FINAL PROJECT REPORT

Increasing atmospheric CO_2 concentration not only has a direct impact on plants but it also affects plant-pathogen interactions, which have gained growing attention in the last years due to economic and health-related problems. Special concern was given thus in the present work to the interaction between powdery mildew and wheat, and the toxigenic fungus, *Fusarium spp.* and wheat; grown under elevated CO_2 level (EC) to assess disease severity changes and food safety risks under future environmental conditions.

Task. 1.1 *Studies for comparative testing of the aggressiveness of various powdery mildew (PM) pathotypes on wheat varieties with different genetic backgrounds, and on the parents and tolerant and susceptible lines of the mapping population, at ambient and doubled CO_2 level. Studying of the impact of interaction between PM infection and CO_2 level on plant resistance characteristics, biomass production and grain yield components.*

A series of experiments were conducted using pathotypes R51 and R76 for artificial infection of 7 winter wheat varieties differing in their resistance to PM (Bezostaya-1, Ukrainka, Apache, Libellula, Mv Regiment, Mv Mambo, Mv Emma). PM inoculation was performed in the tillering stage (at week four) with a known density of conidia on plants grown at either ambient (400 ppm) or elevated (750 ppm) CO_2. Plants inoculated with the two races were separated in different phytotron chambers so that they should not become superinfected by the other race and no natural infection could interact with the treatments.
The disease progress was monitored throughout the experiment and disease progress curves were determined. Responses in photosynthetic processes (net assimilation rate, stomatal conductance, transpiration, effective quantum yield and leaf temperature) to pathogen attack were recorded using a CIRAS-2 portable photosynthesis system (PP Systems, USA). After physiological maturation, the plants were harvested and analysed for morphological, biomass and yield parameters.
For investigations of the mapping population Ukrainka×Mv Hombár, 15 genotypes were used; besides the parent Ukrainka and two types of the other parent, Mv Hombár, 6 tolerant and 6 susceptible lines were selected for the tests. The disease severity of PM infection and the response of different lines in plant biomass were studied in the greenhouse. The plants were grown at either ambient or elevated CO_2 levels. Inoculation was performed with a mixture of PM races (R51+R76) while a natural infection process took also place. Three weeks after inoculation, separate powdery mildew conidia were isolated from the plants of all the genotypes in both greenhouse chambers so that the composition of the PM races could be determined with the Nover differential set.

The results of the identification of the pathotypes showed that R76 was prevalent at the chamber with ambient CO_2 level, while pathotype R51 was predominant at the elevated CO_2 chamber. At both chambers, the pathotypes R47 and R52 were also present at a frequency of 5-5%.
Elevated atmospheric CO_2 level resulted in higher plants, increased total above-ground biomass, enhanced amount of stems and leaves in the PM infected plants. However, neither the tiller number nor the plant grain yield changed significantly in response to high CO_2 compared to the data at the ambient level.
The results showed minor effects of elevated CO_2 on the total plant infection coverage (% of leaf area) in the tests of the mapping population. The resistant parent, Mv Hombár and line R3 did not become infected at any chambers at either CO_2 levels. Among the more tolerant genotypes, there was only a significant difference, on certain sampling days at least, between

the two chambers in two lines; R14 was infected more at high CO_2, while R84 at ambient CO_2. Among the more susceptible genotypes, 3 lines were found to be infected significantly less, and two lines significantly more infected in the chamber with CO_2 enrichment.
The results of the experiments testing separate PM isolates showed that Ukrainka was the only from all the varieties, which exhibited markedly different types of reaction to the two pathotypes. Although at the first period (7-14 days after inoculation) the infection rate was promoted by CO_2 enrichment, from day 24 on, R51 was more virulent, while R76 became less virulent at high CO_2. These findings also explain the results presented above in the Ukrainka×Mv Hombár mapping population, where both types of reaction were recorded, in certain genotypes, similarly to the Ukrainka parent.

Genetic analyses of the Ukrainka×Mv Hombár mapping population provided four QTLs related to powdery mildew resistance originating in Mv Hombár which has an excellent field resistance prevailing for over a decade. These QTLs are located in chromosomes 2A, 1A, 2B and 2D. The QTL identified on the long arm of chromosome 2A proved to be a new major powdery mildew resistance gene. This research gave an important part of the body of PhD dissertation by Judit Komáromi (2016) and provided a basis for the paper of hers and co-workers (Komáromi J, Jankovics T, Fábián A, Puskás K, Zhang Z, Zhang M, Li H, Jäger K, Láng L, Vida G: Powdery Mildew Resistance in Wheat Cultivar Mv Hombár is Conferred by a New Gene, PmHo. PHYTOPATHOLOGY 106:(11) pp. 1326-1334. 2016, IF= 3.119)

Elevated CO_2 level resulted in a different reaction of PM severity of wheat varieties to the two pathotypes (Figure 1). R51 was more virulent at high CO_2 (Bezostaya-1, Ukrainka, Apache, Libellula, Mv Mambo and Mv Emma), while the infection rate of R76 was usually lower or very similar to that at ambient CO_2 (Ukrainka, Apache, Mv Emma, and except days 50 and 60: also Bezostaya-1 and Libellula). The resistant variety, Mv Regiment, however, did not show any disease symptoms at either CO_2 levels.
Despite the fact that pathotype R51 has the most virulence factors, infecting all of the 8 wheat genotypes used in the Nover differential set for identification of PM pathotypes, and R76 infects 7 of 8, R76 caused usually more severe initial disease symptoms at ambient CO_2 level than R51 (Bezostaya-1, Ukrainka, Libellula, Mv Mambo, Mv Emma). In some genotypes, however, R51 took over the leading role of R76 later on. At elevated CO_2, R51 was more successful in certain genotypes (Ukrainka, Apache, Mv Emma), while in Bezostaya-1 and Libellula it was similar to R76.
During the progress of PM infection, the stomatal conductance of infected leaves of wheat varieties increased in general, at both CO_2 levels. Whether the increase was higher or lower at high CO_2, depended on the genotype.
The grain yield of wheat varieties were fairly unchanged by the PM infection, there was only a significant decrease in two varieties, in Bezostaya-1 (-36%) and Mv Mambo (-32%), as a result of infection with R51 at high CO_2.

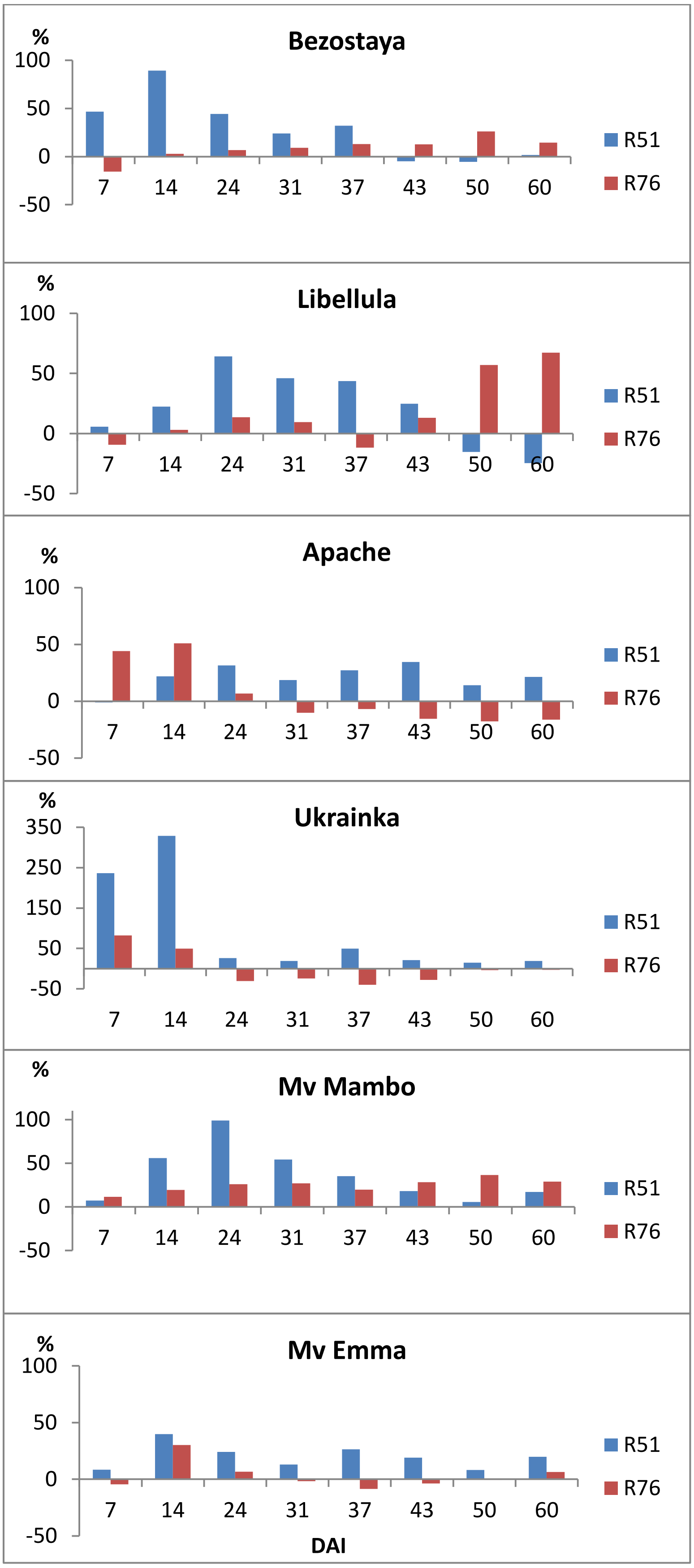


Figure 1. Change in the infection severity of wheat varieties, caused by R51 and R76 in response to elevated CO_2 level (% of the value at NC). *DAI: days after inoculation.*

