Sowing of seed dressed with thiacloprid using a pneumatic drill modified for reducing abrasion dust emissions

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Abstract

Dressed seeds can release a certain amount of abrasion dust containing pesticides during sowing operations. Due to the high sensitivity of honey bees to the exposure of dust containing neonicotinoids insecticides, it is essential to ascertain the quantity of active ingredients (a.i.) released during sowing. The pneumatic precision drills play a key role in the quantity of dust emitted. We developed a prototypal device that, applied to conventional pneumatic drills, operates an effective reduction of abrasion dust emissions. The paper reports the amounts of active ingredient emitted during a field test carried out employing seed treated with the neonicotinoid insecticide thiacloprid. The trials aimed at assessing: the amount of thiacloprid dispersed by a conventional drill; the effectiveness of the prototype to reduce the emissions. The test results provided information on the level of a.i. deposition at ground and of a.i. concentration in the air both in the downwind area and into the sowed field. Basing on such results, it was provided an assessment of the potential exposure of honey bees flying in the area covered by the dust and of the potential amount of a.i. inhalable by the operator during sowing operations. The detected amounts of a.i. seem below the concentrations reported as dangerous for honey bees; however, they can be useful in the study of sub-lethal effects.

Key words: honey bees, pesticides, neonicotinoids, maize, operator's exposure.

Introduction

The phenomenon of emission of dust from dressed seed during sowing has been largely investigated in recent years (Apenet, 2011; Nuyttens *et al.*, 2013). The abrasion dust is spread mainly from the pneumatic drills employed for the precision sowing of maize (*Zea mays* L.) and other crops. The seed treated with pesticides can release small quantities of active ingredient (a.i.) that can reach non-target organisms and the environment. The occurrence of drift dust is harmful for honey bees (*Apis mellifera* L.) and other pollinating insects, especially when the seed are dressed with the insecticides belonging to the class of neonicotinoids and with fipronil (Maini *et al.*, 2010; Sgolastra *et al.*, 2012; Goulson, 2013). For this reason, in 2008 Italian government has suspended the authorization of neonicotinoids (thiamethoxam, imidacloprid and clothianidin) and of fipronil for maize seed dressing. Recently, a different neonicotinoid, the thiacloprid (Sonido™), has been recommended for maize seed treatment, because the a.i. is much more tolerated by honey bees in comparison with other neonicotinoids (Iwasa *et al.*, 2004). Thiacloprid improve crop establishment to reduce damage caused by wireworm (*Agriotes* spp.) feeding (Elbert *et al.*, 2000).

Regarding acute contact toxicity to honey bees, Iwasa *et al.* (2004) report a LD_{50} of 14.6 µg bee^{-1}; other sources report the LD_{50} (oral) equal to 17.3 µg bee^{-1} and LD_{50} (contact) to 38.8 µg bee^{-1} (FAO, 2010). Thiacloprid can cause sub lethal effects at low dose of ingestion, especially when the exposure is associated to other stress factors for the honey bees, like starvation (Laurino *et al.*, 2011) and concomitant infection of *Nosema ceranae* (Vidau *et al.*, 2011). Fungicides have been shown to synergize toxicity of thiacloprid to honey bees in the laboratory, although the same results have not been shown in field studies (Iwasa *et al.*, 2004; Schmuck *et al.*, 2003). Also, sublethal doses of thiacloprid can interfere with the navigation system of the honey bees (Fischer *et al.*, 2014).

Honey bees can come into contact with these compounds in a number of ways (Greatti *et al.*, 2006; Girolami *et al.*, 2009; Krupke *et al.* 2012; Samson-Robert *et al.*, 2014; Bonmatin *et al.*, 2015), including aerial powdering with particulate matter containing pesticide residues, during their flight (Marzaro *et al.*, 2011; Pochi *et al.*, 2012). The honey bees body, covered by hairs that trap electrostatically the airborne particulates, enhances direct exposure of wind dispersed-dust during flight (Prier *et al.*, 2001).

The drift of abrasion dust can be also a potential harm for operators and other humans that inhale the dust during the sowing operations (Biocca *et al.*, 2013).

