Innovative cropping systems to reduce *Fusarium* mycotoxins in wheat

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Fusarium head blight symptoms in wheat (photo: Dimitrios Drakopoulos, Agroscope).

Abstract

Fusarium head blight is a devastating fungal disease of wheat worldwide that causes yield loss and grain contamination with mycotoxins, such as deoxynivalenol and zearalenone. Effective reduction of mycotoxins in grain is crucial in order to improve food and feed safety. To reduce *Fusarium* mycotoxins in high-risk maize-wheat rotations under reduced or no tillage practices, we investigated three innovative cropping systems: (i) "cut-and-carry" biofumigation, (ii) maize intercropping and (iii) cover cropping. We demonstrated that the use of white mustard and Indian mustard as "cut-and-carry" biofumigant crops, intercrops with grain maize and interval cover crops after silage maize substantially reduced mycotoxins (32–76 %) in subsequent wheat. Berseem clover as a "cut-and-carry" biofumigant crop and winter pea as an interval cover crop also greatly decreased mycotoxins (53–87 %) in wheat. "Cut-and-carry" biofumigation and cover crops improved the yield of winter wheat by up to 15 % and spring wheat by up to 25 %, respectively. Based on these findings, we provide a synthesis of alternative cropping systems that effectively reduce *Fusarium* mycotoxins in wheat, thus improving food and feed safety. Nevertheless, the proposed cropping systems may increase production costs, and thus, any economic trade-offs should be further assessed to weigh potential conflicts between food/feed safety goals and economic viability.

Key words: *Fusarium*, mycotoxin, biofumigation, intercrop, cover crop.

Introduction

Fusarium head blight (FHB) is a devastating fungal disease of wheat causing yield loss and grain contamination with mycotoxins, such as deoxynivalenol (DON) and zearalenone (ZEN), which threaten human and animal health (Parry *et al.,* 1995). To minimise the adverse effects on health, the European Commission (European Commission, 2006) has set maximum limits for certain mycotoxins in foodstuffs (e.g. 1250 and 100 µg kg^{-1} for DON and ZEN, respectively, in unprocessed cereals), which are also applied in Switzerland. In most parts of the world, including Switzerland, the predominant species causing FHB in wheat is the fungus *Fusarium graminearum* (Osborne and Stein, 2007). It is an ascomycete with the ability to develop both asexually and sexually (teleomorph *Gibberella zeae*) producing macroconidia and ascospores, respectively, which infect the cereal heads during anthesis in spring (Trail, 2009). Figure 1 shows the life cycle of *F. graminearum* in a maize-wheat rotation.

Suitable crop rotation with non-host species and management of crop residues with deep ploughing are effective agronomic practices to prevent FHB in small-grain cereals (Gilbert and Haber, 2013). However, reduced tillage has several advantages, including the preservation of soil quality and reduced soil degradation (Six *et al*., 2000). In Switzerland, direct payments are provided to farmers employing reduced tillage, which was practiced in approximately 28 % of the open arable land in 2018 (EAER, 2019). Moreover, farmers commonly cultivate wheat after maize since it fits well in the crop rotation in terms of sowing and harvesting periods. Additional crop protection measures against FHB are the selection of less susceptible cultivars and the use of synthetic fungicides. However, most wheat cultivars range between medium to high susceptibility and the efficacy of fungicides can be inconsistent mostly due to the short time frame for application, heterogeneous anthesis and development of resistant fungal strains (Wegulo *et al*., 2015; Beres *et al*., 2018). Moreover, there is currently a tendency towards reduced reliance on synthetic pesticides. In 2017, the Federal Council of the Swiss Confederation adopted a national action plan aiming to define goals and measures for the reduction of pesticide risks (FOAG, 2017). Therefore, novel strategies must be explored to reduce FHB and mycotoxin contamination of the harvested products. In this study, we examined the potential of cropping systems to reduce *Fusarium* mycotoxins in wheat using (i) "cut-and-carry" biofumigation (Drakopoulos *et al*., 2020), (ii) maize intercropping and (iii) cover cropping (Fig. 2).

