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Correlation between physiological tests and field emergence in treated corn seeds

Lucas Caiubi Pereira, Larissa Vinis Correia, Pedro Henrique Felber, Renata Cristiane Pereira, Thaisa Cavalieri Matera, Rayssa Fernanda dos Santos, Alessandro Lucca Braccini*

Department of Agronomy, Universidade Estadual de Maringá, Maringá-PR, Brazil
**Corresponding author: lucascaiubi@yahoo.com.br*

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Abstract: The objective of this work was to evaluate the degree of linear association between field emergence with the results of germination and vigor of corn seeds belonging to different initial vigor and submitted to agrochemical treatment. Following seed treatments with (i) carbendazim/thiram + imidaclopride/tiodicarbe; (ii) piraclostrobine, methyl tiophanate and fipronil; (iii) methyl tiophanate/fluazinam + bifentrine/imidaclopride; and (iv) metalaxyl-m/fludioxonil + thiamethoxam, seeds belonging to four different corn cultivars were assessed for their physiological potential. The strength of the Pearson correlation between germination and vigor tests with field emergence decreased after the chemical treatment, to a degree depending on the slurry composition, initial vigor and the test's substrate.

Keywords: *Zea mays* L.; seed quality; stressful condition; pesticide; insecticide; fungicide

Successful seedling establishment in the field is one of the first critical steps for corn grain production. Besides the use of high-quality seeds, to obtain a given crop density, it is indispensable to accurately calculate seeding rates, a process based on the results of the germination. However, since it is conducted under ideal environmental conditions, this test may be inefficient to account for seeds able germinate, but that fail to develop into viable plants in the field, where the characteristics of the substrate, humidity, and temperature are frequently unfavorable (Baldini et al. 2018).

For that reason, worldwide seed quality control systems simultaneously perform additional physiological evaluations, namely vigor tests, that simulate stressful conditions to have a more realistic prediction of crop emergence in the field (Lovato et al. 2005, Baldini et al. 2018). However, the widespread plant-protection operation of treating corn seeds with pesticides has been pointed out as a factor able to considerably interfere with seed and seedling performance (Yousof et al. 2016), affecting, therefore, the precision of the estimations assessed through physiological tests. In this context, this work aimed at evaluating the degree correlation between field emergence with the results of germination and vigor of corn seeds belonging to different initial vigor and submitted to agrochemical treatment.

MATERIAL AND METHODS

Seedlots belonging to four corn cultivars were tested: Garra Viptera, Maximus TL TG VIPTERA, Impacto, and Formula TL. To minimize the impact that seed size may have on seeding vigor, only seeds of 18/64'' thickness and 22/64'' width (round screen) were used. Preliminary physiological evaluations of each seed lot were performed to assess their initial germination and vigor levels, and the results obtained were herein named high initial physiological quality (HIQ). Then, to obtain seeds of low initial physiological quality (LIQ), a portion of each seed lot was artificially aged in a water-jacketed chamber at 40 ± 1°C and 100% relative

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humidity for 24 h, following the same procedure of the accelerated aging (ISTA 2017).

Seeds were treated in a continuous lot seed-coating device, employing a combination commercial fungicides and insecticides (technologies) as follow: Technology I (slurry volume of 410 mL/100 kg) – carbendazim 150 g/L + thiram 350 g/L (Derosal Plus, 60 mL/100 kg) plus imidacloprid 150 g/L + thiodicarb 450 g/L (Cropstar, 350 mL/100 kg); Technology II (250 mL/100 kg) – pyraclostrobin 25 g/L + methyl thiophanate 225 g/L + fipronil 250 g/L (Standak Top, 250 mL/100 kg); Technology III (680 mL/100 kg) – methyl thiophanate 350 g/L + fluazinam 52.5 g/L (Certeza,180 mL/100 kg) plus bifenthrin 135 g/L + imidacloprid 165 g/L (Rocks, 500 mL/100 kg) and Technology IV (270 mL/100 kg) – metalaxyl-m 10 g/L + fludioxonil 25 g/L (Maxim Advanced, 150 mL/100 kg) plus, thiamethoxam 350 g/L (Cruiser 350 FS, 120 mL/100 kg). Treated and untreated seeds (control) were submitted to the physiological analyses as described below:

Moisture content (MC); germination between paper (GP); germination in the sand (GS), accelerated aging (AA) and cold test (CT) were performed according to ISTA (2017), while the electrical conductivity (EC) was performed using the mass method described in AOSA (2002). Simultaneously, lots were also submitted to a modified accelerated aging test (MAA), where part of the seeds aged in the AA test was germinated in sand bed following the same procedure of the GS test. A modified cold test (MCT) was also performed, and it was conducted similarly to GP, however, before germination, the rolls were wrapped in plastic bags and remained under 10 ± 1°C for seven days. The emergence in the greenhouse (EG) was carried out using four replications of 100 seeds per treatment in 200 × 300 × 60 mm non-sterile sand trays. Water was added to the substrate at the rate of 0.1 times the weight of the dry sand, and this level was maintained during the test by adding water when needed. Normal emerged seedlings were counted on the 15th day after sowing. For the emergence in the field (EF), at a depth of 5 cm, four 100-seed subsamples were distributed in a 2-meter-length furrow spaced 50 cm apart from each other. The precipitation, as well as the temperature, were recorded at a nearby weather station (23°02'S; 52°04'W and altitude of 509 m a.s.l.), and, after 15 days, the percentage of normal seedlings was calculated.

Apart from the emergence in the field, to which the randomized block design was used, the experiment was conducted in a completely randomized layout in a 4 × 2 × 4 factorial scheme (cultivars × vigor × technologies) with four replications. The data obtained were tested for normality (Shapiro-Wilk) and homogeneity (Levene). The variable expressed as a percentage were arcsine $(X/100)^{-1/2}$ transformed to be normalized; however, in tables, the original means were used. Then, the dataset was submitted to analysis of variance ($P < 0.05$), and when significant, the Student-Newman-Keuls (SNK) test was adopted to compare the means. To investigate the degree of association between emergence in the field, and the other physiological tests, the Pearson correlation coefficient ($P < 0.05$) was calculated.

RESULTS AND DISCUSSION

No interaction between the genotypes and the other two factors was found in the three-factorial analysis; however, vigor level and chemical treatments had a significant result in the two-way ANOVA. These results differ from those of Tamindžić et al. (2013), who observed genotypic differences in the physiological response of corn to seed-applied pesticides. However, the authors used different cultivars, higher pesticide doses as well as did not add fungicides to slurries.

The variables GS, CT, and MCT, were not influenced by chemical treatments regardless of the vigor, whereas, for EG and EF, the no impact of the seed-applied pesticides was observed only in HIQ and LIQ, respectively (Table 1). On the other hand, regarding other tests, chemical treatment provided a higher reduction of the physiological potential in LIQ seed lots than in HIQ (Table 1), a fact explained by the artificial aging performed that might have fastened the process of seed deterioration (Silva and Villela 2011). Concerning MC, although between technologies, any difference was observed (Table 1), seed treatment increased seed moisture compared to the control. Nonetheless, regardless the vigor, treatments containing the highest slurries volumes (technologies III and I, respectively) promoted the strongest adverse effects on germination (GP and GS) and vigor (AA, MAA, EG and EF), which may be attributed their high-aqueous content that may have resulted in irreparable membrane damages caused by a faster water uptake (Reedy and Knapp 1990).