Among the strategies adopted to reduce the dust dispersion, a key factor is played by the drill (Apenet, 2011; Foqué *et al.*, 2014). Manufacturers have proposed some devices to decrease dust drift emissions generated by the drill. These include: 1) air deflectors (including "dual pipe deflector" proposed by Syngenta as aftermarket solution applicable to different drill models and similar devices directly applied by seeder manufacturers); 2) the Bayer SweepAir® system (Vrbka *et al.*, 2014); 3) the Bayer AirWasher® system (Chapple *et al.*, 2014).

CREA-ING projected and realized a prototype, based on the recirculation and filtration of the dust (activated carbon, anti-pollen filters - ac-ap-filter) that was successfully tested in field (Pochi *et al.*, 2013) and static

tests (Pochi *et al.*, 2015). Subsequently, the same prototype was equipped with a final stage consisting of an electrostatic filter retaining the finest dust fraction that escape the ac-ap-filter (Biocca *et al.*, 2014). In the present work, such a version of the prototype was tested in real sowing test of maize seed treated with thiacloprid (Sonido™). The test aimed at investigating the drift of the active ingredient contained in the abrasion dust and to provide information on: 1) the effectiveness of the prototype to reduce the emissions; 2) the potential exposure of honey bees flying nearby the sowed field; 3) the potential quantity of a.i. inhalable by the operator during sowing operations.

Materials and methods

The trial was carried out using commercial maize seed (Pioneer Hy-Bred PR32F73) dressed with the insecticide Sonido™, a.i.: thiacloprid, and the fungicides fludioxonil and metalaxyl. According to the manufacturer, the applied dose of thiacloprid was 1.0 mg seed^{-1}.

The sowings took place in the experimental farm of CRA-ING (42°5'51.26"N 12°37'3.52"E; 24 m a.s.l.) in 2014. Two plots of about 2 ha (130 × 150 m) were sowed at the working speed of 1.6 m s^{-1}.

A six-row precision pneumatic drill (Gaspardo, mod. Magica) was employed. The drill was modified with a device that works by partially re-circulating the air generated by the drill's fan. A plastic pipe works as a collector receiving the air expelled by the fan by means of four deflectors pipes and redirecting the airflow into the hoppers (figure 1). The air in excess is forced outward through an opening fitted with an activated carbon filter for automotive use (anti-pollen filter) followed by an electrostatic filter (Expansion Electronics, mod. FE-250) powered by the tractor's battery. The device can be easily removed to restore the conventional configuration of the drill and to allow the comparison between the two different configurations. A patent was issued on the device.

The evaluation of the dust deposition at ground level was made on the basis of the methodology commonly used for this type of test (BBA, 1992), which involves the use of Petri dishes with a 50% solution of acetonitrile and water. In order to reduce the effects of changing wind direction, such a methodology was revised with respect to the layout of the Petri dishes: a series of five Petri dishes spaced 5 m were placed on each plot side (North, South, East and West) at 5, 10 and 20 m from the field edge; hence a total of 60 sampling points was obtained (figure 2).

Data were analyzed with a multifactor ANOVA assuming the sampling plot side and the distance from the field edge as factors. The analyses were performed with the software R (R Core Team, 2013).

Furthermore, different air samplings were made in order to detect the a.i. concentration in the air in significant operative situations.

