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Effects of some chemical treatments on standard germination, field emergence and vigour in hybrid maize seeds

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Abstract

The main aim of this experiment was to investigate the possible effects of some active ingredients and their combinations, commonly adopted for maize seed treatment in Italian agriculture, on seed germination, vigour (cold test) and field emergence. Among the seed treatments, fludioxonil + metalaxyl-M (non-systemic fungicides) with the insecticide mixture tefluthrin (belonging to the pyrethroids class) and methiocarb (belonging to the carbamates), negatively affected field emergence of hybrid maize seeds under favourable and restricted temperature conditions, and the cold test results. The two insecticides showed a significant synergistic detrimental action compared with the effects of each active ingredient. These results confirm the need for precaution when using new formulations with an insecticide mixture. The cold test was confirmed as an excellent test able to highlight a possible loss of seed vigour, which usually is not evident with a standard germination test, and to estimate seed performance in the field, especially under cold temperatures, as often happens when adopting modern crop management techniques like sod seeding and early sowing at the end of winter.

Keywords: cold test, field emergence, fungicides, germination, insecticides, maize, sowing time

Introduction

Many authors consider that the major limitation of the germination test is its inability to detect differences in field performance under sub-optimal conditions among seed lots of high and similar germination (Roberts, 1984). Hence the need for tests able to measure seed vigour, defined by ISTA (2017) as the sum of those properties that determine the activity and performance of seed lots of acceptable germination in a wide range of environments.

Up to now, specialised farming techniques, such as no-tillage and early-spring sowing, have been adopted in maize (*Zea mays* L.) cultivation because of the many agronomic advantages (Johnson *et al.*, 1984; Johnson and Lowery, 1985; Al-Darby and Lowery, 1987; Blandino *et al.*, 2008; Sharratt and Gesch, 2008). Late-winter sowing in northern

Italy is now a common practice in maize (Noli *et al*., 2008; Otto *et al*., 2009), however seed germination and emergence are hindered under cold and wet soils. For these reasons, interest from farmers and the seed industry in the cold test, the vigour test for maize most widely used in North America and North Europe (TeKrony, 1983; Ferguson, 1990; Hampton, 1992), has also been growing significantly in recent years in Italy. Cold test methods differ between laboratories, reproducing the climatic conditions of a specific environment. Van Waes (1995) emphasised that germination at 10°C (ISTA, 2017) does not always accurately discriminate seed lot vigour compared with 5°C (Lovato *et al*., 2005). Moreover, the utilisation of sterile sand instead of field soil as a substrate could exclude any effect of pathogens as a discriminating factor for differences in seed vigour (Lovato *et al*., 2001), as well as satisfactorily predicting field performance (Bruggink *et al*., 1991; Lovato *et al*., 2005; Noli *et al*., 2008).

The maize cultivated area in Italy was 775,000 hectares in 2015 (ISTAT, 2016), 90% of which is concentrated in the northern regions. About 18,000 tons of seed is needed for sowing and 90% of the maize seed distributed on the Italian market has undergone a basic protective treatment with fungicides and approximately 60% of these are then treated with an insecticide product (Assosementi, 2008). Among many factors, the chemical treatments of maize seeds, and especially insecticides, could affect seed vigour (Marchi and Cicero, 2003; Bekarović, 2012; Hejlik, 2012), reducing the ability of seeds to germinate and emerge in cold wet soils.

This experiment therefore aimed to assess how some active ingredients and their combinations, commonly adopted for maize seed treatment in Italian agriculture, can affect seed germination and vigour, measured by the cold test, in comparison with germination in the field at different sowing times.

Materials and methods

The study was performed at the experimental farm of University of Udine, Friuli Venezia Giulia Region, Italy, located 10 km from Udine (46° 04' N, 13° 22' E, altitude 109 m a.s.l. and 0% slope) during two periods: the field trials were conducted in March-May 2016, the laboratory experiments in June-August of the same year.