Task 2.1 *Studying of the impact of doubled CO_2 in the phytotron on the Fusarium head blight (FHB) resistance of wheat varieties with different genetic background and various levels of disease resistance.*

Three winter wheat varieties (Mv Regiment, Mv Emma, Ukrainka, Mv Mambo, Apache) were grown in the phytotron in pots at either ambient (NC, 400ppm) or elevated (EC, 750 ppm) CO_2 level. Two kinds of tests were performed either to examine the resistance against the spread of the fungus from an inoculated spikelet (Type II resistance) or to test the combined resistance to the penetration and spread of the pathogen (Type I+II resistance, Schroeder and Christensen 1963). In the case of single floret inoculation (SFI), one spikelet (usually the 6th–8th from the top of each spike) was injected with a pipette at anthesis with 5 μl inoculum (500,000 conidia mL^{-1}), while in the whole spike inoculation (WSI), a suspension of spores (50,000 conidia mL^{-1}) was sprayed along the entire surface of each spike at anthesis. Inoculated heads were covered with plastic bags for 48 hours to promote the successful establishment of the fungus. The disease progress was monitored from day7 (D7) till day30 (D30), via the visual determination of the number of infected spikelets in each infected spike. The area under the disease progress curve (AUDPC) was calculated from the data according to Campbell and Madden (1990).

The results of the visual evaluation of the disease symptoms showed that infection was more severe in the case of whole spike inoculation than in the case of single floret inoculation in Mv Regiment while in Mv Emma, the infection in SFI at EC was as severe as in WSI at both CO_2 levels (Table 1). Also the AUDPC values exhibited a similar trend. Spike grain yield was usually negatively affected by the more severe infection due to less number of grains which were also smaller. Mv Regiment, however, reacted more with a decrease in the grain number than in the grain size in contrast with Mv Emma.

Table 1. Mean values of the yield components and the records of the disease progress

	GN	GY	TKW	D4	D7	D9	D11	D14	D16	D18	D21	D23	D25	D28	D30	AUDPC
Mv Regiment																
F1 NC	43.4	0.5	10.5	1.0	1.6	3.1	4.7	6.4	8.3	9.9	11.6	12.1	12.4	12.5	12.5	211
F1 EC	40.9	0.4	10.8	0.9	2.0	3.7	5.0	7.5	9.4	10.7	12.1	12.8	13.0	13.5	13.6	228
F2 NC	25.5	0.2	9.3	5.4	7.1	8.9	10.1	11.0	12.2	13.1	13.6	13.9	14.1	14.1	14.1	311
F2 EC	29.2	0.2	8.0	4.5	7.4	9.5	10.3	11.1	12.1	12.8	13.3	13.8	13.9	14.1	14.1	307
Mv Emma																
F1 NC	39.9	0.7	15.8	1.4	3.2	5.1	7.0	8.9	10.9	13.1	15.3	17.1	18.1	18.6	18.8	300
F1 EC	42.0	0.4	8.0	4.1	7.3	10.3	13.1	15.6	17.0	17.2	17.3	17.4	17.5	17.5	17.5	385
F2 NC	33.0	0.3	7.1	4.3	7.5	10.7	13.2	15.6	16.2	16.8	17.3	17.4	17.5	17.6	17.6	385
F2 EC	37.6	0.3	7.6	4.2	7.4	10.5	13.1	15.6	16.6	17.0	17.3	17.4	17.5	17.5	17.5	385

GN = grain number per spike, GY = grain yield per spike, TKW = thousand kernel weight, D4-D30 = 4-60 days after inoculation, AUDPC = area under the disease progress curve. F1 = single floret inoculation, F2 = whole spike inoculation

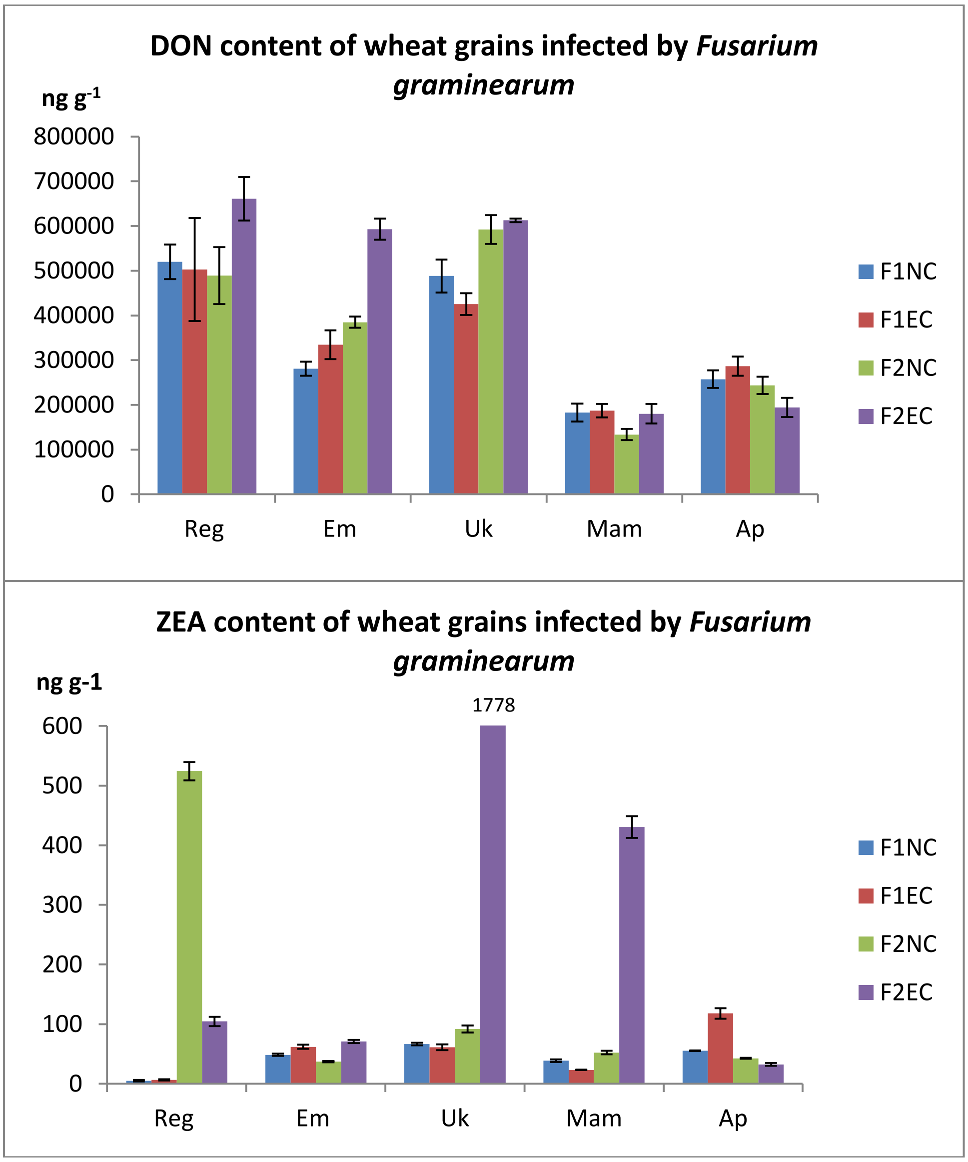


Figure 2. Impact of elevated CO_2 level on the *Fusarium* toxin content of wheat grains infected by *Fusarium graminearum*

Varieties: Mv Regiment (Reg), Mv Mambo (Mam) Mv Emma (Em), Apache (Ap) and Ukrainka (Uk), toxins: deoxynivalenol (DON) and zearalenone (ZEA)
F1 = single floret inoculation, F2 = whole spike inoculation. NC = ambient CO_2, EC= elevated CO_2 level

The results indicate that there is large variation in the resistance to mycotoxin production (Type III resistance) between the varieties (Figure 2) as the range of grain DON content was more dependent on the variety than on the environment or the inoculation treatment, although these latter two parameters also had some influence on the toxin production. The most resistant variety in that sense was Mv Mambo, followed by Apache, while Mv Emma, Mv Regiment and Ukrainka were more in the similar range. Although the ZEA value was high enough in the samples to be harmful even in the lowest quantities, the extreme values of this toxin were found more due to the circumstances (which were sometimes even unknown) than to the genotypes. DON content increased significantly in response to EC in three varieties (Mv Regiment, Mv Emma and Mv Mambo) in WSI, and decreased in one variety, Apache.

ZEA content increased at EC in Mv Emma, Ukrainka, Mv Mambo, while it was lower in Mv Regiment and Apache.

