https://doi.org/10.26651/2017-42-1-1109 0971-4693/94 Euro 20.00
Allelopathy Journal 42 (1): 109-122 (September, 2017) *International Allelopathy Foundation* 2017
Table: 5, Figs : 0

Allelopathic effects of barley and oats on their rhizoplane and rhizosphere *Fusarium* spp.

J. CZABAN*, B. WRÓBLEWSKA and D. LESZCZYŃSKA[1]
Department of Agricultural Microbiology
Institute of Soil Science and Plant Cultivation, State Research Institute,
Puławy-24 100, Poland
E. Mail: janusz. czaban@iung. pulawy. pl

(Received in revised form: July 05, 2017)

ABSTRACT

This study aimed to determine the differences between the colonization of barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.) rhizoplane and rhizosphere by various *Fusarium* species. Two cultivars each of barley and oats were cultivated in 2013 in pure stands and in barley/oats mixture in an organic field experiment. The roots of these plants were freed from adhering soil, then divided into three parts: crowns, upper crown roots and lower crown roots. They were cut into approx 1 cm fragments and were used to isolate the Fusaria using CZID agar medium. Oats, in comparison with barley contained significantly higher percentage of *F. culmorum* and *F. avenaceum* and significantly lower percentage of *F. equiseti* and *F. graminearum* in its rhizoplane. On the other hand, significantly lower CFU numbers of both total fungi and *Fusarium* were found in the rhizosphere soils of oats than in barley.

Key words: Allelopathy, barley/oat mixture, barley roots, cultivars, *Fusarium* sp, oat roots, rhizoplane, rhizosphere

INTRODUCTION

The structure of microbial population in the plant root zone is a key determinant of plant health and productivity (5,17,40). The genus *Fusarium* is one of the fungal constituents of the rhizosphere and rhizoplane of many plants, including cereals. Members of this genus can be recovered from plants as saprophytes, which have important ecological roles in the cycling of nutrients**.** This genus contains many species of agricultural importance because of their ability to parasitise plants by root infection (1,4-7,19,22,35,38). The saprotrophic Fusarias are the first fungi colonising the rhizoplane (22). Thus understanding the ecology of *Fusarium* communities in root zone is highly desirable.

Chemical compositions of plant roots and root exudates vary between plant species and cultivars and this is important in determining the structure of the rhizosphere and rhizoplane bacterial and fungal communities (1,4,5,17,19). Oats produces the antimicrobial substances such as triterpene saponins (avenacins) in roots (30). These compounds when released, are presumed to modify the fungal communities on oat roots. Carter *et al.* (3) found that nearly all fungi from oat roots were resistant to the major saponin, triterpenoid avenacin A-1. On the other hand both avenacin-sensitive and avenacin-resistant fungi were isolated from wheat roots - a non-saponin-producing cereal. It is suggested that avenacin A-1 is present as protective barrier to the infection of oat by saponin-sensitive fungi (3). Besides triterpenoid saponins, oat roots also produce steroidal saponins-avenacosides A and B in grains and leaves (31,32).

*Corresponding author

In Poland, oats is the main component of spring cereal mixtures (used as animal feed) especially in organic agriculture. Sowing oats together with barley increases their competitiveness against weeds and reduces the susceptibility to pathogens. It also stabilizes the yield under varying conditions and increases the nutritive value of feeds prepared from these cereals (20,35,39).

Oat roots, besides saponins, also produce scopoletin (2,16), a coumarin which affects the fungi. Ojala *et al.* (29) reported that scopoletin strongly inhibited the growth of *F. culmorum*. On the other hand, barley synthetizes phenethylamine alkaloid - hordenine (2,27) in its roots, which may play a defensive role against some fungal pathogens. Lovett and Hoult (26) reported that hordenine inhibited the growth of fungal pathogen *Drechslera teres* of barley. Moreover, roots of both barley and oats release other phenolics (18,24). Lanoue *et al.* (24) reported that *F. graminearum* attack on the root system of barley induced the root exudation of *t*-cinnamic acid and four phenolic acids (p-coumaric, ferulic, syringic and vanillic). All of these chemicals inhibited the germination of *F. graminearum* macroconidia on an agar nutrient medium, either as singly or in mixture.

This study aimed to understand the differences between the oats and barley, in colonization of their rhizoplane and rhizosphere by various *Fusarium* species.

MATERIALS AND METHODS

I. Field experiment

Two varieties each of barley (hulled barley 'Skarb', naked barley 'Gawrosz') and oats (hulled oats 'Krezus' and naked oats 'Nagus') were grown in 2013 in an organic field experiment in Chwałowice [51°10′56″N, 21°18′17″E, Mean annual precipitation: 590 mm, 184 m above sea level], Mazowia province, Poland. The field soil was silt loam Eutric Cambisol with pH 5.5 (measured with glass electrode in slurry of 10 g soil and 25 cm^3 of 1M KCl - exchangeable acidity) and 1. 7% organic carbon [determined by the modified Tiurin method according to Czaban *et al.* (9)].

