition of the relative yield goal:

$$RYg = \frac{Yg - BYr}{Yg} \tag{3}$$

where RYg is the relative yield goal (%); Yg is the yield goal (Mg ha^{-1}); and BYr is the break-even yield response to Zn fertilizer application (Mg ha^{-1}).

Next, Equation 2 and Equation 3 are combined:

$$RYoe = \frac{Yg - \frac{Cf}{Pm}}{Yg} \times 100 \quad (4)$$

where $RYoe$ stands for "optimum economic relative yield goal" (%). Finally, the soil Zn availability economic threshold is obtained from a calibration curve that describes this last variable as a function of RY_{OE} (Fig. 4).

Summarizing, the interpretation process has three steps: i) estimation of the RY_{OE} (%) (Equation 4); ii) use of the exponential function calculated by the ALCC method (Correndo *et al.*, 2017) to determine a M3-Zn or DTPA-Zn economic threshold for the estimated RY_{OE} (Fig. 4); and iii) comparison of the economic threshold with the M3-Zn or DTPA-Zn value determined from soil testing.

If agronomists decide to apply the "economic threshold" approach in farm nutrient management, the inputs for RY_{OE}(%) determination (Equation 4) can be easily obtained or estimated from: i) the expected grain price from future, options, and forward contracts (García & Leuthold, 2004); ii) the Zn fertilizer application cost (known at the time of making the decision); and iii) the maize yield goal (depends on hybrid, site, and year), which can be estimated from historic grain yield records, agronomist expertise, and/or from simulation models (Aramburu Merlos *et al.,* 2015).

To compare between the "economic threshold" versus the "sufficiency level" interpretation approach, we performed a sensitivity analysis for the RY_{OE} and, thus, the resulting Zn-availability economic thresholds, considering: i) historical maize prices (2004-2018) in Illinois (USDA, 2019), ii) a fixed Zn fertilizer application cost of $US 25 ha^{-1} (Yara USA, personal communication), and iii) the maize yield of +Zn treatments as yield goal. We used USA dollar prices instead of Argentinean peso prices to avoid overestimating the international maize market variations caused by the high year-on-year inflation rate of the Argentinean peso.

To compare the efficiency of the Zn extractants at different RY_{OE} we calculated the proportion of cases presenting: i) type I error (Zn fertilizer is recommended when it is not needed; Quadrant I), ii) type II error (Zn fertilizer is not recommended when it is needed; Quadrant III), and iii) correctly diagnosed cases (Quadrant II and IV) (Dahnke & Olson, 1990).

Results

The experimental sites had a wide range of soil pH (5.4 to 8.2), M3-P (5.5 to 268.5 mg kg^{-1}), and SOM (11.2 to 73.5 g kg^{-1}) (Table 1). The concentrations of plant-available Zn, based on Mehlich-3 extraction (M3-Zn) ranged between 0.45 and 8.80 mg kg^{-1} were greater than that for DTPA-Zn (range 0.11 to 6.42 mg kg^{-1}), and a very significant ($p<0.0001$) linear regression between the extraction methods determined across all experimental sites accounted for 89% of the variance (Fig. 2). Soil pH was negatively and weakly correlated with DTPA-Zn and M3-Zn (Table 2). The M3-P did not correlate with any other edaphic variable, whereas SOM correlated positively with DTPA-Zn and M3-Zn (Table 2). When pH was included as an independent variable into the model to predict M3-Zn from DTPA-Zn, the determination coefficient was slightly increased ($R^2 = 0.90$; $p < 0.05$) (data not shown).

Maize grain yield ranged from 3.9 to 18.3 Mg ha^{-1} (Table 1) and it responded to Zn fertilizer in 16 of 55 trials (29%) ($p < 0.1$). The average yield response in responsive trials was 1.1 Mg ha^{-1} (11% greater than the control treatment). At responsive trials, soil DTPA-Zn and M3-Zn values ranged from 0.28 to 1.37 mg kg^{-1} and from 0.45 to 2.42 mg kg^{-1}, respectively. Neither the response of grain yield (Mg ha^{-1}) nor relative yield (%) to Zn fertilizer was correlated with yield of Zn-fertilizer (+Zn) treatments ($p = 0.22$ and $p = 0.43$, respectively). There were significant differences ($p < 0.1$) in Zn concentration in grain between the control and the Zn-fertilized treatment in only 4 out of 34 analyzed sites (12%). At those sites, the average response to Zn-fertilizer of Zn concentration in grain was 4.6 mg kg^{-1} (Table S2, Supporting Information).

Based on the results from the stepwise selection method, the multiple regression models to predict relative yield from M3-Zn or DTPA-Zn also included SOM, but not P-M3 nor pH (Table 3). However, both models had very poor predictive capacity, explaining only 30-31% of the variability in relative yield.