Task 1.2. *Phytotron experiments to evaluate the combined effect of drought stress and doubled CO_2 on the PM resistance of wheat varieties with various levels of drought tolerance and disease resistance.*

Four winter wheat varieties (Bezostaya-1, Mv Mambo, Apache and Libellula) were grown in pots in growth chambers either at ambient or elevated CO_2 levels. The six-week old plants were infected at normal water supply (NW, 20-30% soil volumetric water content), mild (MS, 10-15% VWC), or severe drought stress (DS, 5–8%) levels with the inoculum of R76 powdery mildew (PM) pathotype. The stomatal conductance, leaf temperature, net assimilation rate and chlorophyll fluorescence induction parameters were measured, and the development of the visual symptoms were monitored during the disease progress. Plant samples were collected before infection and after the heading of each variety, to determine the antioxidant enzyme activity changes and photosynthetic pigment composition, protein, soluble carbohydrate and starch content of the leaves. After maturation, the plants were harvested and the biomass and yield parameters were determined.

The overall infection rate at normal water level was significantly lower in two varieties (Mann-Whitney test), in Mv Mambo and Libellula, in response to elevated CO_2 level according to the AUDPC (area under the disease progress curve) values (Figure 3).

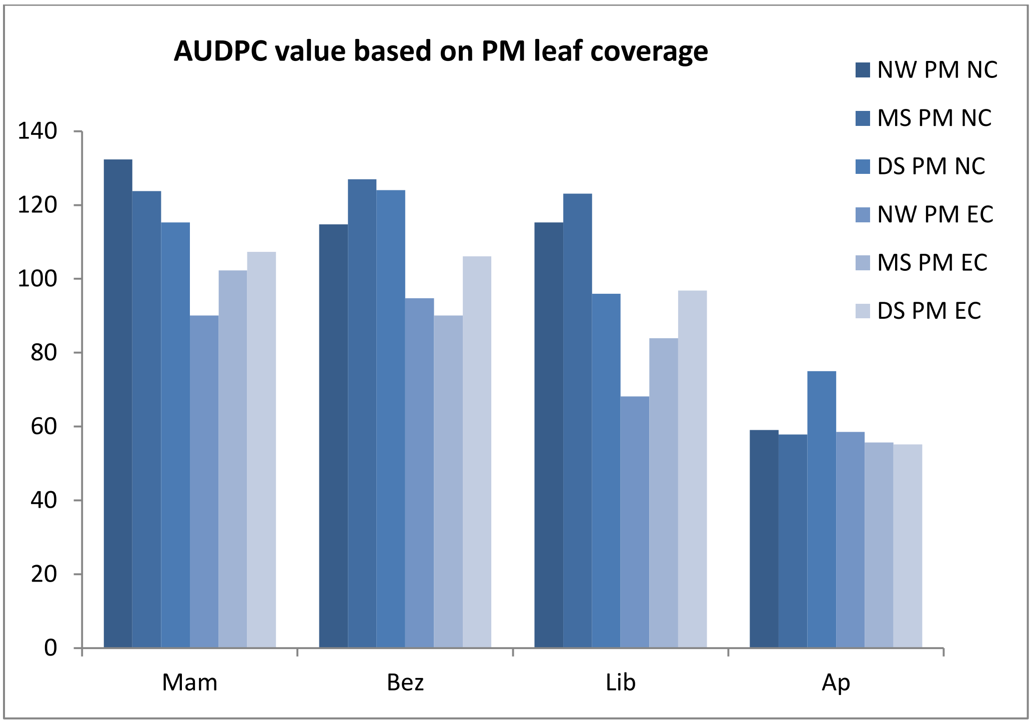


Figure 3. Combined effect of elevated CO_2 level and drought on the powdery mildew infection of wheat.

NW = normal water supply, MS = mild drought stress, DS = severe drought stress, NC, EC =ambient and elevated CO_2, Mam= Mv Mambo, Bez = Bezostaya-1, Lib =Libellula, Ap =Apache

At ambient CO_2 level, in the early phase of infection (14 DAI), mild stress induced only a significant increase in the infection level in Bezostaya-1, while severe drought (DS) made only one variety, Apache, more prone to powdery mildew (Figure 4). At elevated CO_2, Mv Mambo, Bezostaya-1 and Apache had a lower rate of initial infection due to mild or severe drought. The AUDPC values showed that severe drought resulted in a decrease in the overall infection severity in Mv Mambo and Libellula, caused an increase in Apache, while there was no significant change in Bezostaya-1 at ambient CO_2. At elevated CO_2, drought caused higher infection severity in Libellula (Figure 4), but no significant change was recorded in the other varieties.

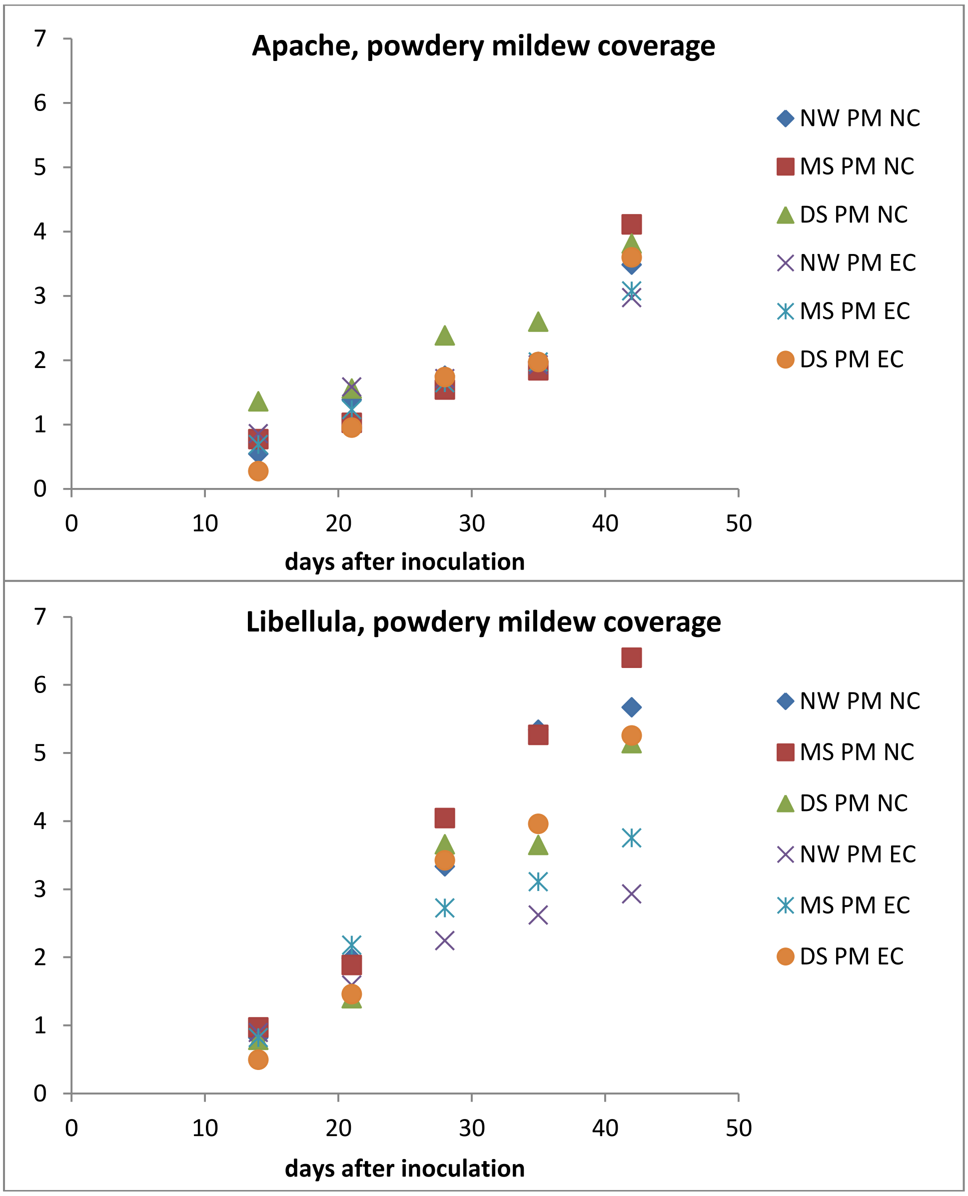


Figure 4. Impact of the water supply and CO_2 level on the powdery mildew infection of wheat.
Explanations as in Figure 3.

Photosynthesis measurements revealed a stimulation of the net assimilation of the flag leaves of the infected plants compared to uninfected plants (Figure 5).

