

# Withdrawal of maize protection by herbicides and insecticides increases mycotoxins contamination near maximum thresholds

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**Abstract** Environmental and economic issues affect decision-making for whether or not to control small infestations of pests and pathogens in crops. Even where no crop yield loss is expected, other risks may be evident, such as the slow accumulation of pathogen inocula. The prevalence of toxins, arising from biotic interactions with fungal diseases, can alter crop quality rather than quantity. Thus, farmer decisions for whether to tolerate pest infestation must take into account several direct and immediate and/or delayed potential risks. Published scientific evidence on the co-occurrence of risk factors resulting from the presence of different pests and pathogens are largely absent, and this has stifled the adoption of integrated pest management. Here, we tested how the withdrawal of herbicide and insecticide protection in maize, alone and in combination, might induce higher prevalence of up to 23 mycotoxins in the crop at harvest. The experiment was conducted over 4 years in 29 fields in the south west of France. The test involved a comparison of paired samples collected from treated and untreated plots. All nine major mycotoxins that were observed in more than 4 % of the samples showed highly variable concentrations both between fields and years. The overall trend following the cessation of

pesticide protection, however, is for higher levels of mycotoxins and up to a six-fold increased in nivalenol mean concentration (to 202.3  $\mu\text{g kg}^{-1}$  of maize seeds) compared to its treated control. Overall mycotoxin concentrations approached 55–67 % of their maximum acceptable rate, a situation of reduced security margin that could lead to economic penalties and market restrictions. We found that the removal of herbicides had a greater impact than that of insecticides on the prevalence of mycotoxins, which differs from the expectation stated in the literature. This finding is further reinforced by the observation that certain species of weeds harbor several species of *Fusarium*. This means that weeds not only play a role as crop competitors but also as reservoirs of inoculum in the field. Our findings illustrate the importance of sanitary evaluation when the implementation of new cropping systems will alter the distribution and occurrence of pests and pathogens.

**Keywords** Agroecology · Biotic interaction · Risk assessment · Pest control · *Fusarium* · *Ostrinia nubilalis*

## 1 Introduction

The rise of environmental and human health concerns relating to agricultural production has led to the incorporation of this issue into the EU agricultural policy, such as Directive 2009/128/EC. However, the transition to the broader adoption of integrated pest control practices seems to be slow. Indeed, in addition to the lack of practical solutions, leading to an imperfect coverage of pest control problems, the change in mentality required to shift to an integrated pest management (IPM) strategy is considerable. Unlike intensive agriculture, in which the usual practice has been to tackle each major pest, on a case-by-case basis, by finding an appropriate chemical solution, IPM strategies involve the use of several different practices, each

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only partially effective in themselves, to make the field crop hostile, or at least less attractive, to major pests or pathogens.

Weeds occupy a key position among the pests of crop fields as they provide a spontaneous and diverse flora in agricultural fields which serves as the basis of ecological food chains, hosting insect species, nematodes, fungi, bacteria, and viruses (Norris and Kogan 2000). Furthermore, weed species may not display disease symptoms when colonized (Altinok 2013). Therefore, transmission to and from such a diverse and timely dormant reservoir can lead to pathogen spillover, with the release of large numbers of parasites (Daszak et al. 2000; Prenter et al. 2004).

Corn borers and several weeds are commonly considered to be among the most noxious pests of maize. Considerable scientific effort has been devoted to studying these pests and finding appropriate ways of their control. However, general set of pests is rarely considered as a whole, or even studied regarding their interactions. As a result, little is known about the responses of particular pest to the presence of other pest species. One clear exception is the amplifying impact of insect foraging galleries on the occurrence of *Fusarium* species (Munkvold et al. 1999; Sobek and Munkvold 1999), potentially resulting in higher levels of mycotoxin contamination (Bakan et al. 2002; Avantaggiato et al. 2002; Folcher et al. 2010).

In this study, we experimentally manipulated weed and insect levels to investigate the indirect effects of a slightly greater presence of these pests on *Fusarium sp.* producing mycotoxins, with the potential to alter maize harvest quality. By removing chemical protection against insects, weeds, or both, we were able to assess not only the direct consequences, in terms of insect or weed population dynamics potentially affecting the yield, but also the indirect impacts on quality due to the contamination of the harvest with larger amounts of mycotoxins. This 4-year experiment is summarized in Fig. 1, which shows a six-fold increase in nivalenol levels in the absence of insecticide and herbicide treatments. Eight of the 23 mycotoxins tested were regularly present, and the change in the range and patterns of their concentrations was just above the threshold for significance when considered separately, although the concentrations of six of these eight compounds were higher in the absence of treatment and approaching their maximum authorized concentrations.

## 2 Materials and methods

### 2.1 Field trials

The experiments were carried out in 29 fields located in an area in which lepidopterans are multivoltine, in the “Midi-Pyrénées” region (South Western France). All the

experimental sites were located in an area of intensive maize production. The experimental sites were chosen on the basis of the previous crop (maize), a known history of regular infestations with maize borers, the presence of a diverse and abundant weed flora, and, to a lesser extent, the use of late sowing dates, which tends to favor synchrony with a second generation of *Ostrinia nubilalis*, the European corn borer, and *Sesamia nonagrioides*, the Mediterranean corn borer, resulting in a higher level of exposure to borer attacks.

