Contents lists available at ScienceDirect

Agricultural Systems

journal homepage: www.elsevier.com/locate/agsy



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HIGHLIGHTS

G R A P H I C A L A B S T R A C T

- To reduce mycotoxins in wheat, the agronomic and economic viability of maize-intercropping and cover cropping were investigated.
- Growing intercrops with maize or interval cover crops in a maize-wheat rotation decreased mycotoxins and maintainedwheat yield.
- Due to increased operating costs, we observed economic trade-offs in the innovative cropping systems.
- Policy makers should support innovation in agricultural systems to enhance food safety while ensuring the economic viability of wheat production.

ARTICLE INFO

Editor: Mark van Wijk

Keywords: Fusarium head blight Wheat Mycotoxin Food safety Intercrop Cover crop



intercropping

Maize-red clove intercropping

ABSTRACT

CONTEXT: The effective control of Fusarium head blight (FHB) in wheat, mainly caused by the toxigenic fungus *Fusarium graminearum*, has a significant impact on food safety worldwide. As maize is one of the main hosts of *F. graminearum*, the risk of infection by this plant pathogen is highest when wheat is grown after maize and infected crop residues are not buried through ploughing.

OBJECTIVES: This study aimed to investigate the agronomic and economic viability of two innovative cropping systems with the goal to reduce the risk of FHB and mycotoxins in subsequent wheat. These systems were maize-intercropping and cover cropping with different plant species before the wheat growing season under reduced tillage practices.

METHODS: For the maize-intercropping study, red clover, sudangrass, phacelia, white mustard and Indian mustard were used as intercrops with grain maize and compared with a sole maize crop in a grain maize-winter wheat rotation under no-tillage or reduced tillage. For the cover cropping study, white mustard, Indian mustard and winter pea were used as interval cover crops in a silage maize-spring wheat rotation under no-tillage and compared with treatments without a cover crop, i.e. herbicide or plough applied after silage maize. The incidence of Fusarium head blight causing species and the accumulation of mycotoxins in grains of wheat as well as the

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https://doi.org/10.1016/j.agsy.2021.103198

Received 11 February 2021; Received in revised form 22 April 2021; Accepted 4 June 2021 Available online 18 June 2021 0308-521X/© 2021 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).





crop yield were monitored. In addition, an economic assessment was conducted by calculating the receipts, operating costs and gross margin for each cropping system.

RESULTS AND CONCLUSIONS: Growing intercrops with maize or interval cover crops in a maize-wheat rotation under reduced tillage decreased mycotoxins in wheat while maintaining wheat yield. The use of white mustard or Indian mustard as intercrops reduced deoxynivalenol in winter wheat by up to 52% compared with maize grown as a sole crop. The use of white mustard, Indian mustard or winter pea as interval cover crops also reduced deoxynivalenol and improved yield in spring wheat by up to 85% and 25%, respectively. Remarkably, the toxin reduction through these cover crops was comparable with that obtained by ploughing. However, due to increased operating costs, we observed economic trade-offs in these innovative cropping systems, i.e. 7–25% reduced gross margin over the entire rotation.

SIGNIFICANCE: Both cereal growers and consumers can benefit from the recommended practices, which considerably lower the risk of mycotoxin contamination in harvest products while maintaining crop yield. To address the economic trade-offs, policy makers should support innovation in cropping systems, enhancing food safety while also ensuring the economic viability of cereal production systems.

1. Introduction

A great challenge of agriculture is to ensure food security while improving food safety through sustainable intensification of agroecosystems (Dardonville et al., 2020; Garnett et al., 2013; Godfray et al., 2010). Maize (Zea mays L.) and wheat (Triticum aestivum L.) are essential food and feed crops contributing substantially to food security and the global economy (FAOSTAT, 2020). Fusarium head blight (FHB) is worldwide one of the most important fungal diseases of wheat that causes significant yield losses (Savary et al., 2019). Severe epidemics in the 1990's had a devastating impact on wheat producers in the United States of America, Canada, Europe, Argentina and China, with yield reductions in Europe reaching up to 50-60% (Singh et al., 2016). But even more concerning than these yield losses is that FHB causes grain contamination with hazardous secondary metabolites (i.e. mycotoxins), such as deoxynivalenol (DON) and zearalenone (ZEN), which are toxic to humans and animals with adverse chronic and acute effects (Escrivá et al., 2015). DON is associated with acute gastrointestinal adverse effects, such as vomiting and feed refusal, while the main effects of longterm dietary exposure to this mycotoxin are suppression of weight gain, anorexia and altered nutritional efficiency (EFSA, 2013). The toxicity of ZEN is linked to reproductive problems in animals and possibly in humans due to estrogenic effects (Marin et al., 2013). Thus, there is a great need to reduce FHB in wheat systems to ensure food safety and security while maintaining the agronomic and economic viability of wheat production.

In most parts of the world, *Fusarium graminearum* is the predominant FHB causing species (Osborne and Stein, 2007). The fungus overwinters on crop residues where its fruiting bodies (i.e. perithecia) develop. Ascospores are discharged from mature perithecia and infect the wheat heads during flowering with the aid of wind, but infection may also occur through water-splashed macroconidia, which are clustered on crop residues in cushion-shaped masses called sporodochia. Crop rotation with non-host species and conventional tillage burying the crop residues are common strategies to control FHB (Parry et al., 1995; Vogelgsang et al., 2019). However, reduced and no-tillage practices that do not bury residues sufficiently are increasingly implemented to preserve soil quality and reduce erosion (Jacobs et al., 2009; Six et al., 2000). Thus, exploration of alternative cropping systems within a maize-wheat rotation is essential to provide integrated agronomic solutions that are economically feasible to effectively control FHB.