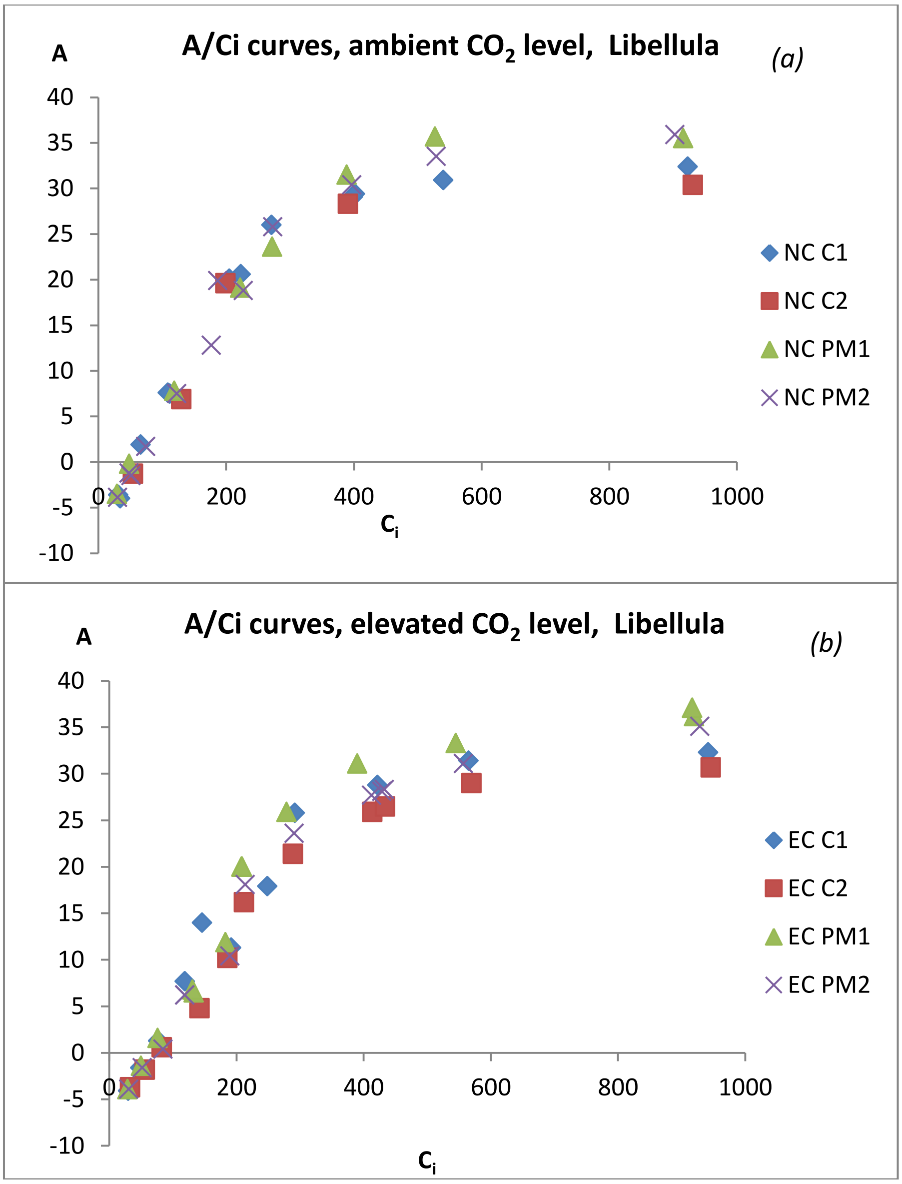


Figure 5. CO_2 response curves of Libellula at ambient (*a*) and elevated (*b*) CO_2 levels.
A = net assimilation (µmol CO_2 s^{-1} m^{-2}), Ci = intercellular CO_2 (µmol mol)
C1, C2 = control (uninfected) plants, PM1, PM2 (plants infected with powdery mildew.

In the period of infection, 6 weeks after planting, drought stress raised the activities of all the antioxidant enzymes (GR – glutathione reductase, APX – ascorbate peroxidase, GST – glutathione-S-transferase, CAT – catalase and POD – guaiacol peroxidase) in Libellula. In Mv Mambo, APX, CAT and POD was increased while GR and GST decreased under mild or severe drought. In Apache and Bezostaya-1, however, the change of the antioxidant enzyme activities depended on the interaction between the CO_2 concentration and the water stress level. In the period after heading, all antioxidant enzyme activities were upgraded by PM infection at either CO_2 level.

The protein content of leaves (DW^{-1}) was found to change in response to drought stress depending on the variety. In Bezostaya-1, there was no significant change at ambient CO_2 level (NC), while at elevated CO_2 (EC), under mild stress (MS) there was a decline in the leaf protein content while strong drought (DS) resulted in a more moderate decrease. In Mv Mambo, drought resulted in a parallel decrease in leaf protein at both CO_2 levels. There were, however, lower protein values at EC than at NC in every treatment (including the control). In Libellula, drought also caused a decline, which was significant already under mild stress at NC, while at EC only under severe drought. In Apache, an increase was found in the leaf protein content under severe drought at NC, while a decrease was recorded under mild stress at EC.

Drought stress usually resulted in increased accumulation of soluble carbohydrates at both CO_2 levels in all the varieties (with an exception of Apache at NC). The increase in soluble carbohydrate content was more pronounced at elevated CO_2 in Bezostaya-1 (MS, DS), Libellula (normal water supply, NW; DS) and in Apache (MS) but there was no difference in the content of soluble carbohydrates between the CO_2 levels in Mv Mambo.

Higher starch content was also found due to drought in three of the four varieties but in Apache, severe drought resulted in a decline at NC. The total carbohydrate content of the leaves increased in response to drought in Mv Mambo and Libellula at both CO_2 levels, in Bezostaya-1 at EC, while in Apache, drought stress (both mild and severe) caused a decline in the total carbohydrate accumulation at NC.

Drought stress induced a decline in the chlorophyll-a, chlorophyll-b and total chlorophyll content of the shoot of all the varieties, at both CO_2 levels. The chlorophyll a/b ratio, being a very conservative trait, was not affected by drought, however it decreased in response to elevated CO_2 under mild and severe drought in Bezostaya-1 and Mv Mambo.

Task 2.2 *Studying of the impact of doubled CO_2 and water supply levels in the phytotron on the Fusarium head blight (FHB) resistance of selected wheat varieties.*

Mv Emma and Mv Regiment plants were grown in phytotron chambers at ambient or elevated CO_2 levels. Inoculation with FHB was carried out on plants under normal water supply level or under drought as described before. The disease progress was monitored until maturation after which the infected spikes were harvested and analysed for yield components.

Based on the statistical analyses performed with Mann-Whitney test on the data, it was found, that in Mv Regiment, in the SFI treatment at NC, drought resulted in lower infection severity regarding both the daily records (D11-D30) and the AUDPC values and also lower DON contamination of the grains. This lead to significantly higher grain number, TKW and grain yield per spike. At EC, the infection was less severe, similarly, and both the DON and ZEA contents decreased significantly. Grain yields were higher here, too, due to the above mentioned features. In WSI in this variety, drought had much smaller effect at NC, on the disease as there was only a decrease between D11-D14, significant only at the $p \leq 0,01$ level compared to normal water supply levels. At EC, drought caused lower disease levels (based on data of D16-D30 and AUDPC) resulting in higher spike grain yields.

In Mv Emma, drought had much less effect compared to Mv Regiment. In SFI at NC, there was only a significant decrease in the infection level in the early phase of infection, on D11. Although the DON content of the grains was less in drought stressed plants, there was no significant change in the yield components. At EC, infection was only lower significantly on D11 while DON concentration of the grains decreased. Grain number increased despite the fact that ZEA content increased. In WSI treatment in Mv Emma, there were only very slight differences at NC in infection severity (D28-D30, $p \leq 0,01$) due to drought, resulting in higher grain number but no change in mycotoxin contents, while at EC, there was no change in any of the above parameters except the grain ZEA content which was higher in response to drought.

Task 1.3. *Studying of the impact of the CO_2 and nutrient supply levels on the powdery mildew resistance of wheat varieties.*

Selected winter wheat varieties (Mv Mambo and Ukrainka) were grown in pots in growth chambers either at ambient or elevated CO_2 levels. Volldünger Linz nutrient solution was applied from week one till heading at V_1-low (1/2×), V_2-normal (1×) or V_3-high (1 ½×) levels, according to the instructions of the manufacturer. The four-week old plants were infected with the inoculum of R51 powdery mildew (PM) pathotype at ambient and doubled CO_2 level and various nutrient supply levels. The disease progress was monitored throughout the experiment and the disease progress curves were determined and the AUDPC (area under the disease progress curve) values were calculated.
The A/Ci curve, stomatal conductance (gs), net assimilation rate (Pn) and chlorophyll fluorescence induction parameters (Fs, Fm', and the calculated effective quantum yield of PSII) were measured using a CIRAS-2 portable photosynthesis system (PP Systems, USA) Estimated chlorophyll measurements were taken on intact plants with Minolta SPAD chlorophyll meter. Plant samples were collected before infection and after the heading of each variety, to determine the antioxidant enzyme activity and photosynthetic pigment composition, protein, soluble carbohydrate and starch content of the leaves.

Powdery mildew progress
V1, the low nutrient treatment resulted in a lower PM infection rate (Figure 6), which was already remarkable 14 and 28 days after infection (Mv Mambo and Ukrainka, respectively). However, the AUDPC values were not significantly different in the case of V1 and V2 for Mv Mambo, and V2 and V3 for Ukrainka. High nutrient level (V3) resulted in significantly higher infection levels than those at the low (V1) nutrient supply level under both the ambient and elevated atmospheric CO_2 concentrations.

Measurements of photosynthesis and chlorophyll content on intact plants
Photosynthesis was stimulated by the nutrient supply as P_N values increased gradually with the increase in the nutrient levels in both varieties at NC (Table 2 and 3). PM infection resulted in a significant decrease in the net assimilation at the ambient CO_2 level only in V2 treatment for Mv Mambo and V3 for Ukrainka, where these changes were also accompanied by a decrease in stomatal conductance and transpiration. The effective quantum yield of PSII did not change significantly in any nutrient treatment or variety. At EC, the impact of PM infection was even less on photosynthesis; lower values of P_N (in addition to decreased g_s and transpiration) were only detected in Ukrainka at the V1 nutrient level. A/Ci curves revealed no significant difference in the photosynthetic capacity in response to PM infection in both varieties at either CO_2 levels in week 8.