Maize seed lots

The analysed seeds were supplied by Pioneer; all belonged to the same genotype, the hybrid P1517W (700 FAO class), and were from the same harvest period (2015), represented by six different lots, one from Italy, one from Chile and four from USA.

The supplier performed germination tests for each seed lot before any seed treatment, obtaining very high and similar germination rates among lots (92-94% as range); despite the different origins, the seeds were considered as a single lot.

Maize seeds were treated with fungicides, insecticides and a combination of the two, following the most conventional technologies adopted by seed companies and in agreement with the seed maize treatments requested by the Italian market. More specifically, six treatments were adopted as source of variation, at the recommended application rates (table 1).

Table 1. Treatment code, active ingredients and application rate as commercial formulation.

Treatment	Active ingredient	Application rate (ml of formulate for 25,000 seeds)
A	Control	-
B	Fludioxonil[1] + Metalaxyl-M[1]	6.25[1]
C	Fludioxonil[1] + Metalaxyl-M[1] + Methiocarb[2]	6.25[1] + 74.8[2]
D	Fludioxonil[1] + Metalaxyl-M[1] + Thiacloprid[3]	6.25[1] + 62.5[3]
E	Fludioxonil[1] + Metalaxyl-M[1] + Tefluthrin[4]	6.25[1] + 75[4]
F	Fludioxonil[1] + Metalaxyl-M[1] + Methiocarb[2] + Tefluthrin[4]	6.25[1] + 74.8[2] + 75[4]

Commercial formulation as follow: [1]Celest XL; [2]Mesurol 500 FS; [3]Sonido; [4]Force 20 CS.

Standard germination test (SG)

The standard germination test was performed according to the criteria established by ISTA (ISTA, 2017). Briefly, four replications of 100 pure seeds per treatment were planted in 200 × 300 × 60 mm sterile sand trays at 25°C in a climatic chamber, with evaluation of germinated (normal and abnormal seedlings) and non-germinated seeds at final counts made seven days after planting and considering 100 seeds × 4 replications × 6 treatments. The amount of water added was about 0.1 times the weight of the dry substrate at the start of sowing and this level was maintained during the trial period by adding water when necessary.

Cold test (CT)

Seeds were exposed to a 7-day cold treatment at 5°C with moist sterile sand (Lovato *et al*., 2001; ISTA, 2017) and then submitted to the SG test. Count(s) were performed at 14 days after planting and interpretation considered 100 seeds × 4 replications × 6 treatments.

Field evaluation

Two field trials were conducted at the experimental farm of the University of Udine, Friuli Venezia Giulia Region, Italy, located 10 km from Udine, during the 2016-sowing season. One trial was sown in the first week of March, an early sowing (ES), simulating a cultivation technique that is becoming established for maize in northern Italy; the other was sown on 20 April, a more typical sowing time (TS) adopted for maize cultivated in the Friuli Venezia Giulia region. The experimental field is characterised by a shallow soil (about 500 mm) with a loamy-sandy texture (average sand, silt and clay, 40, 43 and 17%, respectively) and a moderate continental climate with wet connotation (average yearly rainfall 1523 mm; minimum and maximum temperature 8.0 and 18.4°C, respectively). Rainfall and temperature were monitored during the trial period (figure 1).

Both trials were sown by hand adopting a randomised complete block design with four replications. Each experimental unit included 50 seeds for each treatment arranged in a single 2.5 m row, spaced 50 mm apart in a row 250 mm wide, with a seed depth of 40 mm. Field seedling emergence (when the coleoptile was at least 30 mm from the soil surface) was recorded daily for 30 days after sowing, until it was considered complete. At the end of the experiment, the final percentage field emergence (FE) was obtained.

The thermal time (°Cd) required for seed emergence was also calculated, according to the following formula reported by Wilkens and Singh (2001):

$$°Cd = \sum_{i=l}^{n} (Ta - Tb)$$

where Ta = daily mean temperature, Tb = base temperature (8°C) and n = number of days from sowing.