The implementation of intercropping and cover cropping systems could be one route to control phytopathogens while sustainably intensify agroecosystems (Brooker et al., 2015; Tilman, 2020; Wittwer et al., 2017). Intercropping involves two or more crop species, or genotypes, growing simultaneously, while cover cropping in a rotation implies the cultivation of a certain plant species before or after a cash crop. Both cropping systems have shown potential to increase crop production while maintaining or even enhancing other ecosystem services, such as increasing soil carbon and nitrogen levels (Cong et al., 2015; Schipanski

et al., 2014). Moreover, phytopathogens that develop on crop debris could be controlled by using intercrop or cover crop species with antifungal properties, such as the glucosinolate-derived bioactive substances of mustard (Brown and Morra, 1997; Matthiessen and Kirkegaard, 2006).

In the current study, we investigated the influence of two innovative cropping systems, i.e. 'maize-intercropping' and 'cover cropping' with different plant species before the wheat growing season, on FHB species incidence and accumulation of mycotoxins in grains as well as on wheat grain yield under reduced tillage practices. We also conducted an economic assessment by calculating the receipts, operating costs and gross margin for each alternative cropping system. For the maizeintercropping study, we used red clover, sudangrass, phacelia, white mustard and Indian mustard as intercrops with grain maize and compared these with a sole maize crop. After maize harvest, the crop residues of maize and intercrops were either mulched and left on the soil surface (i.e. no-tillage) or incorporated into the top soil layer after mulching (i.e. reduced tillage). Subsequently, direct sowing of two winter wheat varieties was done. For the cover cropping study, we used white mustard, Indian mustard and winter pea as interval cover crops in a silage maize-spring wheat rotation. Cover crops were mulched and subsequently, two spring wheat varieties were established with direct sowing. We compared the cover cropping systems with two treatments without a cover crop, i.e. herbicide or plough applied after silage maize.

2. Materials and methods

2.1. Experimental design, crop management and treatments

2.1.1. Maize-intercropping

The field experiment was conducted throughout two years and at two different fields at Agroscope-Tänikon in Switzerland (2016-2017: 47°28′50″N 8°54′41″E; 2018–2019: 47°29′01″N 8°54′42″E). A splitsplit-plot design in four blocks was used, comprising two tillage practices (no-tillage, reduced tillage) as whole plots, six maize-(inter)cropping systems (sole maize as well as red clover, sudangrass, phacelia, white mustard and Indian mustard intercropped with maize) as subplots, and two varieties (Levis, Forel) as sub-subplots for the following winter wheat crop (Table S1, Fig. 1 a). The size of each subplot was 108 m^2 (9 m \times 12 m), which was then divided in half for the sub-subplot treatments (4.5 m \times 12 m each). To prevent cross-contamination with ascospores from F. graminearum, adjacent plots with triticale (×Triticosecale) served as buffer zones. The intercrop species used for this study are cover crops commonly grown in Switzerland. Likewise, the chosen maize and wheat varieties were recommended by agricultural extension advisors.

Grain maize (var. Laurinio; KWS, Switzerland) was sown across the entire field (100'000 kernels ha^{-1} , 75 cm distance between maize rows). The intercrops were sown by spreading the seeds at BBCH 13–15 growth stage of maize using a seed broadcaster (APV, Austria). Red clover

(*Trifolium pratense* var. Pastor; Feldsaaten Freudenberger, Germany), sudangrass (*Sorghum* × *drummondii* var. HayKing II Hi-Gest®; Alforex Seeds, USA), phacelia (*Phacelia tanacetifolia* var. Angelia; P.H. Petersen Saatzucht Lundsgaard, Germany), white mustard (*Sinapis alba* var. Admiral; Feldsaaten Freudenberger, Germany) and Indian mustard (*Brassica juncea* var. Vittasso; KWS, Italy) were sown at 20, 40, 8, 20 and 8 kg ha⁻¹, respectively. To facilitate the establishment of the intercrops, a field pass with original harrow was done on the same day to improve

seed-soil contact and control any emerged weeds. For maize production, mineral fertilisers were applied (110 N, 95 P and 220 K kg ha⁻¹). For sole maize, a herbicide treatment (Calaris; active ingredients (a.i.): terbuthylazin and mesotrione; Syngenta, Switzerland) was applied between maize rows at BBCH 30–33. Maize plants were not artificially inoculated with *F. graminearum*, as it was done in the cover cropping study, because intercrops were grown between maize rows, which would have caused significant damage to the plants.



Fig. 1. Maize-intercropping (a). Top: Aerial image of one experimental block during wheat cultivation. The whole plots (two tillage practices) are indicated by the continuous line rectangles, whereas the dashed lines indicate the split of the 12 subplots (maize-(inter)cropping systems) into sub-subplots (two wheat varieties). Bottom: Maize as a sole crop (left), maize-white mustard intercropping (middle) and maize-clover intercropping (right).

Cover cropping (b). Top: Aerial image of the entire experimental field. The whole plots (five cropping systems) were split in half, as indicated by the dashed lines, creating subplots (two wheat varieties). Bottom: Herbicide without cover crop with no-tillage (left), plough without a cover crop (middle) and winter pea with no-tillage (right).

Images from the field experiments at Agroscope-Tänikon, 8356 Ettenhausen, Switzerland.

After the harvest of grain maize with a combine harvester, the following treatments were conducted: 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 (up to 10 cm depth) by employing one single pass with a rotary tiller, mounted with a fixed packer roller. Approximately two weeks after the soil operations, winter wheat (var. Levis, var. Forel; Saatzucht Düdingen, Switzerland) was sown with direct sowing at 180 kg ha⁻¹. In the adjacent buffer plots, inversion tillage with mouldboard plough was applied to bury all maize residues down to 30 cm soil depth, and then winter triticale (var. Larossa; Saatzucht Düdingen, Switzerland) was sown at 180 kg ha⁻¹. For wheat production, mineral fertilisers were applied (140 N, 60 P and 80 K kg ha⁻¹). At BBCH 27–29, herbicides (a mixture of Artist (a.i.: flufenacet and metribuzin) and Chekker (a.i.: amidosulfuron and iodosulfuron); Bayer, Switzerland) were applied. At BBCH 55-57, an insecticide (Karate Zeon; a.i.: lambda-cyhalothrin; Syngenta, Switzerland) was applied against cereal leaf beetles.

