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Field vigor of sunflower seeds after chemical treatment

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ABSTRACT

Sunflower is a globally important oilseed, food and ornamental crop. To prevent the attack of insects and diseases during field emergence, sunflower seeds are usually treated with insecticides and fungicides, respectively. The ways in which they can influence seed germination and vigor are scarcely- known, especially if their effects are transferred to field emergence stage. Also, in sunflower there is no consensus as to which is the best estimator of sunflower seedling field emergence. In order to find out the best estimator, the present study was designed to i) determine the effects of insecticide treatment on sunflower seeds germination and vigor and, ii) estimate sunflower seedling field emergence by a correct association with the laboratory vigor test. Sunflower seeds were treated with tiamethoxam insecticide in commercial doses (6 ml kg^{-1} seeds) and compared with untreated seeds. Laboratory variables evaluated were: radicle emergence (G_{50}), germination percentage (GP) and vigor by Electrical Conductivity (EC). Field vigor was evaluated by applying diverse formulas including chronological days (d) and thermal time (°Cd), using air (°Cd-Air) and soil temperature (°Cd-Soil). GP and G50 were not affected by the insecticide, but vigor was reduced. Only high correlations were detected between laboratory vigor (EC) and the field emergence speed when it was expressed in Mean time of emergence (TME) °Cd-Air, TME (°Cd-Soil) and Days for 50% of maximum emergence (SE50,°Cd-Soil) with coefficients of 0.77, 0.80 and 0.78, respectively. The insecticide did not exert negative effects on sunflower seeds germination. The lower vigor found in the laboratory conditions have been transferred to field conditions, causing a lower speed of seedlings emergence. The association with laboratory vigor test improves when the seedling field emergence variables (TME and SE50), include soil temperature in the thermal time calculation.

1. Introduction

Sunflower is a globally important oilseed, food and ornamental crop (Seiler *et al.*, 2017). In 2017/18 world sunflower production was 18.4 x 10^6 ton, being Ukraine, Russia, the European Union and Argentina, the main producers (USDA, 2018). It was forecast that by 2030, production will increase by 16% and by 2050, 32%, reaching 58.3 X 10^6 ton of oil (Castaño, 2017).

Seedling establishment is the first critical step for sunflower crop production (Ahmad *et al.*, 2009) and it determines the success or failure of the future harvest (Finch-Savage and Bassel, 2016). Among the several biotic stresses for sunflower production, susceptibility to insect pests and diseases is one of the major constraints (Muzammil *et al.,* 2017). To prevent the attack of insects and diseases during field emergence, sunflower seeds are usually treated with insecticides and fungicides (Tamindžić *et al.,* 2013).

Thiamethoxam is the broad-spectrum neonicotinoid insecticide, which is widely used for controlling pests that attack sunflower seeds and seedlings (Kathage *et al.,* 2018). When applied to seeds, neonicotinoids systemically distribute throughout the plant (Stamm *et al.,* 2014) and reduce human exposure and dissemination in to the environment (Afifi *et al.,* 2014). Thiamethoxam interferes with a specific receptor located in the nervous system of the insects called nicotinic acetylcholine receptor, leading to the collapse of the nervous system and consequent death (Carvalho *et al.,* 2011). Also, this insecticide can have detrimental effects on beneficial insects, so an assessment is required to address these potential risks (EFSA, 2013).

Usually thiamethoxam is combined with other insecticides such as imidacloprid and fipronil (Mrđa *et al.,* 2011) or fungicides like metalaxyl (Sudisha *et al.,* 2010). This is the most frequently used fungicide for sunflower seed treatment, controling the downy mildew disease caused by *Plasmopara halstedii* (Miklic *et al.,* 2008).

Despite the benefit that these products have over the control of diseases and insects, many of their active ingredients can affect the physiological quality of the seeds (Shakir *et al.,* 2016). The physiological potential of a seed indicates the capacity to express its vital functions under different environmental conditions, being germination and vigor its most representative attributes (Marcos Filho, 2015). Germination is the proportion of seeds that can originate normal seedlings in optimal conditions of temperature and light for the species (ISTA, 2015). Vigor is "the sum of the properties that determines the activity and performance of the seed lots for an acceptable germination in a wide range of environments" (ISTA, 2015), and it is the best estimator of seeds field performance (Marcos Filho, 2015).

The germination and vigor of sunflower seeds was significantly reduced after treatments with insecticides like thiamethoxam or imidacloprid (Mrda *et al.,* 2011; Krizmanič *et al.,* 2014). However, Grisi et al. (2009) found no adverse effects of insecticides (fipronil and thiamethoxan) on germination or vigor of sunflower seeds. Sajjan et al. (2009) also found no reductions in the germination and vigor of sunflower seeds after treatment with imidacloprid insecticide.