These four cereal cultivars were grown in a pure stand and in 3 barley+oat mixtures ('Skarb' + 'Nagus', 'Skarb' + 'Krezus' and 'Gawrosz' + 'Krezus') on 12 m^2 (4 x 3 m) plots with three replicates. The mixture of 'Gawrosz' and 'Nagus' was excluded because of the low grain yield of a mixture of naked barley and naked oats, hence, not grown by farmers. The previous pure crop was tancy phacelia (*Phacelia tanacetifolia* Benth.). The soil was fertilized with compost (60 t ha^{-1}). Seed rates for barley and oats were 350 and 550 kernels per m^2, respectively, which correspond to: 160, 152, 186 and 175 Kg/ha for 'Skarb', 'Gawrosz', 'Krezus' and 'Nagus', respectively. For the barley/oat mixtures, half of these amounts were used.

II. Fungal analyses

In July 2013 at the heading stage (90 days after sowing), several undisturbed soil blocks with the growing plants were removed with a spade from each field and transferred to the laboratory. The plant roots were then carefully separated from the soil by gentle shaking and leaving the soil layer adhered to the roots. The crowns (tillering nodes) with crown roots were cut off from the seminal roots and from the above-ground parts of the plants. The crown with the roots is called 'root'.

To obtain suspensions of rhizosphere soils, about10 g roots samples with adhering soil of oats and barley growing in pure stands, were shaken for 30 min with 100 ml sterile

water in a mechanical shaker. To reduce the labour in study, Fusaria were studied only in rhizospheres of plants growing in pure stands. The numbers of CFUs (Colony-forming units) of fungi, in the rhizosphere soil suspension were determined by the dilution plate-count method, using Czapek-Dox Iprodione Dichloran Agar (CZID) medium (37), while CFUs of *Fusaria* were determined both on PPA (Peptone PCNB (Pentachloro-nitrobenzene) Agar (26) and CZID agar. The plates were incubated for 4-5 days at 25°C. Afterwards the CFUs of Fusaria were determined and the CFU/g of dry soil (CFU g^{-1} soil) were calculated. The pH of the soil suspensions was measured as explained above. To determine the dry weight, the rhizosphere soils were dried at 60°C.

As the number of Fusaria on PPA (on this medium besides *Fusarium*, only *Gliocladium* fungi were growing) and CZID (on this medium besides Fusaria, other soil fungi were also growing) media were similar, their mean data are presented.

After separation of oat and barley roots, root samples of plants grown in the oat/barley mixtures, were shaken with water (as described above), to follow the same procedure as for the plants grown in pure stands. The roots after removal from the suspensions, were rinsed 15 times by vigorous shaking for 25 seconds (10 times with un-sterile tap water and then 5 times with sterile water) and were dried on sterile filter paper. These cleaned roots were then divided into three parts: (i) crowns and after cutting the remaining root parts approximately in the middle, into (ii) upper and (iii) lower crown roots. All parts of roots were cut into approximately 1 cm fragments and all 720 root pieces were placed on CZID agar medium with sterile tweezers. For each of the 10 experimental series, the root fragments placed on CZID medium, were randomly chosen in equal numbers: 12 pieces of the crowns (4+4+4 for each of the replication, respectively), 30 pieces of upper roots (10+10+10 for each replication, respectively) and 30 pieces of the lower roots (10+10+10 for each replication, respectively). The CZID agar plates with the 1 cm root fragments were incubated at 25ºC for 4-7 days, thereafter, *Fusarium* colonies around the root pieces were identified visually and transferred from CZID agar medium plates to half strength PDA (Potato Dextrose Agar) plates and then from PDA to plates with SNA (Synthetic Nutrient Agar) (34).

Fusarium isolates (30 isolates per each of the 4 experimental series) were also taken from the colonies on the PPA and CZID plates, with incubated suspensions of rhizosphere soils. *Fusarium* species were identified as per Kwaśna *et al.* (23) and Leslie and Summerell (25) based on their morphological (macro- and micro-morphology) and cultural characteristics on two media - half strength PDA and SNA.

III. Fungal analysis of grains used for sowing

To determine the Fusaria load on and in barley and oat grains used for sowing, randomly selected 100 non-disinfected and 100 kernels surface-disinfected with sodium hypochlorite of each cultivar (10), were placed on CZID agar medium. These kernels were processed further as above for the root pieces". While the non-disinfected gave the total of Fusaria on and in seeds, the disinfected seeds gave only the Fusaria within the seeds.

IV. Statistical analysis

The results were subjected to ANOVA. The significance between the means of percentage of *Fusarium* species on the individual parts of roots was evaluated by Tukey's range test. The significance of the differences of the average percentages of various *Fusarium* species between barley and oat roots was calculated by Student's t-test. Statistical significance was at $P = 0.05$.