2.1.2. Cover cropping

The field experiment was conducted throughout two years and at two different fields in Switzerland (2016–2017 at Agroscope-Reckenholz: 47°26′15″N 8°31′38″E; 2017–2018 at Agroscope-Tänikon: 47°28′31″N 8°54′14″E). A split-plot design in four blocks was used, comprising five cropping systems (herbicide without cover crop, plough without cover crop, as well as white mustard, Indian mustard and winter pea as cover crops) as whole plots and two wheat varieties (Digana, Fiorina) as subplots (Table S2, Fig. 1 b). The size of each whole plot was 72 m² (6 m × 12 m), which was then divided in half for the subplot treatments (3 m × 12 m each). To prevent cross-contamination with ascospores from *F. graminearum*, adjacent plots with triticale served as buffer zones. The chosen cover crop as well as the maize and wheat varieties are commonly grown in Switzerland.

Silage maize (var. P8057; Pioneer Hybrid International, USA) was the previous crop and was sown across the entire field at 100'000 kernels ha⁻¹. Herbicides (a mixture of Gardo Gold (a.i.: s-metolachlor and terbuthylazin), Callisto (a.i.: mesotrione) and Banvel 4S (a.i.: dicamba); Syngenta, Switzerland) were applied at BBCH 13-14. To ensure a sufficient level of FHB infection in the field, maize plants were inoculated with a pin method at BBCH 71-73 using conidial suspensions of three F. graminearum isolates ('0410', CBS 121292, Westerdijk Fungal Biodiversity Institute, The Netherlands; '2113', Research Group Crop Breeding and Genetic Resources, Agroscope, Switzerland; '1145', Fungal Collection of Agroscope, Switzerland; all single-spore isolates from wheat in Switzerland). Equal amounts of each isolate were used and the final concentration was adjusted to 10⁶ conidia ml⁻¹ sterile deionised water containing 0.0125% Tween® 20. The pin method involved direct penetration (0.5 cm) of the first visible internode above the crown roots with 4-pins (square pyramid shape) previously dipped in the conidial suspension. Five maize stalks were inoculated from the middle area of the second, third, sixth and seventh maize row, resulting in ten inoculated stalks for each subsequent wheat subplot.

After the harvest of silage maize, the maize residues were mulched across the entire field. Subsequently, white mustard (var. Salsa; Limagrain, Belgium), Indian mustard (var. Vittasso; KWS, Italy) and winter fodder pea (var. Arkta; Feldsaaten Freudenberger, Germany) were sown at 33, 8.8 and 143 kg ha⁻¹, respectively, using direct sowing. For the 'herbicide without cover crop' treatment, a herbicide (Roundup Profi; a. i.: glyphosate; Leu + Gygax AG, Switzerland) was applied. For the 'plough without cover crop' treatment, maize residues were buried into the soil by inversion tillage with mouldboard ploughing (down to 30 cm depth). White mustard and Indian mustard were mulched before the first frost, while winter pea was mulched in the beginning of the following spring. Subsequently, spring wheat (var. Digana and var. Fiorina; Saatzucht Düdingen, Switzerland) was sown at 210 kg ha⁻¹ after seedbed

preparation. The fertiliser inputs for the maize and wheat crops as well as the insecticide application for wheat were the same as described above in the maize-intercropping experiment.

2.2. Measurements

2.2.1. Maize-intercropping

Prior to maize harvest, the aboveground biomass of each intercrop species was determined by collecting the plant material from an area of 0.42 m² (0.7 m \times 0.6 m) from the 2nd and 10th intercrop row and merging it into one composite sample per subplot, comprising 12 intercrop rows. The biomass (t ha⁻¹) was determined after drying the samples at 105 °C for 48 h. For the maize grain yield (t ha⁻¹), an area of 12 m² (8 m \times 1.5 m) was harvested per subplot using a combine harvester. To determine the seed moisture content, a representative sample of 2 kg was drawn directly from the harvester. For the grain yield and seed moisture content in wheat, an area of 9 m^2 was harvested using a plot combine harvester (Wintersteiger, Austria). For seed health tests and mycotoxin analysis, wheat grain subsamples of 5 g and 150 g were drawn, respectively, using a riffle divider (Schieritz & Hauenstein AG, Switzerland). To determine the proportional incidence of FHB causing species (%), seed health tests with 100 grains per sub-subplot were conducted as described in Vogelgsang et al. (2008). This measurement was based on macro- and microscopic observations of the developed fungal colonies (Leslie and Summerell, 2006).

For mycotoxin analysis, the wheat grain subsamples were milled (Cyclotec[™] 1093, Foss Tecator, Sweden; 1-mm mesh size) and flours were stored at -20 °C until mycotoxin extraction. For each sample, a subsample of 5 g wheat flour was extracted with 20 ml Milli-Q water: acetonitrile (16:84) solvent solution and shaken for 2 h. Water was purified by a Milli-Q gradient A10 water purification system (MilliporeSigma, USA). Afterwards, each extract was filtered through a folded paper filter (Whatman, 595 1/2, 125 mm; GE Healthcare Ltd., UK) and collected in a vial. Then, 2 ml of each extract passed through a 3-ml ISOLUTE® SPE tube (Biotage, Sweden) containing 0.3 g alox:celite (1:1), which was fitted with 20-µm frits at the bottom and the top of each tube, and mounted on a Visiprep SPE Vacuum Manifold (12-port; Supelco, USA). A volume of 200 µl cleaned extract was added into LCvials with a Hamilton syringe and the eluate was evaporated completely with an airstream using a Visidry Drying Attachment (12port; Supelco, USA) at 40 °C. Finally, a volume of 1 ml Milli-Q water: methanol (90:10) solution was pipetted into each vial and crimped. The following mycotoxin standards were included in the analysis: nivalenol (NIV), deoxynivalenol (DON), 3- and 15-acetyldeoxynivalenol (Ac-DON), fusarenon-x (Fus-X), diacetoxyscirpenol (DAS), HT-2, T-2 (>97%, premixed standard, Trilogy Analytical Laboratory Inc., USA), monoacetoxyscirpenol (MAS; >98%, Fermentek, Israel) and zearalenone (ZEN; Sigma-Aldrich, Switzerland). Hereinafter, Ac-DON stands for the sum of 3- and 15-acetyldeoxynivalenol. For each run, reference sample flours (Trilogy Analytical Laboratory Inc., USA) of wheat grain naturally contaminated with either DON or ZEN were included, while a wholewheat flour (organic product; COOP, Switzerland) without any detected mycotoxins was used for the matrix-matched calibration curve of each standard. Mycotoxins were analysed with liquid chromatography (1260 Series, Agilent, Germany) mass spectrometry (6470 Triple Quad, equipped with JetStream ESI source, Agilent, USA), and the contents in wheat grain are presented in $\mu g \; kg^{-1}$ (see Table S3 for instrument pa rameters). The limit of detection (LOD) and limit of quantification (LOQ) of each analyte are provided in Table S4. When values were below LOQ or LOD, values were replaced by LOQ+2 or LOD+2, respectively.