Figure 1. Modified drill with the recycling-filtering device. (1) hoppers' tight lids replacing the normal lids; (2) collector of pipes coming from the drill's fan; (3) main collector pipe; (4) recycling pipes from each hopper to main collector; (5) box containing the anti-pollen filter with activated carbon filter; (6) electrostatic filter and filtered air outlet.
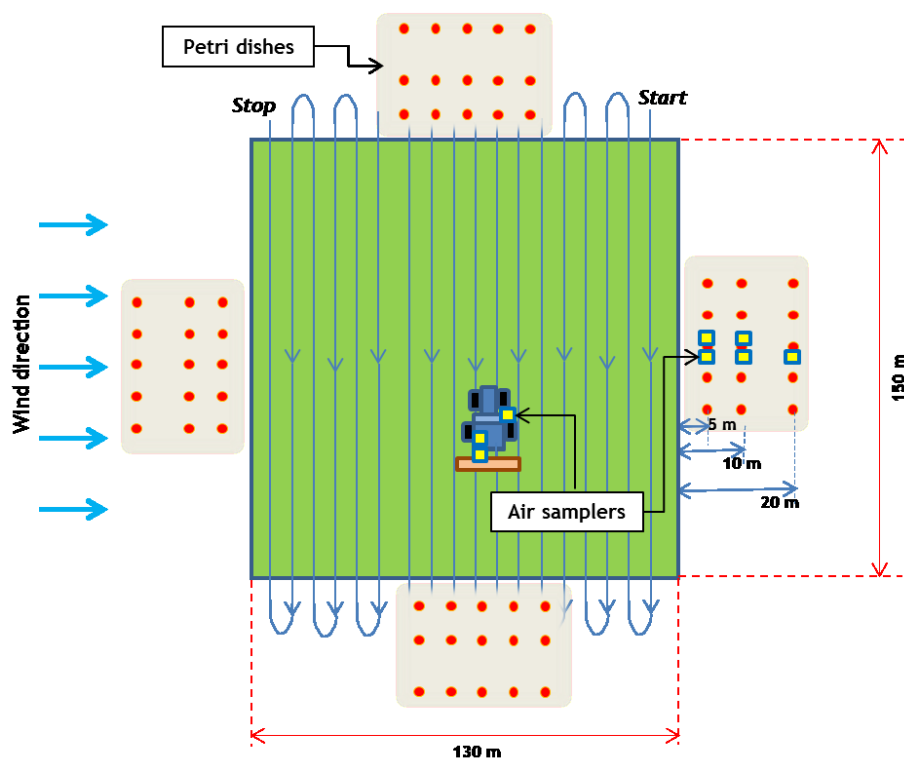
Figure 2. Layout of field trials, showing Petri dishes and air samplers position (not in scale).

The first air sampling was made on the test plot side that, at the beginning of the test, was downwind, placing five low volume air pumps (TCR Tecora, mod. Bravo) at the same distances adopted for the rows of Petri dishes: two pumps were placed at 5 m from the field edge, two pumps at 10 m, one pump at 20 m.

They operated at a constant flow of 15 L min^{-1} and were equipped with 0.45 μm PTFE Millipore diskette filters, without any sampling head. The height of sampling was 2.0 m from the ground.

Further air samplings were carried out by means of three personal air samplers equipped with conical sampling heads. One sampler (Zambelli, mod. Ego Plus), operating at 4 L min^{-1}, was placed on the tractor, outside the cab, near its door, at a height of about 2 m from the ground, with the aim of providing information about the air concentration of thiacloprid inside the field during

the sowing and of determining the potential quantity of a.i. inhalable by the operator when the sowing is operated with the cab's door open. Two more samplers (SKC with a Dorr-Oliver sampling head and one Zambelli Ego Plus) were placed inside the cab. These operated at 2 and 4 L min^{-1}, respectively.

Finally, a personal sampler (Zambelli, mod. Ego Plus) fixed on the chest of the operator and operating at 4 L min^{-1}, was employed to sample the air during the loading of seed in the hoppers of the drill. The operation consisted of the loading of the six sacks of seed set out for each test and was carried out twice.

The conditions of air sampling are summarized in table 1.

During the tests, the micrometeorological conditions were continuously monitored by a portable meteorological station (Kestrel, mod. 4500) (table 2).

Table 1. Details of air sampling methods.

Sampler	Number of samplers	Position	Operation	Sampled air volume (average) L
Personal	2	Inside the cab	Sowing	158
Personal	1	Outside the cab	Sowing	208
Personal	1	On the operator	Seed loading	16
Ambient	5	Field edge, downwind	Sowing	850

Table 2. Micrometeorological conditions during the tests.

Drill	Duration min	Air temperature °C	Relative Humidity %	Wind speed m s^{-1}			Prevailing wind direction
				Average	Max	Min	
Prototype	60	25	62.3	0.6	1.4	0.0	North, East
Conventional	52	28	46.4	1.3	3.2	0.4	South

The determination of active ingredients in the samples was carried out at CRA-PAV. Active substances were extracted from the samples with acetonitrile. Solutions were vibrated in ultrasonic bath for 10 min and then filtered with 0.45 μm filters. The analyses were carried out by means of HPLC coupled to a MSD (Mass Spectrometry Detector) operating with an ES+ (Electrospray Ionisation Interface, positive mode) and the relative methods were validated in compliance with GLP (Good Laboratory Practice) procedures. The instrumentation consisted of Waters Alliance 2695 Separation Module and 2695 Autosampler, Micromass 4 micro Triple Quadrupole Mass Spectrometer with Electrospray Ionisation (ESI) probe, Waters X-Terra MS column C18, 5 μm 150×4.6 mm, flow 0.3 mL min^{-1}, gradient elution with water (0.1% acetic acid) and 10% acetonitrile (0.1% acetic acid) up to 90%, in MRM (Multiple Reaction Monitoring) mode. The mass spectrometer detector was tuned in the MRM mode at the maximum sensitivity for each of the parent ions m/z and polarity, two product ions fragmentations for each were followed and detected.