Materials and methods

"Cut-and-carry" biofumigation

Field experiments were conducted in 2016–2017 and in 2017–2018 at Agroscope-Reckenholz in Zurich. A maize-wheat rotation resulting in high FHB disease pressure was simulated by artificially inoculating maize stalks with *F. graminearum*. The field experiment was arranged in four blocks and experimental plots were randomised within each block. Subplots included the two winter wheat varieties Levis and Forel. For the "cut-and-carry" biofumigation treatments, mulch layers from different cover crops were applied in autumn onto the

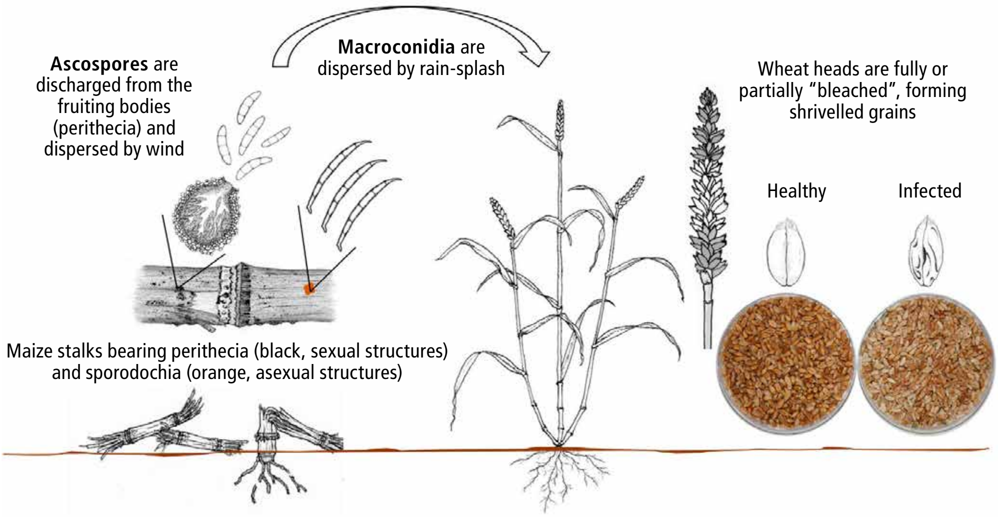
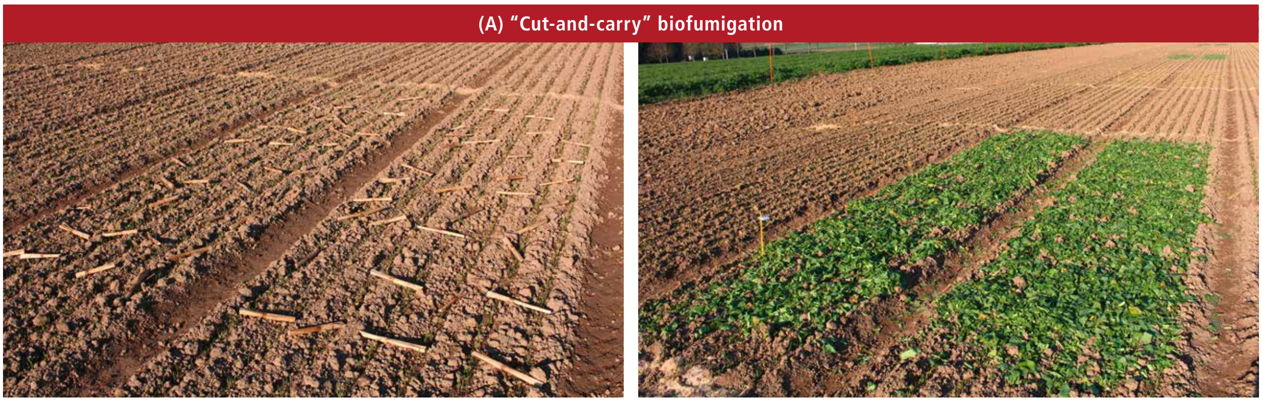