The simple Pearson's correlation was used to determine the differences between the "barley *Fusarium* communities" and "oat *Fusarium* communities" by determining the correlation coefficients: (i) within barley plants, (ii) within oat plants and (iii) between barley and oat plants, growing both in pure stand and in barley/oat mixtures. These coefficients of Pearson's correlation were calculated between the percentages of all 9 detected *Fusarium* species in the *Fusarium* communities on the surfaces of: crowns + upper roots + lower roots and separately on crowns, upper roots and lower roots. Further, the Pearson's correlation was used to evaluate the relationships: (i) between the fungal CFU numbers and the rhizosphere soil pH, (ii) between the percentages of *Fusarium* species in the fungal communities on the roots and in the rhizosphere soils as well as (iii) among the percentages of individual *Fusarium* species on barley roots and on the oat roots separately.

RESULTS AND DISCUSSION

Isolation of Fusaria

From 720 root pieces (free of soil) of barley and oats (Table 1), 1286 *Fusarium* isolates were selected (212, 558, 516 from crown, upper roots and lower roots, respectively). Among these, 9-*Fusarium* species were identified. The most common species were *F. oxysporum* (351 isolates), *F. culmorum* (338), *F. equiseti* (271), *F. solani* (146) and *F. avenaceum* (97). The less common were: *F. graminearum* (33), *F. sporotrichioides* (29), *F. crookwelense* (11) and *F. tricinctum* (7). Three fungal isolates were not identified due to lack of sporulation, but their colony characteristics suggested that they belonged to the *Fusarium* genus.

From the pieces of barley roots from all experiments, we obtained 599 *Fusarium* isolates (98, 259 and 242 from the crowns, upper roots and lower roots, respectively). From the pieces of oat roots from all experiments 687 ones (114, 299 and 274 from the crowns, upper roots and lower roots, respectively). This showed that oat roots had higher number of Fusaria isolates than barley roots by 15%,16%,15% and 13% for all roots, crowns, upper and lower roots, respectively.

Among the isolates from the barley roots, the most abundant was *F. oxysporum* - 187, followed by *F. equiseti* - 147, *F. culmorum* - 104, *F. solani* - 80, *F. avenaceum* - 29, *F. graminearum* - 26, *F. sporotrichioides* - 15, *F. crookwelense* - 6, *F. tricinctum* - 3 and *Fusarium* sp. - 2. Among the isolates from the oat roots the most abundant was *F. culmorum* - 222, followed by *F. oxysporum* -165, *F. equiseti* - 136, *F. avenaceum* - 69, *F. solani* - 66, *F. sporotrichioides* - 13, *F. graminearum* - 6, *F. crookwelense* - 5, *F. tricinctum* - 4 and *Fusarium* sp. - 1.

These results are consistent with data from studies on Fusaria in rhizoplanes of other plants. *F. oxysporum*, *F. solani* and *F. equiseti* were the main *Fusarium* species in the rhizoplanes of pea, tomato, maize and wheat (19). *F. oxysporum* and *F. equiseti* in the rhizoplane of sugarcane (19). *F. oxysporum* and *F. solani* in the rhizoplanes of lentil, sesame, broad-bean, cucumber, tomato and cotton (1,19,22), *F. culmorum*, *F. oxysporum*, *F. avenaceum* and *F. equiseti* in the rhizoplane of oriental goat's rue (6) and *F. equiseti*, *F. culmorum* and *F. oxysporum* in rhizoplane of spring wheat (28). Zohri *et al.* (41) reported the presence of *F. oxysporum* as the most abundant, followed by *F. solani*, *F. culmorum*

and *F. equiseti* on the rhizoplanes of barley, basil, broad bean, Egyptian clover, fenugreek, jimsonweed, maize, rosemary, sunflower, Syrian bean caper and wormwood.

In comparison to the plants grown in pure stand, more *Fusarium* isolates were obtained from the roots of plants grown in barley/oat mixtures (by 38% and 25% on average in barley and oats, respectively). In both oat cultivars, the proportions of *F. oxysporum* in the fungal communities from the plant mixtures were 2 times lower than *Fusarium* communities in the pure stands. Similarly, we observed higher percentages of *F. culmorum* in the *Fusarium* communities in oat rhizoplanes from the plant mixtures than from the pure stands. However in contrast to *F. oxysporum*, these differences were not statistically significant at $P < 0.05$. Besides Fusaria, the *Gliocladium roseum* and *G. catenulatum* were also isolated from the roots of all studied barley and oat cultivars (results not presented).