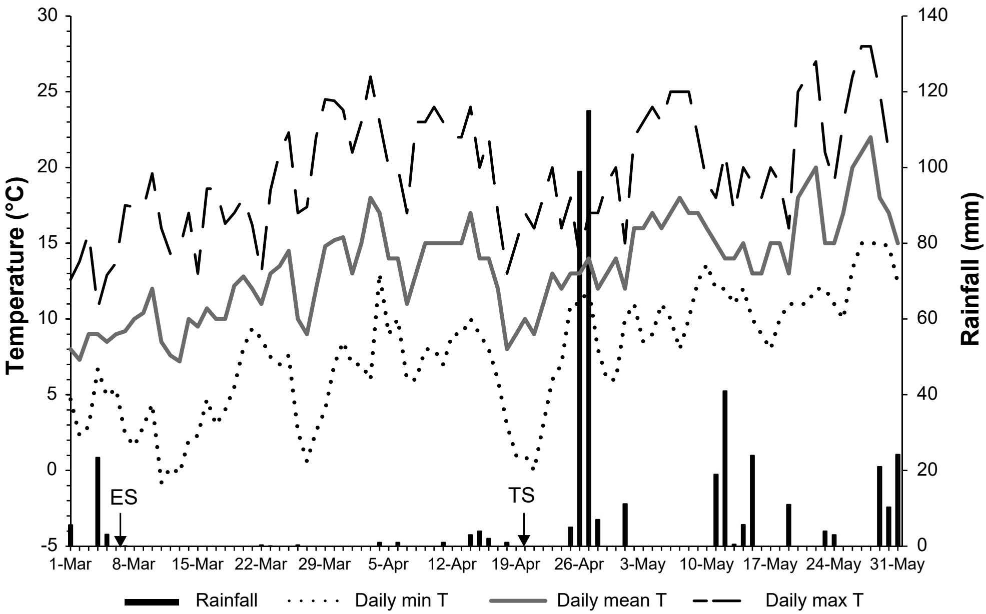

Figure 1. Meteorological data during the field trials at Udine (Italy) in 2016. Rainfall and minimum (Min T), mean (Mean T) and maximum (Max T) air temperature (daily values) are reported. (TS = typical sowing time and ES = early sowing time).

Statistical analysis

Angular transformation was applied to all percentage values obtained from the experiments and then submitted to statistical analysis using one-way analysis of variance (ANOVA) through the CoStat 6.4 software (CoHort Software, Monterey, USA). When statistically significant differences among treatments were evidenced, a Student–Newman–Keuls (SNK) test at $P \leq 0.05$ was adopted to separate the means. The mean values reported as percentage were obtained after inverse transformation.

Because seed lots had been produced in different countries, a comparison of this source of variation was made before any chemical seed treatment, in order to evaluate a possible effect on standard germination and cold test results. No significant differences among lots were found, with mean values of 94 and 77%, for standard germination and cold test, respectively.

Pearson correlation analysis was also performed for germination under laboratory conditions and the field emergence data set.

Results

Laboratory test

Very limited differences in germination were observed between the six seed treatments studied under standard laboratory conditions (table 2). The control treatment (A), with 94% germination, differed statistically from the lowest germination of seeds given treatments C, D and F (89, 89 and 88%, respectively). Treatments B and E showed intermediate germination, without significant differences from the other treatments.

After the cold test, more marked differences among seed treatments were observed in seed germination percentage, with A, E and B showing statistically higher values (77.5, 77 and 76.5%, respectively) than C and D (70.5 and 69.5%, respectively). Treatment F had the lowest germination percentage (62%) in the trial (table 2).

The ratio of cold germination/standard germination, which can be considered an index of seed vigour, confirmed the seeds with treatments A, E and B as the most vigorous, followed by C and D, with seeds of treatment F the least vigorous in the trial.

Table 2. Standard laboratory germination and germination after the cold test of maize seeds given different fungicide and/or insecticide treatments (table 1).