The M3-Zn ALCC model (Equation 5) was not modified when M3-Zn was estimated from DTPA-Zn, using the relationship presented in Fig. 2 ($p = 0.89$). Similarly, the DTPA-Zn ALCC model (Equation 6) did not differ when DTPA-Zn was estimated from M3-Zn ($p = 0.99$) (Fig. 4).

$$M3 - Zn = e^{-5.91 + 4.72 \times arcsin\sqrt{(RYoe/100)}};\ r = 0.47 \quad (5)$$

$$DTPA - Zn = e^{-8.59 + 6.17 \times arcsin\sqrt{(RYoe/100)}};\ r = 0.51 \quad (6)$$

Using the "sufficiency level" approach, the critical thresholds of plant-available Zn in soil necessary to achieve a fixed 97% relative yield goal, were 1.97 mg kg^{-1} for M3-Zn (from 1.64 to 2.37 mg kg^{-1} considering a 95% confidence interval) (Fig. 3A) and 1.02 mg kg^{-1} for DTPA-Zn (from 0.81 to 1.29 mg kg^{-1} considering a 95% confidence interval) (Fig. 3B).

Using the "economic threshold" interpretation model, developed from Equations 5 and 6, for a RY_{OE} goal ranging from 90% to 99%, the M3-Zn threshold varied from 0.98 to 2.79 mg kg^{-1}, while the DTPA-Zn threshold varied from 0.41 to 1.61 mg kg^{-1} (Fig. 4). Both DTPA-Zn and M3-Zn methods presented a similar proportion of correctly diagnosed cases (avg. 74%) (Fig. 5). When the RY_{OE} goal increased from 90 to 99%, the proportion of correctly diagnosed cases decreased from 85 to 67% for DTPA-Zn and from 85 to 62% for M3-Zn. On average, for both Zn extraction methods, the observed proportion of type I error was 12% and the proportion of type II error was 14%, for a RY_{OE} goal ranging between 90 and 99%.

Discussion

Edaphic properties

The values of pH, SOM, DTPA-Zn, and M3-Zn (Table 2) were within the range reported by Sainz Rozas *et al.* (2015) for agricultural soils in Argentina. A linear correlation between M3-Zn and DTPA-Zn (Fig. 2) has been described before by multiple authors (Table S3, Supporting Information). However, the slope and the intercept of the linear models varied markedly across studies (Table S3, Supporting Information). The lack of uniformity in the methodologies used between those studies may have hampered the comparison of the results, owing to the operationally defined characteristics of the extraction procedures (Quevauviller, 1998). In our study, the slope of the linear model (1.35; Fig. 2) was less than 25% of the values described by other researchers, whereas the regression coefficient was within the median (0.86) and the third quartile (0.94) of the previously described values. The significantly larger concentrations of available Zn measured as M3-Zn relative to DTPA-Zn (Fig. 2) is a consequence of the greater acidity of the M3 extractant, which causes greater solubilization and desorption of Zn from clays, iron and aluminum oxides, stabilized organic molecules, and secondary minerals (Harter, 1991).

The correlation between edaphic variables (Table 2), the weak negative linear correlations (-0.38 and -0.27) between soil pH and tests (DTPA-Zn and M3-Zn) of soil available Zn can be explained by the fact that the solubility of Zn minerals and the Zn desorption from clays and oxides decrease with increasing pH (Harter, 1991). The correlation between soil pH and M3-Zn is weaker than that observed between soil pH and DTPA-Zn, which is probably a consequence of the near-neutral buffered pH of the DTPA extractant solution. Therefore, considering soil pH during Zn diagnosis would be more important when using M3-Zn compared with employing DTPA. A correlation between SOM and soil tests of available Zn (Table 2) has been reported by other authors (Catlett *et al.*, 2002; Barbieri *et al.*, 2017) and is partially a consequence of the large cation exchange capacity of SOM, which leads to an increase in the size of the soil exchangeable Zn pool (Wijebandara *et al.*, 2011).

Grain yield and grain Zn concentration response to Zn fertilizer

The maximum values of soil DTPA-Zn and M3-Zn concentration (6.42 mg kg^{-1} and 8.80 mg kg^{-1}, respectively; Table 1) were much less than greenhouse toxicity thresholds reported for maize (11 mg kg^{-1} DTPA-Zn, Takkar & Mann, 1978; 318 mg kg^{-1} M3-Zn, Borkert *et al.,* 1998). None of the evaluated trials presented significant negative yield response to Zn fertilizer application (Table 1). Therefore, the possibility of Zn phytotoxicity can be disregarded.

The lack of correlation between the potential yield of maize (grain yield for the +Zn treatment) and a yield response to fertilizer has already been reported for Zn (Barbieri *et al.,* 2017) and other nutrients that reach the root surface by diffusion and root-interception (Dodd & Mallarino, 2005). Plants growing in highly productive environments have greater growth and consequently a greater Zn demand, but also a larger capacity for soil exploration. Therefore, the greater plant demand for Zn is compensated by the greater access to soil Zn. As a result, a greater potential yield is not necessarily associated with a greater yield response to Zn fertilizer.