Table 1. *Fusarium* spp. (%) in *Fusarium* communities on surface of crowns, upper crown roots and lower crown roots of barley and oats grown in pure stands or in barley/oat mixtures

Fusarium species	Barley				Oats			
	Crowns	Upper roots	Lower roots	Means	Crowns	Upper roots	Lower roots	Means
	Gawrosz (pure stand) (11 + 36 + 43 = 90)*				Krezus (pure stand) (19 + 43 + 51 = 113)*			
F. culmorum	9. 1	8. 3	4. 7	7. 4	21. 1	23. 3	23. 5	22. 6
F. equiseti	45. 5	27. 8	23. 3	32. 2	15. 8	20. 9	13. 7	16. 8
F. oxysporum	0	19. 4	46. 5	22. 0	42. 1	25. 6	41. 2	36. 3
F. solani	27. 3	25. 0	23. 3	25. 2	0	9. 3	9. 8	6. 4
F. avenaceum	0	0	2. 3	0. 8	15. 8	11. 6	11. 8	13. 1
F. graminearum	18. 2	8. 3	0	8. 8	0	2. 3	0	0. 8
F. sporotrichioides	0	0	0	0	5. 3	0	0	1. 8
F. crookwellense	0	2. 8	0	0. 9	0	2. 3	0	0. 8
F. tricinctum	0	5. 6	0	1. 9	0	4. 7	0	1. 6
Fusarium sp.	0	2. 8	0	0. 9	0	0	0	0
	Gawrosz (mixture with Krezus) (23 + 55 + 53 = 131)*				Krezus (mixture with Gawrosz) (35 + 82 + 53 = 170)*			
F. culmorum	21. 7	29. 1	28. 3	26. 4	57. 1	37. 8	52. 8	49. 2
F. equiseti	60. 9	27. 3	7. 5	31. 9	11. 4	19. 5	11. 3	14. 1
F. oxysporum	8. 7	30. 9	43. 4	27. 7	11. 4	18. 3	18. 9	16. 2
F. solani	4. 3	9. 1	1. 9	5. 1	5. 7	14. 6	7. 5	9. 3
F. avenaceum	4. 3	1. 8	3. 8	3. 3	11. 4	4. 9	5. 7	7. 3
F. graminearum	0	0	3. 8	1. 3	0	3. 7	1. 9	1. 9
F. sporotrichioides	0	1. 8	7. 5	3. 1	2. 9	0	0	1. 0
F. crookwellense	0	0	3. 8	1. 3	0	1. 2	0	0. 4
F. tricinctum	0	0	0	0	0	0	0	0
Fusarium sp.	0	0	0	0	0	0	1. 9	0. 6
	Skarb (pure stand) (24 + 40 + 39 = 103)*				Nagus (pure stand) (20 + 47 + 64 = 131)*			
F. culmorum	25. 0	25. 0	10. 3	20. 1	45. 0	25. 5	28. 1	32. 9
F. equiseti	45. 8	27. 5	20. 5	31. 3	15. 0	12. 8	14. 1	14. 0
F. oxysporum	8. 3	20. 0	41. 0	23. 1	35. 0	25. 5	37. 5	32. 7
F. solani	0	25. 0	15. 4	13. 5	5. 0	14. 9	10. 9	10. 3
F. avenaceum	4. 2	2. 5	2. 6	3. 1	0	14. 9	7. 8	7. 6
F. graminearum	8. 3	0	5. 1	4. 5	0	0	0	0. 0
F. sporotrichioides	0	0	5. 1	1. 7	0	4. 3	1. 6	2. 0
F. crookwellense	4. 2	0	0	1. 4	0	2. 1	0	0. 7
F. tricinctum	4. 2	0	0	1. 4	0	0	0	0
Fusarium sp.	0	0	0	0	0	0	0	0
	Skarb (mixture with Nagus) (23 + 78 + 48 = 149)*				Nagus (mixture with Skarb) (15 + 71 + 46 = 132)*			
F. culmorum	4. 3	17. 9	25. 0	15. 7	60. 0	33. 8	47. 8	47. 2
F. equiseti	26. 1	23. 1	16. 7	22. 0	0	25. 4	19. 6	15. 0