Treatment	Standard laboratory germination (%)	Cold test (%)
A	94.0a	77.3a
B	92.0ab	76.5a
C	89.0b	70.3b
D	88.5b	69.5b
E	91.5ab	76.5a
F	87.5b	62.0c

Note: Means followed by the same letter are not significantly different (SNK at the 5% level).

Field trials

The TS field trial demonstrated the highest emergence for treatments A and B (94 and 92%, respectively), statistically higher than E (90%) and C (87.5%). Treatments D and F had the lowest emergence percentages in the field (84 and 81.5%, respectively) (table 3).

The ES trial showed the highest rates of emergence for treatments A, B and E (78.2, 77.2 and 76%, respectively) followed by treatments C and D (71.5 and 70.7%), with the lowest registered in treatment F (64.2%) (table 3). In general, the treatment ranking and germination rates are similar to those obtained by the vigour test.

The favourable climate during the test (figure 1) exerted negligible stress on seedling emergence, as shown by the mean emergence rate in TS trial (88.5%) very close to that obtained for SG (90.4%). Conversely, seedling emergence in the late-winter sowing trial (ES) was affected by low temperatures during the sowing-emergence period, with a mean emergence very close to that of the cold test and about 20% lower than that obtained with standard germination (table 2).

Table 3. Percentage of seed emergence under field trials of maize seeds given different fungicide and/or insecticide treatments (table 1).

Treatment	Emergence (%)	
	Typical sowing time	Early sowing time
A	94.0a	78.3a
B	91.8ab	77.3a
C	87.8cd	71.5b
D	85.0de	70.8b
E	89.3bc	76.0a
F	83.0e	64.3c

Note: Means followed by the same letter are not significantly different (SNK at the 5% level).

Seedling emergence ended 23 and 13 days after sowing, corresponding to a thermal time of 77.3 and 66°Cd in ES and TS trials, respectively. Wilkens and Singh (2001) indicated that seedling emergence from a germinated seed is dependent on sowing depth (40 mm in our trial) and thermal time accumulation, as reported in their CERES model formula. Using this formula, we obtained 69°Cd as thermal time requirement for seedling emergence, a value very close to our data, given that during the field trial period we registered 6-9°C thermal time per day.

Pearson correlation analysis results under standard and earlier sowing time

The TS trial showed a positive and significant correlation with CT emergence (0.74**), a value in agreement to those obtained by other authors with similar trials (Lovato *et al*., 2005; Noli *et al*., 2008) (figure 2A).

The very favourable climate condition for emergence in TS is confirmed by the average (88%) and low variability (78-95%) of emergence, very close to the values obtained in SG (91% as average and 86-96% as range) (table 3). This also explains the positive and significant correlation (0.58**) between SG and TS obtained (figure 2A), despite being less than the previous one and substantially in agreement with the results obtained by Lovato *et al*. (2005). Under critical field conditions as in the ES trial, with low temperatures during the emergence period (figure 1), the correlation between CT and ES resulted as very high ($R^2 = 0.86$); conversely SG and ES showed a very weak relationship ($R^2 = 0.36$) (figure 2B). A very poor but still significant correlation (r = 0.53**) was found between CT and SG (data not shown), confirming the results of Lovato *et al*. (2005).

Seed treatment effects

The fludioxonil + metalaxyl-M seed treatment (B) did not affect germination and very similar results were obtained using these fungicides in combination with tefluthrin (treatment E), with the exception of a slight germination reduction in the TS field trial with respect to the non-treated control (table 3).