The study was carried out in natural conditions, in four consecutive summers, from 2008 to 2011. Each trial was carried out on an area of about 400 m<sup>2</sup> divided into four adjacent plots, as follows: one plot with neither insecticide nor herbicide treatments (NN (for none-none) control), one plot with insecticide and herbicide treatments (IH treatment), one plot receiving only the herbicide treatment (H treatment), and one plot receiving only the insecticide treatment (I treatment). Thus, whole experiment included 116 microplots (29 experimental sites × 4 plots for each site) suitably designed for within-site paired-sample comparison analysis.

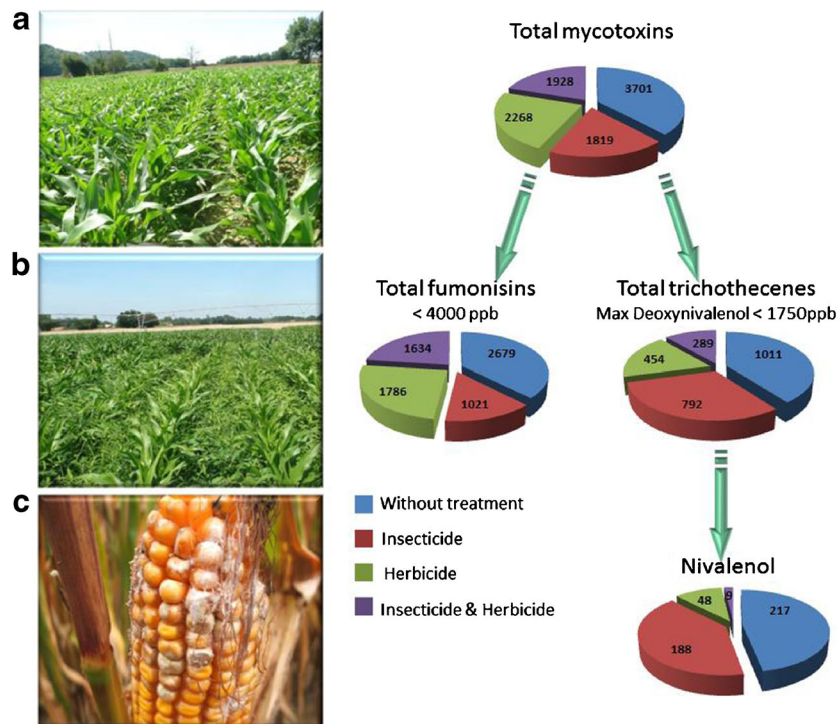
The 29 sites (8 for 2008 and 7 for each of the other years) belonged to Haute-Garonne (21 sites), Tarn & Garonne (5 sites), and Gers (3 sites). These areas were located in relative close proximity with a mean distance between plots of 3.6 km [range 1.8–139.0 km].

### 2.2 Sampling for the evaluation of pest occurrence

During cultivation, two visual control ratings were carried out for weed monitoring (the first between 45 and 55 days after maize emergence, the second close to harvest) as follows: number of each weed/square meter and total percentage ground cover. During the sampling close to harvest, grassy weeds found in the two plots without herbicide treatment (NN and I) were collected, for identification of the fungal species present on weeds. The components of the mycoflora were identified by “Laboratoire Qualtec” (Vandoeuvre 54, France). Identification to species level was possible for eight species, with all other cases of *Fusarium* identified only to the genus level (*Fusarium sp.*).

At harvest, the infestation of the crop with caterpillars of either of the two borers studied was checked by dissecting 20 stalks and ears from each plot. The relative frequencies and densities of European corn borer and corn stalk borer caterpillars were recorded.

For further laboratory analysis, 1.2 kg samples of kernels were taken from 20 mixed ears collected randomly from each plot (i.e., 4.8 kg per field). These samples were divided, as follows: 1 kg for mycotoxin analyses and 0.2 kg for mycoflora analyses. These samples were stored at 4 °C, to prevent the postharvest development of fungi between the field and the laboratory.



**Fig. 1** Mean contamination levels of maize seeds expressed as parts per billion of the various mycotoxin fractions as a function of herbicide and insecticide treatment status over 29 fields. *Left panel: a* field maize without weeds, *b* field maize with weeds resulting from an absence of herbicide treatment, and *c* ears showing injury due to *Fusarium* complex infestation. In the absence of an effect of herbicide or insecticide treatment on mycotoxin contamination, pie charts should present equal quarters. Instead, mycotoxin concentrations were highest in the harvest

from untreated plots, followed by plots on which weeds were not controlled, then plots with no insect control, and, finally, on plots treated with both herbicide and insecticide. The difference between the four treatments was more marked for trichothecenes and, particularly, for nivalenol. Regulation (EC) N°1126/2007 is fixing fumonisins B<sub>1</sub> and B<sub>2</sub> <4000 ppb and deoxynivalenol <1750 ppb so that contaminations reach 55 and 67 %, respectively, of their maximum acceptable thresholds in the situation of reduced control of weeds and insects