F. oxysporum	17. 4	33. 3	43. 8	31. 5	13. 3	18. 3	19. 6	17. 1
F. solani	13. 0	17. 9	0	10. 3	6. 7	16. 9	6. 5	10. 0
F. avenaceum	8. 7	0	4. 2	4. 3	0	2. 8	4. 3	2. 4
F. graminearum	30. 4	6. 4	6. 3	14. 4	0	0	2. 2	0. 7
F. sporotrichioides	0	0	4. 2	1. 4	13. 3	0	0	4. 4
F. crookwellense	0	1. 3	0	0. 4	0	2. 8	0	0. 9
F. tricinctum	0	0	0	0	6. 7	0	0	2. 2
Fusarium sp.	0	0	0	0	0	0	0	0
	Skarb (mixture with Krezus) (17 + 50 + 59 = 126)*				Krezus (mixture with Skarb) (25 + 56 + 60 = 141)*			
F. culmorum	11. 8	6. 0	16. 9	11. 6	36. 0	19. 6	25. 0	26. 9
F. equiseti	76. 5	18. 0	8. 5	34. 3	28. 0	28. 6	18. 3	25. 0
F. oxysporum	5. 9	32. 0	42. 4	26. 8	0	23. 2	25. 0	16. 1
F. solani	0	24. 0	10. 2	11. 4	12. 0	1. 8	6. 7	6. 8
F. avenaceum	0	20. 0	11. 9	10. 6	16. 0	21. 4	18. 3	18. 6
F. graminearum	0	0	0	0. 0	0	1. 8	0	0. 6
F. sporotrichioides	5. 9	0	8. 5	4. 8	8. 0	3. 6	5. 0	5. 5
F. crookwellense	0	0	0	0	0	0	0	0
F. tricinctum	0	0	0	0	0	0	1. 7	0. 6
Fusarium sp.	0	0	1. 7	0. 6	0	0	0	0
	Barley (means)				Oat (means)			
F. culmorum	14. 4 a	17. 3 ab	17. 0 ab	16. 2	43. 8 c	28. 0 bc	35. 4 c	35. 7
F. equiseti	51. 0 c	24. 7 b	15. 3 a	30. 3	14. 0 ab	21. 4 ab	15. 4 a	16. 9
F. oxysporum	8. 1 a	27. 1 b	43. 0 c	26. 1	20. 4 ab	22. 2 b	28. 4 b	23. 7
F. solani	8. 9 ab	20. 2 b	10. 2 ab	13. 1	5. 9 a	11. 5 ab	8. 3 a	8. 6
F. avenaceum	3. 4	4. 9	5. 0	4. 4	8. 6	11. 1	9. 6	9. 8
F. graminearum	11. 4	2. 9	3. 0	5. 8	0	1. 6	0. 8	0. 8
F. sporotrichioides	1. 2	0. 4	5. 1	2. 2	5. 9	1. 6	1. 3	2. 9
F. crookwellense	0. 8	0. 8	0. 8	0. 8	0	1. 7	0	0. 6
F. tricinctum	0. 8	1. 1	0	0. 6	1. 3	0. 9	0. 3	0. 8
Fusarium sp.	0	0. 6	0. 8	0. 5	0	0	0. 4	0. 1

* - values in the parentheses are numbers of *Fusarium* isolates from crowns + upper crow roots + lower crown roots = Total isolates;

The mean values in the lowest part of the table marked with the same letters do not significantly differ at $P = 0.05$

Fusarium communities

Distinct differences in percentages of various *Fusarium* species were found between the *Fusarium* communities on barley and oat roots, both in pure stands as well as in the barley/oat mixtures. The barley roots, as compared to the oat roots, had: (i). lower percent of *F. culmorum* on all the root parts, (ii). higher percent of *F. equiseti* on the crowns and less distinct differences (iii). higher percent of *F. graminearum*, but (iv). lower percentage of *F. avenaceum* on all the root parts.

The mean percentages (for crowns, upper and lower roots of all experimental series) of *F. culmorum* (16% for barley vs 36% for oats), *F. equiseti* (30% vs 17%), *F. avenaceum* (4% vs 10%) and *F. graminearum* (6% vs 1%), presented in the lowest part of Table 1, significantly differed between these two plant species at $P < 0.05$. Further in barley roots *F. oxysporum* (%) gradually increased but the *F. equiseti* (%) decreased from the crowns, upper roots and lower roots (Table 1). The mean percentages of *F. equiseti* and *F. oxysporum* for the individual root fractions significantly differed from each other in barley ($P < 0.05$). In contrast, in oats percentage of both *F. oxysporum* and *F. equiseti* were similar on all the root parts and did not differ from each other (Table 1).

Simple Pearson correlation between the percentages of various *Fusarium* species ($n = 15$) showed that on the barley roots *F. equiseti* was, negatively correlated with *F. oxysporum* ($r = -0.83$, at $P<0.01$) and *F. culmorum* was negatively correlated (at $P< 0.05$) with *F. solani* ($r = -0.57$). On the oat **r**oots *F. culmorum* was negatively correlated (at

P< 0. 05) with *F. equiseti* (*r* = -0. 53), *F. oxysporum* (*r* = -0. 53) and especially (at *P*< 0. 01) with *F. avenaceum* (*r* = -0. 68) suggesting competition between these species. The evidence of such competition between the *F. culmorum* and *F. avenaceum* on stem bases of wheat has been reported (35).

Similar differences between the "barley *Fusarium* communities" and "oat *Fusarium* communities" on residues of these plants, has been reported (12,13). The mean percentage of both *F. equiseti* and *F. graminearum* were significantly higher on the residues of barley than on the oat ones (18-19% versus 6-7% for *F. equiseti*, and 9-12% versus 3-4% for *F. graminearum*) (12,13). However, the mean percentage of *F. avenaceum* and *F. culmorum* were significantly lower (10% versus 20-21% for *F. avenaceum* and 4-6% versus 13% for *F. culmorum*). Similarly, Watkins and Boosalis (43) reported that oat stubbles supported the colonization of *F. culmorum*, whereas, barley stubble supported the colonization of *F. equiseti*. Fernandez *et al.* (14) found that *F. equiseti* was most common *Fusarium* species from subcrown internodes of barley. Fernandez and Holzgang (11) reported that *F. culmorum* was the most common species on sub crown internodes and crown/lower culms of oats. In all these cases, it appears that the same factors were responsible for the selection of the *Fusarium* species.