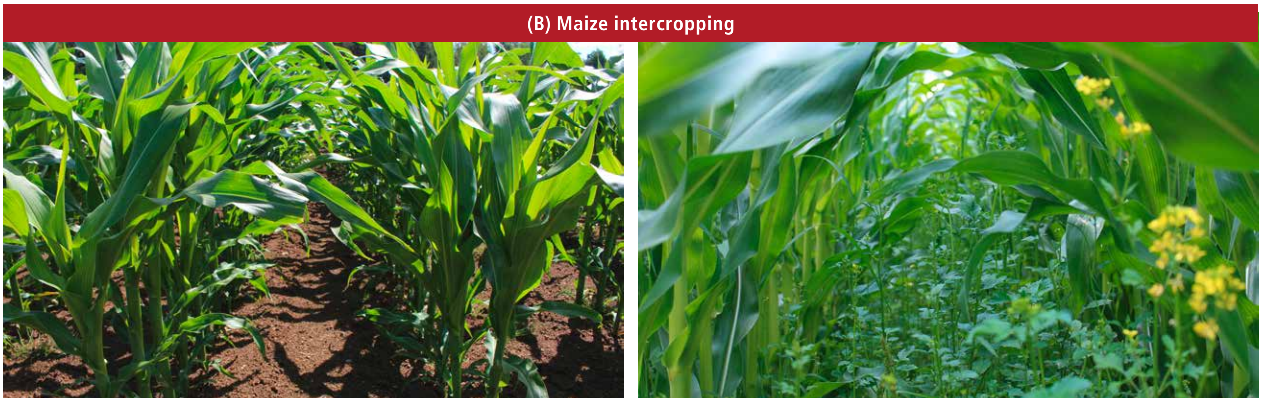
Figure 1 | **Life cycle of *Fusarium graminearum* in a maize-wheat rotation** (drawings: Jonas Lehner, Agroscope; photos: Dimitrios Drakopoulos, Agroscope).

inoculated maize stalks after wheat sowing. Specifically, fresh aboveground biomass of white mustard (*Sinapis alba*, var. Admiral), Indian mustard (*Brassica juncea*, var. Vittasso) and berseem clover (*Trifolium alexandrinum*, var. Tabor) were collected from different fields, cut to pieces of 4–6 cm and manually applied to each subplot. Approximately 19 tonnes of mulch material were applied per hectare, providing sufficient coverage of the maize stalks. *F. graminearum* infected maize stalks without any application of mulch layers served as a control treatment. One spore trap (Fig. 3) with a *Fusarium*-selective agar medium was placed in each plot during wheat anthesis to catch airborne ascospores discharged from perithecia, the fruiting bodies of *F. graminearum*. For each treatment, the sum of developed *Fusarium* colony forming units (CFU) from three time points during wheat anthesis was calculated. The disease incidence was determined by counting the number of heads with typical FHB symptoms, that is, ten heads from ten different locations observed per wheat subplot. Wheat