### 2.3 Fungal species identification and analysis on harvests

Fungal species were identified by morphological identification, according to the reference protocol entitled “Detection and identification of *Fusarium spp.* and *Microdochium nivale* on cereal grains by isolation, semi-selective mycological and microbiological study” (Ref. MH-16.3: Version B according to Ios et al. 2004). Dichlorane-chloramphenicol peptone agar and potato dextrose agar culture media were used. The distinction between 21 *Fusarium* species was possible (*F. poae*, *F. tricinctum*, *F. sporotrichioides*, *F. langsethiae*, *F. chlamydsporum*, *F. avenaceum*, *F. equiseti*, *F. scirpi*, *F. acuminatum*, *F. heterosporum*, *F. sambucinum*, *F. culmorum*, *F. graminearum*, *F. crookwellense*, *F. lateritium*, *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, *F. anthropilum*, *F. oxysporum*, *F. solani*).

### 2.4 Mycotoxin analysis

Analysis of mycotoxins covered trichothecenes A (DAS, 15- monoacetoxyscirpenol, T-2 toxine, HT-2 toxine, verrucarol), B (deoxynivalenol, 15-O-acetyl, 3-acetyl, L- epoxy deoxynivalenol, fusarenon X, nivalenol) and D

(roridin A, verrucarin A); zearalenone and associated metabolites (zearalenol  $\alpha$ , zearalenol  $\beta$ ); fumonisins (B<sub>1</sub> and B<sub>2</sub>); ochratoxins (A and B); and others (citrinin, patulin). These mycotoxins were analyzed using HPLC-MS/MS (high-performance liquid chromatography–mass spectrometry) while aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>) were evaluated by HPLC/fluorimetry FNF (fluorene-NSI-fluorene). All the measures were conducted on the analytical platform of the “Laboratoire de Développement et d’Analyses” (Ploufagan 22, France). Multiple reactions monitoring as well as identification and quantification were carried out for two or three transitions for each mycotoxin. The AFNOR V03-110 protocol from the French agency for product standardization was used. Details of the protocol applied are available in Folcher et al. (2010).

Concentrations that were observed could be compared to the maximum thresholds given by Commission regulation (EC) No 1126/2007 (OJ 2007). Depending on the final use made of the maize seed, the maximum acceptable levels vary for example from 750 to 1750 ppb for deoxynivalenol and from 200 to 4000 ppb for fumonisins B<sub>1</sub> and B<sub>2</sub> (see European Regulation (EC) No 1126/2007 (OJ 2007)). Threshold values has not yet been fixed for nivalenol in EU regulation.

## 2.5 Statistical analysis

### 2.5.1 Statistical comparisons

Student's *t* tests were used for comparisons of (i) Gaussian variables (i.e., European corn borer and corn stalk borer densities) (ii) mean numbers of weeds and weed ground cover, after arcsine square root transformation to normalize the distributions, and (iii) Ln ( $x + 1$ ) transformed variables for each chemical family of mycotoxins (also to normalize their distributions).

### 2.5.2 Model development

The statistical methodology followed those developed in Folcher et al. (2012): i.e., generalized linear models (Nelder and Wedderburn 1972) for the ranking of explanatory variables by order of mycotoxin levels, and Akaike information criterion (Venables and Ripley 2002) for selecting the best models.

We focused on the development of the final mycotoxin models, with the standard diagnostic checks on the Ln ( $x + 1$ ) transformed residuals from our final fitted model. We then used Shapiro–Wilks tests to assess the normality of the residuals, to check the validity of the models selected. The explanatory variables accounted for experimental site (field trial locations), the proportion of grains infected with mycotoxin-producing *Fusarium spp.*, and levels of weed, European corn borer and corn stalk borer infestations. Statistical analyses were carried out for the following variables ( $n=29$  for each variable, insecticide and herbicide treatments and controls): Comm (location of field trials), LaRP (populations of *O. nubilalis* and *S. nonagrioides* larvae counted in all plants) and quadratic I(LaRP<sup>2</sup>) to account for asymptotic mycotoxin levels limit with fungal density, Stri and Ln (Stri) (deoxynivalenol and trichothecene levels), Fum and Ln (Fum) (fumonisin B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub> levels), Zear and Ln (Zear) (zearalenone and metabolite levels), Niva and Ln (Niva) (nivalenol levels), SMyc and Ln (SMyc) (total mycotoxin levels), FuProd (percentage of grains infected with *F. verticilloides* and *F. proliferatum*, both of which are major fumonisin producers), TriProd (percentage of grains infected with *F. graminearum* and *F. culmorum*, both of which are major trichothecene producers), FTZProd (percentage of grains infected with *Fusarium spp.*, considering all the species present), and Sad2 (weed counts for each set of conditions).

