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Growth Analysis for Studying the Effect of the Fungal Pathogen *Diaporthe helianthi* on the Achene Dry Matter Accumulation per Head in Sunflower Hybrids

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Diaporthe helianthi Munt. Cvet. et al. (anamorph: *Phomopsis*) is a dangerous pathogen of sunflower, causing important yield losses. No information is as yet available, however, on the changes taking place in the achene yield-forming process, which eventually lead to the reduction in yield.

The basic assumption was that by tracing the growth of the achene yield – using the growth analysis method – it would be possible to determine how the pathogenic fungus *Diaporthe (=D.) helianthi* – affected the dry matter accumulation process. The achene dry matter mass per head (ADM) was recorded at nine sampling dates during the achene filling period in five sunflower hybrids, in plants inoculated with the pathogen or treated with fungicide. Simultaneously with sampling, scoring was carried out for symptom dynamics.

The results proved that, as the result of inoculation with *D. helianthi,* considerable changes took place in the achene dry matter accumulation process. The estimated growth characteristics of a second-order polynomial fitted to the ADM mass data: maximum yield per head (Y_{MAX}), average of absolute growth rate (AGR_{AVG}), maximum of absolute growth rate (AGR_{MAX}), maximum point of the absolute growth rate ($XAGR_{MAX}$) and average of relative growth rate (RGR_{AVG}), were considerably smaller in the inoculated treatment than in the sprayed treatment.

Keywords: sunflower, *Diaporthe helianthi,* achene dry matter accumulation.

The achene yield formed in the sunflower heads is the resultant of the external and internal effects influencing the plant. Under ideal growing conditions, sunflowers utilise all their surplus energy for the development of the achenes. In many cases, however, the conditions are less than optimum due to drought or excessive rainfall, temperature stress or disease.

Sunflower are a crop whose yield stability is greatly influenced by diseases. Of the 16–18 pathogens which infect Hungary's most important oil crop, *D. helianthi* caused the greatest damage in the second half of the 1990s (Békési, 1999).

The pathogen was first identified in the southern part of Hungary by Németh et al. (1981). From then on it spread rapidly in sunflower-producing areas, sometimes causing devastating epidemics. Most recently it caused economically important yield losses between 1997 and 1999. The yield decreased to 1.1–1.2 t/ha on the most heavily infected areas.

If sufficient ascospores are available in spring and the environmental conditions are favourable for infection, the greatest yield losses can be expected in plant stands infected before the flowering. The aggressive cellulose- and lignin-degrading enzymes and phyto-

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toxins produced by the pathogen damage the vascular tissues of the crop, thus breaking the incorporation of assimilates into the achenes. The diseased plants are subject to forced ripening, leading to a reduction in head diameter, shrivelled achenes and the breakdown of oil synthesis. As a consequence, there is a decline in the seed and oil yields per hectare.

In recent years, a number of papers have been published in Hungary on the use of growth analysis for the mathematical analysis of crop production, with special regard to maize (Berzsenyi, 1996; Berzsenyi et al., 1999; Berzsenyi and Lap, 2000a; 2000b).

The most obvious application of growth analysis, according to Berzsenyi (2000), is to determine the effect of environmental factors on the performance of different genotypes.

Gardner et al. (1985) regarded diseases as plant growth factors.

Csikász (1998) and Csikász et al. (2002a, 2002b) successfully applied growth analysis to model the dry matter accumulation of sunflower achenes. In the course of this work it was found that the achene formation process could be described by fitting growth functions to the data of ADM measurements carried out at regular periods during the achene-filling period, and by calculating growth characteristics from the parameters of the functions.

Treitz et al. (2002) used growth analysis to demonstrate various forms of tolerance to *D. helianthi* in sunflower hybrids.

The experiments were planned on the assumption that, by tracing the development of the achene yield, it would be possible to determine how the pathogenic fungus *D. helianthi* modified the process of dry matter accumulation.