The physiological and metabolic ways in which insecticides can influence seeds germination and vigor are scarcely-known (Moraes Dan *et al.,* 2012), especially if their effects are transferred to field emergence stage. Some authors indicate the existence of induction to the formation of free radicals and subsequent oxidative stress (Braguini, 2005). Others relate phytotoxic effects with abnormal radicular growth of seedlings (Silveira *et al.,* 2001; Nunes, 2006; Dan *et al.,* 2011). In sunflower, the treatment with insecticide (thiamethoxam) and fungicide (metalaxyl) delayed the emergence of radicles through an increase in the duration of phase II of germination (Szemruch, 2011). The movement and degradation of the insecticide active ingredients in the seed tissues can be different from those found within the seedlings (Srinivas *et al.,* 2017). So the insecticide effects on seed physiological quality could be changed at seedling field emergence stage.

Currently, there is no consensus about which is the best estimator of sunflower seedling field emergence, adding more complexity. Different calculations, that vary according to the literature or protocols used by seed companies, were finding (Finch-Savage and Bassel, 2016). Some variables used in sunflower are seedling emergence percentage and the shoots and roots length and weight (Mrđa *et al.,* 2011; Zhao *et al.,* 2016). In maize and soybean, seedling height, stems diameter, leaf area and the Maguire index have been used (Mondo *et al.,* 2013; Gonçalves Avelar *et al.,* 2015).

In order to find out the best estimator the present study was designed to i) determine the effects of insecticide treatment on sunflower seeds germination, vigor and seedling emergence, ii) estimate the sunflower seedling field emergence by a correct association with the laboratory vigor test.

2. Materials and methods

2.1 Seed Treatments

Sunflower seeds from commercial hybrid D01 by Dow AgroSciences were treated with insecticide tiamethoxam 35% (6 ml kg^{-1} seeds) diluted in water (9 ml kg^{-1}seeds). Ten days after harvest, a seed sample of 200 g was placed in an experimental machine (Cimbria Heyde type), later adding the insecticide. Non treated seeds were considered as control. These were stored in kraft-paper bags at 25°C and 30-50 % relative humidity until performing laboratory and field evaluations.

2.2 Laboratory tests

Radicle emergence was examined by placing four replications of 50 seeds of each treatment in 9-cm-diameter Petri dishes on two pieces of WhatmanN°1 filter paper, moistened with 2.5 ml of distilled water. Afterwards, the Petri dishes were wrapped up in plastic film and placed in a chamber at continuous 25°C (ISTA, 2015) with 12 hours of alternating light/dark. The numbers of seeds with emerged radicles (>2mm) were counted at 24, 28, 32, 36, 42, 48, 52, 56 and 60 hours from sowing. These moments were determined observing the time from which the radicles emergence in sunflower is evident, according to previous work (Szemruch *et al.,* 2013). The time required for the emergence of 50% of radicles (G50) was calculated according to Ranal and García de Santana formula (2006), expressed in hours for 50% of maximum radical emergence (1).

$$\text{G50}= \frac{\left\lfloor \left(ER/2\right) - R_1 \right\rfloor \times \left(H_2 - H_1\right)}{R_2 - R_1} + H_1$$

where ER is final percentage of seeds with radicle emerged, H_1 refers to the hours from the beginning of the radicle emergence period, H_2 corresponds to the hours to the end of the radicle emergence period, R_1 is the number of radicles emerged counted at H_1 and R_2 is the number of radicles emerged counted at H_2.

Germination percentage (GP) was calculated through the counting of normal seedlings, on the tenth day after being sown. Four replications of 50 seeds were sown in sand boxes and placed in the germination chamber (ISTA, 2015) at 25°C and 12 hours alternating light/dark, prior Pre-chilling for 48 h. Abnormal, dead and fresh seeds were evaluated.

Seed vigor was evaluated by Electrical conductivity (EC) test. Four replicates of 50 pre-weighed dehulled seeds were placed in 38 ml distilled water at 25 °C for 24 h. The seeds that were stored in the chamber were kept out of the chamber for 24 h. prior to being analyzed. Then, they were measured using a conductivity meter according to Braz et al. (2008) and expressed as $\mu S.cm^{-1}. g^{-1}$. Electrical conductivity assesses the degree of damage in cell membranes as a result of seed deterioration (ISTA, 2015). The highest deterioration results in lower membrane capacity repair and greater amount of solute released during imbibition (Marchos-Filho *et al.,* 2015). Thus, high levels of electrolytes released into the solution (and higher values of electrical conductivity) involve lower seed vigor (ISTA, 2015).