The saturated model developed for the various chemical families of mycotoxins were:

$$\begin{aligned} & \text{Ln}(\text{Mycotox chemical families} + 1) \\ & = \text{Comm} + \left\{ \begin{array}{c} \text{TriProd} + \text{FuProd} \\ \text{FTZProd} \end{array} \right\} + \text{Sad2} + \text{LaRP} + \text{I}(\text{LaRP}^2) \end{aligned} \quad (1)$$

### 2.5.3 Co-occurrence, diversity, and similarity between samples

We used the Jaccard index to assess the co-occurrence of weeds and *Fusarium* species. Deviation from the expectations of random association was assessed under a geometric distribution law with an adapted threshold taking into account the prevalence of the organisms compared and a correction for multiple comparisons. We investigated whether there was conservatism of co-occurrence of species disjunction across sites, by comparing the observed Jaccard index of similarity ( $J = i/r$  where  $j$  is the number of cases where both the weed and the *Fusarium* species of a particular pair are present at a site and  $r$  is the combined presence for the same pair of species) with that expected for a random distribution. Based on the two-tailed Jaccard index values obtained, we determined whether relationships between pairs of species displayed a significant excess or deficit with respect to expectations. All statistical analyses were performed with *R* software (2.14.1 version on line <http://www.r-project.org>, March 2014).

## 3 Results and discussion

### 3.1 Treatment efficacy

#### 3.1.1 Control of foraging insects

Insecticide treatment halved the numbers of larvae in maize ears and stalks, for both European corn borer and corn stalk borer. We observe that this decrease was significant for the densities of European corn borer and corn stalk borer on stalks ( $t=2.75$ ,  $Df=97.87$ ,  $P=0.007$  and  $t=2.14$ ,  $Df=98.55$ ,  $P=0.035$ , respectively), but not for occurrence on ears ( $t=1.59$ ,  $Df=99.38$ ,  $P=0.115$  and  $t=1.09$ ,  $Df=92.35$ ,  $P=0.277$ , respectively). This is consistent with the two to three generations of these borers produced annually in the south of France, avoiding insecticide spraying campaigns from a full control of the damage and putative indirect effects of *Fusarium spp.* contamination.

#### 3.1.2 Control of weeds

In our study, differences in weed infestation profile were observed between fields and between years. At each site, the herbicide program was adapted according to the precise situation and the principal species to be controlled. Overall, we observe that comparisons between treated and untreated plots, whether based on total weed biomass or percent coverage, showed that treatments were effective, as they resulted in weed levels less than half those of the



**Table 1** Major weed species ranked by their prevalence, regularity across the 4 years of the experiment, and mean density across the 29 maize field sites

Weed species	Prevalence as rate of site occupancy	Regularity across years (max. 4)	Mean density $\pm$ std error (plant m <sup>-2</sup> )
<i>Chenopodium album</i> L.	0.810	4	10.5 $\pm$ 0.9
<i>Convolvulus arvensis</i> L.	0.762	4	3.6 $\pm$ 0.3
<i>Echinochloa crus-galli</i> (L.) P. Beauvois	0.667	4	10.5 $\pm$ 0.6
<i>Solanum nigrum</i> L.	0.476	4	5.0 $\pm$ 0.6
<i>Polygonum aviculare</i> L.	0.429	4	24.7 $\pm$ 6.4
<i>Elytrigia repens</i> (L.) Nevski	0.381	4	13.2 $\pm$ 2.0
<i>Datura stramonium</i> L.	0.381	4	7.8 $\pm$ 1.3
<i>Sonchus oleraceus</i> L.	0.286	2	2.4 $\pm$ 0.6
<i>Digitaria sanguinalis</i> (L.) Scopoli	0.286	3	5.0 $\pm$ 0.7
<i>Portulaca oleracea</i>	0.238	3	4.8 $\pm$ 1.3
<i>Anagallis arvensis</i> L.	0.238	3	3.6 $\pm$ 0.8
<i>Setaria pumila</i> (Poir.) Roemer & Shultes	0.238	2	3.5 $\pm$ 0.8
<i>Mercurialis annua</i> L.	0.143	2	3.8 $\pm$ 1.4
<i>Rubus</i> spp.	0.095	3	<sup>a</sup> 32.6 $\pm$ 14.0
<i>Veronica arvensis</i> L.	0.095	2	8.5 $\pm$ 3.9
<i>Potentilla reptans</i> L.	0.095	3	3.0 $\pm$ 1.5

Less common species: *Capsella bursa-pastoris* (L.) Medicus, *Sonchus arvensis* L., *Amaranthus retroflexus* L., *Coryza sumatrensis* (Retz) E. Walker and the seldom found: *Trifolium* sp., *Linaria vulgaris* Miller, *Viola tricolor* L., *Polygonum persicaria* L., *Poa annua* L., *Sonchus asper* (L.) Hill, *Daucus carota* L., *Cirsium arvense* (L.) Scopoli, *Lamium purpureum* L. & *Senecio vulgaris* L.