Results

As to the effectiveness of the prototype, the results show that the prototype strongly reduced the emissions of dust and a.i.. The figure 3 shows the depositions of a.i. at ground level expressed as average of the four sides. The results indicate an overall reduction of 93.4%.
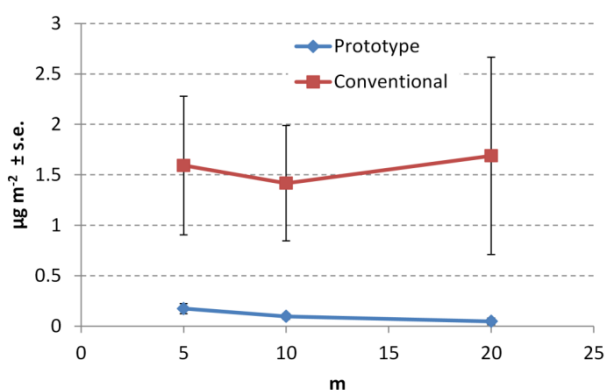

Figure 3. Amounts (μg m^{-2} ± s.e.) of active ingredient collected at soil level during the sowing with the prototype and the conventional drill: average deposition of four sides.

Table 3. Results of ANOVA test on the comparison between prototype and conventional drill referred to the residues of active ingredient detected at soil level.

	Df	F value	P
Drill	1	17.98	5.13×10^{-5}
Side	3	14.14	1.04×10^{-7}
Distance	2	0.05	0.95
Drill × side	3	15.24	3.47×10^{-8}
Drill × distance	2	0.08	0.93
Side × distance	6	0.37	0.89
Drill × side × distance	6	0.27	0.94

The ANOVA test (table 3) shows the high significance of the drill effect (effect of the prototype) and of the side of sampling (effect of wind direction). Interestingly, the differences determined by the sampling distances from the field edge did not result significant: i.e. the amounts collected at 20 m from the field edge are comparable with the residues at 5 m. This was particularly evident in the test with conventional drill (figure 3), characterized by a slightly higher wind velocity that could affect the behavior of dust deposition referring to the distance from the sowed field.

These results confirm the good performances provided by the prototype in previous tests conducted with different active ingredients (Pochi *et al*., 2013; 2015), likely because the electrostatic filter is more efficient towards the finest dust fraction.

Considering the a.i. concentrations in the air downwind the sowed plot, the prototype seems even more effective in reducing them than it was for the depositions at ground level. In fact, the values observed during the sowing with the prototype were always under the limit of detection (LOD) (i.e. 0.3 ng filter^{-1}) and the corresponding percent reduction of residues in the air is equal to 100%. The values of the a.i. air concentrations are reported in table 4. Such a high reduction level is probably due to the action of the electrostatic filter that retains the finest particles of dust (which can float in the air) that escaped the ac-ap-filter (figure 4).

The potential exposure of honey bees to abrasion dust containing thiacloprid was calculated as described in Pochi *et al*. (2012) and in Tremolada *et al*. (2010). This approach allows to assess the amount of a.i. that can contaminate (by contact) an insect flying during the sowing of dressed maize seed. It was hypothesized that each honey bee flies in a sort of virtual tunnel, the dimensions of which depend on the bee's body cross-

Table 4. Average air concentration of thiacloprid (ng m^{-3} ± s.e.) in the different samplings.