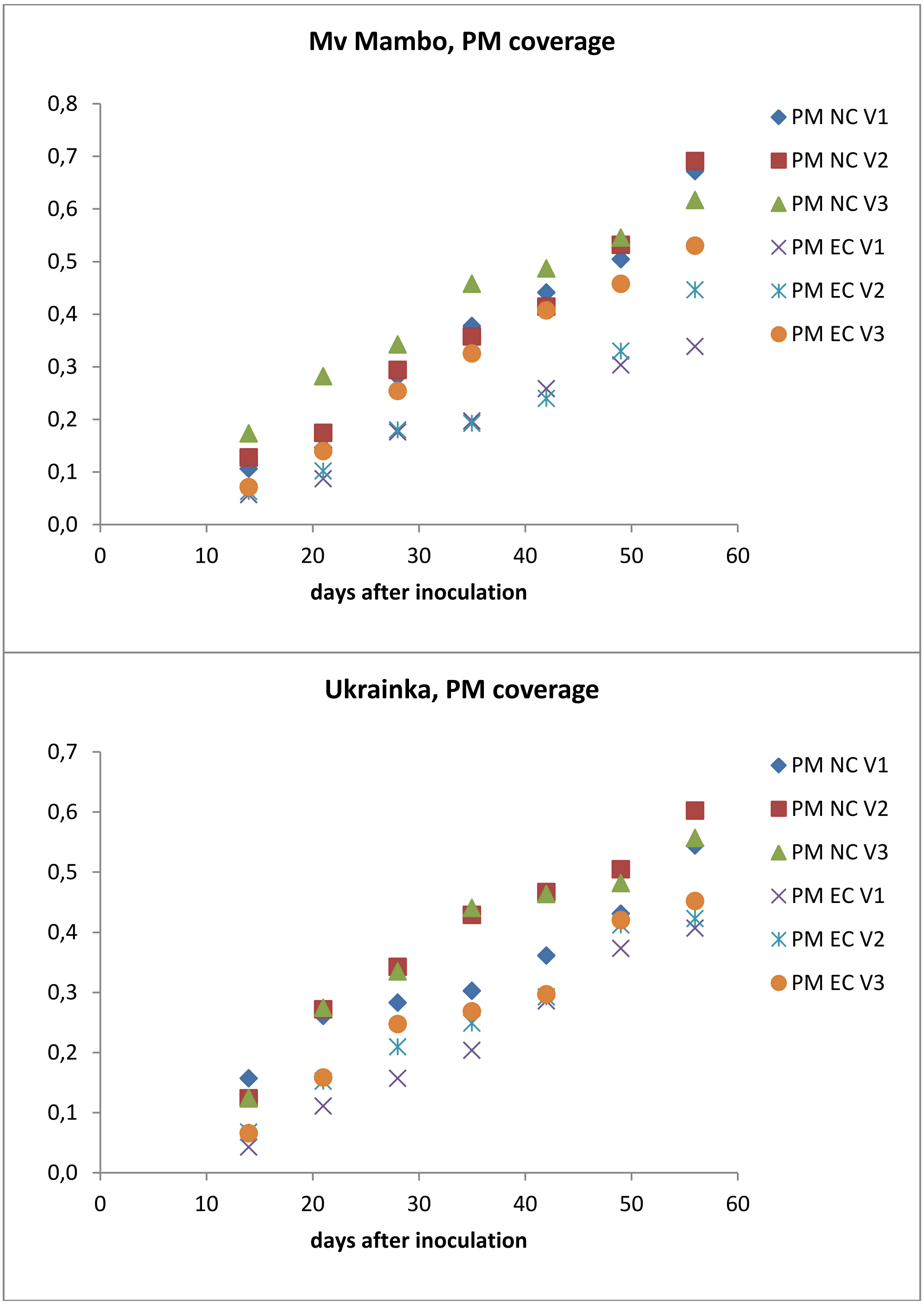


Figure 6. Combined effect of nutrient supply and CO_2 levels on powdery mildew infection in wheat

NC, EC =ambient and elevated CO_2, V1-V3 = nutrient supply levels

Table 2. Impact of elevated CO_2 and various nutrient supply levels on the photosynthetic parameters in week 8 after planting, Mv Mambo

	mean values						standard deviations					
Mv Mambo	**EVAP**	**GS**	**TL**	**CI**	**PN**	**Φ**	**EVAP**	**GS**	**TL**	**CI**	**PN**	**Φ**
NC												
V1 C	3.43	471	16.18	322.8	14.3	0.530	0.55	78	0.49	9.0	1.1	0.024
V1 PM	3.37	447	16.76	315.8	14.2	0.500	0.40	55	0.75	9.8	1.3	0.025
V2 C	3.19	505	16.73	318.1	14.8	0.519	0.32	65	0.29	4.2	0.3	0.022
V2 PM	2.71	373	16.60	309.3	13.8	0.492	0.19	33	0.25	5.8	0.4	0.012
V3 C	2.58	509	16.53	314.2	16.0	0.563	0.13	34	0.40	2.4	0.4	0.014
V3 PM	2.40	441	16.56	308.6	15.5	0.542	0.30	71	0.30	6.6	1.9	0.052
EC												
V1 C	2.10	393	16.50	621.9	19.2	0.539	0.32	115	0.50	20.6	1.4	0.033
V1 PM	2.14	361	16.81	618.5	19.6	0.534	0.17	19	0.42	4.7	0.5	0.004
V2 C	2.26	454	16.43	640.5	18.3	0.501	0.15	96	0.64	6.0	1.9	0.044
V2 PM	2.05	345	16.81	632.4	16.5	0.468	0.25	46	0.61	13.1	2.1	0.045
V3 C	2.83	462	16.38	634.3	19.0	0.538	0.35	124	0.33	18.5	1.1	0.024
V3 PM	2.37	402	16.38	633.5	18.1	0.510	0.11	67	0.82	14.9	1.0	0.027

EVAP= transpiration, g_s= stomatal conductance, T_L = leaf temperature, C_i =intercellular CO_2, P_N = net photosynthesis, Φ = effective quantum yield of PSII. NC, EC = ambient and elevated CO_2 levels, V1-V3 = nutrient supply levels, C = control, uninfected plants, PM = plants infected with powdery mildew

Table 3. Impact of elevated CO_2 and various nutrient supply levels on the photosynthetic parameters in week 8 after planting, Ukrainka

	mean values						standard deviations					
Ukrainka	**EVAP**	**GS**	**TL**	**CI**	**PN**	**Φ**	**EVAP**	**GS**	**TL**	**CI**	**PN**	**Φ**
NC												
V1 C	2.98	385	16.85	305.1	14.3	0.491	0.33	89	0.45	7.6	1.8	0.048
V1 PM	3.13	437	17.05	316.3	13.5	0.412	0.38	85	0.33	22.4	2.5	0.123
V2 C	2.90	450	16.38	313.6	15.8	0.543	0.49	111	0.33	15.9	0.8	0.015
V2 PM	2.75	434	17.18	306.8	15.6	0.545	0.15	32	0.39	4.2	0.6	0.017
V3 C	2.84	624	16.55	322.3	16.2	0.539	0.16	58	0.15	6.0	0.6	0.045
V3 PM	2.24	387	16.98	309.9	13.2	0.506	0.42	135	0.17	21.1	0.9	0.051
EC												
V1 C	2.32	459	16.56	632.0	20.2	0.534	0.24	82	0.45	12.4	1.2	0.025
V1 PM	2.00	304	17.13	608.6	18.2	0.452	0.27	60	0.18	21.7	1.3	0.073
V2 C	2.35	431	16.49	631.0	18.8	0.494	0.64	125	0.50	22.3	1.3	0.080
V2 PM	2.68	481	16.43	642.8	18.7	0.496	0.22	50	0.53	10.4	1.3	0.023
V3 C	2.59	471	16.47	644.5	17.8	0.493	0.43	108	0.31	17.6	0.8	0.143
V3 PM	2.72	426	16.33	626.4	19.3	0.427	0.23	81	0.50	8.4	2.2	0.080

For explanations see Table 2.

Measurements of photosynthesis and chlorophyll content on intact plants

Photosynthesis was stimulated by the nutrient supply as P_N values increased gradually with the increase in the nutrient levels in both varieties at NC (Table 2 and 3). PM infection resulted in a significant decrease in the net assimilation at the ambient CO_2 level only in V2 treatment for Mv Mambo and V3 for Ukrainka, where these changes were also accompanied by a decrease in stomatal conductance and transpiration. The effective quantum yield of PSII did not change significantly in any nutrient treatment or variety. At EC, the impact of PM infection was even less on photosynthesis; lower values of P_N (in addition to decreased g_s and transpiration) were only detected in Ukrainka at the V1 nutrient level. A/Ci curves revealed no significant difference in the photosynthetic capacity in response to PM infection in both varieties at either CO_2 levels in week 8.

In week 8, the chlorophyll content of the latest fully developed leaves was not significantly different in various nutrient treatments. There was no difference between the control and PM infected leaves at V1 level either. At V2 and V3, however, PM infection resulted in a slight decrease in the chlorophyll content in Ukrainka. In Mv Mambo, however, there was no change in chlorophyll due to PM infection. A similar trend was found also at EC in Ukrainka as PM infection caused a slight decrease in the leaf chlorophyll content at the V2 and V3 nutrient levels.

