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Impact of tillage on the incidence of *Fusarium* spp. in soil

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Abstract

The influence of several long-term conventional and conservation tillage treatments on the incidence and the diversity of *Fusarium* spp. in soil was studied. Soil samples were randomly collected from naturally contaminated field trials and Fusarium species were isolated by using the dilution plate method. The identification of the species was done by direct microscopic observation on *Fusarium*-specific media. The isolation frequency of *Fusarium* species and the total number of colony forming units was affected by the sampling year and the cultivated crop and showed significant differences between the tillage treatments. Moldboard plough-treatments resulted in a lower diversity of Fusarium species than the chisel plough and rotary tiller treatments. Besides the tillage system the tillage depth also appeared to affect the *Fusarium* populations. The deeper the tillage the lower was the number of isolated *Fusarium* spp. Twenty *Fusarium* species were identified over both years of investigation. In conservation tillage plots a higher diversity of *Fusarium* species was found than in the moldboard plough-based tillage plots. A correlation between *Fusarium* species producing the mycotoxin deoxynivalenol (DON) isolated from soil and the DON-content of grain could not be observed. However, these investigations indicate that conservation soil tillage results in conditions which increase the incidence of *Fusarium* species in soil.

Introduction

Fusarium species are ubiquitous in soil and are important plant pathogens world-wide (Domsch and Gams, 1970). *Fusaria* exist in soil as colonisers of living plants or plant residues within the soil or adjacent to the soil surface (Burgess, 1981) and are able to persist as conidia, chlamydospores, or mycelium (McMullen and Stack, 1983). It is a genus, which can sufficiently maintain inoculum in soil due to an efficient use of transient substrates (Mitchell, 1979).

Tillage practices are valuable methods of disease control and are appreciated as an integral part of sustainable agriculture. In general, tillage systems that bury crop residues are favourable measures to reduce disease but the effect of tillage treatment on the development of plant pathogens is variable and the resulting interactions are complex (Conway, 1996). Under fundamentally different environmental conditions tillage systems vary in their effect on the microbial soil community. Conservation-tillage implements, such as rotory tiller or chisel plough, leave more than 30% of the soil surface covered by crop residues after planting. In the case of conventional tillage with a moldboard plough the crop residues are buried and exposed to efficient microbial degradation in the moist soil environment (Bockus and Shroyer, 1998). Due to the fact that conservation farming practices have major advantages such as the reduction of costs, the improvement of soil structure and water infiltration, thus ensuring the reduction of soil erosion, reduced tillage and stubble retention have become popular practices in present agriculture.

The interaction of tillage and *Fusarium*-infection is a matter of particular interest. In wheat and maize growing areas an increase of *Fusarium* species is a

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controversial issue. They can cause additional risks particularly because of the production of mycotoxins. These mycotoxins lead to food- and feed-borne intoxication in humans and animals, thus several guidelines in Europe, USA and Canada recommend maximum levels for contamination with toxins such as deoxynivalenol (DON) (Codex Alimentarius Commission, 2002).

Contradictory data on the effect of tillage on *Fusarium* spp. are available. Under different depth and type of implement used conservation tillage decreased (Damm, 1998a, b; Weber et al., 2001), increased (Bailey and Duczek, 1996) or had no effect (Arnold-Reimer, 1994; Swan et al., 2000; Wildermuth et al., 1997) on *Fusarium* foot and root rot. Most studies on *Fusarium* head blight or ear rot documented a significant increase (Beck and Lepschy, 2000; Dill-Macky and Jones, 2000; Yi et al., 2001) or no increase of infections in case of conservation tillage treatments (Bahle and Leist, 1997; Flett and McLaren, 1998; Miller et al., 1998; Steinkellner et al., 2002).

Comparative studies on the effects of soil management on *Fusarium* populations in soil are rare. The objective of this study was to determine the effect of long-term conventional and conservation tillage treatments on the incidence and the diversity of *Fusarium* spp. in soil.