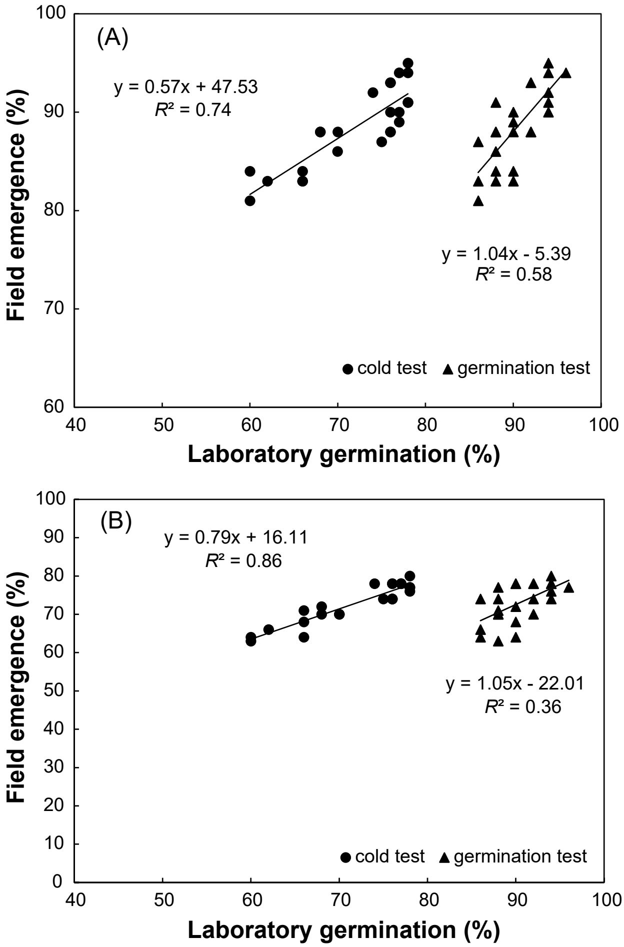

Figure 2. Relationship between standard laboratory germination (▲) and germination after cold test (●) with (**A**) typical sowing time (TS) and (**B**) early sowing time (ES) field emergence.

The effects of the above fungicides in combination with methiocarb (treatment C) and with thiacloprid (treatment D) were significantly detrimental to seed germination compared wtih the non-treated control in the SG trial (table 2). These treatments significantly reduced germination with respect to B and E treatments in CT (table 2) and TS trials (table 3), as well as with respect to the non-treated control.

Seeds treated with fungicides in combination with insecticides (fludioxonil + metalaxyl-M + tefluthrin + methiocarb) (treatment F) showed the lowest germination rates in all trials. However, whereas the percentage in SG was not statistically different from the other values with the exception of the control (table 2), the E treatment showed the significantly lowest germination with respect to all other treatments in CD (table 2) and also TS, in the latter with the exception of the D treatment (table 3).

No differences were observed between SG germination and TS of the non-treated control (both 94%), confirming the absence of soil microflora that could affect field seedling emergence (tables 2 and 3).

Discussion

The absence of differences between SG germination and TS emergence of the non-treated control (tables 2 and 3), removes any possible disagreement between cold test germination and field emergence, when sand is used as substrate for the cold test, as in this trial, instead of raw soil, as recommended by the classical manual in order to simulate field conditions. Indeed, as reported by many authors the cold test with sand is preferable to soil when treated seeds are tested (Noli *et al*., 2008) due to difficulty in standardising the procedure, as the microflora is extremely variable among soils (Burris and Navratil, 1979; Bruggink *et al*., 1991). As confirmed by the experiment of Noli *et al*. (2008), in which the worse climatic condition and the presence of soil-borne pathogens affected the non-treated seed lots, determining a no significant correlation between SG and field emergence.

This experiment showed that the treatments without systemic fungicides (fludioxonil + metalaxyl-M) or with these fungicides and tefluthrin (insecticide belonging to the pyrethroids class) caused no appreciable negative effects on seed germination and vigour. On the contrary, seed treatments involving the above fungicides in combination with methiocarb, insecticide belonging to the carbamates and used in maize as a broad-spectrum pesticide and bird repellent, or in combination with thiacloprid, a neonicotinoid insecticide, depressed seed vigour, highlighted in particular by the vigour test.