<sup>a</sup> Expressed in percent soil cover for *Rubus*

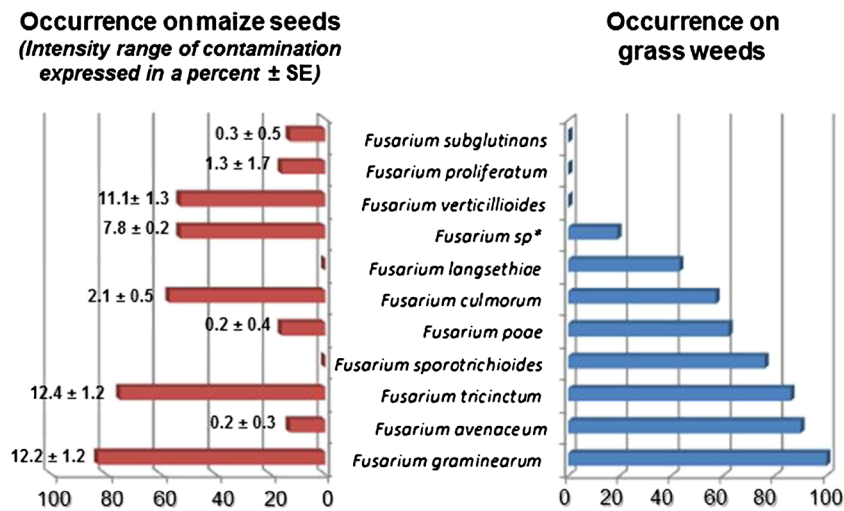
control plots. Significant decreases were observed both for mean weed number (33.19  $\pm$  7.22 in control plots vs. 16.88  $\pm$  2.80 in treated plots,  $t=2.11$ , Df=73.74,  $P=0.039$ ) and percent cover (0.30  $\pm$  0.04 % in control plots vs. 0.12  $\pm$  0.03 % in treated plots,  $t=3.75$ , Df=71.91,  $P=0.0004$ ). The main species identified were those commonly found in the area concerned (Table 1), and they belonged to the principal families of weeds usually encountered in maize fields. Our results show that the pesticides were effective and that it was possible to distinguish between the different treatments. They also indicate that the effects of these treatments were not complete, with weeds and borers nevertheless detected on the various plots.

### 3.2 Impact of relaxing pesticide control on *Fusarium* and toxins

#### 3.2.1 Impact on the prevalence of the various *Fusarium* species, weeds, and maize grains

Our comparison made with the *Fusarium* species infecting standing weeds (rather than the residues) (Fig. 2) with those infecting maize seeds (Fig. 2) identified a ubiquitous set of species common to both lists. For instance, *F. graminearum*,

*F. culmorum*, and *F. tricinctum* were abundant on both grass weeds and maize grains. For those species, weeds were potential reservoirs of inoculum. However, as also shown by Altinok (2013) and Postic et al. (2012), most of the *Fusarium* species identified were present on only one of the two lists. Subject to the limitations associated with sample size, this finding suggests that the weed species present in the field would variously act as a reservoir amplifying the amount of inoculum for the different *Fusarium* species found on the crop. As suggested by Jones (1986), it seems more likely that weeds compete directly with maize for water and nutrient, and this competition can weaken the maize plants in situations of limited irrigation or during dry periods before the tassel stage. Rodriguez-del-Bosque (1996) observed that competition with weeds affected *Aspergillus* contamination only during droughts. *F. verticillioides* has been reported to follow a pattern similar to that for *A. flavus*, with more severe ear damage under drought stress. We did not experimentally measure the expression of water stress in the maize crops studied and the sites differed in their water retention capacities, which were not correlated with the prevalence of *Fusarium*. Perspectives in continuity to our work would be exploring further the causality of the co-occurrence of



**Fig. 2** Respective occurrence of *Fusarium* species found either on the maize harvest seeds or on the grass weeds collected in the field plots: the mean intensity range of contamination measured on maize seed is also provided. Asterisk covers both unidentified and mixtures of several

*Fusarium* strains. Species followed but not observed included the following: *F. sporotrichioides*, *F. langsethiae*, *F. chlamyosporum*, *F. equiseti*, *F. scirpi*, *F. acuminatum*, *F. heterosporum*, *F. sambucinum*, *F. crookwellense*, *F. lateritium*, *F. anthophilum*, *F. oxysporum*, *F. solani*

*Fusarium*-weed species pairs, and focusing more closely in the effects of natural vegetation bordering fields when exploring fungal dynamics.