Materials and methods

Experimental design

The site is located at Ansfelden in Upper Austria and represents the semi-humid climate area with a mean annual temperature of 9.1 °C and average of 800 mm annual rainfall. The growing period takes about 230 days. Weather data of this area are illustrated in Figure 1. The soil type is characterised as a deep medium-heavy calcium-free loose sedimentary brown earth. Details of chemical and physical characteristics of the soil were documented by Liebhard (1993a, b, 1994) and Liebhard et al. (1994).

The incidence of *Fusarium* spp. was investigated on an existing long-term trial in a long plot design in 2000 and 2001. Each tillage treatment was replicated 4 times (60 × 9 m per replication). The rotation sequence (winter wheat, sugar beet, winter wheat and maize) and eight tillage treatments were applied to the same plots each year since 1980. The tillage factors were moldboard plough (to a depth of 30 cm, 24 cm and 17 cm), chisel plough (to a depth of 30 cm, 24 cm and 17 cm), rotary tiller (to a depth of 10 cm) and rotary tiller alternating with moldboard plough (24 cm). Winter wheat (*Triticum aestivum* L. cv. 'Juventus') was sown in October 1999 and soil-sampling was performed before harvest in July 2000. Frost-susceptible mustard (*Brassica juncea* L.) was grown after wheat as a cover crop. Maize (*Zea mays* L. cv. 'Altess') was sown in April 2001 and soil samples were taken out in October 2001. Weed and pest control and fertilisation were according to standard field management practices.

Soil sampling and isolation of Fusarium *spp.*

At harvest for each plot 10 soil cores were randomly collected within the rows to a depth of 10 cm and bulked. The samples were air-dried and stored at 4 °C until processed within 2 weeks. The isolation of *Fusarium* spp. was done by the dilution plate technique using Nash-Snyder medium (Rush et al., 1992; Windels, 1992). Subsamples of soil (3 replicates, 50 g each) were added to 100 mL of 0.15% water agar and mixed thoroughly. Further dilution series were made using 1 mL aliquots of the above mentioned solutions in 9 mL of 0.15% water agar. 1 mL of the final dilution (1:10 000, based on preliminary tests) was transferred to each of three petri dishes containing Nash-Snyder medium and soil particles were dispersed by circular agitation of the petri dishes. The petri dishes were incubated at 22 °C below UV-light with a photoperiod of 16 hr light/8 hr dark. After 12–14 days the total number of fungal and bacterial colonies were counted and expressed as colony-forming units per g of air-dried soil (CFU/g soil). All *Fusarium* colonies and fungal colonies of uncertain identity were transferred to potato-dextrose-medium, carnation-leaf-agar (Nelson et al., 1983) as well as low nutrient agar (Nirenberg, 1976) for further identification.

Identification of Fusarium *spp.*

The identification of *Fusarium* spp. was done by direct microscopic observation and was based upon morphological characters according to Gerlach and Nirenberg (1982) and Nelson et al., (1983). *Fusarium*-populations were also expressed as colony-forming units per g of air-dried soil (CFU/g soil).