2.2.2. Cover cropping

The grain yield, proportional incidence of FHB causing species in grains and mycotoxins content in grains of wheat were determined as described for the maize-intercropping experiment.

2.3. Gross margin analysis

For the entire crop rotation of the maize-intercropping and cover cropping experiments, a gross margin analysis was conducted as follows (see also Supplementary material 2):

GM = R - OC

GM stands for the gross margin, R for the receipts and OC for the operating costs.

The seed prices of the intercrops, cover crops and wheat were retrieved from UFA-Samen (2019), while the prices of the herbicide and insecticide products were obtained from the cantonal plant protection offices of Thurgau and Zurich in Switzerland (BBZ-Arenenberg and Strickhof, 2019). The fertilisation costs were obtained from Landor (2019). The costs related to machinery operations were retrieved from Gazzarin (2018) and include the engine performance per hour, the operation time per ha in min, the machine cost ha⁻¹ and the imputed labour costs. The selling prices of grain maize and wheat grain were obtained from Swissgranum (2019), while the selling price of silage maize at 30% dry matter was obtained from Agridea (2019). In Switzerland, direct payments are given for the implementation of reduced tillage practices and no-herbicide use. However, direct payments were not included for the gross margin analysis of this study because growers who adopt innovative cropping systems (e.g. use of intercrops or cover crops) within a maize-wheat rotation are not entitled to these subsidies as of yet. In addition, agricultural policies frequently change and regulations are usually country-specific. Calculations were performed in Swiss Francs (CHF).

For the maize-intercropping experiment, the average values of R and OC were calculated for the sole maize and the different maize-intercropping systems under reduced tillage or no-tillage, pooled across both wheat varieties and the two experimental years. For the cover cropping experiment, the calculations were conducted for each cropping system, pooled across both wheat varieties and the two experimental years.

2.4. Statistical analysis

Data were analysed with the software Genstat 19^{th} edition (VSN International Ltd., UK) and figures were plotted with Prism 8 (GraphPad Software Inc., USA). For both maize-intercropping and cover cropping experiments, data were analysed separately for each experimental year. Normal distribution and equality of variances across groups were verified with the Shapiro-Wilk and Bartlett's tests, respectively. To reach or approach a normal distribution and homoscedasticity, the response variables were transformed accordingly (Supplementary material 3). Untransformed data are presented in tables and figures. For post hoc comparisons, Fisher's protected LSD test was used ($\alpha = 0.05$).

2.4.1. Maize-intercropping

A split-split-plot ANOVA was performed to test the effects of tillage, maize-intercropping and wheat variety on the response variables in wheat, i.e. grain yield, incidence of FHB causing species in grains and mycotoxins in grain. The treatment structure (fixed effects) was defined as 'tillage \times maize-intercropping \times wheat variety' and the block structure (random effects) was defined as 'block / whole plot / subplot / subplot'. A non-parametric test (Kruskal-Wallis one-way ANOVA on ranks) was performed to test the effect of maize-intercropping on maize grain yield as well as the year effect on the response variables in maize and wheat. A parametric one-way ANOVA was performed to test the effect of the year on the dry aboveground biomass of intercrops.

2.4.2. Cover cropping

A split-plot ANOVA was performed to test the effects of cropping system and wheat variety on the response variables in wheat, i.e. grain yield, incidence of FHB causing species in grains and mycotoxins in grain. The treatment structure (fixed effects) was defined as 'cropping system \times wheat variety' and the block structure (random effects) as 'block / whole plot / subplot'.

3. Results

3.1. Maize-intercropping

3.1.1. Maize yield and intercrop biomass

In both experimental years, there was no significant difference in maize yield between the tested maize-intercropping systems and the sole maize (Table 1). The year effect on the aboveground dry biomass (t ha⁻¹) of intercrops was significant for phacelia (2016: 0.74; 2018: 0.21; p = 0.001) and white mustard (2016: 1.14; 2018: 0.71; p = 0.009).

3.1.2. Mycotoxins and Fusarium head blight causing species incidence in wheat grains

DON, 3- and 15-Ac-DON were the only mycotoxins detected throughout both experimental years. Overall, the average DON content and incidence of *F. graminearum* in grains in 2019 were 11- and 3-fold higher, respectively, compared with 2017 (Table S5). In contrast, the incidences of *F. avenaceum* and *F. poae* were higher in 2017 than in 2019 (Table S5). Ac-DON was detected only in 2019 whereas ZEN was detected only in 2017 (Table S5).