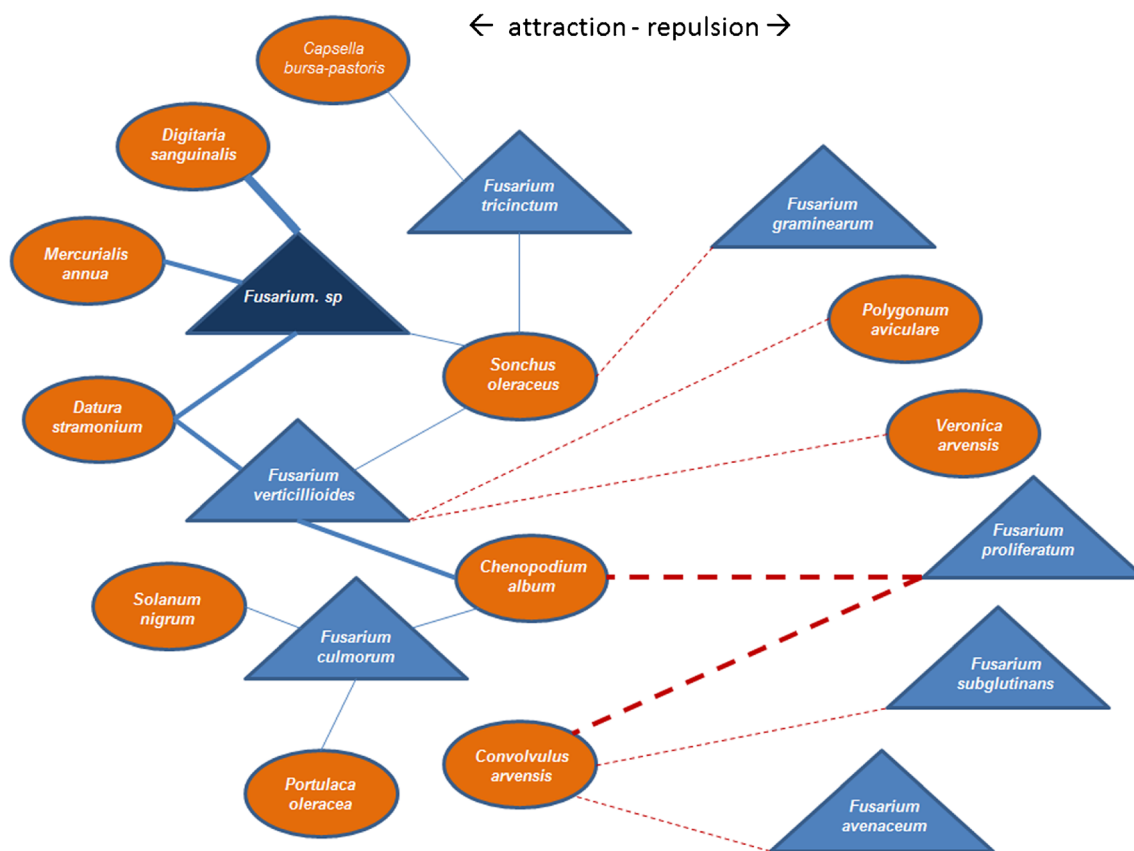
### 3.2.2 Impact of each treatment on the occurrence of mycotoxins

Comparisons in our experiment of herbicide-treated and control plots and of insecticide-treated and control plots presented high fluctuations both between years and among sites. Accordingly, they revealed no strong statistical link to the contamination of the maize harvest with mycotoxins when considered separately. Each mycotoxin considered alone, only the nivalenol concentration varied significantly according to herbicide treatment status (concentration =  $202.33 \pm 79.24$  ppb in non-treated plots vs.  $33.53 \pm 12.50$  ppb in treated plots;  $t=2.10$ ,  $Df=103.81$  and  $P=0.038$ ), whereas the relationship between the prevalence of *F. avenaceum* as responsible for nivalenol contamination and herbicide status was at the verges of significance (mean frequency = 0.015 in control plots versus 0.002 in herbicide-treated plots ( $t=1.85$ ,  $Df=63.59$  and  $P=0.070$ )). The observed results for nivalenol concentration were confirmed by a complementary analysis conducted with a quasi-Poisson model (applied to the initial data, without transformation,  $\chi=0.32$ ,  $Df=3$ ,  $P=0.044$ ) accounting for the overdispersion of the data (Venables and Ripley 2002) for mycotoxin contaminations. The quasi-Poisson model revealed another significant effect of herbicide treatment, on fusarenone X concentration. Again, the levels of this mycotoxin were significantly lower on treated plots ( $11.38$

$\pm 6.98$  ppb in control plots vs.  $0.69 \pm 0.69$  ppb in treated plots,  $\chi=0.34$ ,  $Df=3$ ,  $P=0.047$ ).

By contrast with the impact of herbicide withdrawal, we observed that contaminations were little affected by withdrawal of the insecticide treatment. This observation was not consistent with the widely reported effects of insect borers on mycotoxin prevalence. Instead, it was consistent with the low overall prevalence of borers in the assay (less than 0.7 larvae per plant) and with the preliminary finding that, for insects present for long periods of time (as in this area, in which insects are multivoltine), insecticide treatment significantly protected stalks, without further decreasing injury levels on maize ears.

When mycotoxins are considered over the 4 years of the experiment and pooled per chemical family as in Fig. 1, the trends gets clearer: the fumonisins show a mean 64 % increase of their mean concentrations from 1634 to 2679 ppb in maize seeds with herbicide and insecticide withdrawals while trichothecenes present a 250 % increase from 289 to 1011 ppb. For trichothecenes, the withdrawal of herbicide has slightly more impact than that of insecticide while fumonisins show the opposite tendency. When the maximum concentrations observed are expressed as a proportion of the respective maximum tolerated rates of 4000 ppb for fumonisins and 1750 ppb for deoxynivalenol, the contaminations reach respectively 67 and 55 % of the upper maximum limits. With the relatively low weed infestations and limited insect injuries, all the mycotoxins were thus at concentrations compatible with the EU regulation rules although in a situation of reduced security margin prior to economic penalties and market restrictions.