However, overall, none of the technologies compromised the sales potential of seeds, since the results of germination (GP or GS) were higher than 85% (Table 1), the minimum rate for sale of corn seeds lots in Brazil (MAPA 2013). As shown

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Table 1. Mean values of moisture content (MC); germination between paper (GP); germination in sand (GS); accelerated aging (AA); modified accelerated aging (MAA); electrical conductivity (EC); cold test (CT); modified cold test (MCT); emergence in greenhouse (EG) and emergence in field (EF) of corn seeds of high (HIQ) and low initial quality (LIQ) belonging to four cultivars (Garra Viptera, Maximus TL TG VIPTERA, Formula TL, and Impacto) submitted to four industrial seed treatments

Treatment	MC (%)		GP (%)		GS (%)		AA (%)		MAA (%)	
	HIQ	LIQ	HIQ	LIQ	HIQ	LIQ	HIQ	LIQ	HIQ	LIQ
Control	13.2^{Ba}	14.3^{Bb}	98^{Aa}	95^{Ab}	97^{Aa}	91^{Ab}	84^{Aa}	72^{Ab}	90^{Aa}	75^{Ab}
Tec I	14.4^{Aa}	15.2^{Ab}	95^{Ba}	87^{Cb}	98^{Aa}	89^{Ab}	80^{Ba}	56^{Cb}	78^{Ba}	65^{Cb}
Tec II	14.6^{Aa}	15.3^{Ab}	95^{Ba}	90^{Bb}	98^{Aa}	91^{Ab}	78^{Ba}	65^{Bb}	91^{Aa}	70^{Bb}
Tec III	14.2^{Aa}	15.4^{Ab}	94^{Ba}	86^{Cb}	97^{Aa}	90^{Ab}	79^{Ba}	45^{Db}	72^{Ca}	62^{Cb}
Tec IV	14.3^{Aa}	15.5^{Ab}	96^{Ba}	89^{Bb}	96^{Aa}	88^{Ab}	81^{Ba}	67^{Bb}	92^{Aa}	69^{Bb}
	EC (µS/cm g)		CT (%)		MCT (%)		EG (%)		EF (%)	
	HIQ	LIQ	HIQ	LIQ	HIQ	LIQ	HIQ	LIQ	HIQ	LIQ
Control	115.13^{Ba}	175.12^{Ab}	91^{Aa}	88^{Ab}	97^{Aa}	93^{Ab}	97^{Aa}	82^{Ab}	96^{Aa}	85^{Ab}
Tec I	170.37^{Aa}	210.37^{Bb}	90^{Aa}	85^{Ab}	98^{Aa}	92^{Ab}	98^{Aa}	72^{Bb}	92^{Ba}	84^{Ab}
Tec II	177.78^{Aa}	218.34^{Bb}	92^{Aa}	83^{Ab}	96^{Aa}	91^{Ab}	97^{Aa}	80^{Ab}	95^{Aa}	83^{Ab}
Tec III	179.12^{Aa}	278.76^{Cb}	90^{Aa}	86^{Ab}	95^{Aa}	92^{Ab}	99^{Aa}	75^{Cb}	90^{Ba}	84^{Ab}
Tec IV	178.37^{Aa}	208.21^{Bb}	89^{Aa}	82^{Ab}	97^{Aa}	94^{Ab}	98^{Aa}	80^{Ab}	94^{Aa}	86^{Ab}

According to Student-Newman-Keuls (SNK) test, means followed by the same uppercase letter in the column belong to the same group at 5% probability, while means followed by the same lowercase letters in the line do not differ from each other by the *F*-test at a 5% probability level

in Table 2, GP showed the lowest correlation value, indicating that it was not the most suitable test to provide a reliable relationship with the emergence in the field, mainly regarding treated seeds. The same was reported by Baldini et al. (2018) and Lovato et al. (2005), who further stated that due to its near-optimal temperature and humidity, condition hardly found in the field, seed lots of