Our results for the Zn concentration of grain (Table S2, Supporting Information) confirm previous findings (Cakmak & Kutman, 2018; Zhao *et al.*, 2019) for wheat (*Triticum aestivum L.*) and rice (*Oryza sativa L.*), where Zn application at early crop stages improved grain yield, without affecting Zn concentration in grain. Cakmak & Kutman (2018) also reported that Zn concentration in grain of maize was less responsive to Zn application (9%) than were those in wheat and rice (86% and 27%, respectively). In those sites where grain Zn concentration was increased by Zn-fertilizer (n=4), soil Zn availability was greater than the Zn sufficiency thresholds determined for grain yield. At all the sites, Zn concentration in grain was less than the maize grain optimum Zn target for human health (38 mg kg^{-1}; Bouis & Welch, 2010).

Multiple regression models to predict maize grain yield response to Zn fertilizer

The weak coefficient of determination between relative yield and soil tests for available Zn (Table 3) suggests that other factors, which are not accounted for in these tests, determine Zn availability for maize. Some factors, which may have affected Zn availability and are not accounted for through DTPA-Zn or M3-Zn are: 1) Zn availability in deeper soil layers (> 20 cm), 2) Zn released by residues from previous crops, 3) Zn buffering capacity (Nair, 2019), and 4) limitations in Zn diffusion and plant uptake, determined by soil moisture (Marschner, 1993), temperature (Barrow, 1986; Almas *et al.*, 2000), and/or biological activity (Zhang *et al.*, 2017).

The inclusion of SOM into multiple regression models with DTPA-Zn or M3-Zn slightly improved their capacity to predict relative grain yield (Table 3). However, both models had a similar and poor predictive capacity ($R^2 = 0.31$ and 0.30, respectively). These results are consistent with the fact that SOM is correlated with both methods used to estimate soil Zn availability (Table 2). The small improvement in yield prediction was probably a consequence of SOM increasing Zn diffusion rate through desorption of Zn and formation of soluble complexes. Thus, Han *et al.* (2011) reported a large correlation coefficient between Zn uptake by soybean (*Glycine max* (L). Merr.) and organically bound Zn ($r = 0.92$; $p < 0.001$), and between this last variable and SOM ($r = 0.95$; $p < 0.001$). Based on the small values of the correlation coefficients of the DTPA-Zn or M3-Zn multiple regression models, their use for diagnosing maize grain yield response to Zn fertilizer would be unsatisfactory.

Zn-DTPA and Zn-M3 sufficiency level thresholds

The fixed critical thresholds for M3-Zn and DTPA-Zn calculated at a 97% relative yield goal were (1.97 mg kg^{-1} and 1.02 mg kg^{-1}, respectively). As opposed to the unsatisfactory results from the previously described regression models, the use of the "sufficiency level" approach, allowed successfully differencing soils into two categories: deficient or adequate Zn supplying capacity (69% correctly diagnosed cases for both methods) (Fig. 3). Previous greenhouse correlation studies reported similar DTPA-Zn thresholds for maize as the one determined in our study: 0.8 mg kg^{-1} (Lindsay & Norvell, 1978), 0.5 to 1.0 mg kg^{-1} (Lindsay & Cox, 1985), 1.2 mg kg^{-1} (Rashid & Rafique, 1989), and

1.35 mg kg^{-1} (Khoshgoftarmansh *et al.*, 2012). Differences between these thresholds are probably based on soil types and contrasting soil properties, the statistical method, and the relative yield goal used during the calibration (Tang *et al.*, 2009; Zamuner *et al.*, 2016).

In a field calibration study, Barbieri *et al.* (2017) determined a DTPA-Zn threshold of 0.99 mg kg^{-1} for the 97% relative yield goal, similar to our value. In contrast to DTPA-Zn, our study is the first M3-Zn field calibration for maize. Melgar *et al.* (2001) and Ruffo *et al.* (2016) reported non-significant relationships between M3-Zn and yield response to Zn fertilizer. The lack of association in these previous studies was probably a consequence of the small number of locations (Melgar *et al.,* 2001:n=14; Ruffo *et al.,* 2016: n=10) and the limited range of M3-Zn concentrations (from 0.7 to 2.1 mg kg^{-1} and from 0.9 to 2.9 mg kg^{-1}, respectively) determined in those studies.

Zn-DTPA and Zn-M3 economic thresholds

The sensitivity analysis resulted in different RY_{OE} scenarios with contrasting grain prices and grain yields goals, leading to different soil Zn availability economic thresholds (Table 4). Of 25 scenarios considered, three presented threshold values below the 95% confidence interval for a fixed 97% relative yield goal, while 13 were above that range. That is to say that in 64% of the scenarios any economic profit was lost by using the 95% confidence interval of a fixed 97% relative yield goal (sufficiency level): 12% by overestimating the soil Zn economic threshold and 52% by underestimating the soil Zn economic threshold. These results demonstrate how differences between seasons and regions on maize productivity and grain price make it unsuitable to establish a single Zn threshold for all economic-productive situations.