For the harvest 2017, the average DON and ZEN contents across the entire field were 450 and 180 µg kg⁻¹, respectively (Table S5), while the values of all other measured mycotoxins were below detection or quantification limits (Table S4). The treatments with maize-white mustard and maize-Indian mustard decreased DON by 58% and 32%, respectively, compared with sole maize (590 µg kg⁻¹; $p \le 0.05$; Fig. 2 a). The treatments with maize-white mustard and maize-white mustard and maize-phacelia decreased ZEN content by 47% and 34%, respectively, compared with sole maize (240 µg kg⁻¹; $p \le 0.05$; Fig. 2 b). No significant differences were observed between no-tillage and reduced tillage on DON (Fig. S1 a) and ZEN (Fig. S2) contents. The average incidence of FHB causing species in wheat grains across the entire field was 20% with the most dominant species in FHB-infected grains being *F. graminearum* (82%) followed by *F. poae* (7%) and *F. avenaceum* (5%) (Fig. S3 a).

For the harvest 2019, the average contents of DON and Ac-DON in grain across the entire field were 5'040 and 150 µg kg⁻¹, respectively (Table S5), while the values of all other measured mycotoxins were below the quantification or detection limits (Table S4). The lowest DON contents were observed after maize-Indian mustard and maize-clover, the highest after maize-phacelia and maize-sudangrass, while maize white mustard and sole maize led to intermediate values ($p \le 0.05$; Fig. 2 c). There was a three-way interaction among tillage, maize-intercropping and wheat variety on Ac-DON (p = 0.019; Table S6). Within Levis, no significant differences were observed among the tested maize-intercropping systems regardless of the tillage treatment. In contrast, within Forel, use of maize-clover intercropping and sole maize resulted in the highest and lowest Ac-DON contents (170 and 50 µg kg⁻¹, respectively) under no-tillage ($p \le 0.05$), whereas no differences were

Table 1

Maize-intercropping. Effect of maize-(inter)cropping system (SM: sole maize; C: clover; S: sudangrass; PH: phacelia; WM: white mustard; IM: Indian mustard) on *maize grain yield* (t ha⁻¹) for each experimental year (maize harvests 2016 and 2018). No significant differences were observed between maize-intercropping systems and sole maize (p > 0.05); \pm represent the standard error of the mean (n = 8).

-							
		SM	С	S	PH	WM	IM
	2016	12.0 \pm	11.7 \pm	10.5 \pm	$10.9 \ \pm$	10.8 \pm	11.0 \pm
		0.3	0.4	0.7	0.5	0.8	0.5
	2018	10.0 \pm	$\textbf{9.3}\pm\textbf{0.8}$	$\textbf{9.7}\pm\textbf{0.3}$	$\textbf{9.2}\pm\textbf{0.4}$	$\textbf{9.6} \pm \textbf{0.2}$	$\textbf{9.2}\pm\textbf{0.3}$
		0.2					



Fig. 2. Maize-intercropping. Effect of maize-(inter)cropping system (SM: sole maize; C: clover; S: sudangrass; PH: phacelia; WM: white mustard; IM: Indian mustard) on deoxynivalenol (DON) (a) and zearalenone (ZEN) (b) content in wheat grain in harvest 2017 and on DON content in wheat grain in harvest 2019 (c). ZEN was detected only in 2017. Average values of two tillage practices (no-tillage, reduced tillage) and two wheat varieties (Levis, Forel) are presented. Different letters indicate significant differences according to Fisher's protected LSD test for post hoc comparisons ($\alpha = 0.05$) and bars represent the standard error of the mean (n = 16).

Table 2

Maize-intercropping. Effect of maize-(inter)cropping system (SM: sole maize; C: clover; S: sudangrass; PH: phacelia; WM: white mustard; IM: Indian mustard) on *wheat grain yield* (t ha⁻¹) within wheat variety (Levis, Forel) and tillage practice (NT: no-tillage; RT: reduced tillage) in harvest 2017. Different letters indicate significant differences according to Fisher's protected LSD test for post hoc comparisons ($\alpha = 0.05$) and \pm represent the standard error of the mean (n = 4).

Wheat	Tillage	Maize-(inter)cropping						
variety		SM	С	S	PH	WM	IM	
Levis	NT	6.42 ± 0.3 ab	5.52 ± 0.6 b	6.92 ± 0.2 a	5.92 ± 0.2 ab	5.49 ± 0.2 b	5.43 ± 0.6 b	
	RT	$\begin{array}{c} 7.10 \\ \pm \ 0.2 \end{array}$	6.56 ± 0.5	6.31 ± 0.4	$\begin{array}{c} 7.22 \\ \pm \ 0.2 \end{array}$	6.94 ± 0.5	6.81 ± 0.3	
Forel	NT	6.47 ± 0.4	5.25 ± 0.3	6.51 ± 0.3	6.26 ± 0.3	5.60 ± 0.6	6.19 ± 0.5	
	RT	5.97 ± 0.4	± 0.4	6.28 ± 0.4	± 0.4	6.20 ± 0.5	6.06 ± 0.4	

observed under reduced tillage. No differences were observed between no-tillage and reduced tillage on DON content (Fig. S1 b). The average incidence of FHB causing species in wheat grains across the entire field was 49% and *F. graminearum* was even more dominant in FHB-infected grains (93%) than it was in 2017 (Fig. S3 b).

3.1.3. Wheat yield

For the harvest 2017, a three-way interaction was observed among tillage, maize-intercropping and wheat variety on wheat yield (p = 0.027; Table S6). For Levis, maize-sudangrass resulted in the highest

yield (6.92 t ha⁻¹) compared with the other intercropping systems under no-tillage ($p \le 0.05$), whereas the effect of maize-intercropping on yield was not significant under reduced tillage (Table 2). For Forel, the effect of maize-intercropping on yield was not significant for any tillage regime (Table 2).

For the harvest 2019, maize-intercropping with clover, phacelia and Indian mustard resulted in slightly lower yield for the subsequent wheat crop compared with the sole maize (6.75 t ha^{-1}), whereas intercropping with white mustard or sudangrass did not significantly affect wheat yield (Table 3).