In week 11, the chlorophyll content of the flag leaf was lower in plants infected by PM in all the treatments in both varieties at NC, compared to uninfected plants. This was also true at EC (except for V3 in Ukrainka)

Analyses of leaf components

Based on the analyses performed on the collected leaf samples, it was found that elevated nutrient supply (V2, V3) resulted in a higher chlorophyll-a and total chlorophyll content in plants of Ukrainka at NC but no difference was found at EC and in Mv Mambo at either CO_2 levels in week four (at the time of PM infection). In the heading period, PM infection induced a significant decrease in the chlorophyll-a, chlorophyll-b and total chlorophyll contents and resulted in a lower chlorophyll a/b ratio in Mv Mambo in most treatments at both CO_2 levels. A similar trend was found for Ukrainka, except for the chlorophyll a/b ratio which decreased at NC but increased at EC in the V2 and V3 treatments.

In week four, at the time of PM infection, the protein content of the latest fully developed leaves was significantly raised by the increase in the nutrient supply in Ukrainka (V2, V3 at NC; V3 at EC) while in Mv Mambo, the leaf protein values were more stable, not affected by the nutrient supply. In the heading period, however, there were lower leaf protein values in Mv Mambo in plants infected by PM than in uninfected plants.

At the time of infection, the nutrient supply had no effect on the leaf soluble carbohydrate content of leaves and neither was there a clear tendency recorded for the leaf starch content in response to the nutrient treatments. In the heading period, however, a decrease in the soluble carbohydrate content was found in the infected plants in Ukrainka at NC (V2, V3) and at EC in V2, while an elevated soluble carbohydrate content was recorded in V3. In Mv Mambo, a considerable increase in the soluble carbohydrates was recorded in most treatments at both CO_2 levels, as a result of PM infection (except V3 at EC). PM infection induced higher shoot starch accumulation in both varieties at both CO_2 levels in most nutrient treatments (except for V2 and V3 in Ukrainka at NC, where a decrease was recorded in response to the infection with PM). Elevated CO_2 level also had a stimulating effect on the starch production in most treatments in both varieties.

Changes in the antioxidant enzyme system

In the four week old plants, just before PM infection, most enzymes had very low activities with the exception of glutathione-reductase (GR). In Ukrainka, even the activity of this enzyme exhibited no change in response to elevated CO_2 level, similarly to ascorbate peroxidase (APX), catalase (CAT) and non-specific peroxidase (POD). Glutathione-S transferase (GST), however, showed a slight decrease in the V1 and V2 nutrient treatment at EC. Similarly, no significant differences could be found in Mv Mambo for most enzymes, as only CAT activity increased at EC. No significant influence of the nutrient supply could be detected on the antioxidant enzyme system either at this early stage of plant development.

PM infection induced various changes in the antioxidant enzyme system by the heading period. In Ukrainka, also elevated CO_2 level had an influence on certain enzyme activities. GR activity significantly decreased in the V1 and V2 treatments due to EC, both in control plants and those infected with PM. At EC, however, PM infection resulted in slightly higher GR activities in V1 and V2 than at NC. GST activity was lower in the infected plants than in the control at both CO2 levels. GST activity was also less in response to EC. PM infection resulted in the increased activity of APX at NC and in V1 at EC. CAT activity was influenced by the CO2 level only, as it was lower in both the control and infected plants at EC. POD activity declined due to PM infection at both NC and EC. The nutrient supply level itself, however, did not have any influence on the antioxidant enzyme system in this variety.

In Mv Mambo, the activity of GR decreased in response to PM infection in V2 and V3 at NC, while in the infected plants at EC, it was similar to that at NC. GST activity declined considerably due to PM infection irrespective of the CO2 level. PM induced, however, an increase in the APX activity at both CO2 levels. CAT activity was significantly reduced by PM infection in V2 and V3 at NC, but no significant change was detected at EC. At EC, however, low nutrient supply in control plants resulted in a very low CAT activity, which increased gradually to ambient levels with the increase in the nutrient supply. There was an increase in the enzyme activity in response to increased nutrient supply also in the infected plants, reaching even higher values than those at the ambient level. POD activity changed the less as it decreased significantly only in the infected plants grown at EC, in V1 and V2 treatments.

Based on the results, it can be concluded that the nutrient supply had a crucial effect on the disease severity and progress. Its higher level stimulated photosynthesis and also influenced the composition of leaf components. It increased the leaf protein content in one variety but did not affect the soluble carbohydrate and starch content of the leaves and the activity of antioxidant enzyme system in the young plants at the time of artificial infection.

Task 2.3. *Studying of the impact of the CO_2 and nutrient supply levels on the resistance of selected wheat varieties to Fusarium graminearum.*

Mv Regiment and Mv Emma plants were grown in the phytotron in PGB-96 growth chambers. The growth conditions and infection processes were as described before. There were 12 pots (48 plants) per treatment and variety. Nutrient supply was applied as follows: Volldunger Linz nutrient solution at low (0.5x), control (1x) and high (1.5x) levels (according to the instructions of the manufacturer).

Grain toxin content

DON content was principally not influenced by the nutrient supply, as there was no clear tendency associated with it (Figure 7). Elevated CO_2 level, however, caused an increase in the DON content at the WSI treatment in Em while the difference was only significant at V2 in

Reg. ZEA content in SFI increased, decreased or did not change depending on the variety or nutrient supply level (Figure 8), though in Em, it had an extremely high value in V3 due to EC. In WSI, ZEA concentration was lower at EC in all the nutrient levels in Reg but in Em, it was only lower in V1 while it was higher at EC in V2 and V3.

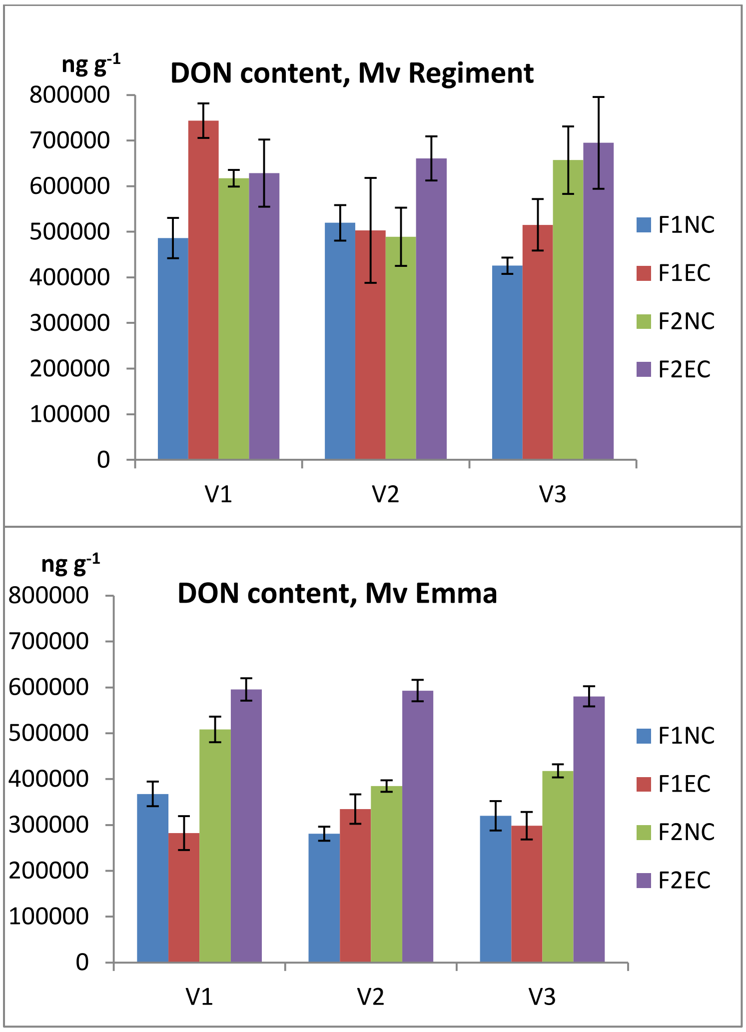


Figure 7. DON content of wheat grains infected by *Fusarium graminearum* at various nutrient supply levels.

V1-V3 = nutrient supply levels, F1 = single floret inoculation, F2 = whole-spike inoculation. NC = ambient CO_2*, EC= elevated* CO_2 *level*

Relationships between infection severity, mycotoxin production and grain yield
In Reg, in SFI, not only toxin contents were higher but also the *Fusarium* infection was more severe at EC in V1, resulting in more dry spikelets and lower yield (due to both less grain number per spike and lower TKW values). In the V2 treatment, the infection was significantly more severe between D14-16 and there were also significantly more dry spikelets (between D14-28), resulting in less grains per spike. The change in the DON and ZEA contamination of the grain due to EC was contradictory in V3, which resulted in similar infection success and grain yield at both NC and EC. The greatly increased ZEA values caused by WSI in V2 and V3 treatments at NC could not be explained by any known factors, there was, however no change in the infection level and spike grain yield in response to EC in any of the nutrient treatments.

