Supplementary Material

Efficacy of Different Fungicide Spraying Techniques on the Infestation with *Kabatiella zeae* and Formation of *Fusarium* Mycotoxins in Forage Maize

Tim Birr, Andreas Tillessen, Joseph-Alexander Verreet, Mario Hasler and Holger Klink

Table S1. Dry matter yield (± *SD*; dt/ha), DON and ZEN concentrations (± *SD*; µg/kg) in forage maize samples at silage maturity of the cultivar "SY Werena" in the two fungicide-untreated controls without (T1) and with (T2) silk channel inoculation of main ears with macroconidia of *Fusarium culmorum* seven days after silk emergence at BCCH 65 at the three trial locations Barkhorn, Hemdingen, and Hohenschulen.

Treatment	Barkhorn			Hemdingen			Hohenschulen		
	Yield (dt/ha)	DON (µg/kg)	ZEN (µg/kg)	Yield (dt/ha)	DON (µg/kg)	ZEN (µg/kg)	Yield (dt/ha)	DON (µg/kg)	ZEN (µg/kg)
T1 – Non-inoculated	180.1 ± 9.1	2598 ± 776	498 ± 252	189.8 ± 8.4	4453 ± 2204	585 ± 192	131.1 ± 12.1	3834 ± 2640	478 ± 198
T2 – Inoculated	176.4 ± 8.5	28,355 ± 4233	1966 ± 438	190.2 ± 6.5	14,992 ± 2368	2260 ± 791	136.8 ± 6.7	25,493 ± 4237	2254 ± 874

DON = deoxynivalenol; ZEN = zearalenone.

Table S2. Disease severities of *Kabatiella zeae* (± *SD*; percentage of leaf area affected by symptoms) on the upper, middle (L-2 to L+2) and lower leaf segment of forage maize at BBCH 65, BBCH 75 and BBCH 85 of the cultivar "SY Werena" depending on different fungicide spraying techniques (treatments T2 – T5) at the three trial locations Barkhorn, Hemdingen, and Hohenschulen. Treatments: T2 – Fungicide-untreated control, T3 - Overhead spraying technique, T4 - Dropleg spraying technique, T5 - Combination of T3 and T4. The fungicide Prosaro® was applied (T3 – T5) with 1.0 L/ha and a spray volume of 400 L/ha at BBCH 65.

Treatment	Leaf segment	Disease severity *K. zeae* (%)								
		Barkhorn			Hemdingen			Hohenschulen		
		BBCH 65	BBCH 75	BBCH 85	BBCH 65	BBCH 75	BBCH 85	BBCH 65	BBCH 75	BBCH 85
T2 – Untreated control	Upper	1.0 ± 0	2.6 ± 0.1	13.6 ± 0.8	1.0 ± 0.1	2.6 ± 0.2	7.6 ± 0.9	1.6 ± 0.6	10.1 ± 4.1	25.6 ± 6.0
	Middle	1.1 ± 0.1	1.9 ± 0.2	4.7 ± 0.2	1.9 ± 0.3	3.7 ± 0.8	4.6 ± 0.6	2.7 ± 1.5	6.1 ± 3.2	15.6 ± 1.9
	Lower	1.0 ± 0	1.8 ± 0.2	3.4 ± 0.8	2.0 ± 0.2	3.4 ± 0.5	3.5 ± 0.5	1.7 ± 0.5	2.8 ± 0.6	4.5 ± 1.3
T3 – Overhead spraying technique	Upper	1.0 ± 0	1.4 ± 0.3	7.0 ± 1.5	1.0 ± 0	2.1 ± 0.1	4.5 ± 0.4	1.2 ± 0.3	3.0 ± 1.7	9.9 ± 3.5
	Middle	1.0 ± 0.1	1.6 ± 0.2	2.6 ± 0.3	1.6 ± 0.3	2.8 ± 0.3	3.0 ± 0.3	1.7 ± 0.9	3.0 ± 1.8	6.7 ± 4.1
	Lower	1.0 ± 0	1.5 ± 0.2	2.4 ± 0.1	1.8 ± 0.1	2.7 ± 0.2	2.8 ± 0.3	1.2 ± 0.3	2.2 ± 0.9	2.9 ± 0.9
T4 – Dropleg spraying technique	Upper	1.0 ± 0.1	2.3 ± 0.3	14.2 ± 1.5	1.0 ± 0	2.4 ± 0.1	6.9 ± 0.2	1.3 ± 0.4	6.5 ± 2.4	13.9 ± 3.3
	Middle	1.0 ± 0	1.6 ± 0.1	4.1 ± 0.6	1.5 ± 0.2	2.8 ± 0.3	3.1 ± 0.3	1.7 ± 0.5	2.9 ± 0.8	4.9 ± 1.8
	Lower	1.0 ± 0	1.4 ± 0.2	2.5 ± 0.5	1.8 ± 0.2	2.5 ± 0.3	2.6 ± 0.3	1.2 ± 0.2	2.1 ± 0.7	2.9 ± 0.9
T5 – Combination of T3 and T4	Upper	0.9 ± 0.1	1.3 ± 0.1	9.9 ± 1.3	1.0 ± 0	2.1 ± 0.1	4.0 ± 0.2	0.9 ± 0.2	2.5 ± 0.9	6.7 ± 1.7
	Middle	1.0 ± 0	1.4 ± 0.1	2.7 ± 0.1	1.5 ± 0.2	2.5 ± 0.2	2.6 ± 0.2	1.3 ± 0.3	1.8 ± 0.7	4.6 ± 1.0
	Lower	1.0 ± 0	1.0 ± 0.1	2.0 ± 0.1	1.5 ± 0.3	2.2 ± 0.2	2.3 ± 0.2	1.2 ± 0.2	1.6 ± 0.3	2.1 ± 0.4