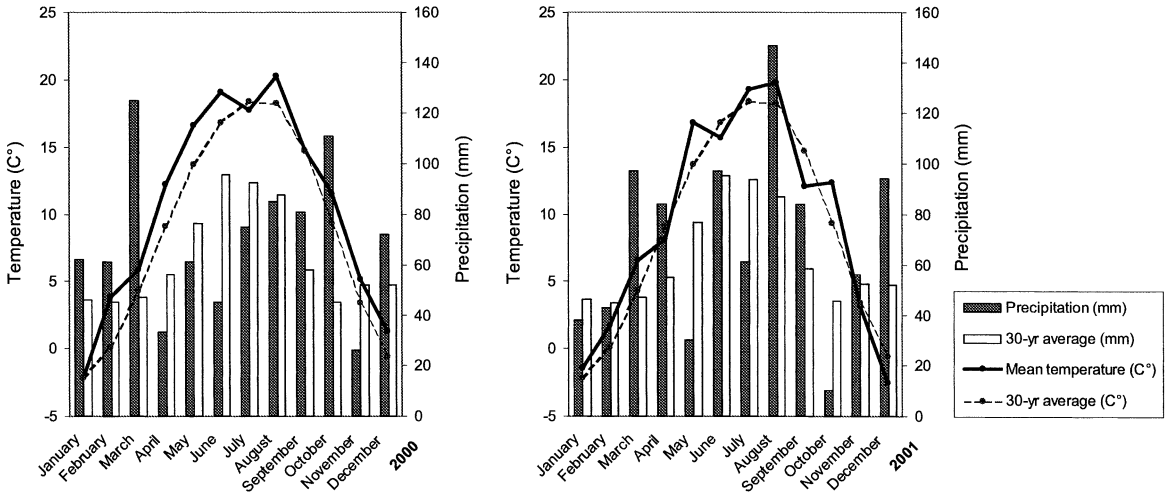

Figure 1. Monthly temperature and precipitation during 2000–2001 and 30-yr average. Data obtained from official measuring point Linz/Hörsching, Central Institute of Meteorology and Geodynamics (ZAMG).

Analysis of deoxynivalenol concentrations

Final-harvest grain samples from each test plot were taken for assessment of deoxynivalenol (DON) contamination of winter wheat in 2000 and maize in 2001. The DON content of each sample was analysed by IFA Tulln, Centre for Analytic, Tulln. Samples consisting of 250 g of grain were ground and DON was determined by gas chromatography with electron-capture detection after extraction with acetonitrile/water and a clean-up with using a Mycosep^{TM} – column (Weingärtner et al., 1997).

Statistical analyses

Analysis of variance was done after a variance check by Bartlett's test and if required data transformation $x' = SQRT(1/2 + x)$. Mean values were compared using least significant difference (LSD) test ($P < 0.05$). Non-transformed means are reported. Pearson correlation coefficients were calculated among DON producing *F. culmorum* and *F. graminearum* (CFU/g soil) and DON contamination of grain (μg/kg). DON (μg/kg) and CFU/g soil data for the year 2000 were subjected to $x' = SQRT(1/2+x)$ transformation prior to analysis and non-transformed data were shown. These analyses were performed using Statgraphics Plus 5.0 and SPSS 9.0 for Windows.

Results

The number of total colony forming units (fungal and bacterial colonies; CFU/g soil) and *Fusarium* spp. was higher in 2000 when wheat was grown (Figure 2) than in 2001 when maize was cultivated (Figure 3) and showed differences between the tillage treatments. The total colony forming units ranged from about 67700 to 112500 CFU/g soil in 2000 and from 24100 to 92500 CFU/g soil in 2001. The proportion of *Fusarium* spp. within the tillage treatments was between 11% and 62% and ranged from about 8300 to 70000 CFU/g soil in 2000 and from 5000 to 42500 CFU/g soil in 2001. Moldboard based plots at 24 cm and 17 cm showed higher *Fusarium* populations in 2001 than in 2000. Chisel plough plots to a depth of 24 and 17 cm showed higher populations in 2000 than in 2001. Based on the analysis of variance the isolation frequency of total colony forming units and *Fusarium* spp. was significantly influenced by tillage treatments (data for 2000 and 2001 are averaged, Table 1). In moldboard plough treatments significantly lower total colony forming units were isolated. By comparison the lowest value, 49583 CFU/g soil in moldboard plough 30 cm treatment, was only about the half of the highest value, 101250 CFU/g soil in chisel plough 24 cm. There was no treatment x year interaction.

The effect of tillage became more clear when looking at the incidence of *Fusarium* spp. Meaned over both years the significantly lowest CFU/g soil were found in moldboard plough 30 cm treatments, followed by moldboard plough 24 cm treatment and