Our results demonstrated that, based on the relationship between M3-Zn and DTPA-Zn, either soil test can be predicted from the other without affecting the calibration (Fig. 4). These results indicate that a field calibration to determine M3-Zn thresholds would not be necessary for regions where DTPA-Zn has already been calibrated, or vice versa, if a regression model that associates M3-Zn and DTPA-Zn is available. However, there are discrepancies in the literature on the slope and intercept of these models (Table S3, Supporting Information). Therefore, further studies are necessary to explain the reasons behind the differences in DTPA-Zn versus M3-Zn models among studies or soils.

Despite the weak correlation between relative yield and the test value for available Zn in soil (Equations 5 and 6), the economic threshold approach (for a RY_{OE} between 90-99%) allowed a correct diagnosis of the grain yield response to Zn fertilizer in 65-85% of the sites (Fig. 5). Therefore, prior to sowing, when interpreting soil Zn test results and making decisions on whether or not to apply Zn fertilizer, it would be advantageous to define the RY_{OE} (Equation 4) and improve soil Zn testing by using the "economic threshold" approach (Fig. 4). When Zn-fertilizers are to be applied for maize, the main options are foliar-Zn or soil-Zn applications (Ruffo *et al,* 2016; Barbieri *et al.,* 2017). Foliar-Zn applications allow deficiencies to be corrected in the growing crop, at growth stages V_6-$V_{T,}$ whereas soil-Zn applications (at or before sowing) allow the application of large Zn rates, leading to a residual Zn availability especially in non-fixing soils (Alloway, 2009).

Conclusions

Our results demonstrated that Mehlich-3 and DTPA extractants for Zn had a similar capacity to predict maize yield. Their capacity was only slightly or not improved by the inclusion of pH, extractable P, or SOM in the predictive equation. Either soil test concentration of available Zn can be predicted from the other without affecting the field calibration results. The M3-Zn threshold we determined for maize (1.97 mg kg^{-1} for a fixed 97% relative yield goal) is the first obtained by field calibration, under a wide range of edaphic and climatic conditions. However, we suggest the use of an "economic threshold" instead of using a fixed critical threshold for DTPA-Zn or M3-Zn when deciding whether a foliar Zn fertilizer should be applied. The "economic threshold" approach could be also applicable to other low-mobility nutrients such as P. This threshold can be determined from the results of field calibration studies combined with readily available economic and productive data, allowing to account for economic, productive, and environmental factors into the farm fertilizer management, and making current agricultural systems more sustainable. Moreover, the "economic threshold" approach could be used as input for decision support systems, simulation systems, and site-specific management.

Supporting Information

Table S1 General information for 55 maize Zn-fertilizer application trials: location, soil type (USDA Soil Taxonomy), textural class, sowing date (SD), plant population (PP), and previous crop.

Table S2 Sowing date (SD), plant population (PP), and grain Zn concentration of the control and the

Zn fertilized treatments.

Table S3 Results from previously published linear regression models to predict zinc Mehlich 3 (mg kg^{-1}) from zinc DTPA (mg kg^{-1})

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Data accessibility statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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TABLES

Table 1 Soil properties and grain yield dataset for 55 maize Zn-fertilizer trials: soil DTPA extractable Zn (DTPA-Zn), Mehlich-3 extractable Zn (M3-Zn), extractable P (P-M3), pH, soil organic matter (SOM), grain yield (GY) on No Zn-fertilizer applied (Control) and Zn-fertilizer (+Zn) treatments, and ANOVA p-value for the GY comparison between Control and Zn-fertilized. SD: standard deviation, VC: variation coefficient.

Trial	DTPA-Zn	M3-Zn	P-M3	pH	SOM	GY Control	GY +Zn	p-value
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}		g kg^{-1}	Mg ha^{-1}	Mg ha^{-1}	
1	5.16	8.80	25.7	5.9	45.3	15.3	15.4	0.91
2	1.26	1.87	8.8	5.7	31.8	12.5	13.5	0.09
3	0.65	1.46	11.2	5.8	38.4	11.0	11.2	0.60
4	1.67	1.57	17.1	5.7	33.2	8.8	9.0	0.67
5	0.47	1.09	12.7	5.8	35.8	11.8	12.9	0.09
6	1.05	1.81	32.4	6.2	45.9	10.1	10.5	0.64
7	0.59	1.50	28.2	6.3	43.2	10.9	11.3	0.23
8	0.58	1.20	5.5	6.4	37.5	11.4	11.6	0.46
9	3.11	4.37	71.9	6.5	62.0	16.4	16.9	0.25
10	6.42	8.73	69.5	5.8	25.5	6.1	6.2	0.36
11	1.04	2.42	66.2	7.4	21.5	11.1	11.0	0.57
12	0.59	2.31	32.5	7.9	19.7	10.6	11.3	0.45
13	0.78	2.18	21.7	6.0	23.8	11.1	12.1	0.08
14	1.37	2.08	9.2	5.6	29.0	12.3	13.3	0.07
15	2.10	4.60	68.0	5.9	57.1	18.3	17.7	0.38
16	3.20	4.81	60.2	6.0	53.8	12.3	12.0	0.32
17	2.15	3.14	60.2	6.0	53.8	6.3	6.2	0.34
18	1.13	1.65	27.0	6.3	19.9	7.2	8.3	0.08
19	2.81	4.34	256.8	5.6	19.8	10.2	10.6	0.43
20	2.19	3.36	31.0	6.0	32.1	12.6	11.9	0.24