The insecticide mixture tefluthrin and methiocarb (in treatment F with fludioxonil + metalaxyl-M) had a very negative effect on seed vigour and germination (tables 2 and 3), evidencing a synergistic detrimental action with respect to the individual effects. This effect, also found in the standard germination test, was significantly marked in the vigour test (cold test) and in the field trial, especially with an early sowing time with limiting temperatures. These results confirm those of Marchi and Cicero (2003); treatment of corn seed with fludioxonil + metalaxyl (the same fungicides used in the present experiment) did not alter the seed vigour, however, insecticides (phosphorothioate and pyrethroid) and combinations of fungicides and insecticides increased the electrical conductivity of the maize soaking solution.

Lipophilicity, together with the mobility of the active ingredient, determined by a limited molecular weight (< 300) (Briggs, 1997) and good solubility, is one of the most important chemical properties for uptake of insecticide compounds applied as seed treatments. Assessed in terms of octanol water partition coefficient (where log Kow is octan-1-ol/water partition coefficient), it measures the affinity of compounds for the lipid phase of plant tissues (e.g. plasmamembrane, waxes, cutin, suberin) (Salanenka and Taylor, 2011). Moderately lipophilic compounds (log Kow around 2) are systemic because they permeate the lipid phase of cell membranes, unlike hydrophilic compounds that are not able to pass through lipid barriers (Briggs *et al*., 1982). Among the insecticides used in our experiment, thiacloprid, belonging to the neonicotinoid family, was the only systemic one, as confirmed by its moderate non-ionic lipophilic characteristics (log Kow 1.26), limited molecular weight (252) and good water solubility (185 mg L^{-1}). The slight negative effect of treatment D (tables 2 and 3) on seed vigour is probably due to direct contact with the embryo occurring during the imbibition phase, during which the active ingredient was able to pass through the lipid barriers of the maize seed coat (Briggs *et al.*, 1982). In fact, maize, belonging to the Poaceae family, is characterised by a selective permeability of the seed coat, which means that the cutinised-suberised membrane, located between the pericarp and aleurone layer of caryopses, is only permeable to non-ionic moderately lipophilic compounds (Taylor and Salaneka, 2012; Dias *et al.*, 2014). In contrast, tefluthrin, belonging to the pyrethroid family, confirms its non-systemic nature with high log Kow (greater than 5), high molecular weight (418) and very limited water solubility (0.02 mg L^{-1}). It is strongly retained in the plant lipid constituents (Edgington, 1981) and consequently not able to interact with the maize embryo, as evidenced by the similar values of germination and vigour of treatment E to the untreated control (tables 2 and 3). The situation is different for methiocarb, belonging to the azotorganic carbamate family, which although being considered without systemic activity (in seed dressing it is used only in maize), has a low but not negligible solubility (27 mg L^{-1}) and a molecular weight (225) and a log Kow (2.92) very similar to those of Cumarin 151 (230 and 2.1, respectively). The latter is a lipophilic fluorescent non-ionic compound used in studies as a tracer to act as a model of systemic seed treatment active ingredient, which effectively showed a diffusion in sweetcorn seeds (Dias *et al.*, 2014). The hypothesis is therefore that methiocarb is able to overcome the pericarps of the maize caryopsis, slightly affecting, as in treatment C, seed germination and vigour (tables 2 and 3). Methiocarb, used in combination with tefluthrin as in our experiment (treatment F), may have conveyed the latter active ingredient within the seed pericarp, synergistically accentuating the negative effects on seed germination and vigour. For these reasons, we are fully in agreement with Loeffler *et al.* (1988) of extreme caution regarding the utilisation of new formulations on the market, particularly concerning the mixture of insecticides, which could affect seed vigour.

The standard germination test does not provide a reliable prediction of maize performance in the field under critical conditions, conversely, the cold test has been confirmed as an excellent vigour test to predict seed performance, especially in limiting temperature conditions, as often happens with modern crop management techniques like early sowing and sod seeding (Lovato *et al.*, 2005; Noli *et al.*, 2008).

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