3.2. Cover cropping

3.2.1. Mycotoxins and Fusarium head blight causing species incidence in wheat grains

Overall, the average contents of DON, Ac-DON and NIV in wheat grain were 37-, 18- and 2-fold higher in 2018, respectively, compared with 2017 (Table S7). Also, the average incidence of *F. graminearum* and *F. avenaceum* in grains were 7- and 5-fold higher in 2018, respectively, compared with 2017 (Table S7). Contrarily, the average incidence of *F. poae* was 2-fold higher in 2017 compared with 2018 (Table S7).

For the harvest 2017, the average contents of DON, Ac-DON and NIV in grain across the entire field were very low (i.e. 70, 10 and 40 μ g kg⁻¹, respectively; Table S7), and thus the tested cropping systems did not show any significant effect. The values of the other measured mycotoxins were below the quantification or detection limits (Table S4). The average incidence of FHB causing species in wheat grains across the entire field was 8% with the most dominant species in FHB-infected grains being *F. poae* (41%) followed by *F. graminearum* and *F. avenaceum* (both 23%) (Fig. S3 c). The use of winter pea as a cover crop resulted in higher incidence of *F. avenaceum* (7%) compared with the other cropping systems (0–1%; $p \le 0.05$).

For the harvest 2018, a significant interaction between cropping system and wheat variety was observed for DON (p = 0.031; Table S8). For the variety Digana, the use of plough, white mustard, Indian mustard and winter pea decreased DON compared with the herbicide treatment by 69%, 44%, 50% and 85%, respectively (p < 0.05; Fig. 3 a). For the variety Fiorina, the use of plough or cover crops (white mustard, Indian mustard or winter pea) decreased DON by 58 to 82% compared with the herbicide treatment ($p \le 0.05$; Fig. 3 b). The plough and cover crop treatments decreased Ac-DON by 69 to 86% compared with the herbicide treatment ($p \le 0.05$; Fig. S4). The average contents of DON, Ac-DON and NIV in grain across the entire field were 2'580, 180 and 70 μ g kg⁻¹, respectively (Table S7), while the values of the other measured mycotoxins were below the quantification or detection limits (Table S4). The average incidence of FHB causing species in wheat grains across the entire field was 28% with the most dominant species in FHB-infected grains being F. graminearum (46%) followed by F. avenaceum (37%) and F. poae (7%) (Fig. S3 d). The highest incidence of F. avenaceum was observed with the use of winter pea (20%) and the lowest after the plough treatment (4%), while intermediate incidences were found after the herbicide, white mustard and Indian mustard treatments (7%, 10% and 11%, respectively; p < 0.05).

Table 3

Maize-intercropping. Effect of maize-(inter)cropping system (SM: sole maize; C: clover; S: sudangrass; PH: phacelia; WM: white mustard; IM: Indian mustard) on *wheat grain yield* (t ha⁻¹) in harvest 2019. Average values of two tillage practices (no-tillage, reduced tillage) and two wheat varieties (Levis, Forel) are presented. Different letters indicate significant differences according to Fisher's protected LSD test for post hoc comparisons ($\alpha = 0.05$) and \pm represent the standard error of the mean (n = 16).

SM	С	S	PH	WM	IM
$\begin{array}{c} \text{6.75} \pm 0.1 \\ \text{ab} \end{array}$	$\begin{array}{c} 6.06 \pm 0.2 \\ d \end{array}$	$\begin{array}{c} \textbf{6.92} \pm \\ \textbf{0.2 a} \end{array}$	$\begin{array}{c} \textbf{6.18} \pm \textbf{0.2} \\ \textbf{cd} \end{array}$	$\begin{array}{c} 6.47 \pm 0.2 \\ bc \end{array}$	$\begin{array}{c} \textbf{6.17} \pm \textbf{0.1} \\ \textbf{cd} \end{array}$



Fig. 3. Cover cropping. Effect of cropping system (HWCC: herbicide without cover crop; PWCC: plough without cover crop; WM: white mustard; IM: Indian mustard; WP: winter pea) on deoxynivalenol (DON) content in wheat grain of var. Digana (a) and var. Fiorina (b) in harvest 2018. Different letters indicate significant differences according to Fisher's protected LSD test for post hoc comparisons ($\alpha = 0.05$) and bars represent the standard error of the mean (n = 4).

3.2.2. Wheat yield

In 2017, yield was similar among cropping systems, whereas in 2018, the use of cover crops (white mustard, Indian mustard or winter pea) improved yield by 7 to 25% ($p \le 0.05$) compared with the herbicide or plough treatments (Table 4).

3.3. Gross margin analysis

3.3.1. Maize-intercropping

The use of sole maize generated the highest gross margin for maize (CHF 1'934 ha⁻¹), which was 19 to 31% higher than with the intercropping systems (Table 5). The differences on the gross margin of maize between sole maize and intercropping systems are explained by treatment-specific operating costs and maize yield variations. For example, the operating costs for the sowing of the intercrops ranged from CHF 167 to 398 ha⁻¹ depending on the crop species, whereas the costs for the herbicide treatment of the sole maize was CHF 136 ha⁻¹.

For wheat under no-tillage, the maize-sudangrass intercropping yielded the highest gross margin (CHF 1'651 ha⁻¹) (Table 5). In contrast, under reduced tillage, sole maize resulted in 1 to 11% higher gross margin for wheat compared with the intercropping systems, which ranged from CHF 1'392 to 1'550 ha⁻¹. Compared with no-tillage, the use of reduced tillage resulted in additional operating costs (CHF 160 ha⁻¹). However, due to higher wheat yields, reduced tillage generated 2 to 28% higher wheat gross margins compared with no-tillage, except for maize-sudangrass where the opposite occurred.

For the entire maize-wheat rotation under no-tillage, the highest gross margin was generated by sole maize (CHF 3'471 ha⁻¹), with the maize-intercropping systems ranging from CHF 2'609 to 2'987 ha⁻¹ (Table 5). Similarly, under reduced tillage, the highest gross margin was also generated by sole maize (CHF 3'505 ha⁻¹), with the maize-intercropping systems ranging from CHF 2'886 to 3083 ha⁻¹.