**Fig. 3** Observed statistical deviation from neutral co-occurrence between weeds and *Fusarium* over 29 maize fields located in South West France. The lines connect the weed species and the fungus in each pair considered. They highlight the situations of deficit or excess compared

to independent random distribution between weeds and *Fusarium*, dashed red lines for repulsion according to Jaccard's index values, and solid blue lines for attraction. The levels of significance, with  $P < 0.05$ , 0.01, and 0.001 are shown as lines of increasing thickness

### 3.3 Interactions between variables

#### 3.3.1 Co-occurrence patterns of diversity for weed-*Fusarium* species pairs

We determined whether species occurrences were neutral with respect to the presence of other taxa. We did this by quantifying the deviation from neutrality of relationships between pairs of species, with one of the species concerned being a major broadleaf or grass weed and the other being a *Fusarium* spp. We found both situations with excess conservatism of the co-occurrence of a weed species with a *Fusarium* species and, conversely, situations of weed/*Fusarium* species disjunction across sites. Divergence, corresponding to a lower than expected similarity index, was found for *F. proliferatum* with either *Conyza arvensis* or *Chenopodium album*, and, to a lesser extent, *Polygonum aviculare* and *Veronica arvensis* also had lower than expected rates of co-occurrence with *F. verticillioides* (red dashed lines in Fig. 3). Conversely, ten

weed/*Fusarium* pairs presented higher than expected levels of similarity (solid blue lines in Fig. 3). Several of these weed species/*Fusarium* pairs had already been identified by Postic et al. (2012) or Altinok (2013). Our observations are not consistent with an anticipated negative interaction due to the antifungal effects of the broadleaf weed *Datura stramonium* extract (Usha et al. 2009). Our measurement remains purely statistical, so pairwise associates may reflect both causality or/and similar responses to any external environmental condition or agricultural practice. These results should therefore be seen as preliminary and used to guide more direct evaluations. These potential association/repulsion relationships extend beyond the simple situation in which *Fusarium* is found at the surface of a host but no distinction is made between its passive presence and real trophic relationships. Our findings do not demonstrate a trophic relationship either, but are supported by much greater statistical power, with more than 100 observations at 29 different sites.

### 3.3.2 Relationships between mycotoxin levels, lepidopterans, *Fusarium* spp., and weeds

Using generalized linear models and Akaike's information criterion, we showed that interactions occurred between variables. The extremely low levels of zearalenone (5 to 19 ppb) led to the removal of Eq. 1 (saturated model). The other final models obtained were each specific to the chemical family of mycotoxins targeted.

$$\begin{aligned} \text{Trichothecene model : } & \ln(\text{Stri} + 1) \\ & = \text{Comm} + \text{SAd2} + \text{LaRP} \end{aligned} \quad (2)$$

$$\begin{aligned} \text{Fumonisin model : } & \ln(\text{SFum} + 1) \\ & = \text{Comm} + \text{FuProd} + \text{SAd2} + \text{LaRP} + I(\text{LaRP}^2) \end{aligned} \quad (3)$$

$$\begin{aligned} \text{Nivalenol model : } & \ln(\text{Niva} + 1) \\ & = \text{SAd2} + \text{LaRP} + I(\text{LaRP}^2) \end{aligned} \quad (4)$$

$$\begin{aligned} \text{Total mycotoxin model : } & \ln(\text{SMyc} + 1) \\ & = \text{Comm} + \text{FTZProd} + \text{SAd2} + \text{LaRP} \end{aligned} \quad (5)$$

Details of the coefficients obtained and of the associated probabilities are compiled in Table 2. Trichothecene levels were best "explained" by weed number, the populations of *O. nubilalis* and *S. nonagrioides* larvae present on the entire plant, and the location of field trials (Eq. 2 and Table 2). Fumonisin levels were best described by weed number, the location of field trials, the percentage of grains infected with the subset of *Fusarium* spp. producing fumonisin, the populations of *O. nubilalis* and *S. nonagrioides* larvae present on entire plants, and the associated quadratic term (Eq. 3 and Table 2). Nivalenol level varied with weed number and the

densities of *O. nubilalis* and *S. nonagrioides* (and the borer quadratic factor) on entire plants (Eq. 4 and Table 2). However, the residuals of this last model were not normally distributed ( $P < 10^{-4}$ ), rendering this model less reliable than the three others.

Literature has mainly focused on the importance of borers on the prevalence of *Fusarium*, so it is worth noting the similar presence of weeds as explanatory variable (*Sad2*) in each of the four final models (Eqs. 2–5).