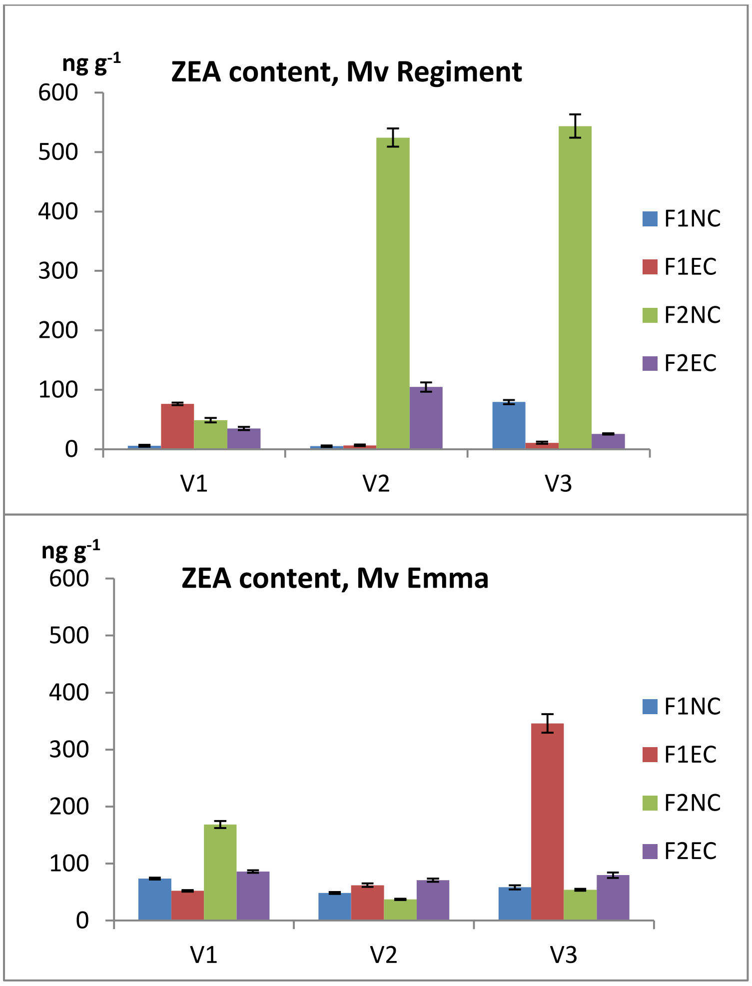


Figure 8. ZEA content of wheat grains infected by *Fusarium graminearum* at various nutrient supply levels.

V1-V3 = nutrient supply levels, F1 = single floret inoculation, F2 = whole-spike inoculation. NC = ambient CO_2*, EC= elevated* CO_2 *level*

In Em, due to the lower DON and ZEA production, SFI resulted in less severe infection at EC in V1. Nevertheless, the number of dry, uninfected spikelets increased significantly between D14-16, which lead to less number of grains per spike. In V2, the higher DON and ZEA levels caused higher dying of the spikelets (D14, D18-21), which, however, might have hindered the infection process, resulting in a lower infection rate and less damage to yield at EC. In V3, the infection rate did not change significantly, however, the grain ZEA content increased and the grain number per spike decreased. In this variety, infection caused by WSI was more severe at EC in both V1 and V3, while it was similar to that at NC in V2. As the DON content increased in response to EC at all the nutrient supply levels, a decline was found in the yield parameters.

Comparison between *Fusarium graminearum* and *Fusarium culmorum* with respect to infection severity and reactions of wheat varieties to elevated CO_2 level

The impact of elevated CO_2 level was invetigated on twelve winter wheat genotypes, representing different *Fusarium* head blight (either type I or type II) resistances. Two types of inoculation methods were performed. Whole spike inoculation was carried out at full flowering of the main spike of six wheat plants per genotype per isolate, the entire ear was sprayed with inoculum of *F. graminearum* or *F. culmorum*. From stocks, the isolates were diluted to the concentration of 50000 conidia mL^{-1}. In the case of single spikelet inoculation, three different methods were carried out in the experiment. The spikes at the early flowering stage were injected with *F. graminearum* or *F. culmorum* isolates (concentration: 500000 mL^{-1}). The location of the inoculated spikelet varied, either at the top, in the middle or at the basal part (1/4, 1/2 or 3/4) of the ear. After all types of inoculations, the spikes were covered with plastic bags for 2 days.

Infection development was evaluated after inoculation in intervals of 2-3 days. The day of infection (showing any symptom of Fusarium head blight) was recorded for each spikelet. The ears were evaluated until the 40th day after injection. Area under disease progress curve was calculated from the data. One wheat genotype was excluded from the analysis because of insufficient germination and plant development.

The difference of infection severity of plants grown at elevated or ambient CO_2 level was less detectable under circumstances which favour the development of *Fusarium*, such as susceptible wheat genotypes, basal location of single floret inoculation or the more aggressive pathogen (eg. Type II resistance of cv. Mv Karizma is weak, so the disease progress was very similar at both CO_2 levels and for both *Fusarium* species, Figure 9).

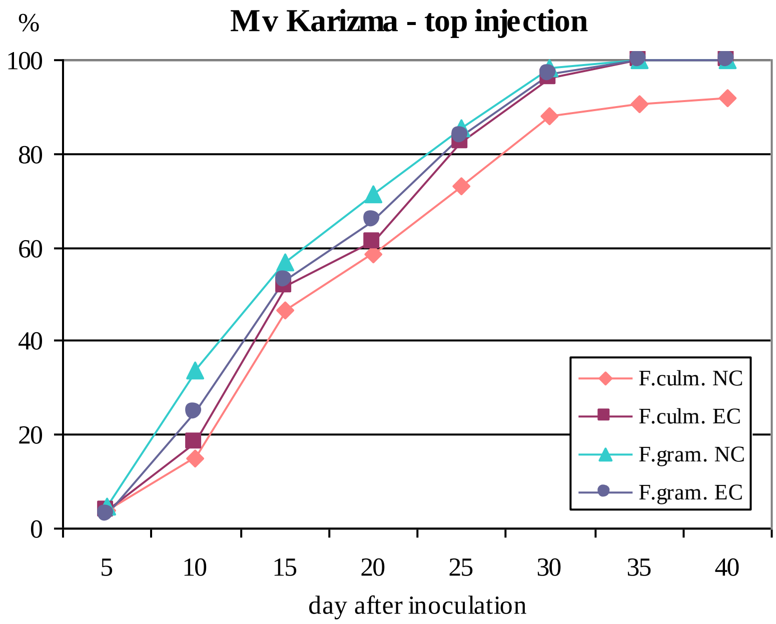
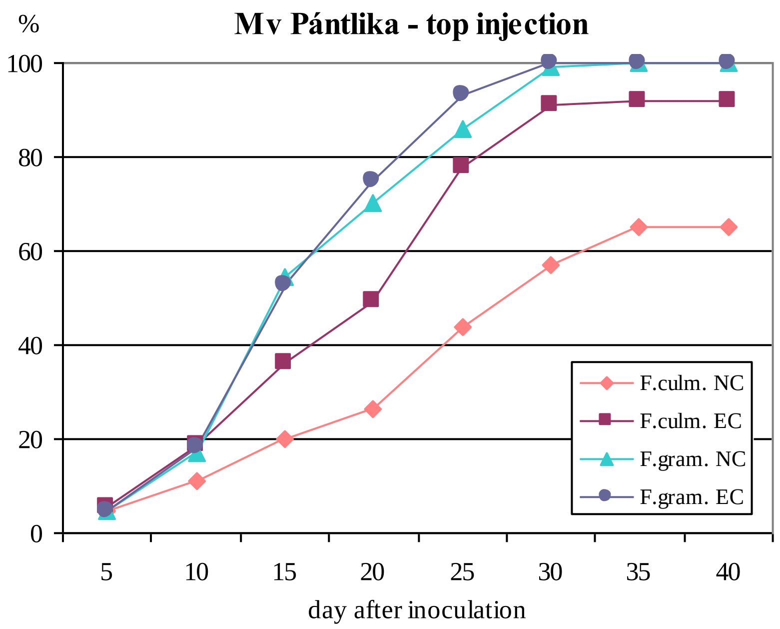


Figure 9. Infection progress of *F. culmorum* and *F. graminearum* in Mv Karizma grown at ambient or elevated CO_2 level

Among wheat genotypes Mv Pántlika had the highest level of resistance, in previous experiments it proved to be constantly moderately resistant against the spread of *Fusarium* within the spike through the rachis. In this trial the top injection with *F. culmorum* inoculum was the most suitable to present the effect of elevated CO_2 level (Figure 10). In the whole spike inoculation the difference in the final infection was not so remarkable, but the disease progress varied between plants grown at ambient or elevated CO_2 levels.