3.3.2. Cover cropping

Overall, the gross margin of the entire rotation for the cover cropping was substantially lower compared with the maize-intercropping (Tables 5 and 6). For the cover cropping, the highest gross margin over the entire rotation was generated by the herbicide treatment without growing a cover crop (HWCC; CHF 1'263 ha⁻¹) followed by maize-white mustard-wheat, maize-winter pea-wheat, maize-Indian mustard-wheat and maize-plough without a cover crop-wheat (CHF 1'035 ha⁻¹) (Table 6). The differences between the gross margins are explained by the treatment-specific operating costs which were higher for the cover crops and plough treatments compared with the HWCC treatment. The

Table 4

Cover cropping. Effect of cropping system (HWCC: herbicide without cover crop; PWCC: plough without cover crop; WM: white mustard; IM: Indian mustard; WP: winter pea) on *wheat grain yield* (t ha⁻¹) for each experimental year (harvests 2017 and 2018). Average values of two wheat varieties (Digana, Fiorina) are presented. In harvest 2017, no significant effect of cropping system was found (p = 0.395). Different letters indicate significant differences according to Fisher's protected LSD test for post hoc comparisons ($\alpha = 0.05$) and \pm represent the standard error of the mean (n = 8).

	HWCC	PWCC	WM	IM	WP
2017 2018	$\begin{array}{c} 4.82\pm0.3\\ 3.99\pm0.1\\ c\end{array}$	$\begin{array}{l} 4.89\pm0.2\\ 4.18\pm0.1\\ bc\end{array}$	$\begin{array}{l} 4.63\pm0.4\\ 4.46\pm0.2\\ abc\end{array}$	$\begin{array}{l} 4.20\pm0.2\\ 4.69\pm0.1\\ ab\end{array}$	$\begin{array}{l} 4.76\pm0.3\\ 4.98\pm0.2\\ a\end{array}$

cost for the sowing of the cover crops ranged from CHF 185 to 416 ha⁻¹, while the plough treatment cost CHF 425 ha⁻¹. Nevertheless, cultivation of winter pea after maize yielded the highest receipts for the wheat crop (CHF 2'533 ha⁻¹). The lowest receipts were observed for HWCC (CHF 2'293 ha⁻¹).

4. Discussion

The effective control of FHB in cereal-based rotations is an important component of agronomic and economic sustainability worldwide. To the best of our knowledge, this is the first study demonstrating the potential of intercropping with maize to control FHB in a subsequent cereal crop. Compared with maize grown as a sole crop, the intercropping of white mustard with maize substantially decreased DON and ZEN contents in the following winter wheat crop. The mechanisms by which intercrops can affect 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 involves the production of antifungal metabolites based on the release of glucosinolate-derived substances (Brown and Morra, 1997; Drakopoulos et al., 2020; Manici et al., 1997). Nevertheless, a significant reduction of mycotoxins through maize-mustard intercropping was only

Table 5

Maize-intercropping. Effect of maize-(inter)cropping system (SM: sole maize; C: clover; S: sudangrass; PH: phacelia; WM: white mustard; IM: Indian mustard) under no-tillage or reduced tillage on *receipts, operating costs* and *gross margin* of grain maize, wheat grain and the entire crop rotation (maize-wheat) in Swiss Francs ha⁻¹. Average values of two experimental years (2016–2017, 2018–2019) and two winter wheat varieties (Levis, Forel) are presented.

	SM	С	S	PH	WM	IM
No-tillage						
Receipts						
Grain maize	4'011	3'832	3'676	3'656	3'714	3'674
Wheat grain	3'179	2'716	3'293	2'891	2'894	2'890
Operating costs						
Grain maize	2'077	2'297	2'339	2'108	2'147	2'125
Wheat grain	1'642	1'642	1'642	1'642	1'642	1'642
Gross margin						
Grain maize	1'934	1'535	1'336	1'548	1'567	1'549
Wheat grain	1'537	1'074	1'651	1'249	1'252	1'248
Entire rotation	3'471	2'609	2'987	2'797	2'818	2'797
Reduced tillage						
Receipts						
Grain maize	4'011	3'832	3'676	3'656	3'714	3'674
Wheat grain	3'373	3'303	3'351	3'337	3'307	3'194
Operating costs						
Grain maize	2'077	2'297	2'339	2'108	2'147	2'125
Wheat grain	1'802	1'802	1'802	1'802	1'802	1'802
Gross margin						
Grain maize	1'934	1'535	1'336	1'548	1'567	1'549
Wheat grain	1'571	1'501	1'550	1'535	1'505	1'392
Entire rotation	3'505	3'036	2'886	3'083	3'072	2'942

Table 6

Cover cropping. Effect of cropping system (HWCC: herbicide without cover crop; PWCC: plough without cover crop; WM: white mustard; IM: Indian mustard; WP: winter pea) on *receipts, operating costs* and *gross margin* of silage maize, wheat grain and the entire crop rotation (maize-treatment-wheat) in Swiss Francs ha⁻¹. The treatment-specific operating costs refer to costs derived from the use of cover crops, herbicide or plough applications. Average values of two experimental years (2016–2017, 2017–2018) and two spring wheat varieties (Digana, Fiorina) are presented.

	HWCC	PWCC	WM	IM	WP
D					
Receipts					
Silage maize	2'460	2'460	2'460	2'460	2'460
Wheat grain	2'293	2'359	2'363	2'312	2'533
Operating costs					
Silage maize	1'659	1'659	1'659	1'659	1'659
Treatment	132	425	296	332	526
Wheat grain	1'699	1'699	1'699	1'699	1'699
Gross margin					
Silage maize	801	801	801	801	801
Wheat grain	594	660	664	613	834
Entire rotation	1'263	1'035	1'170	1'082	1'109

observed under moderate disease pressure (harvest 2017) and not under a severe epidemic (harvest 2019). Moreover, in the maize crop of 2018, the aboveground biomass of white mustard was considerably lower compared with that of 2016. Therefore, a good establishment of the intercrop appears to be crucial to effectively reduce mycotoxins in subsequent wheat.