According to this approach, in which the least parameterized saturated models are constructed, variables with low levels of variability, i.e., prevailing climatic conditions and choice of tillage system, are unlikely to be accounted for. However, the effects of tillage could be considered as follows: levels of grain contamination with trichothecenes produced by *F. graminearum* and *F. culmorum* appear to be significantly higher on un-plowed than on plowed plots ( $P = 0.001$ ). This observation is consistent with the known form in which these fungi overwinter, as chlamydozoospores in the soil, not being exposed to the air or parasites. Conversely, the contamination of grains with the fumonisins produced by *F. verticillioides*, *F. proliferatum* and, to a lesser extent, *F. subglutinans* would not vary between plots, because the overwintering form of the fungus, i.e., macro- and microconidia would have been buried by plowing *Fusarium* mycelium with crop residues into the soil. We can further hypothesize that the climate was more favorable to fungi producing fumonisins, such as *F. verticilloides*, which is responsible for 11.15 % of grain contamination (vs. 1.34 % for *F. proliferatum*) in particular (Fig. 2). Indeed, the field trials were carried out in the south west of France, where temperatures are favorable for *F. verticilloides*, which has an optimal temperature range for development of 25 to 30 °C, and an optimal temperature of 20 °C for toxin production (Marin et al. 1995; Thibault et al.

**Table 2** Interactions between variables according to the final fumonisin, trichothecene, nivalenol, and total mycotoxin models (2008–2011 data)

Explanatory variables	Fumonisin		Trichothecenes		Nivalenol		Total mycotoxins	
	Coefficients	<i>P</i>	Coefficients	<i>P</i>	Coefficients	<i>P</i>	Coefficients	<i>P</i>
Site	a	$<10^{-4}$	a	0.0003	Rejected	–	a	$<10^{-4}$
SAd2	0.011	0.063	0.016	0.0135	0.0179	$<10^{-4}$	0.010	0.1346
LaRP	2.569	$<10^{-4}$	0.565	0.1681	2.2442	0.0025	1.449	0.0002
FuProd	6.222	$<10^{-4}$	Rejected	–	Rejected	–	–	–
TriProd	Rejected	–	Rejected	–	Rejected	–	–	–
FTZProd	–	–	–	–	Rejected	–	3.086	$<10^{-4}$
I(LaRP <sup>2</sup> )	–0.290	0.182	Rejected	–	–0.5365	0.0084	Rejected	–

Models were selected on the basis of Akaike information criterion (Venables and Ripley 2002)

*SAd2* number of weeds per square meter, *LaRP* number of european corn borer + corn stalk borer larvae per plant, *FuProd* frequency of grains infected with fungi producing fumonisins, *TriProd* frequency of grains infected with fungi producing trichothecenes, *FTZProd* frequency of grains infected with fungi producing fumonisins, trichothecenes, and zearalenone, *I(LaRP<sup>2</sup>)* quadratic factor associated with the number of european corn borer + corn stalk borer larvae per plant

<sup>a</sup> One coefficient per site



1997). Fungi producing trichothecenes need lower temperatures, ranging between 20 and 25 °C, with an optimal temperature of 15 °C for toxin production (Caldwell et al. 1970; Brennan et al. 2005; Magan 2006). So, fumonisin levels are probably enhanced by the combined effects of plowing and local air temperature.

This experiment was based on the hypothesis that weeds and insects cause different kinds of crop damage. The weed canopy has several effects, modifying ecosystem microclimate, increasing the size of the reservoir for pests and pathogens, and direct competition with the crop. By contrast, insect pests principally damage plant tissues (both through herbivory and boring activities). The impacts of these two groups of pests are not exclusive and there may even be a strong interaction, providing fungi with opportunities to develop, colonize the crop, and, ultimately, affect crop quality. We therefore aimed to investigate whether decreasing the use of one or several pesticides to control weeds and insect pests resulted in an increase in the contamination of maize with mycotoxin-producing *Fusarium* fungi. Our findings confirm that cropping practices (previous crop, tillage) and climate may contribute to the development of different species of the *Fusarium* complex present in the field. Nevertheless, we found decreasing crop protection against both weeds and insects impacted the prevalence of mycotoxins but still within their range of acceptable contaminations as fixed by regulation. Exposition to nivalenol and, possibly, fusarenes were the most pronounced. It is worth noting that the relatively small number of insect attacks observed in this experiment precludes drawing firm conclusions about the impact of reducing insecticide use. Last, and although our experiment has been conducted over four consecutive years, this trial does not rule out the possibility of a progressive build-up of fungal inoculum within the field, over several seasons.

#### 4 Conclusion

Consumers express overwhelming support for a “sustainable agriculture” that uses less or no pesticide, but balk at associated increases in food prices and/or lower quality. In temperate agricultural systems, current agronomic practice is for simple, intensive cropping systems, supported by pesticides to assure crop protection. Our experimental results demonstrate that a slight increase of weed and insect infestations, resulting in our case from the simple withdrawal of herbicides and insecticides, have visible impacts on the prevalence of total mycotoxins in the harvest; with a spectacular, six-fold increase in nivalenol. All mycotoxin concentrations observed over the 4 years and 29 field sites of our experiment remained under their maximum acceptable rates; nevertheless, their concentrations approached 55–67 % of the regulatory limits significantly reducing the margin of security. We suggest that potential

integrated pest management strategies should be evaluated not only for their potential loss of yield but also for their potential impact on the quality of the harvest. Furthermore, these studies should explicitly focus on the interactions within and between pests and pathogens.

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