21	0.99	1.61	15.5	5.9	30.4	11.6	12.9	0.01
22	3.13	4.36	10.3	5.8	62.1	9.0	8.8	0.41
23	2.88	3.58	12.0	6.0	69.7	8.6	8.3	0.51
24	2.58	3.41	8.2	6.0	61.6	8.2	8.3	0.20
25	1.75	2.85	34.0	6.0	56.9	8.6	8.9	0.52
26	1.98	2.64	62.2	5.4	55.6	14.3	14.7	0.64
27	1.05	1.68	17.8	6.2	58.4	10.3	9.9	0.34
28	1.77	2.46	8.7	5.9	60.0	10.9	10.9	0.99
29	1.94	2.67	12.7	5.9	73.5	11.5	11.4	0.64
30	1.89	3.22	49.1	5.9	54.8	12.2	12.6	0.58
31	0.37	3.10	18.3	6.4	29.1	7.2	8.2	0.15
32	0.11	1.79	19.6	8.2	11.2	9.5	10.2	0.20
33	0.98	2.10	25.0	5.9	43.4	9.0	9.2	0.60
34	1.15	2.42	27.3	5.5	34.0	12.1	13.7	0.03
35	0.23	0.45	18.4	8.1	17.1	4.6	6.5	0.08
36	0.89	1.20	29.9	6.0	20.0	8.7	8.8	0.93
37	0.27	0.69	12.8	5.8	19.8	8.2	9.2	0.08
38	0.88	1.20	71.5	6.9	25.5	9.1	9.7	0.05
39	0.28	0.47	19.8	8.0	44.3	10.4	10.7	0.06
40	0.44	1.15	29.3	6.0	35.3	12.4	12.3	0.83
41	4.67	7.50	46.1	5.8	40.7	10.5	10.1	0.30
42	1.73	2.69	34.8	5.8	28.0	9.9	9.5	0.25
43	4.02	6.89	26.5	6.3	31.3	9.2	9.4	0.78
44	0.83	2.44	29.4	6.9	22.8	7.7	6.2	0.21
45	0.42	1.10	45.5	7.0	20.5	8.6	9.8	0.06
46	0.79	1.49	14.8	6.4	25.3	9.1	10.2	0.02
47	0.28	0.64	71.5	6.9	25.5	11.1	11.9	0.02
48	0.96	2.40	268.3	7.2	20.0	4.1	3.9	0.66
49	0.98	1.57	38.6	5.6	28.0	9.8	11.3	0.09

50	0.74	1.27	81.3	5.9	34.0	12.6	13.6	0.06
51	0.65	1.28	65.5	5.9	28.3	12.7	13.1	0.50
52	2.28	3.35	27.0	5.8	61.0	13.2	13.4	0.91
53	1.99	2.22	31.5	5.9	30.0	8.8	8.9	0.66
54	1.18	1.74	23.0	5.8	28.3	13.6	13.6	0.96
55	0.65	1.17	14.4	6.5	18.0	6.1	5.9	0.53
Average	1.56	2.62	40.7	6.2	36.9	10.3	10.7	
SD	1.30	1.86	48.2	0.7	15.8	2.7	2.7	
Median	1.05	2.18	27.3	6.0	32.1	10.4	10.7	
Min. value	0.11	0.45	5.5	5.4	11.2	4.1	3.9	
Max. value	6.42	8.80	268.3	8.2	73.5	18.3	17.7	
VC (%)	83.37	71.08	118.0	10.7	42.6	26.0	25.5	

Table 2 Correlation coefficients between extractable zinc soil tests [DTPA extractable Zn (DTPA-Zn) and Mehlich-3 extractable Zn (M3-Zn)] and edaphic properties [pH, available phosphorus (P-M3), and soil organic matter (SOM)].

r/p†	pH	P-M3	SOM	DTPA-Zn	M3-Zn
pH	1	ns	<0.01	<0.01	0.04
P-M3	0.07	1	ns	ns	ns
SOM	-0.38	-0.20	1	<0.01	0.03
DTPA-Zn	-0.38	0.13	0.38	1	<0.01
M3-Zn	-0.27	0.16	0.29	0.94	1

† The lower left part of the table shows the correlation coefficients (r), while the upper right part presents the probability values (p-values). ns = not significant.

Table 3 Multiple regression models to predict maize relative yield (RY) from DTPA extractable zinc (DTPA-Zn, mg kg^{-1}), Mehlich-3 extractable zinc (M3-Zn, mg kg^{-1}), soil organic matter (SOM, g kg^{-1}), pH, and extractable phosphorus (P-M3, mg kg^{-1}). Data outside the range of plant response (RY>100%) was excluded (n = 38).