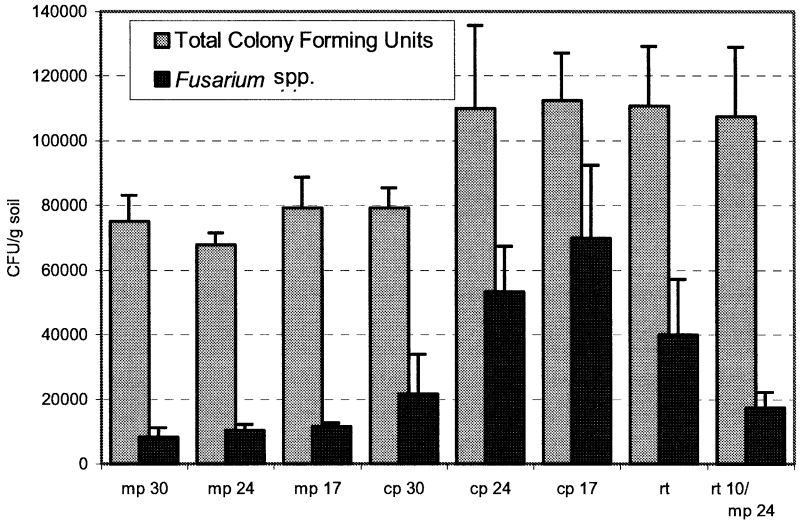

Figure 2. Isolation frequency of total colony forming units and *Fusarium* spp. under different tillage treatments in wheat 2000 (mp = moldboard plough, cp = chisel-plough, rt = rotory tiller; 30, 24, 17 = depth of treatment in cm).

rotory tiller/moldboard plough 24 cm treatment. The significantly highest frequency was found in the chisel plough 24 cm and 17 cm. With 47917 and 50833 CFU/g, respectively, this is more than a seven fold increase compared to the moldboard plough 30 cm treatment (6667 CFU/g soil). The incidence of *Fusarium* spp. was not influenced by the year and there was no interaction between treatment and year.

In sole consideration of the deoxynivalenol producing *Fusarium* species *F. culmorum* and *F. graminearum* (averaged data for 2000 and 2001) the highest isolation frequency was also determined in chisel plough 24 cm and 17 cm treatments (Table 1).

Twenty *Fusarium* species were identified during the two years. The most frequent species, *F. equiseti* (31.5% of total *Fusarium* isolates), *F. oxysporum* (13.1%), *F. merismoides* (10.3%), *F. solani* (9.6%), *F. graminearum* (7.6%), *F. culmorum* (5.9%), *F. sporotrichoides* (3.7%), *F. sambucinum* (1.9%), *F. tricinctum* (1.7%), *F. poae* (1.2%), *F. avenaceum* (1.0%), *F. proliferatum* (1.0%), *F. sacchari* (0.8%), *F. verticilloides* (0.5%) and *Fusarium* spp. (10.2%) are listed in Figure 4. Additional *Fusarium* spp. identified included *F. aquaeductuum*, *F. bactridioides, F. crookwellense, F. flocciferum, F. graminum, F. semitectum* and a few species of uncertain identity. The distribution of species showed major differences between the two trial years and between the tillage treatments. In 2000, more *Fusarium* species were isolated from soil. A higher diversity of Fusarium species was found in conservation tillage plots (chisel plough, rotary tiller) than in the moldboard plough-based tillage plots. In 2000 the predominant *Fusarium* spp. isolated from soil were *F. equiseti* (16.5%), *F. graminearum* (14.2%), *F. merismoides* (11.2%) and *F. culmorum* (10.1%). In 2001 there was a change of the predominant species to *F. equiseti* (47.3%), *F. oxysporum* (16.9%), *F. solani* (14.9%) and *F. merismoides* (9.3%).

In 2000 when wheat was grown the deoxynivalenol producing species *F. graminearum* and *F. culmorum* were found in soil from most plots. In 2001 when maize was cultivated these same *Fusarium* species were isolated in only four plots. Nevertheless, DON could be detected in grain collected from most plots. A correlation between DON-producing *Fusarium* species (CFU/g soil) and DON-content of wheat or maize grain could not be observed (Figures 5 and 6).