It is possible that the oat saponins- triterpenoid avenacins produced by oat roots - were important in promoting the *F. culmorum*. Tsurushima (39) reported that oat roots secrete antifungal compounds (triterpenoid glucosides – avenacins). Perhaps, *F. culmorum* and *F. avenaceum* are less sensitive to these oat saponins than *F. equiseti* and *F. graminearum*. Czaban *et al.* (8) reported that *F. culmorum* was slightly sensitive to triterpenoid saponins from common soapwort (*Saponaria officinalis* L.), whereas, triterpenoid saponins from field scabious (*Knautia arvensis* (L.) J.M. Coult.), alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) stimulated the growth of this fungus. Contrarily, the growth of wheat pathogen *Gaeumannomyces graminis* var. *triticii* was inhibited by these saponins. Kiecana *et al.* (21) reported that the percentages of *F. culmorum* and *F. avenaceum* among 688 *Fusarium* isolates from roots of 39 oat genotypes were higher (20 and 5 times, respectively) than the percentage of *F. equiseti*. It supports this hypothesis. Oat roots, besides saponins also produce and secrete scopoletin (2,16), which strongly inhibits the growth of *F. culmorum* in pure culture (31). However, due to high proportion of *F. culmorum* in oat rhizoplane, scopoletin may not be a crucial factor in the selection of *Fusarium* species on oat roots.

The reason for the differences between the barley and oats in colonisation of their roots by *F. oxysporum* and *F. equiseti* is difficult to explain. As mentioned above, in barley roots the *F. equiseti* was negatively correlated with *F. oxysporum* and an opposite trends occurred in percentage of *F. oxysporum* (gradual increase from crown to lower roots) and *F. equiseti* (gradual decrease). On the other hand, the percentage of these fungi remained the same on all oat root parts, although the oat crown roots were about 2-times longer than barley crown roots (data not presented)**.** It is possible, that these differences between the plant species were not accidental, as the above trend on barley roots occurred in all 5- barley experiments, both in pure stands and also in barley/oat mixtures. The lower part of the roots is younger than the upper, hence, the trend seen in the barley roots is logical and consistent with the results of Kurakov and Kostina (23**)**, who reported that *F. oxysporum* colonised the rhizoplane of tomato and cucumber as the first *Fusarium* species.

Table 2. The simple Pearson's correlation coefficients among *Fusarium* communities within barley plants, within oat plants and between barley and oat plants, in pure stands and in mixtures

Plant		Barley					Oats				
		Skarb (alone)	Gawrosz (alone)	Skarb + Nagus M	Skarb + Krezus M	Gawrosz + Krezus M	Nagus (alone)	Krezus (alone)	Nagus + Skarb M	Krezus + Skarb M	Krezus + Gawrosz M
Barley	Skarb (alone)	1	**0.815****	**0.716****	**0.819****	**0.881****	**0.652****	**0.629****	0.465	**0.660****	0.512*
	Gawrosz (alone)	**0.815****	1	**0.719****	**0.731****	**0.688****	0.367	0.379	0.166	0.474	0.194
	Skarb + Nagus M	**0.716****	**0.719****	1	**0.605****	**0.733****	0.555*	**0.659****	0.350	0.483	0.400
	Skarb + Krezus M	**0.819****	**0.731****	**0.605****	1	**0.868****	0.474	0.494	0.171	**0.610****	0.275
	Gawrosz + Krezus M	**0.881****	**0.688****	**0.733****	**0.868****	1	**0.642****	**0.650****	0.457	**0.736****	0.568*
Oat	Nagus (alone)	**0.652****	0.367	0.555*	0.474	**0.642****	1	**0.871****	**0.791****	**0.677****	**0.803****
	Krezus (alone)	**0.629****	0.379	**0.659****	0.494	**0.650****	**0.871****	1	0.574*	**0.605****	**0.611****
	Nagus + Skarb M	0.465	0.166	0.350	0.171	0.457	**0.791****	0.574*	1	**0.667****	**0.952****
	Krezus + Skarb M	**0.660****	0.474	0.483	**0.610****	**0.736****	**0.677****	**0.605****	**0.667****	1	**0.730****
	Krezus + Gawrosz M	0.512*	0.194	0.400	0.275	0.568*	**0.803****	**0.611****	**0.952****	**0.730****	1

M: Mixture. The shaded data represent the correlations within barley plants (upper rows) and within oat plants (lower rows)

Besides its percentage among the *Fusarium* communities was the highest in the apical rhizoplane zone, nearest to the root tip and gradually decreased to the older parts of the roots. Similarly, the population of *F. oxysporum* was maximal at short distance (1-2 cm) from the tip in cotton (23).