2. 3 Field evaluations

The experiment was sown on 05/31/2017 on a Typic Argiudoll soil in the experimental field of Agronomy, University of Lomas de Zamora (34° 45°

S; 58º 29’ W). One hundred seeds were sown in each 1.5 × 1 m plot with rows separated by 0.15 m and at 5 cm soil deep. These were free from weeds, diseases and pests and without fertilization and supplementary irrigation. Field vigor was evaluated counting the emerged seedlings at14, 17, 19, 21, 23, 28, 30, 33, 37, 39, 43, 45 y 47 days after sowing, considering the following variables:

i) Final percentage of emerged seedlings (FPES), resulting from the relationship between the total number of seeds sown, relative to those which indeed showed cotyledons above the soil surface (emergence state according to Schneiter and Miller, 1981).

ii) Days for 50% of maximum emergence (SE50), was calculated by the following formula:

$$\text{SE50} = \frac{\left[\left(E_{MAX}/2\right) - E_1\right] \times \left(D_2 - D_1\right)}{E_2 - E_1} + D_1$$

where:

SE50: days to reach 50% of seedlings emergence

E_{MAX}: maximum number of seedlings emerged

D1: beginning of the interval measured in days where 50% of seedlings emergence occurs

D2: end of the interval where 50% of seedlings emergence occurs

E1: number of emerged seedlings in D1

E2: number of emerged seedlings in D2

iii) Mean field emergence rate (ER) calculated as the inverse of SE50 and expressed in 1.d^{-1}

iv) Mean time of emergence (TME) was calculated according to Nakagawa formula (Nakagawa, 1999) and expressed in days (d).

v) Speed of field emergence (SFE) was calculated according to Maguire’s formula (Maguire, 1962) and expressed by seedling per chronological day (i.e., seedlings.d^{-1}). Also SE50, ER, TME and SFE were calculated expressing the chronological days in thermal time, calculated as the sum of the average air temperature - the base temperature, to obtain SE50 (°Cd-Air), ER (1.°Cd-Air^{-1}), TME (°Cd-Air) and SFE (seedlings.°Cd-Air^{-1}). The thermal time was calculated according to Aguirrezábal et al. (2003), with a base temperature of 6 °C. The last four variables were also analyzed considering the soil temperature in the thermal time calculation to express SE50 (°Cd-Soil), ER (1.°Cd-Soil^{1}),TME (°Cd-Soil) and SFE (seedlings.°Cd-Soil^{-1}).

vi) Days to V2 and V4 stages, according to Schneiter and Miller (1981) phenological stages.

vii) Seedling height in V2 and V4 stages, was calculated from soil surface until the apical bud, according to Schneiter and Miller (1981) phenological stages.

2.4 Statistical analysis

Laboratory and field tests were studied by means of a complete randomized design (CRD) with 4 replicates. Analysis of variance and LSD tests were performed with a 5 % significance level. Between laboratory and field data Pearson coefficients correlation were calculated. Percentage values were transformed using angular transformation. Infostat statistical software was used (Di Rienzo *et al.*, 2008).

3. Results

3.1 Effects of insecticide on germination and vigor laboratory test.

There were no effects from tiamethoxam on sunflower time radicle emergence (G50) or germination percentage (GP) (Table 1). EC was significantly increased in seeds treated with insecticide, indicating less vigor under laboratory conditions (Table 1).

3.2 Seedling field emergence

Table 1. Comparison between sunflower seed treated whit insecticide and control for laboratory tests: mean time required for the emergence of 50% of radicles (G50), germination percentage (GP) and seed vigor by electrical conductivity (EC).

	Control	Insecticide	C.V.** (%)
Radicle emergence G50 (hours)	42.0 ± 2.23 A*	37.9 ± 4.95 A	9.6
Germination GP (%)	99.0 ± 1 .0 A	97.0 ± 2.50 A	2.0
Vigor EC (μS.cm^{-1}. g^{-1})	40.1 ± 2.08 B	48.4 ± 3.22 A	6.1

* Different letters in each line indicate significant differences (*P*< 0.05). Values are mean ± SD. **C.V. = coefficient of variation.

Table 2. Comparison between sunflower for D01 hybrid seed treated whit insecticide and control for field vigor evaluations, final percentage of emerged seedlings (FPES), days for 50% of maximum emergence (SE50), mean field emergence rate (ER), mean time of emergence (TME) and speed of field emergence (SFE), expressed in chronological days (d) and thermal time (°Cd-Air and °Cd-Soil), days to V2 and V4 stages (dV2, dV4) and seedling height in the same stages (SdH-V2, SdH-V4). Abbreviations: Sd= seedlings.