(A) "Cut-and-carry" biofumigation



(B) Maize intercropping



(C) Cover cropping

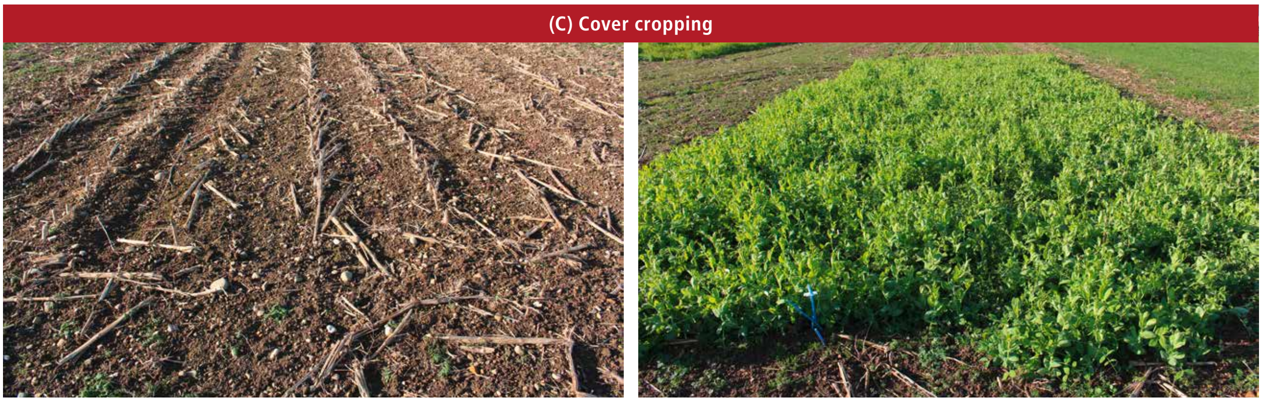


Figure 2 | **Cropping systems to control Fusarium head blight and reduce mycotoxins in wheat. (A) "Cut-and-carry" biofumigation: Untreated stalks as a control treatment (left); mulch layer of Indian mustard covering the maize stalks (right). (B) Maize intercropping: Maize sole crop as a control treatment (left); maize-white mustard intercropping (right). (C) Cover cropping after silage maize: Herbicide without a cover crop as a control treatment (left); winter pea (right)** (photos: Dimitrios Drakopoulos, Agroscope).

was harvested using a plot combine harvester to determine the grain yield at 12 % seed moisture. The mycotoxins DON and ZEN in wheat grain were quantified using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocols.

Maize intercropping

Field experiments were conducted in 2016–2017 and in 2018–2019 at Agroscope-Tänikon in Ettenhausen, Switzerland, using a split-split-plot design in four blocks. This design comprised two tillage regimes as whole plots (no-tillage and reduced tillage); five maize intercropping systems (red clover (*Trifolium pratense*, var. Pastor), sudangrass (*Sorghum × drummondii*, var. Hay-King II Hi-Gest®), phacelia (*Phacelia tanacetifolia*, var. Angelia), white mustard (var. Admiral), Indian mustard (var. Vittasso)) and a sole maize crop (no intercropping) as subplots as well as two winter wheat varieties (Levis and Forel) as sub-subplots. Grain maize (var. Laurinio) was sown across the entire field, and the seeds of the intercrops were spread at BBCH stages 13–15 of maize with a seed broadcaster. After harvesting the grain maize with a plot combine harvester, the following tillage treatments were applied: For no tillage, the maize and intercrop residues were mulched on the soil surface; for reduced tillage, the crop residues were mulched and then incorporated into the top soil layer (~10 cm depth) in a single pass with a rotary tiller. Subsequently, winter wheat was established with direct sowing. The disease incidence was determined by counting the number of heads with developed symptoms per wheat sub-subplot. The wheat grain yield was determined as described above. The mycotoxins in grains were measured by liquid chromatography–mass spectrometry.

Cover cropping

Field experiments were conducted at Agroscope-Reckenholz and Agroscope-Tänikon in 2016–2017 and in 2017–2018, respectively. A split-plot design in four blocks was used. This design comprised five cropping systems (herbicide without cover crop, ploughing without cover crop, white mustard (var. Salsa), Indian mustard (var. Vittasso) and winter pea (*Pisum sativum*, var. Arkta)) as whole plots and two spring wheat varieties (Digana and Fiorina) as subplots. Silage maize (P8057) was cultivated prior to the tested cropping systems. To ensure a sufficient level of FHB infection in the field, 20 maize plants per plot were inoculated with *F. graminearum* using the pin method at BBCH 71–73. After harvesting the silage maize, the residues were mulched across the entire field. For the "herbicide without cover crop" treatment, glyphosate was applied. For the "ploughing without cover crop" treatment, maize residues were buried into the soil with a mouldboard plough (~30 cm depth). The cover crops were established with direct sowing. White mustard and Indian mustard were mulched before the first frost, while winter pea, as a winter cover crop, was mulched at the beginning of the following spring. Subsequently, spring wheat was sown by direct sowing. The disease incidence, grain yield and mycotoxins in wheat were measured as described above.