The aim of investigations carried out in 2002 was to detect differences in the ADM accumulation of hybrids infected with *D. helianthi* or protected with fungicide.

Materials and Methods

The experiment was set up in the University of Kaposvár, Forage Research Institute, in Iregszemcse. Five test genotypes were sown in a randomised block design with four replications in 9-metre plots, each containing 4 rows (25.2 m^2) on 9 May 2002. The sunflower hybrids tested were: NA/2, NA/3, NA/4, NA/5, NA/6.

Two types of treatment were applied:

1. Artificial stem inoculation. The *D. helianthi* isolate was provided by the Plant Pathology and Physiology Laboratory of the Institute. Inoculation was carried out using the method of Kovács and Tüske (1984), which involves making an incision in the stem and inserting a mycelium disc with a diameter of 5 mm into the wound on the border of the epidermis-cortex and the pith, after which the wound is covered with moist cotton-wool and a strip of aluminium foil to ensure the moisture required for the initiation of infection. Sixty plants were inoculated on each plot. The time of inoculations was July 20th for hybrids NA/4 and NA/5 and on July 22nd for NA/2, NA/3 and NA/6, when the hybrids exhibited 10% flowering.

2. Spraying with a 1.0 l/ha dose of Alert S fungicide was carried out when the sunflower plants were in the 6–8-leaf stage and in the star bud (R1) stage (Schneiter and Miller, 1981).

Sampling for the determination of ADM mass was begun one week after the end of flowering. Samples were taken from each plot every six days during the achene-filling period, on a total of 9 occasions. Each sample consisted of heads from 3 plants. The achenes were threshed from the heads by hand, then dried. After reaching the air-dry state, the dry matter mass was measured using an analytical balance.

In order to study symptom dynamics, the plots per hybrids were scored on a 0–9 scale in each treatment and each replication at the same time as the samples were taken for ADM mass determination.

The grades on this scale reflected the progress and intensity of the symptoms, as follows:

0 – no symptoms
1 – the first Diaporthe-spots appeared on the leaves
2 – elliptical spots measuring at most 5 cm appeared on the stems at the leaf-stem nodes
3 – the stem lesions measured 10–15 cm, but affected only the epidermis and not encirclants the stem
4 – the lesion lengths measured 20–30 cm and the piths were also damaged
5 – stems with encircling lesions
6 – necrosis covered 40–50% of the stems
7 – the necrosis had spread to 60–75% of the stems
8 – 80–90% of the stems were infected
9 – the whole plant had wilted or the stem had broken at the site of infection.

The second order polynomial $lnY=P_0+P_1X+P_2X^2$, reported by Hunt and Parsons (1974) and Hunt (1982), was fitted to the ADM data recorded for the individual hybrids, where X was the serial number of the sample (representing 6-day units), and Y was the achene dry matter mass per head (g/head) at the Xth sampling. Curve fitting was carried out for each 4 replications. The closeness of fit was checked with orthogonal polynomials (Sváb, 1981). The parameters of the growth functions (P_0, P_1, P_2) were then used to calculate the characteristics of the growth curves for each hybrid.

The estimated characteristics of the growth curves were as follows:

– maximum yield (g/head): Y_{MAX}

$Y_{MAX} = \exp (P_0–P_1^2/4P_2)$

– average of absolute growth rate (g/6 days): AGR_{AVG}

$AGR_{AVG} = (1/8)\{Y(9)–Y(0)\} = (1/8)\{\exp (P_0+9P_1+81P_2)–\exp (P_0+P_1+P_2)\}$

– maximum point of absolute growth rate: X_{AGRMAX} (value expressed as the number of samplings)

$X_{AGRMAX} = [P_1–\sqrt{(–2P_2)}]/(–2P_2)$

– maximum of absolute growth rate (g/6 days): AGR_{MAX}

$AGR_{MAX} = \sqrt{(–2P_2)} \exp (P_0–P_1^2/4P_2–0{,}5)$

– average of relative growth rate (g/6 days): RGR_{AVG}

$RGR_{AVG} = P_1+10P_2$

Analysis of variance (ANOVA) was used to evaluate changes in the various characteristics in each treatment and for each hybrid.