Model	Dependent variable	Predictor	Parameter	Standard error	p-value	Partial R^2	Adj. R^2
Model 1	RY (%)	Intercept	86.43	2.04	<0.0001		0.31
		DTPA-Zn	1.26	0.57	0.03	0.17	
		SOM	0.17	0.06	0.006	0.14	
		P-M3			ns†		
		pH			ns		
Model 2	RY (%)	Intercept	85.92	2.10	<0.0001		0.30
		M3-Zn	0.80	0.40	0.05	0.15	
		SOM	0.18	0.06	0.003	0.15	
		P-M3			ns		
		pH			ns		

† ns = not significant.

Table 4 Sensitivity analysis of zinc economic thresholds based on DTPA (mg Zn kg^{-1}) and Mehlich-3 (M3) (mg Zn kg^{-1}) extractants, for different prices of maize, and production systems.

Grain yield Zn-fertilizer applied (+Zn) treatment (Mg ha^{-1})	Historical (2004-2018) interior maize prices at Illinois ($US Mg^{-1})														
	Min: 75.3			Q1: 99			Q2: 160			Q3: 179.8			Max: 333		
	RY_{OE}†	DTPA	M3	RY_{OE}	DTPA	M3	RY_{OE}	DTPA	M3	RY_{OE}	DTPA	M3	RY_{OE}	DTPA	M3
Min§: 3.9	90.2	0.42#	1.00#	94.8	0.73#	1.52#	95.4	0.79#	1.62#	96.6	0.96	1.87	98.1	1.26	2.32
Q1‡: 8.9	95.7	0.83	1.68	97.8	1.18	2.20	98.0	1.25	2.29	98.5	1.41#	2.52#	99.2	1.69#	2.90#
Q2: 10.7	96.4	0.93	1.83	98.1	1.28	2.34	98.3	1.34#	2.43#	98.8	1.51#	2.65#	99.3	1.78#	3.01#
Q3: 12.5	96.9	1.01	1.95	98.4	1.36#	2.46#	98.6	1.43#	2.54#	98.9	1.58#	2.75#	99.4	1.85#	3.10#
Max¶: 17.7	97.8	1.20	2.23	98.9	1.55#	2.71#	99.0	1.61#	2.79#	99.3	1.75#	2.98#	99.6	2.00#	3.29#

† RY_{OE} = optimum economic relative yield goal.

‡ Qn = quartile

§ Min = minimum value

¶ Max = maximum value

Critical soil zinc test threshold that is outside the 95% confidence interval estimated for 97% relative yield goal.

FIGURE CAPTIONS

Figure 1 Map indicating the location of the experimental sites conducted from 2009 – 2017 in Argentina.

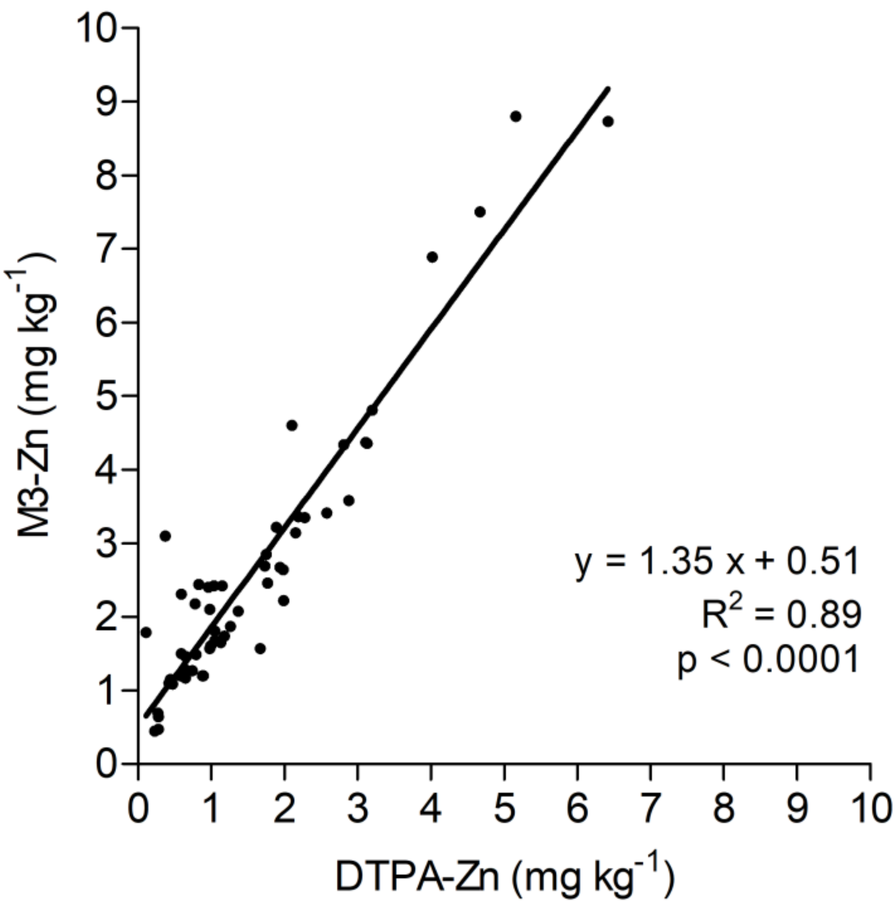
Figure 2 Relationship between Mehlich-3 (M3-Zn) and DTPA (DTPA-Zn) extractable Zn, for 55 trials of soils in Argentina from maize fields.