Figure 3 | Spore trap with a *Fusarium*-selective medium adjusted to the same height as the flowering wheat heads (design: Hans-Rudolf Forrer, Agroscope; photo: Dimitrios Drakopoulos, Agroscope).

Data analysis

Analysis of variance was performed to test for significant differences between the examined treatments within the experimental year of each study. Post hoc comparisons were performed using Fisher's protected least significant difference (LSD) test ($\alpha = 0.05$). The correlation between ascospore deposition and DON content in grain were investigated using Spearman's correlation.

Results

"Cut-and-carry" biofumigation

In wheat harvest 2017, mulch layers of white mustard, Indian mustard and berseem clover reduced the DON content in grain by 37–53 % compared with the control treatment. The "cut-and-carry" biofumigation treatments also reduced the ZEN content by 65–75 % and increased the grain yield by 3–7 % compared with the control (Table 1). In wheat harvest 2018, the "cut-and-carry" biofumigation treatments reduced the DON and ZEN contents in grain by 50–58 % and 67–87 %, respectively. Compared with the control, white mustard, Indian mustard and berseem clover increased the grain yield by 8–15 % (Table 1). The effects of the "cut-and-carry" biofumigation treatments in terms of mycotoxins and grain yield were similar for both tested varieties. Strong correlations were observed between ascospore deposition in spore traps and DON content in grain for both wheat varieties (Levis: $r_s = 0.796$; Forel: $r_s = 0.840$; Fig. 4).

Maize intercropping

None of the tested intercropping systems significantly reduced the maize grain yield compared with the sole maize crop, which amounted to 12 and 10 t ha^{-1} in 2016 and in 2018, respectively. In wheat harvest 2017, the highest DON content in grain was observed after sole maize, while the maize-white mustard and maize-Indian mustard intercropping systems decreased DON by 58 % and 32 %, respectively. Similarly, the highest ZEN content was observed after sole maize, while maize-white mustard and maize-phacelia decreased ZEN by 47 % and 34 %, respectively (Table 2). The effect of maize intercropping on wheat yield was not significant ($p = 0.455$). In wheat harvest 2019, the lowest DON contents were observed after maize-Indian mustard and maize-clover. The ZEN content in grain was below the detection limit for all treatments. Compared with sole maize, intercropping with clover, phacelia and Indian mustard resulted in 7–9 % lower yield for the subsequent wheat crop, whereas intercropping with white mustard and sudangrass did not significantly reduce the wheat yield (Table 2). The effects of the maize intercropping systems in terms of mycotoxins and grain yield in wheat were similar for both tested wheat varieties and tillage regimes.

Cover cropping

In the wheat harvest 2017, the FHB disease pressure was low. Consequently, both the DON content in grain (0.05–0.10 mg kg^{-1}, $p = 0.727$) and the yield (4.2–4.9 t ha^{-1}, $p = 0.395$) of spring wheat were similar among the tested cropping systems. In contrast, in the wheat harvest 2018, growing white mustard, Indian mustard or winter pea after silage maize decreased the DON content in grains of the subsequent spring wheat by 54–74 % compared with the herbicide treatment without a cover crop. There were no significant differences between cover crops and the plough treatment on the DON content in wheat grain. In addition, all cover crop treatments improved the grain yield of spring wheat by 13–25 % (Table 3). In both the 2017 and 2018 harvests, the ZEN content in grain was below the detection limit. The effects of the cover cropping systems in terms of mycotoxins and grain yield in wheat were similar for both tested varieties.