The simple correlation analysis of the proportions of nine *Fusarium* species on the three parts of barley and oat roots showed (Table 2) the correlation coefficients (n=27) within the *Fusarium* communities in barley rhizoplane (range from 0.61 to 0.88, median 0. 73, 100 % coefficients are significant at P<0. 001) or within the compositions of *Fusarium* communities in oat rhizoplane (range 0.57 - 0.95, median 0.70, 90 % coefficients significant at P < 0. 001 + 10 % coefficients significant at P <0. 01) are distinctly higher than the correlation coefficients between "barley *Fusarium* communities" and "oat *Fusarium* communities" (range 0.17 - 0.74, median 0.48, 32% are significant at P < 0.001 + 16% significant at P <0.01). This confirms that "barley *Fusarium* communities" both in the rhizoplanes of plants growing in pure stand and in mixtures, were more similar to each other than to "oat *Fusarium* communities" and vice versa.

The main differences between the "barley *Fusarium* communities" and "oat *Fusarium* communities" were found in the crowns (Table 3), whereas their differences on upper and lower roots were much smaller.

Table 3. The simple Pearson's correlation coefficients among *Fusarium* communities on crowns, upper crown roots and lower crown roots, within barley plants, within oat plants and between barley and oat plants, in pure stands and in mixtures

Parameters	Range	Median	% coefficients significance at	
			P<0.01	P<0. 05
Correlation coefficients between *Fusarium* communities of crowns (n = 9)				
Within "barley communities"	0. 45-0. 97	0. 73	30	40
Within "oat communities"	0. 25-0. 93	0. 69	30	20
"Barley communities" with "oat communities"	-0. 30-0.71	0. 22	0	8
Correlation coefficients between *Fusarium* communities of upper roots (n = 9)				
Within "barley communities"	0. 58-0. 91	0. 78	50	10
Within "oat communities"	0. 63-0. 97	0. 85	70	10
"Barley communities" with "oat communities"	0. 39-0. 95	0. 74	32	28
Correlation coefficients between *Fusarium* communities of lower roots (n = 9)				
Within "barley communities"	0. 66-0. 97	0. 87	70	20
Within "oat communities"	0. 66-0. 98	0. 80	60	30
"Barley communities" with "oat communities"	0. 25-0. 95	0. 77	44	24

Fusaria in oat and barley rhizospheres

Significantly more CFU (Colony forming units) numbers of both total fungi (approx 5 times) and Fusaria (approx 6 times) were found in the rhizosphere soils of barley than in those of oats (Table 4).

This confirms the occurrence of some antifungal compounds in the rhizosphere soil of oats. In comparison to barley rhizosphere, the antifungal effects of oat root exudates similarly reduced the CFU number of both total fungi and Fusaria, as the percentage of *Fusarium* out of total fungi were similar in the rhizosphere of all oat and barley cultivars (2.2 - 3.1 %). Hence CFU numbers of both group of fungi were correlated (r = 0.986, P<0. 05, n = 4).

The lower pH of the rhizosphere soils of oats than barley (Table 4) may also be a reason for the difference in CFUs of both the fungal groups between barley and oats Significant correlation between the fungal numbers and pH of the soil suspensions (r = 0.983, $P < 0.05$ and r = 0.994, $P < 0.01$, n = 4 for the total CFU numbers of fungi and CFU numbers of *Fusarium*, respectively) were noticed. Gollany and Schumacher (15) reported that the apical zone of oat roots causes the acidification of rhizosphere soil in comparison to roots of barley.

The *Fusarium* species distribution in the rhizosphere soils (Table 4) slightly reflected the distribution of *Fusarium* species in the rhizoplane (Table 1). The *Fusarium* communities in barley rhizosphere soils, were richer in *F. equiseti* and poorer in *F. culmorum* than oat rhizospheres. Further, *F. graminearum* was found only in the rhizosphere of barley 'Skarb', but *F. avenaceum* was found in rhizospheres of both oat cultivars, although the highest percentage of *F. avenaceum* was found in rhizosphere of barley 'Gawrosz' (Table 4). The distribution of the nine *Fusarium* species in the rhizosphere soil of barley varieties, was significantly positively correlated with the distribution of these species in the corresponding rhizoplanes of 'Skarb' (r = 0. 77 at P<0. 05), 'Gawrosz' (r = 0. 83 at P<0. 01) and 'Nagus' (r = 0. 86 at P<0. 01).

Table 4. Total fungi and Fusaria in the rhizosphere soils of barley and oats and the proportions of various *Fusarium* spp in the *Fusarium* rhizosphere communities and pH of rhizosphere soils