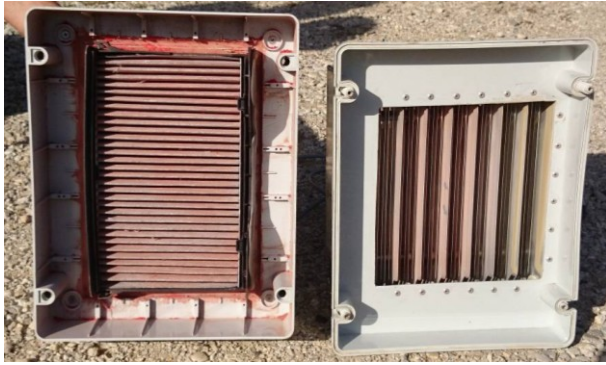| Sampler position	Distance m	Drill Conventional	Prototype	Reduction %
Sampling area downwind the test plot	5	10.5 ± 4.6	< LOD	100
Sampling area downwind the test plot	10	7.8 ± 0.8	< LOD	100
Sampling area downwind the test plot	20	8.6	< LOD	100
Outside the cab	-	317.4	47.3	85
Inside the cab	-	17.0 ± 2.0	< LOD	100
Seed loading	-	13712.5 ± 645.8		-

Figure 4. The two filtering stages of the prototype after the sowing test. Left: the ac-ap-filter which retained most of the dust. Right: the steel plates of the electrostatic filter coated with a thin layer of very fine dust.

section and the length of flight. The volume of the virtual tunnel represents the volume of air intercepted by the bee during the flight and can be obtained by multiplying the flight distance by the cross section area (nearly 12.6 mm^2, resulting from the approximation of the honey bee body to a cylinder with base diameter of 4 mm) (Tremolada *et al.*, 2010).

In a second hypothesis, we assumed that also electrostatic forces, mainly generated by wings movements, can attract the dust during the flight, the volume of intercepted air increases. In this case we considered a radius of the front section equal to 10 mm, with a resulting front area of 314.2 mm^2.

Referring to a 500 m long flight, the volume of air intercepted by a bee will be 0.0063 m^3 in the first case and 0.16 m^3 in presence of electrostatic attraction. Basing on the results reported in table 4, such air volumes have been multiplied by the a.i. concentration according to two hypotheses of flight: 1) the bees fly in the area downwind the sowed field at 0-20 m distance from its side; in this case, the a.i. air concentrations (only for conventional drill) do not seem to be affected by the distance (up to 20 m) from the field edge (table 4). Therefore, we assumed that, at a flying height of about 2 m (i.e. sampling height) and in the given meteorological test conditions, the average concentration of a.i. was 9.0 ng m^{-3}, corresponding to the average of the values in table 4 (conventional drill); 2) the bees fly in just sowed areas of the field, within the cloud of dust raised by the drill, where the a.i. concentrations recorded by the air sampler on the tractor (outside the cab) reached 317 ng m^{-3} with the conventional drill and 47.3 with the prototype (table 4).

In the table 5 are reported the results of the potential exposure from combining the hypotheses on flight and on the volume of air explored by the bees.

As regards the potential quantity of a.i. inhalable, the loading of the six sacks of seed needed by each test required, respectively 4.68 min and 3.38 min (average time: 4.03 min). The air concentration of thiacloprid resulting from the analysis of the filters was 13.7 µg m^{-3} (ng L^{-1}). Considering that a man at work meanly breathes in 20 L min^{-1} of air, the amount of inhalable thiacloprid can be referred to different time intervals. In the table 6 are reported the results of the inhalable amounts calculated on the basis of:
- time of sampling (4 min for 6 sacks of seed);
- time needed for two full loadings of the hoppers (32.2 min, 48 sacks of seed), that would suffice to sow for a day (eight hours).

In general, the samplings carried out inside the cab (referred as "sowing with closed cab" in table 6) provided negligible quantities of a.i.. Also in this case, the employing of the prototype considerably reduced the a.i. air concentrations: with the prototype, no a.i. was detected inside the cab and a 85% reduction was recorded by the sampling outside the cab.

Considering the seed loading and the sowing with open cab, the inhalable amount of thiacloprid after a working day would be 11.8 µg.

Table 5. Potential values of by contact exposure to thiacloprid of a honey bee flying for 500 m, under different hypotheses of flight conditions and of exploring air volumes.

Flight position	Drill	Honey bee's front section mm^2	Exposure ng bee^{-1}
5-20 m from field edge	Conventional	12.6	0.1
		314.2	1.4
Inside the field	Conventional	12.6	2.0
		314.2	49.9
Inside the field	Prototype	12.6	0.3
		314.2	7.4

Table 6. Potential values of exposure to thiacloprid of an operator during sowing operations.