	Control	Insecticide	C.V.** (%)
FEPS (%)	87 ± 2 A	89 ± 3 A	3.01
SE50 (d)	18 ± 1 A*	22 ± 1 B	3.99
ER (1.d^{-1})	0.057 ± 0.001 A	0.046 ± 0.002 B	3.44
TME (d)	20 ± 1 B	24 ± 1 A	2.80
SFE (Pl/d)	5 ± 0.1 A	4 ± 0.2 B	3.70
SE50(°Cd-Air)	94.1 ± 4.42 B	112.3 ± 7.02 A	5.69
ER (1.°Cd-Air^{-1})	0.0105 ± 0.0005 A	0.085 ± 0.0005 B	6.07
TME(°Cd-Air)	109.5 ± 5.74 B	138.5 ± 4.65 A	4.22
SFE (Sd.°Cd-Air^{-1})	0.86 ± 0.031 A	0.72 ± 0.061 B	6.24
SE50(°Cd-Soil)	105.7 ± 3.82 B	129.0 ± 4.65 A	3.63
ER (1.°Cd-Soil^{-1})	0.0093 ± 0.0005 A	0.0085 ± 0.0006 A	6.08
TME(°Cd-Soil)	120.7 ± 4.19 B	145.7 ± 2.99 A	2.73
SFE (Sd.°Cd-Soil^{-1})	0.65 ± 0.04 B	0.75 ± 0.01 A	4.48
dV2	17 ± 2 B	22 ± 2 A	11.0
dV4	30 ± 2 B	33 ± 0 A	6.0
SdH-V2	4.5 ± 0.39 A	4.5 ± 0.22 A	7.03
SdH-V4	5.9 ± 0.42 A	6.3 ± 0.42 A	6.83

* Different letters in each line indicate significant differences (*P*< 0.05). Values are mean ± SD. **C.V. = coefficient of variation.

Regarding seedling field emergence, no differences were found in the FEPS between seeds treated with tiamethoxam and control (Table 2). In contrast, treatment with thiamethoxan significantly reduced the speed of field emergence expressed in chronological days SE50, ER, TME y SFE (Table 2). Similar reductions were observed when the variability due to temperature was eliminated, that is, when the speed of field emergence was expressed in thermal time, SE50 (°Cd-Air), ER (1.°Cd-Air^{-1}), TME (°Cd-Air) and SFE (seedlings.°Cd-Air^{1}) (Table 2).

Tiamethoxam reduced the speed for field emergence even when the thermal time included the soil temperature (°Cd-Soil) (Table 2), except in the case

Table3. Pearson coefficients correlation between laboratory vigor (Electrical Conductivity) and field evaluations: mean time of emergence (TME) and days for 50% of maximum emergence (SE50), expressed in thermal time (°Cd-Air and °Cd-Soil) for D01 hybrid. * p values < 0.05, ns= not significant.

	Electrical Conductivity	
Coefficients		p Value
TME (°Cd-Air)	0.77	0.024*
TME (°Cd-Soil)	0.80	0.017*
SE50 (°Cd-Air)	0.69	0.056 ns
SE50 (°Cd-Soil)	0.78	0.022*

of ER ($1.°Cd\text{-}Soil^{-1}$) where the differences were not significant. The seeds treated with insecticide required more chronological days to reach V2 and V4 stages (dV2 and dV4) (Table 2). In contrast, the seedlings height (SdH-V2 and SdH-V4) was not modified with the treatment in those stages (Table 2).

3.3 Laboratory vigor test (EC) and seedling field emergence association.

Only high correlations were detected between laboratory vigor (EC in seeds without pericarp) and field emergence speed when it was expressed in TME (°Cd- Air), TME (°Cd-Soil) and SE50 (°Cd-Soil) with correlation coefficients of 0.77, 0.80 and 0.78, respectively (Table 3).

4. Discussion

4.1 Effects of insecticide on germination and vigor laboratory test.

Sunflower time radicle emergence (G50) was not affected by tiamethoxam application, indicating this treatment did not modify sunflower germination phases. Different results were obtained in previous works, in which the combination between the insecticide (thiamethoxam) and fungicide (metalaxyl) delayed the emergence of radicles (Szemruch *et al.*, 2011). It is probable that in such combination, the fungicide rather than the insecticide has generated the delay in emergence radicles. So it should be analyzed if there is a synergistic detrimental effect of two active ingredients, such as has been found in corn seeds (Baldini *et al.*, 2018).