Discussion and conclusions

An effective management of *Fusarium* mycotoxins is crucial in order to improve food and feed safety of cereal products. An ecological intensification of agroecosystems should integrate crop protection measures that pose no risks to the environment and human health. We investigated three pre-harvest field strategies to control FHB and reduce mycotoxins in wheat through innovative cropping systems under reduced or no tillage practices. The novel "cut-and-carry" biofumigation strategy with

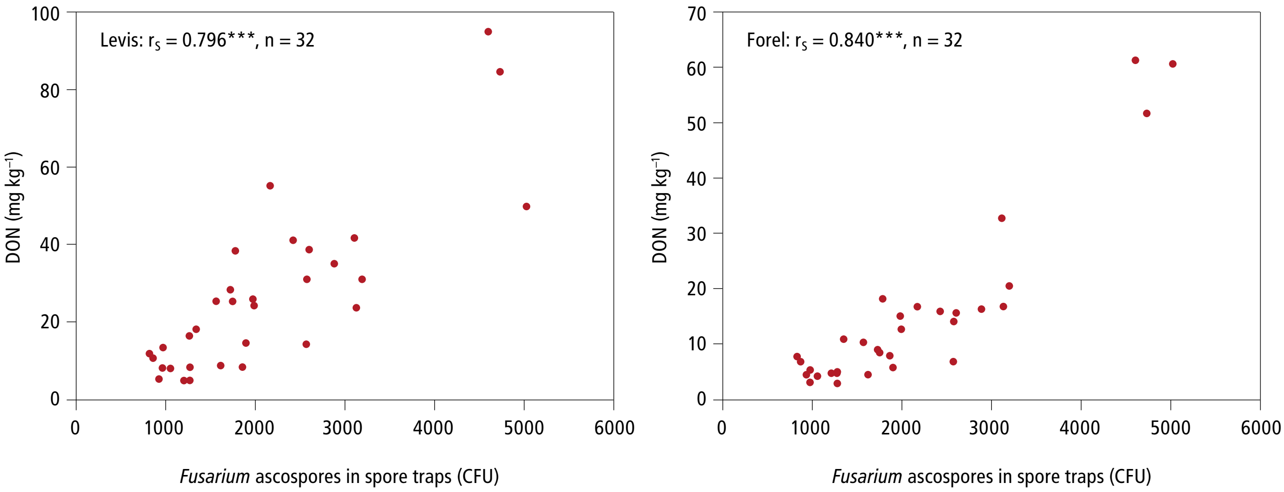


Figure 4 | **Correlation between deoxynivalenol (DON) content in grain and ascospore deposition (*Fusarium* colony forming units (CFU)) for the wheat varieties Levis (left) and Forel (right) in the "cut-and-carry" biofumigation study. The Spearman's rank correlation coefficient (r_s) was calculated (***$p < 0.001$). Data from two experimental years (2016–2017 and 2017–2018) and four treatments were used for the analysis (three "cut-and-carry biofumigation" treatments and the control; n = 32).**

Table 1 | Effects of *"cut-and-carry" biofumigation* treatments on the deoxynivalenol and zearalenone contents in grain and yield in wheat harvests 2017 and 2018. For each treatment, the percentage of increase/decrease compared with the control is provided. Average values from two wheat varieties (Levis and Forel) are reported (n = 8) and different letters in parentheses indicate significant differences between treatments ($\alpha = 0.05$).