Table 2. Pearson correlation coefficient between emergence in field and germination between paper (GP); germination in sand (GS); accelerated aging (AA); modified accelerated aging (MAA); cold test (CT); modified cold test (MCT); electrical conductivity (EC) as well as emergence in greenhouse (EG) of corn seeds of both high (HIQ) and low initial physiological quality (LIQ) belonging to four corn cultivars (Garra Viptera, Maximus TL TG VIPTERA, Formula TL and Impacto) and submitted to four industrial seed treatments

	Treatment	GP		GS		AA		MAA	
		HIQ	LIQ	HIQ	LIQ	HIQ	LIQ	HIQ	LIQ
Emergence in field	control	0.834	0.754	0.865	0.823	0.825	0.811	0.873	0.876
	Tec I	0.654	0.654	0.854	0.634	0.814	0.810	0.856	0.856
	Tec II	0.785	0.669	0.855	0.712	0.816	0.802	0.889	0.867
	Tec III	0.689	0.654	0.811	0.645	0.804	0.801	0.878	0.857
	Tec IV	0.794	0.687	0.837	0.773	0.802	0.811	0.889	0.865
		CT		MCT		EC		EG	
		HIQ	LIQ	HIQ	LIQ	HIQ	LIQ	HIQ	LIQ
	control	0.431	0.335	0.534	0.443	–0.743	–0.675	0.853	0.834
	Tec I	0.434	0.345	0.545	0.434	–0.634	–0.564	0.783	0.745
	Tec II	0.467	0.352	0.467	0.487	–0.723	–0.643	0.814	0.813
	Tec III	0.434	0.245	0.389	0.367	–0.673	–0.539	0.745	0.710
	Tec IV	0.443	0.353	0.457	0.345	–0.726	–0.673	0.824	0.745

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different deterioration level, often, do not differ in germination (Yousof et al. 2016).

According to Hinkle et al. (2003), a strong linear relationship between two variables show values higher than 0.8; however, in physiological tests of rice (Lorentz and Nunes 2013) and onion seeds (Gonçalves et al. 2017) suggested as acceptable values above 0.6 and 0.7, respectively. Nevertheless, due to the natural vigor and germination differences that may exist across seed lots within the same crop, there are no fixed reference values for interpreting the Pearson coefficient in the area of seed testing, demanding that a dataset analysis specific reading for a given sample set. In this work, corroborating Lovato et al. (2005), both AA and MAA tests were the most effective for predicting maize seedling emergence in the field (Table 2), followed by EC and EG.

Jointly with the temperature, the relative humidity is considered the most important aspect to affect seed viability and longevity (Boligon et al. 2011). For this reason, the use of the AA test in tropical cropping systems is a key indicator to estimate seed performance in the field (Bittencourt and Vieira 2006). In this work, Figure 1 corroborates this statement, since, over the conduction of the EF test, the accumulated rainfall was 105 mm, the mean relative humidity was 78% and the temperatures minimum and maximum were, respectively, 15°C and 32°C (Figure 1), values considered standard for the macroregion of Maringá-PR, Brazil, in November (MAPA 2017).

In this study, both AA and MAA tests allowed technologies to be differentiated into different vigor levels, with the imidacloprid-based technologies I and III (which also had the highest slurry volumes) standing out as the most harmful (Table 1). Such as in this work, imidacloprid has been reported to impair vigor and germination of horticultural as well as in large-field crops such as soybean and wheat (Taylor and Salanenka 2012, Abati et al. 2014, Shakir et al. 2016). In this sense, investigating the movement of traced compounds in corn seeds, Dias et al. (2014) and Yang et al. (2018) reported that as a non-ionic moderately lipophilic compound, imidacloprid is considered to be able to pass the barrier formed by the cutinised-suberised of seed membranes during the imbibition, exposing the embryo to a direct contact with the insecticide. This same ability to pass through seeds membrane has been documented in systematic antifungal molecules; however, at commercial doses, these products have not been reported to impair seed quality (Baldini et al. 2018).