Variables studied	Barley cultivars		Oat cultivars	
	'Skarb'	'Gawrosz'	'Krezus'	'Nagus'
CFU of total fungi	3388 x 10^3 b	1887 x 10^3 b	410 x 10^3 a	596 x 10^3 a
CFU of Fusaria	83. 2 x 10^3 b (93.9 and 72.5 on PPA and CZID, respectively)	57. 8 x 10^3 b (44.9 and 70.7 on PPA and CZID, respectively)	10. 0 x 10^3 a (7.2 and 12.8 on PPA and CZID, respectively)	13. 2 x 10^3 a (11.7 and 14.7 on PPA and CZID, respectively
Fusarium % in total fungal CFUs	2.5%	3. 1%	2. 4%	2. 2%
Number of *Fusarium* isolates from rhizosphere soils	31	34	34	30
pH of rhizosphere soils	5. 5 b	5. 3 ab	4. 9 a	5. 0 a
Proportions (%) of various *Fusarium* species in the rhizosphere soils				
F. culmorum	6. 5	0	20. 6	26. 7
F. equiseti	32. 3	44. 1	11. 8	3. 3
F. oxysporum	19. 4	17. 6	11. 8	30. 0
F. solani	32. 3	17. 6	41. 2	23. 3
F. avenaceum	0	14. 7	11. 8	6. 7
F. graminearum	3. 2	0	0	0
F. sporitrichioides	0	0	2. 9	6. 7
F. crookwellense	0	0	0	0
F. tricinctum	6. 5	2. 9	0	3. 3
Fusarium sp.	0	2. 9	0	0

CFU : Colony forming units

In oat varieties, both the rhizospheres and rhizoplanes (in pure stand and in mixture with 'Skarb') of 'Nagus' were richer in *F. culmorum* but poorer in *F. avenaceum* than those of 'Krezus' (Tables 1 and 4).

Table 5. The *Fusarium* species present on of barley and oat seeds used for sowing

Fusarium species	Barley				Oats			
	'Skarb'		'Gawrosz'		'Krezus'		'Nagus'	
	ND*	D**	ND	D	ND	D	ND	D
F. poae	5	1	3	1	17	14	3	0
F. avenaceum	2	0	1	0	7	2	0	0
F. equiseti	0	0	1	0	2	0	0	0
F. tricinctum	0	0	0	0	2	1	0	0
F. sporotrichioides	0	0	0	0	2	0	0	0
F. graminearum	0	1	0	0	0	0	0	0
F. culmorum	0	0	0	0	0	0	1	0
All *Fusarium* species	7	2	5	1	30	17	4	0

*ND : Not disinfected, **D : Disinfected

Fusaria on seeds of oats and barley

The barley and oat seeds used for sowing in our experiment, were examined for external contamination and internal infection by Fusaria. It was done to evaluate whether the above differences between barley and oats in the distribution of the *Fusarium* species in their rhizoplanes and rhizospheres was a reflection of the contamination of the barley seeds by *F. equiseti* and *F. graminearum* and of the oat seeds by *F. culmorum* and *F. avenaceum*. The seeds taken for sowing in 2013 were only slightly contaminated with Fusaria (4-8% of the non-disinfected kernels and 0-2% of the kernels disinfected with hypochlorite), except for the hulled oats 'Krezus' (30% non-disinfected kernels and 17% of the disinfected ones). The most abundant *Fusarium* species on and in the barley and oat seeds was *F. poae* which was not found in the plant rhizoplanes. Further, *F. avenaceum* was present on the kernels of 'Gawrosz' and 'Skarb' and on the seeds of 'Krezus', while, *F. equiseti* occurred on the kernels of 'Gawrosz' and 'Krezus', *F. culmorum* was found only on 'Nagus' and *F. graminearum* only on 'Skarb' seeds. *F. sporotrichioides* and *F. tricinctum* were found only on 'Krezus' seeds (Table 5). Perhaps the contamination of the oat seeds with *F. avenaceum* could be important to some degree in further colonization of the plant roots with this fungal species. In comparison to 'Nagus', both the roots and the rhizosphere soil of 'Krezus' were richer in *F. avenaceum* (Tables 1 and 2) and *F. avenaceum* was found only on the kernels of 'Krezus' (Table 5).

CONCLUSIONS

The oat rhizoplane, in comparison to barley rhizoplane, was characterized by significantly higher proportions of *F. culmorum* and *F. avenaceum* and lower proportions of *F. equiseti* and *F. graminearum*, both in pure stands and in barley/oat mixtures. The CFU numbers of both total fungi and Fusaria were significantly lower in the rhizospheres of oats, than in those of barley, suggesting the presence of some significant antifungal substance(s). The composition of the rhizosphere *Fusarium* communities generally reflects the composition of their rhizoplanes. In barley, an opposite trend of percentage of *F. oxysporum* (gradual increase from crown to lower roots) and *F. equiseti* (gradual decrease) along the roots was noticed. There were higher number of *Fusarium* isolates from the

rhizoplanes of the plants growing in the barley/oat mixtures than from the pure stands. The oat *Fusarium* communities from plants growing in mixtures were poorer in *F. oxysporum* than in pure stands. Analysis of *Fusarium* communities on and in the seeds used for sowing, suggested that *Fusarium* fungi (especially *F. culmorum* and *F. equiseti*) colonising the root surfaces of the plants, originated from the soil and not from the seeds. All these observations suggested the allelopathic effects of oats and barley on the composition of *Fusarium* communities in their root zone.

ACKNOWLEDGEMENTS

This work was supported by the Polish Ministry of Agriculture and Rural Development - Project No. PKre-029-16-16/13 (740).

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