Discussion

Tillage and stubble management may have significant short-term as well as long-term effects on soilborne fungi. In this study we utilised rotation sequences and tillage treatments that were applied for the last twenty years. We determined that both the total number of microbial colonies and of *Fusarium* species was significantly affected by the sampling year. In addition to the total number, the distribution of individual *Fusarium* spp. across both years was clearly different in our study. A similar change in frequency of isolation of *Fusarium* species in succeeding years is also

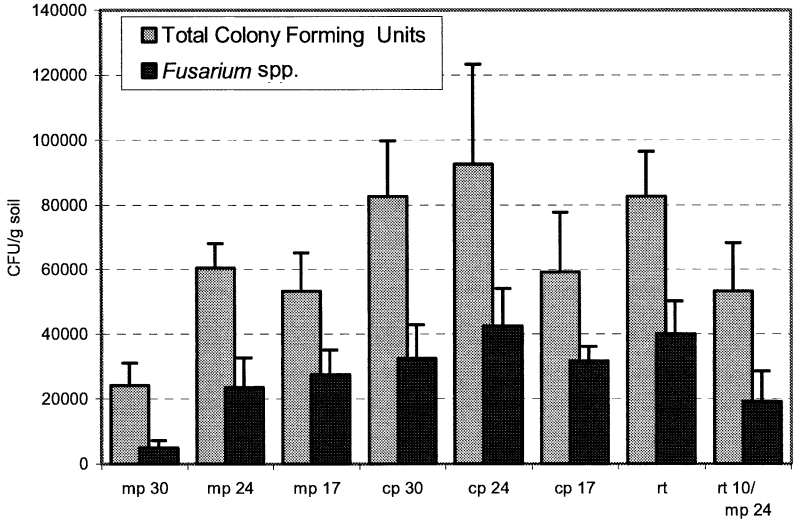

Figure 3. Isolation frequency of total colony forming units and *Fusarium* spp. under different tillage treatments in maize 2001 (mp = moldboard plough, cp = chisel-plough, rt = rotory tiller; 30, 24, 17 = depth of treatment in cm).

documented for *Fusarium* spp. causing stalk rot and grain infection in maize (Lew et al., 2001; Lipps and Deep, 1991). The clear differences observed between and within the plots in our study could have been influenced by climatic factors. Environmental conditions, such as climate, vegetation, soil moisture, soil type, fertility are known to affect microbial soil populations and especially the range of *Fusarium* isolated from soil (Bockus and Shroyer, 1998; Burgess, 1981). In this regard Cook (1973, 1981a) reported that *Fusarium* spp. survive better in soil in dry than in wet conditions and may prefer the combination with higher temperature. In our study the weather conditions were clearly different in both years. Soil-sampling was done in June 2000 and in October 2001. The year 2000 was extremely warm. In particular the mean temperature between February and June was 2.3 °C to 3.7 °C above average. The mean precipitation between January and March was 135% to 265%, between April and June 41% to 80% of the 30-yr monthly average. By comparison in 2001 the mean temperature in March, May and October was clearly higher than normal (2.3 °C to 3.1 °C) and lower in April, June and September (−1.0 °C to −2.6 °C). The highest precipitation in the mean growing period was measured in April (153% of the 30-yr monthly average), August (169%) and September (144%). Isolations from roots of wheat and maize and additional sampling dates are required to further clarify the effect of climatic factors and crop species on Fusarium populations.

The number of total colony forming units was higher in 2000 when wheat was grown than in 2001 when maize was grown. Moldboard plough treatments resulted in a lower number of CFU/g soil than chisel plough treatments and rotory tiller treatments. In contrast to the number of total colony forming units there were differences in isolation frequency of *Fusarium* spp. between both years in relation to the tillage treatments. In fact moldboard plough treatments resulted in a lower number of *Fusarium* spp. in soil than chisel plough treatments and rotory tiller treatments. But moldboard plough based plots were also more likely to show higher populations in 2001 than in 2000, whereas chisel plough 24 and 17 cm plots showed higher populations in 2000 than in 2001. This may have been a reflection of the incidence of individual *Fusarium* species in these plots across both years. Regrettably, the trial site reported in our study did not include a no-till treatment. The different range of *Fusarium* spp. between the individual tilled plots may be due to different abilities for survival of the species in different soil layers. *Fusarium* spp. can survive for periods of at least two years up to 10 years under suitable conditions (Burgess et al., 2001; Garrett, 1970).