	Control	"Cut-and-carry" biofumigation		
		White mustard	Indian mustard	Berseem clover
2017				
Deoxynivalenol	12.9 mg kg^{-1} (a)	– 40 % (b)	– 37 % (b)	– 53 % (b)
Zearalenone	0.6 mg kg^{-1} (a)	– 75 % (b)	– 71 % (ab)	– 65 % (ab)
Grain yield	8.1 t ha^{-1} (a)	+ 7 % (b)	+ 3 % (ab)	+ 4 % (ab)
2018				
Deoxynivalenol	55.4 mg kg^{-1} (a)	– 50 % (b)	– 58 % (b)	– 56 % (b)
Zearalenone	0.2 mg kg^{-1} (a)	– 76 % (bc)	– 67 % (b)	– 87 % (c)
Grain yield	6.5 t ha^{-1} (a)	+ 8 % (b)	+ 15 % (b)	+ 14 % (b)

mulch layers applied onto infected maize residues substantially decreased mycotoxin contamination and improved the grain yield of wheat. More specifically, mulch layers of white mustard, Indian mustard and berseem clover consistently decreased the mycotoxins DON and ZEN in both harvest years (by up to 58 % and 87 %, respectively) and increased yield by up to 15 %. Mustard plants are widely grown as cover crops, as they provide a broad range of agronomic benefits, such as biofumigation, weed control and soil preservation (Snapp *et al.*, 2005). Upon tissue disruption, the release of glucosinolate-breakdown products (i.e. isothiocyanates) inhibits the growth of several microbial species, including the mycotoxigenic species *F. graminearum* (Drakopoulos *et al.*, 2019). Isothiocyanates are among the most bioactive substances of mustard against soil-borne pathogens, pests and weeds (Brown and Morra, 1997). The phytochemical profile of clover, such as berseem clover, indicates the presence of several bioactive compounds, such as flavonoids, phenolic acids, clovamides and saponins (Oleszek *et al.*, 2007; Kolodziejczyk-Czepas, 2012). Besides their antifungal effects, "cut-and-carry" green manures are an excellent source of nitrogen, improving the inherent soil fertility and soil organic carbon stocks. Sorensen and Grevsen (2016) reported that the aboveground biomass of annual legume crops and perennial green manure crops yielded 200 and 400–500 kg nitrogen per hectare, respectively, over a growing season. Thus, growers can benefit from the "cut-and-carry" approach with berseem clover by simultaneously fertilising their cash crops and controlling residue-borne pathogens through biofumigation. For adequate coverage of maize residues in one hectare, growers should use the aboveground biomass of white mustard or Indi-

Table 2 | Effects of *maize intercropping* treatments on the deoxynivalenol and zearalenone contents in grain and yield in wheat harvests 2017 and 2019. For each intercropping treatment, the percentage of increase/decrease compared with the sole maize crop is provided. Average values from two tillage practices (reduced and no tillage) and two wheat varieties (Levis and Forel) are reported (n = 16). Different letters in parentheses indicate significant differences between treatments ($\alpha = 0.05$).

	Sole maize	Intercropping				
		Red clover	Sudangrass	Phacelia	White mustard	Indian mustard
2017[1]						
Deoxynivalenol	0.59 mg kg^{-1} (a)	– 22 % (ab)	– 7 % (ab)	– 18 % (ab)	– 58 % (c)	– 32 % (b)
Zearalenone	0.24 mg kg^{-1} (a)	– 31 % (abc)	– 12 % (ab)	– 34 % (bc)	– 47 % (c)	– 15 % (abc)
2019[2]						
Deoxynivalenol	4.9 mg kg^{-1} (ab)	– 10 % (b)	+ 14 % (a)	+ 22 % (a)	+ 9 % (ab)	– 13 % (b)
Grain yield	6.7 t ha^{-1} (ab)	– 9 % (d)	+ 3 % (a)	– 7 % (cd)	– 3 % (bc)	– 7 % (cd)

[1] The effect on grain yield was not significant ($p > 0.05$).
[2] Zearalenone was below the detection limit (0.1 µg kg^{-1}).