Table S3. Dry matter yield (± *SD*; dt/ha) of forage maize of the cultivar "SY Werena" depending on different fungicide spraying techniques (treatments T2 – T5) at the three trial locations Barkhorn, Hemdingen, and Hohenschulen. Treatments: T2 – Fungicide-untreated control, T3 - Overhead spraying technique, T4 - Dropleg spraying technique, T5 - Combination of T3 and T4. The fungicide Prosaro® was applied (T3 – T5) with 1.0 L/ha and a spray volume of 400 L/ha at BBCH 65.

Treatment	Dry matter yield (dt/ha)		
	Barkhorn	Hemdingen	Hohenschulen
T2 – Untreated control	176.4 ± 8.5	190.2 ± 6.5	136.8 ± 6.7
T3 – Overhead spraying technique	194.3 ± 9.0	200.7 ± 9.1	159.1 ± 11.7
T4 – Dropleg spraying technique	192.5 ± 3.8	198.0 ± 4.5	151.9 ± 5.7
T5 – Combination of T3 and T4	203.2 ± 7.7	205.3 ± 9.9	164.9 ± 9.3

Table S4. Concentrations of DON and ZEN (± *SD*; µg/kg) in forage maize samples at silage maturity of the cultivar "SY Werena" depending on different fungicide spraying techniques (treatments T1 – T5) at the three trial locations Barkhorn, Hemdingen, and Hohenschulen. Treatments: T1 – Fungicide-untreated control, T2 – Fungicide-untreated control, T3 - Overhead spraying technique, T4 - Dropleg spraying technique, T5 - Combination of T3 and T4. Treatments T2 to T5 were inoculated with macroconidia of *Fusarium culmorum* seven days after silk emergence by silk channel inoculation of forage maize main ears. Treatment T1 was not inoculated with *Fusarium* spores in order to measure the success of the silk channel inoculation by comparing DON and ZEN concentrations between the two fungicide untreated controls T1 and T2. The fungicide Prosaro® was applied (T3 – T5) with 1.0 L/ha and a spray volume of 400 L/ha two days after inoculation at BBCH 65.

Treatment	Mycotoxin	Mycotoxin concentration (µg/kg)		
		Barkhorn	Hemdingen	Hohenschulen
T1 - Untreated control [1]	DON	2598 ± 776	4453 ± 2204	3834 ± 2640
	ZEN	498 ± 252	585 ± 192	478 ± 198
T2 – Untreated control [2]	DON	28,355 ± 4233	14,992 ± 2368	25,493 ± 4237
	ZEN	1966 ± 438	2260 ± 791	2254 ± 874
T3 – Overhead spraying technique [2]	DON	12,354 ± 2062	4716 ± 1891	14,557 ± 2400
	ZEN	991 ± 118	649 ± 178	1424 ± 632
T4 – Dropleg spraying technique [2]	DON	7479 ± 1602	1781 ± 763	3699 ± 2253
	ZEN	657 ± 306	296 ± 78	592 ± 254
T5 – Combination of T3 and T4 [2]	DON	8102 ± 1883	2233 ± 871	4727 ± 2468
	ZEN	556 ± 237	261 ± 102	511 ± 371

[1] Without *Fusarium* inoculation. [2] With *Fusarium* inoculation seven days after silk emergence. DON = deoxynivalenol; ZEN = zearalenone.