Negative correlations were found between EF and the EC (Table 2), indicating that the value increase of one variable is associated with a decreasing of the other. This same performance has also been described for other crops, and it is related to the nature of the test, in which deteriorated seeds, when soaked in water, leak larger amounts of electrolyte or cell metabolites because of their inefficient mechanism of membrane repair (Diniz et al. 2013). Among the slurries tested, the EC results of technologies I and III stood out as the most harmful ones, whereas untreated seeds exhibited the lowest values of it (Table 1). It should be pointed, however, that commercial formulations of pesticides are expected to have intrinsic electrical conductivity (Cunha et al. 2017), meaning that part of the increased

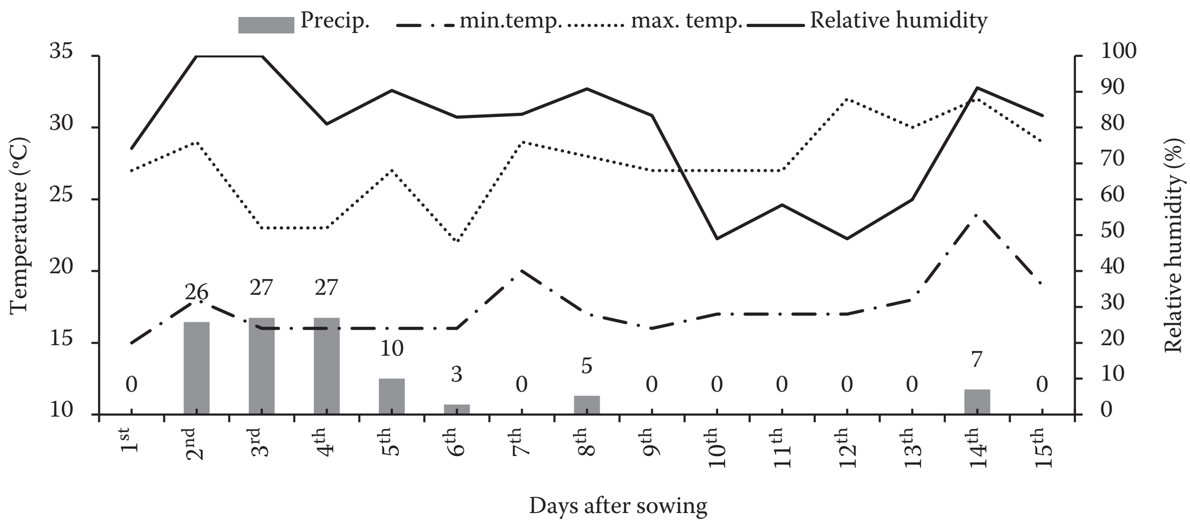

Figure 1. Meteorologic data indicating the relative humidity, minimum and maximum temperatures and rainfall precipitation for the 15-day period of the emergence in the field test (Maringá-PR, Brazil, from November 3 to 17, 2017)

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EC values after treatment may be primarily related to slurries composition than to any deterioration caused by their use.

Regarding CT and MCT, poor correlations were found with EF, results that contrast with those reported by Baldini et al. (2018) and that might be primarily related to the cool tolerance (Lovato et al. 2005) of the cultivars, as they are also recommended for winter cultivation, but also to the fact that non-limiting temperature value (lower than 15°C) was recorded in field. Overall, treated seeds showed higher *r*-values in soil or sand-based tests (GS, MAA, and EG) than in the paper-based ones (GP, AA, and CT) (Table 2). This same behavior was also observed in the means test, in which higher physiological potential was observed in sand-based tests (Table 1). According to Taylor and Salanenka (2012), this might be attributed to the ability of the sand and soil structure to dissolved chemicals with the percolating water, which diminishes the concentration of active ingredients near the seeds, reducing, thus, the risk of phytotoxicity. In conclusion, the strength of correlation between physiological tests and field emergence in corn seeds decreased with a chemical treatment to a degree dependent on the slurry composition (volume), lot vigor, and the test's substrate.

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