EC values were increased by insecticide treatment and reduced sunflower seed vigor. The EC assessed the degree of damage in cell membranes as a result of seed deterioration (ISTA, 2015). Tiamethoxam produced oxidative reactions which deteriorated the cell membrane promoting a high release of electrolytes into the solution and then lower vigor in seeds (Ford *et al.*, 2011). The generation of oxidation reactions by the insecticide thiamentoxam should be investigated in sunflower seeds.

4.2 Seedling field emergence

Regarding seedling field emergence, thiametoxam increases seedling height, weight and root length in corn (Horii *et al.*, 2007; Tamindžić *et al.*, 2013). This was associated with an improvement in the phenolic content and antioxidant seed activity induced by the insecticide (Horii *et al.*, 2007). However, in soybean, the insecticide thiamethoxam reduced the seedling length (Abati *et al.*, 2018). Thiametoxam application in Oilseed rape reduced the root growth but these alterations became insignificant as seedlings developed in the field (Huang *et al.*, 2015). In sunflower tiamethoxam did not affect the final percentage of emerged seedlings (FPES), or their height (SdH-V2 and SdH-V4),but it did reduce their emergence speed. This was evident in most of the variables of the emergence speed measured, both in days and in thermal time (Table 2), considering not only the air temperature but also the soil temperature. It is likely that the lower vigor found in the laboratory conditions has been transferred to field conditions, causing a lower speed of seedling emergence. The negative effects of chemical seed treatments on seedling growth would be related to the physico-chemical properties of root uptake (Taylor and Salanenka, 2012). In oilseed rape, thiamethoxam adverse effects on seedlings were associated with reducedsize of the roots due to decrease in the mobilization of reserves during field emergence (Huang *et al.*, 2015).The adverse effects of neonicotinoids on field seedling growth seem to be species specific and should be evaluated in sunflower roots including morphological and molecular aspects.

4.3 Laboratory vigor test (EC) and seedling field emergence association

FPES was not adequate to estimate field vigor of sunflower seeds, due to the lack of association with EC (data not shown). However, a high correlation between field emergence percentage of sunflower seeds and germinative energy (number of seedlings emerged after 4 days in the germination trial) was found by Liović et al. (2008). The same analysis, for 5 sunflower lots, indicated a high association between field emergence percentage and EC (in seeds without pericarp) according to Albuquereque et al. (2001). FPES only measures the number of seedlings emerged in relation to the total sown, but not the speed with which they emerged, so it would not be a good estimator of sunflower field vigor. A high and significant correlation between field emergence speed (in seedlings.day^{-1}) and the EC (in sunflower seeds with pericarp) were measured (Anfinrud and Schneiter, 1984). On the other hand, for Braz and Rossetto (2009) the number of seedlings.day^{-1} was little associated with EC (in seeds without pericarp). A significant variability in field vigor estimations caused by differences in soil conditions (Anfinrud and Schneiter, 1984; Mondo *et al.,* 2013; Santorum *et al.,* 2013) and the meteorological environment (Marcos-Filho, 2015) has been described, including temperature variations, water content, soil texture and structure and the presence of pests or plant competition. Also Murcia et al. (2002) showed that changes in sowing date, and therefore in the environment, can modify the association between field emergence percentage of sunflower seeds and vigor tests results. One way to become independent of temperature effect, and minimized the environmental conditions, is the estimation of field vigor replacing the number of chronological days by thermal time (°Cd, tbase = 6ºC). In this sense, our experiments demonstrated the high associations between laboratory vigor (EC) and field emergence speed when it was expressed in TME (°Cd- Air), TME (°Cd-Soil) and SE50 (°Cd-Soil). This contributes to minimizing environmental variability and it could help in the improvement and standardization of sunflower vigor test. In addition, these relations improve when considering the soil temperature in the thermal time calculation with 0.80 and 0.78 correlation coefficients for TME (°Cd-Soil) and SE50 (°Cd-Soil), respectively. It would be interesting to advance in methodologies that can also minimize the environmental variability (Finch-Savage and Bassel, 2016). The field vigor estimation represents a substantial contribution, due to its potential effects on plants development and yield (Pommel *et al.*, 2002, Egli and Rucker 2012).

Conclusions

The insecticide thiamethoxam did not exert negative effects on sunflower seeds germination. The vigor in the laboratory was significantly reduced and this effect was transferred to the field stage with lower speed of seedling emergence. The association with laboratory vigor test improves when the seedling field emergence variables (TME and SE50), include soil temperature in the thermal time calculation.

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