Twenty *Fusarium* species were identified in the 2 years of this study. These *Fusarium* spp. are typical representatives of *Fusaria* in soil (Burgess, 1981; Domsch and Gams, 1970; Rodriguez-Molina et al., 2000) and correspond to the predominate *Fusarium* species investigated in Austrian grain (Adler

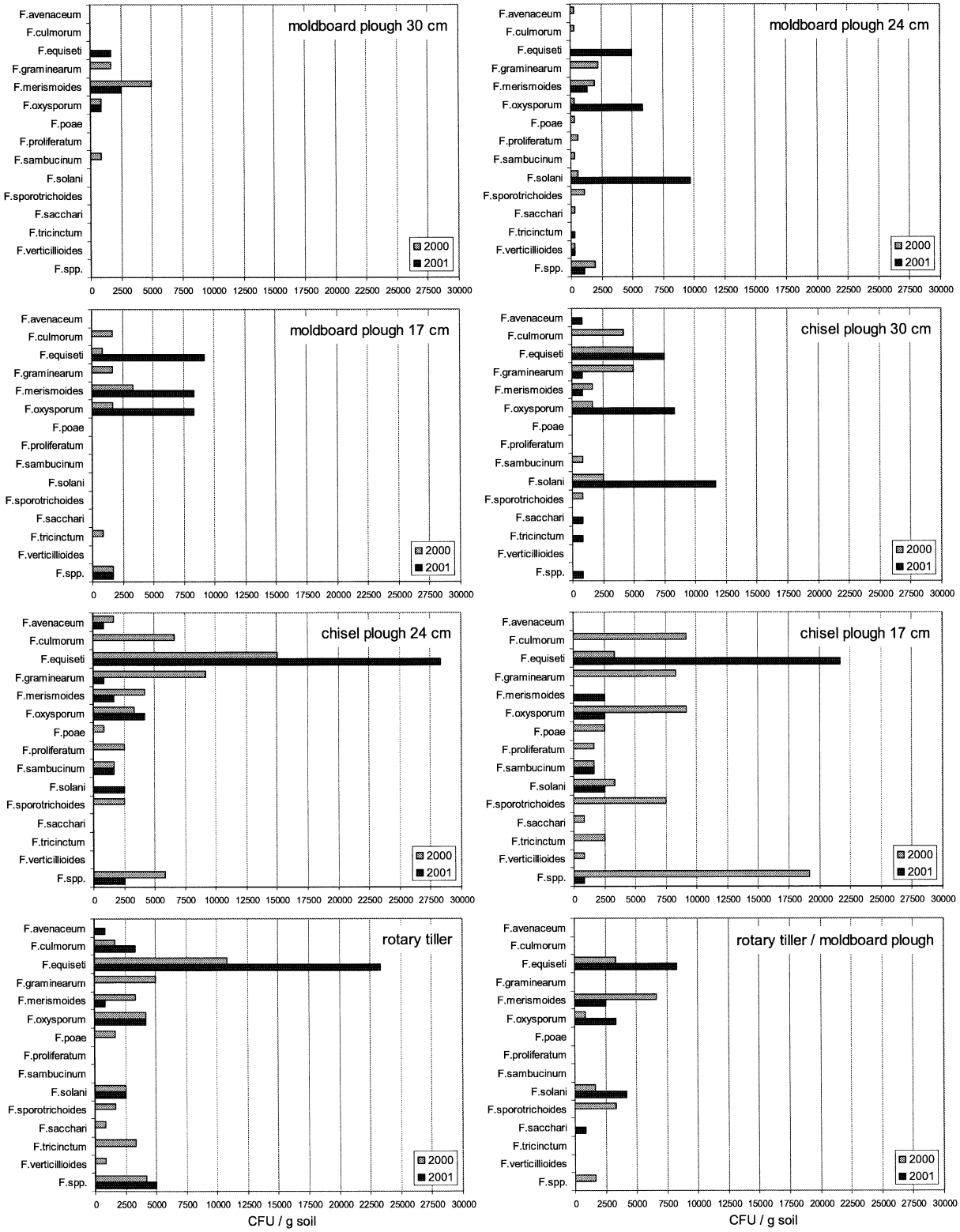

Figure 4. Diversity of *Fusarium* spp. under different tillage treatments.