Figure 3 Relationship between maize yield (RY) in control plots relative to those receiving Zn-fertilizer (Relative Yield) and soil zinc Mehlich-3 (M3-Zn) (A) or zinc DTPA (DTPA-Zn) (B) content (0–20 cm) across 55 trials using the modified arcsine-logarithm calibration curve (Correndo *et al.*, 2017). CT: critical soil zinc test threshold estimated at a 97% RY. $CI_{95\%}$: 95% confidence interval for CT.

Figure 4 Relationship between soil test DTPA-Zn or M3-Zn thresholds (0–20 cm) and maize optimum economic relative yield goal (RY_{OE}) (%) determined by the modified arcsine-logarithm calibration curve. Soil DTPA-Zn results are also presented as estimated from M3-Zn using the equation in Fig. 2 (M3-Zn = 1.35 DTPA-Zn + 0.51, R^2 = 0.89), and vice versa.

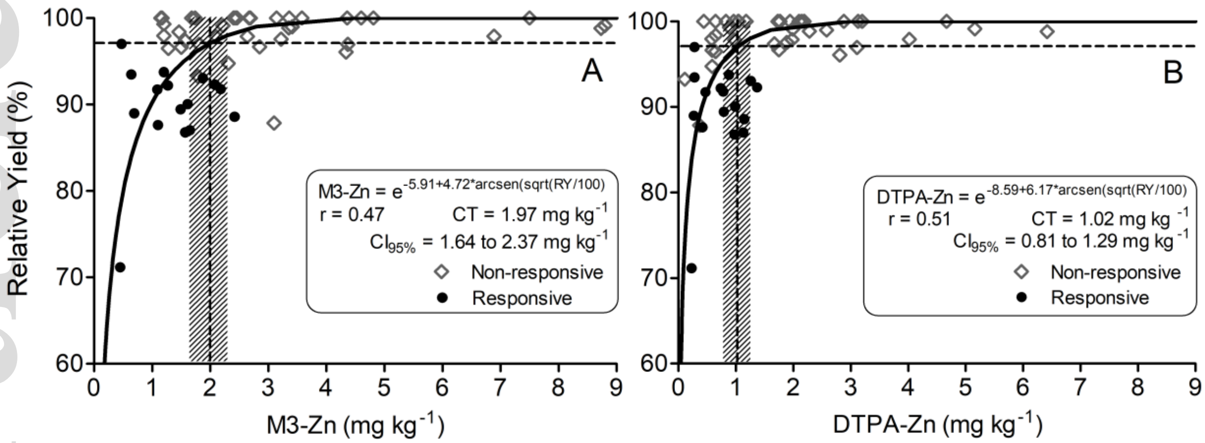
Figure 5 DTPA-Zn and M3-Zn correctly diagnosed cases (CD) (trials in quadrants II and IV), type I error (trials in quadrants I, recommends zinc when not needed), and type II error (trials in quadrants III, zinc not recommended but needed) (Fig. 3) versus optimum economic relative yield goal (RY_{OE}) (%).

Figure 1.



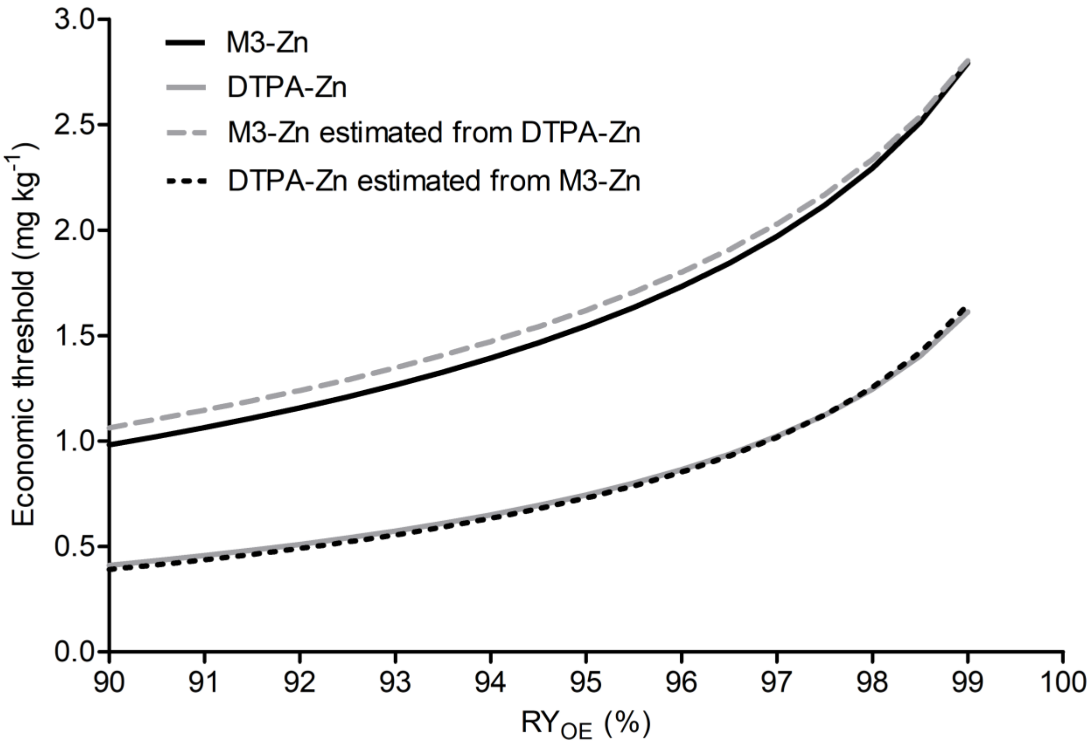
M3-Zn (mg kg-1)
DTPA-Zn (mg kg-1)
0
1
2
3
4
5
6
7
8
9
10
y = 1.35 x + 0.51
R2 = 0.89
p < 0.0001