Operation	Drill /sampling	A.i. air concentration µg m^{-3}	Inhaled a.i. during the sampling hours µg	Inhaled a.i. in 8 hours of work µg day^{-1}
Seed loading	-	13.71	1.1	8.7
Sowing	Conventional/open cab	0.32	0.33	3.1
Sowing	Conventional/closed cab	0.017	0.018	0.16
Sowing	Prototype/open cab	0.047	0.049	0.45
Sowing	Prototype/closed cab	0.0	0.0	0.0

Discussion

In the present work we assessed the amounts of thiacloprid emitted by drills during the sowing of maize seed dressed with Sonido™. The trials were carried out employing the same pneumatic precision drill configured as a conventional drill and equipped with an innovative device capable to reduce the emissions of dust and active ingredient.

The percent reduction of a.i. drift, in the downwind area, consequent to the adoption of the prototype varied from about 93% for ground deposition (passive sampling) up to 100% for air concentrations (active sampling).

As to the a.i. concentration in the air, the presence on the prototype of a filtering stage with electrostatic action on the dust seems to significantly improve its performance with reference to the first version with only a cap-filter, in particular reducing the emission of the finest dust fraction. Consequently, during the sowing with prototype, no residues of a.i. were found in air samplings in the area downwind the sowed field and a consistent reduction (about 85.5%) was observed in the air sampled into the sowed field by means of the samplers placed on the tractor. The available data did not suffice for statistical analysis, but the observed reductions represent a very encouraging result that, anyway, needs to be validated in tests on a wind tunnel under controlled wind conditions (Biocca et al., 2015).

Considering the recorded values of wind speed, differences were observed, between the two tests, the magnitude of which (0.7 m s^{-1} in average) should not significantly affect the sampling. Anyhow, its most likely effect should be an underestimation of the differences of a.i. amounts detected in the two tests, according to the following considerations: 1) test with prototype: the lower wind speed probably allowed the persistence (in the air) and the deposition (at ground), in the sampling area, of dust amounts higher than those that could have been observed under higher wind speed conditions; 2) test without prototype: the higher wind speed favored the dispersion of the dust, as testified by the behavior of the ground depositions (figure 3), whose values seem to increase with the distance from the field edge. With lower wind speed we would have probably observed higher values at the lower distance (5 m).

The values of a.i. deposition detected at ground in the downwind areas can be useful to specific studies on the assessment of risk of contamination of honey bees during their foraging activity on the flora surrounding the sowed areas (Sgolastra et al., 2012; Pistorius et al., 2015). The presence of a.i. in the air represents an additional cause of contamination and some hypotheses were done in order to estimate the amount of a.i. that can come in contact with the honey bees during their flights. The values obtained are very distant from the LD_{50} reported for thiacloprid, (Iwasa et al., 2004; FAO, 2010). However, after experimental validation, they can be useful in the study of sub-lethal effects, also in association with the above data of deposition at ground.

As regards the exposure of the operator, the loading of the seed represented the most critical moment, because of the dispersion of abrasion dust from the border of the hoppers. The a.i. potentially inhaled was estimated as 8.7 µg day^{-1}. Moreover, the use of tractors without cab or with cab open is still frequent. As a consequence, the driver, during his working time, can be exposed to the dust and its contents. In the present case the resulting amounts of thiacloprid potentially inhaled were 3.1 µg day^{-1} and 0.045 µg day^{-1}, respectively with conventional drill and with prototype (about 85% reduction). Closing the cab strongly reduces the amounts, but is not sufficient to exclude some contamination with conventional drill, also depending on the efficiency status of the filter equipping the cab air system. With the prototype the analysis did not detect residues of thiacloprid. Also in this case all found values are far away from the AOEL, Acceptable Operator Exposure Level - Systemic (0.02 mg kg_{bw}^{-1} day^{-1}) - PPDB, 2011. Nevertheless, agricultural workers are continuously exposed to residues of various chemicals contained in dust and aerosols dispersed in succeeding operations that require the manipulation of incoherent (granular, powder, liquid) materials. The exposure usually occurs according to seasonality. For each a.i., it can be defined a characteristic period (e.g., the sowing of maize falls in the period March-April) when the workers (especially if they work as contractors) are occupied in a specific operation. Then they pass to the subsequent operation/a.i. In such conditions, according to a principle of precaution, it will be necessary putting in place all necessary measures to reduce the level of exposure.

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