Table 1. Effect of tillage on the incidence of CFU/g soil; means of 2000 and 2001 assessments

Tillage	total colony forming units*	*Fusarium* spp.	*Fusarium culmorum* & *Fusarium graminearum***
moldboard plough 30 cm	49583 a	6667 a	833
moldboard plough 24 cm	64166 ab	16944 ab	1250
moldboard plough 17 cm	66250 ab	19583 bc	1667
chisel plough 30 cm	80833 b	27083 bc	5000
chisel plough 24 cm	101250 b	47917 d	8333
chisel plough 17 cm	85833 b	50833 d	8750
rotary tiller	96667 b	40000 cd	5000
rotary tiller/moldboard pl. 24 cm	80417 b	18333 ab	0
F-ratio tillage	2.48 ($P = 0.030$)	6.03 ($P = 0.000$)	
F-ratio year	16.44 ($P = 0.000$)	0.09 ($P = 0.768$)	
F-ratio tillage*year	1.20 ($P = 0.321$)	1.31 ($P = 0.268$)	

ANOVA for SQRT(1/2+x); means followed by different letters are significantly different
*fungal and bacterial colonies
**data inhomogenicity; transformation for *F. culmorum* & *F. graminearum* has been found not feasible

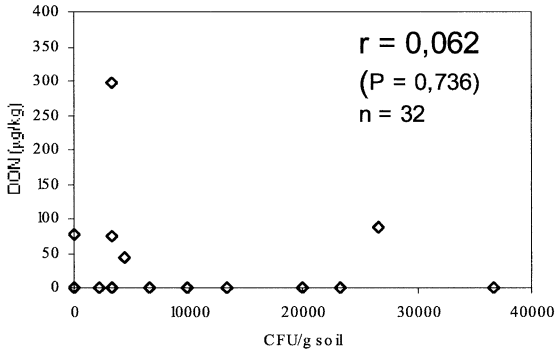

Figure 5. Correlation between DON-producing *Fusarium* spp. (CFU/g soil) and DON-contamination (μg/kg) of winter wheat 2000.

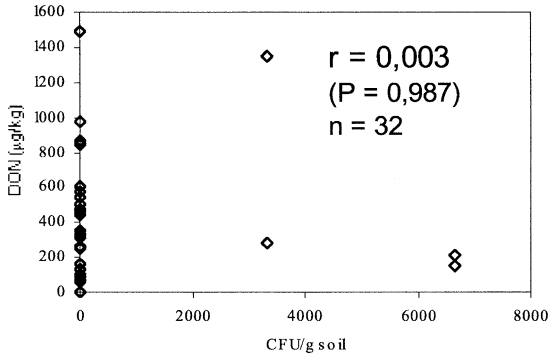

Figure 6. Relationship between DON-producing *Fusarium* spp. (CFU/g soil) and DON contamination (μg/kg) of maize 2001.

et al., 1990; Lew et al., 2001, Shala-Mayerhofer and Glauninger, 2002). The chosen soil plate method enables investigations of soil samples on a big scale (Parkinson, 1973), but it has to be considered that the recovery of soilborne fungal species is influenced by sampling procedure, transit and storage conditions, modes of persistence of the fungus and isolation technique (McMullen and Stack, 1983; Windels, 1992).

In our study, while the moldboard plough-treatments generally resulted in a lower diversity of *Fusarium* species than the chisel plough and rotary tiller-treatments (Figure 4), this was in contrast with the moldboard plough 24 cm treatment. Different degrees of weed infestation as a result of tillage treatment might have contributed to this. For example, moldboard plough 24 cm plots clearly showed a higher infestation with *Cirsium arvense* (Glauninger et al., 2002). Besides the tillage system, the tillage depth seems to play a decisive role for the presence of *Fusarium* spp. in soil in our study. The deeper the tillage the lower was the isolation frequency and the diversity of total colony forming units and *Fusarium* spp. in the upper 10 cm soil layer. Furthermore, tillage method and depth have an effect on the shift in the *Fusarium* spp. Both quality and quantity of crop residues entering the soil influence soil microorganisms and microbial processes in soil (Kandeler et al., 1999). Liebhard (1993a) has shown that non-turning tillage systems result in an increase of organic matter and that the humus content decreases with increasing tillage depth. Thus, microbial biomass is higher in the top soil layer of reduced tillage plots than in plough based tillage plots (Kandeler and Böhm,