Figure 2.



Relative Yield (%)
100
90
80
70
60
0 1 2 3 4 5 6 7 8 9
M3-Zn (mg kg-1)
A
M3-Zn = e-5.91+4.72*arcsen(sqrt(RY/100)
r = 0.47 CT = 1.97 mg kg-1
CI95% = 1.64 to 2.37 mg kg-1
Non-responsive
Responsive
DTPA-Zn (mg kg-1)
B
DTPA-Zn = e-8.59+6.17*arcsen(sqrt(RY/100)
r = 0.51 CT = 1.02 mg kg-1
CI95% = 0.81 to 1.29 mg kg-1
Non-responsive
Responsive

Figure 3.



M3-Zn
DTPA-Zn
M3-Zn estimated from DTPA-Zn
DTPA-Zn estimated from M3-Zn
Economic threshold (mg kg⁻¹)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
90
91
92
93
94
95
96
97
98
99
100
RYOE (%)

Figure 4.

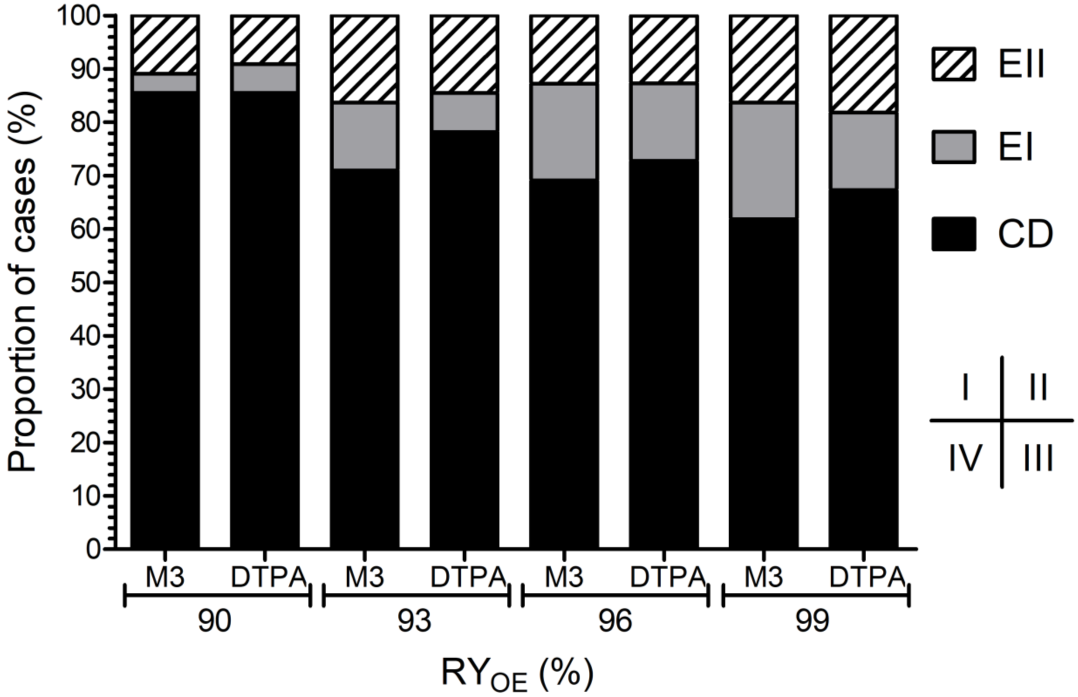

100
90
80
70
60
50
40
30
20
10
0
Proportion of cases (%)
M3
DTPA
M3
DTPA
M3
DTPA
M3
DTPA
90
93
96
99
RYOE (%)
EII
EI
CD
I
II
IV
III

Figure 5.