One of the most effective control strategies to manage FHB and reduce mycotoxins is the adoption of a suitable crop rotation (Champeil et al., 2004; Shah et al., 2018). Here, we demonstrated that growing interval cover crops, i.e. white mustard. Indian mustard and winter pea. after silage maize reduced DON and Ac-DON in spring wheat under no tillage compared with wheat from plots without a cover crop. Remarkably, the decrease in mycotoxins through cover crops was comparable to the plough treatment, by which the maize residues were buried into the soil. Besides the mycotoxin reduction, growers could also benefit from the implementation of reduced tillage practices by reducing production costs, improving soil quality and preventing soil erosion (Zikeli and Gruber, 2017). Leplat et al. (2013) discussed the importance of choosing the preceding crop in the development of FHB in wheat and suggested assessing the role of less frequently studied cover crops, such as Indian mustard. Furthermore, Li et al. (2019) demonstrated that diversified crop rotations improved the resilience to biotic stresses while ensuring consistent crop yields. Following our approach, the inclusion of an intercrop or an interval cover crop in a maize-wheat rotation promotes the diversification of agroecosystems and does reduce FHB and mycotoxins. Nevertheless, legume cover crops have been shown to serve as alternative hosts for Fusarium species (Šišić et al., 2018; Walder et al., 2017); indeed, we found that winter pea increased the incidence of F. avenaceum. Thus, agricultural practices targeting particular Fusarium species might create ecological niches that could be filled by other species within the FHB complex (Vogelgsang et al., 2019; Xu and 2009). In our maize-intercropping experiments, Nicholson, F. graminearum was by far the most dominant FHB species in wheat, whereas in the cover cropping experiments, a higher species diversity was observed. This phenomenon could alter the range of accumulated fungal metabolites in grain, frequently referred to as emerging mycotoxins, which merits further research (Gruber-Dorninger et al., 2016).

With respect to implementation of this practice, it is essential that none of our tested intercropping systems significantly reduced the maize grain yield and only marginally reduced the wheat yield in 2019 with three out of the five intercrop species. Hence, we suggest that intercropping is most often not leading to a decrease in grain production. Even more encouraging is that the cover cropping is also not negatively affecting the yield of the following spring wheat and if anything, it can lead to an increase in yield as shown in this study. Hence, these

innovative cropping systems have the potential to improve food safety while ensuring food production. However, the profitability of these systems is a crucial factor influencing growers' decisions to adopt alternative cropping systems. Although the inclusion of intercrops or cover crops brings benefits to the agroecosystem, as we show here and has been shown also for reduction of soil erosion, provision of nitrogen and increased soil organic matter, it can also lead to additional costs (Snapp et al., 2005). We did find an economic trade-off associated with maize-intercropping and cover cropping systems. The main reasons for the reduced gross margins of these innovative cropping systems were the increased operating costs, in particular the sowing of intercrops or cover crops (see Tables 1 and 2). Nevertheless, the differences in the gross margin between the cropping systems 'silage maize-herbicide without cover crops-spring wheat' and 'silage maize-cover crops-spring wheat' were rather small due to higher wheat yields with the use of cover crops, indicating the real potential for cover cropping as a sustainable means to control FHB. Overall, it is important to note that the gross margin of the entire rotation for cover cropping was substantially lower compared with maize-intercropping. The main reasons for the latter are the lower vields of spring wheat than winter wheat as well as the lower revenue from silage maize compared with grain maize.

In the intercropping experiments, we found similar DON (detected in both years) and ZEN (detected in 2017) contents in wheat grain between no-tillage and reduced tillage. Hence, our findings suggest that growers could apply no-tillage (i.e. mulching) over reduced tillage (i.e. mulching plus rotary tiller) without increasing the probability for elevated mycotoxin contamination. However, compared with no-tillage, reduced tillage resulted in most cases in higher wheat yields and therefore higher receipts, outweighing the associated increased operating costs. Pittelkow et al. (2015) demonstrated that no-tillage can frequently reduce crop yields compared with conventional tillage, but its negative impact can be minimised when combined with residue retention and appropriate crop rotation. In fact, we observed equal or higher wheat yields for the cover cropping systems compared with the plough treatment without growing a cover crop under no-tillage.

5. Conclusions

We have demonstrated that mustard as an intercrop with maize as well as winter pea and mustard as interval cover crops in a maize-wheat rotation decreased mycotoxins in wheat. However, in years with severe FHB disease pressure (maize-intercropping in 2018-2019 and cover cropping in 2017–2018), significant mycotoxin reductions were observed only with growing interval cover crops and not with intercropping. Thus, even greater crop diversification efforts are necessary to effectively manage mycotoxins in cereal-based rotations. Certainly, growers will face some challenges in adopting these innovative cropping systems because they will need to carefully weigh potential trade-offs between farm profitability and food safety goals. Hence, only adjusted agricultural policies could support growers in the use of intercropping and cover cropping systems in maize-wheat rotations under reduced tillage regimes. Ultimately, these policies could serve as a bridge towards augmented diversification of cropping systems with the overall goal of improving food safety while ensuring the economic viability of cereal production systems.

Data availability statement

All raw data are available from the authors on request.

Author contributions

SV, DD and AK conceived and designed the experiments. DD performed and coordinated the experiments and wrote the first draft of the manuscript. SV, JS, HRF and TDB provided inputs with respect to the interpretation of the results and manuscript preparation. AZ and AK provided inputs with respect to the economic aspect of the study. FEW and TDB provided inputs with respect to the mycotoxin analysis.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgements

The authors warmly thank Irene Bänziger for her excellent technical assistance. We also thank the students Pascal Weber, Anja Marty and Sofie Bolt for their help in the field and the laboratory. This work was supported by the MycoKey project "Integrated and innovative key actions for mycotoxin management in the food and feed chain", funded by the Horizon 2020 Research and Innovation Programme (grant agreement No. 678781) and the Swiss State Secretariat for Education, Research and Innovation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.agsy.2021.103198.

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