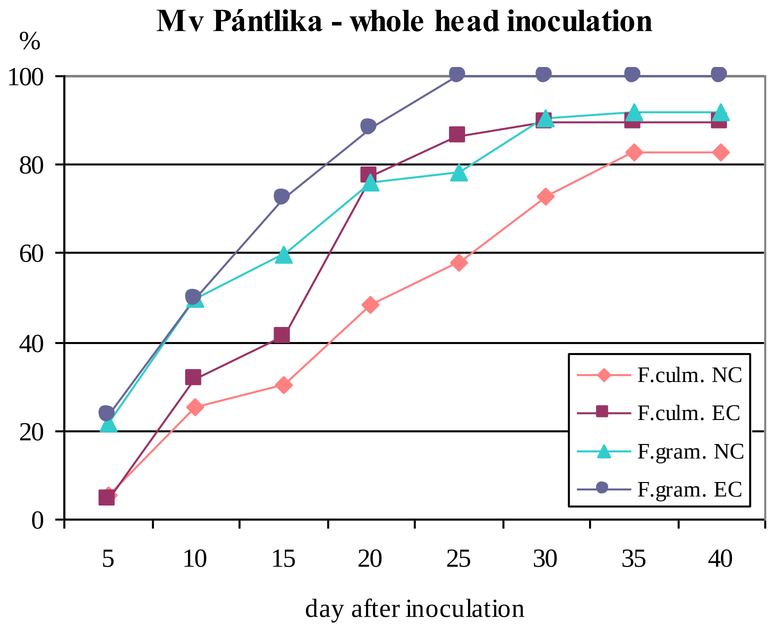


Figure 10. Infection progress of *F. culmorum* and *F. graminearum* in Mv Pántlika grown at ambient or elevated CO_2 level

In the case of two wheat varieties (Mv Menüett and Mv Kokárda), it was observed that at the ambient CO_2 level, the number of plants, exhibiting no infection, no or limited spread of infection only to the neighbouring spikelets from the injection point, increased compared to the plants grown at elevated CO_2 level (Figure 11-12). In average, this phenomenon resulted in relevant difference in the disease progress and infection level on the 40th day after inoculation. In SFI of Mv Menüett, the difference was more pronounced when the plants were inoculated with *F. culmorum* isolate, which has lower aggressiveness in spreading through the rachis. In the case of whole spike inoculation of Mv Kokárda plants, the difference of infection levels was more expressed in the case of the inoculation with *F. graminearum*.

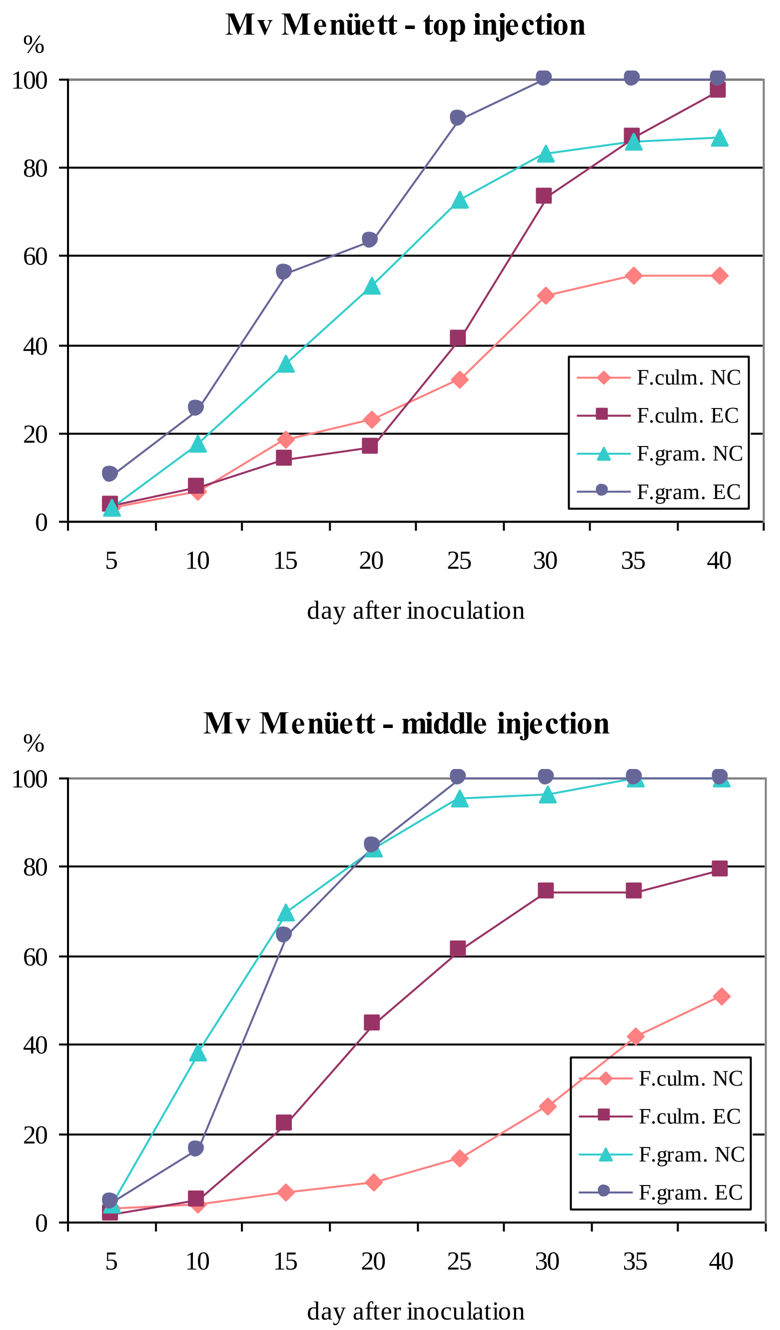


Figure 11. Infection progress of *F. culmorum* and *F. graminearum* in Mv Menüett grown at ambient or elevated CO_2 level

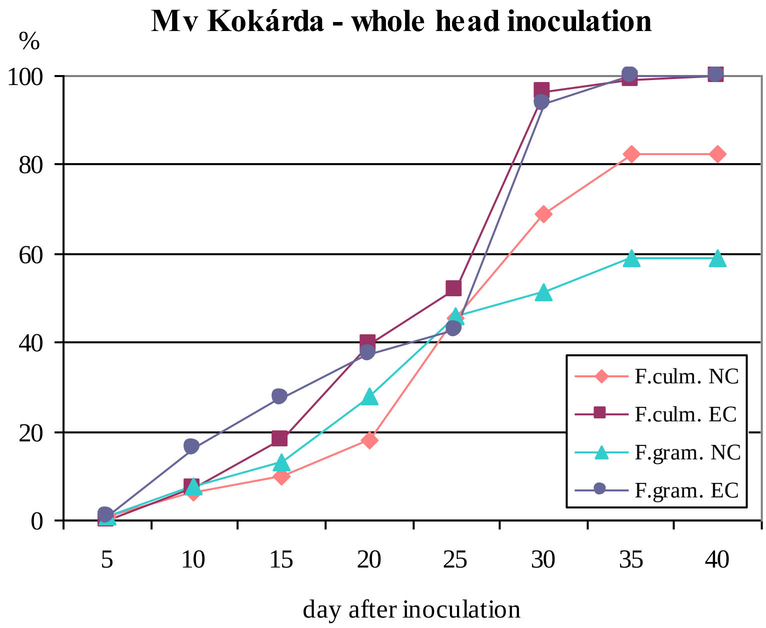


Figure 12. Infection progress of *F. culmorum* and *F. graminearum* in Mv Kokárda grown at ambient or elevated CO_2 level

3-factor REML analysis (GenStat18) of the AUDPC (area under the disease progress curve) values revealed that the genotype and *Fusarium* had statistically proved effect on the spread of the infection, in every type of inoculation techniques. The factor CO_2 had an effect in the case of top injection, and only in interaction with the wheat genotypes. Based on the pairwise comparison (Mann-Whitney test, SPSS16) of AUDPC values of plants grown at elevated and ambient CO_2 level, significant differences were found in two cases of top injection, in the wheat breeding line Mv22-14 infected with *F. culmorum* and in Mv Ménrót infected with *F. graminearum* (Figure13). In both cases the decreased disease progress in response to elevated CO_2 level.

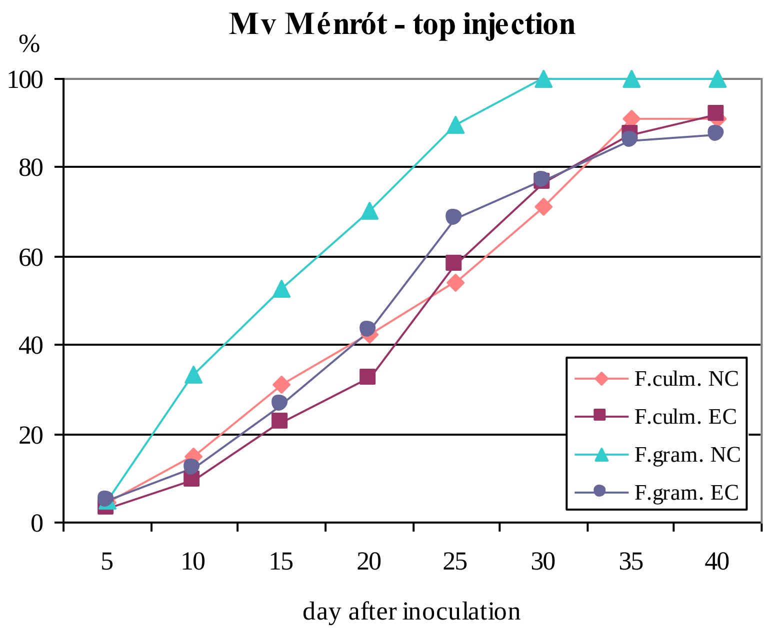


Figure 13. Infection progress of *F. culmorum* and *F. graminearum* in Mv Ménrót grown at ambient or elevated CO_2 level