Table 3 | Effects of *cover cropping* systems and ploughing without cover crop treatment on the deoxynivalenol content in grain and yield in wheat harvest 2018. For each treatment, the percentage of increase/decrease compared with the herbicide without cover crop treatment is provided. Average values from two wheat varieties (Fiorina and Digana) are reported (n = 8). Different letters in parentheses indicate significant differences between treatments ($\alpha = 0.05$).

	Without cover crop – herbicide	Without cover crop – ploughing	Cover cropping		
			White mustard	Indian mustard	Winter pea
Deoxynivalenol[1]	5.4 mg kg^{-1} (a)	– 75 % (b)	– 57 % (b)	– 54 % (b)	– 74 % (b)
Grain yield	4.0 t ha^{-1} (a)	+ 5 % (ab)	+ 13 % (abc)	+ 18 % (bc)	+ 25 % (c)

[1] Zearalenone was below the detection limit (0.1 µg kg^{-1}).

an mustard grown in one hectare, whereas for berseem clover, half a hectare is sufficient. Although the "cut-and-carry" approach may increase production costs in the short term, its long-term agronomic benefits, such as reduced *Fusarium* mycotoxins and increased soil fertility, are expected to compensate for the initial economic trade-off. Finally, in the "cut-and-carry" biofumigation experiments, we observed a strong positive correlation between ascospore deposition in spore traps and DON content in wheat. Hence, the number of *Fusarium* colonies in spore traps during wheat anthesis is a reliable predictor of DON contamination risk.

Moreover, we showed that maize intercropping reduced mycotoxins in subsequent winter wheat, but only under moderate disease pressure (2017) and not under very high disease pressure (2019). In 2017, compared with the sole maize crop, the use of white mustard and Indian mustard as intercrops decreased DON in wheat by up to 58%. The mechanisms by which intercrops affect the disease dynamics may include changes in the microclimate, alterations of wind, rain and/or vector dispersal, changes of host morphology and physiology as well as direct pathogen inhibition (Boudreau, 2013). The main direct disease-suppression mechanism of mustard is related to the release of glucosinolate-derived substances, which have antifungal properties (Brown and Morra, 1997; Manici *et al.*, 1997). Another positive finding was that none of the tested intercropping systems significantly reduced the maize grain yield when intercrops were sown at the BBCH 13–15 growth stage of maize.

One of the most effective cultural practices to manage FHB is the adoption of suitable crop rotation (Champeil *et al.*, 2004; Shah *et al.*, 2018). In fact, we found that growing interval cover crops (white mustard, Indian mustard or winter pea) in silage maize-spring wheat rotation reduced DON in wheat by up to 74 %. Remarkably, the decrease in mycotoxins with cover crops was comparable to that with the plough treatment whereby *Fusarium*-infected maize residues were buried in deep soil layers. However, in Switzerland, spring wheat is less frequently cultivated than winter wheat due to its lower yields. Therefore, the cultivation of spring wheat after growing a cover crop could be supported by agricultural policies.

In summary, we shed light on alternative crop protection strategies to reduce the risk of *Fusarium* mycotoxins in wheat using innovative cropping systems under reduced or no tillage systems. White mustard and Indian mustard could be successfully cultivated as "cut-and-carry" biofumigant crops, cover crops and intercrops of maize to reduce mycotoxins in wheat, with the former two agronomic practices being more effective and consistent than the latter. Berseem clover as a "cut-and-carry" biofumigant or green manure crop and winter pea as a cover crop are not only effective against *Fusarium* mycotoxins but can also improve soil fertility and soil organic carbon stocks. In the context of sustainable crop protection, cereal growers and consumers could benefit from the proposed pre-harvest strategies, as they reduce the risk of mycotoxin contamination in harvested products, thereby improving grain yield and quality. As novel cropping systems, such as "cut-and-carry" biofumigation, may increase production costs, any economic trade-offs should be further assessed. These trade-offs could be addressed by adjusted agricultural policies to avoid potential conflicts between food safety goals and farm profitability. ■

Acknowledgements

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