1996). Furthermore, microbial degradation of crop residues could be of considerable importance in relation to the results of the present study. Yi et al. (2002) showed that decomposition of crop residues decreases with increasing incorporation depth, associated with a decline in CFU and *Fusarium graminearum*. Findings by Rodriguez-Molina et al. (2000) also indicated that population densities of *Fusarium* spp. decrease with increasing soil depth. The persistence of *Fusarium* spp. in the upper soil layer might be favoured, as it is linked to the existence of plant debris and humus (Nash et al., 1961). In addition, the tillage system and the tillage depth at our trial site may have influenced soil factors such as texture (Liebhard, 1993a), the availability of nutrients (Liebhard, 1993b), soil density, pore volume, pore size (Liebhard, 1994) and soil water supply (Liebhard et al., 1994). Such physiochemical characteristics of soil fundamentally affect the activity, ecology and population dynamics of micro-organisms in soil (Stotzky, 1997). Further studies are needed for a better understanding of these interactions.

Although data of the deoxynivalenol producing *Fusarium graminearum* and *F. culmorum* (Thrane, 2001) are not homogenous and therefore were not statistically analysed (Table 1), they suggest a lower population of these species after stubble incorporation by moldboard-plough. Both years showed clear differences in the detection of the two deoxynivalenol (DON) producing species *F. graminearum* and *F. culmorum* and the DON-contamination of grain. In 2000 these *Fusarium* species were found in soil from most plots and DON-contamination of wheat could be detected in most plots. In 2001 these *Fusarium* species were isolated only in a few plots, but DON-contamination of maize was detected in most of the samples. While the recommended maximum level (0.5 mg/kg) for contamination with DON (Lew et al., 2001) was never reached in our wheat samples, several maize samples exceeded this limit. Our results show that a higher population of *F. graminearum* or *F. culmorum* in the soil is not linked with a higher DON-contamination of grain, there being no correlation between the presence of mycotoxin producing species in soil and the DON-content of grain. The origin of *Fusarium* infections are predominantly crop residues on the soil surface, where *F. graminearum* produces sexually perithecia or asexually sporodochia and where *F. culmorum* sporulation is entirely asexual (Cook, 1981b). Organic soil matter with mycelium and chlamydospores in soil are considered to be only of minor importance in relation to inoculum source (Obst and Paul, 1993). Accompanying studies on *Fusarium*-infestation of wheat and maize on this trial site (Steinkellner et al., 2002) have shown a significantly higher incidence of *Fusarium* head blight in chisel ploughed plots than in all other plots (disease incidence ranged from 2.3% to 8.7%) and no effect of tillage on ear rot (disease incidence ranged from 6.5% to 8.5%). The wheat and maize cultivars used for this study are rated as moderately susceptible to *Fusarium*-infestations. The results also have shown no correlation between disease incidence and DON-contamination of grain. This is not surprising, for as mentioned by Gang et al. (1998), Mesterházy et al. (1999) and Walker et al. (2001), the DON content depends on the capacity of fungal strains for DON production. Furthermore climatic factors might have an important influence on DON-contamination. Findings by Mesterházy et al. (1999) have shown that DON-contamination changes more extensively than *Fusarium* infection with the year of testing.

Our results contribute to the understanding of the interaction of tillage and *Fusarium* spp. in soil. Mouldboard ploughing reduces the *Fusarium* populations in soil and especially results in a lower occurrence of pathogenic species, even though the population of *Fusarium* spp. in soil is not a quantitative indicator for *Fusarium* infection and DON contamination of the grain. Beyond this, tillage treatments have an impact on the activity, ecology and population dynamics of micro-organisms in soil and can also affect the development of plant pathogens.

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