Results

Analysis of symptom dynamics

The results obtained after artificial inoculation of stem *(Table 1)* demonstrate that the intensity of the infection progress proportionately with the time of sampling for all the hybrids. The pith of hybrids NA/3, NA/4, NA/5 and NA/6 exhibited severe damage from the 4th scoring date. Hybrid NA/4 showed the greatest extent of infection by the end of the growing season.

Table 1

Stem infection caused by *Diaporthe helianthi,* grouped according to hybrids, sampling dates and treatments (scores)

Hybrid		NA/2		NA/3		NA/4		NA/5		NA/6	
Scoring		Treatment		Treatment		Treatment		Treatment		Treatment	
Code	Time	I	S	I	S	I	S	I	S	I	S
1	05. Aug	0	0	0	0	0	0	0	0	0	0
2	11. Aug	0	0	2	0	2	0	2	0	2	0
3	17. Aug	2	0	3	1	3	1	3	1	3	1
4	23. Aug	3	0	4	1	4	1	3	1	4	1
5	29. Aug	3	1	5	1	5	1	4	1	5	1
6	04. Sept	4	1	5	2	6	1	5	1	6	1
7	10. Sept	4	1	6	2	7	1	7	1	7	2
8	16. Sept	4	1	7	2	8	1	7	2	7	2
9	22. Sept	4	1	7	2	8	2	7	2	7	2

I = inoculated with *Diaporthe helianthi*
S = sprayed with fungicide

In genotype NA/2 the symptoms developed slowly and spots did not appear around the site of infeciton until the 3rd scoring date. At the next two scoring dates this genotype had a score of 3, while from the 6th scoring date onwards the intensity of infection was given a score of 4.

All the genotypes responded favourably to the fungicide treatment. No symptoms were observed on the hybrids until the second scoring, and only leaf spots appeared up to the 5th date. Infection spread to the stem from the 6th to 8th scoring date, depending on the genotype. The score given to hybrid NA/2 was never greater than 1, and even on NA/4 stem lesion was only observed at the last scoring date.

Growth analysis

In all cases the second order polynomial could be fitted reliably to log-transformed data. Results of curve fitting gave a practically good description of the ADM accumulation

Table 2

Average values of the calculated parameters and growth characteristics for each hybrid and treatment

Hybrid and treatment	P_0	P_1	P_2	R_2	Y_{MAX} (g/head)	AGR_{AVG} (g/6 days)	AGR_{MAX} (g/6 days)	X_{AGRMAX} *	RGR_{AVG} (%)
NA/2 I	3.168	0.361	–0.026	0.871	84.6	5.27	11.64	2.63	10.20
NA/2 S	3.227	0.412	–0.031	0.914	101.6	6.20	15.14	2.67	10.70
NA/3 I	3.342	0.234	–0.017	0.810	63.0	2.80	7.04	1.41	6.10
NA/3 S	3.351	0.319	–0.023	0.871	87.4	5.16	11.26	2.32	9.10
NA/4 I	3.515	0.235	–0.017	0.845	78.1	3.66	8.53	1.71	6.60
NA/4 S	3.352	0.397	–0.031	0.911	100.8	4.84	15.33	2.34	8.30
NA/5 I	3.596	0.248	–0.018	0.861	87.8	4.37	9.89	1.74	7.00
NA/5 S	3.656	0.310	–0.023	0.919	112.6	6.19	14.48	2.12	8.40
NA/6 I	3.378	0.285	–0.023	0.834	72.4	2.87	9.33	1.57	6.00
NA/6 S	3.425	0.323	–0.023	0.902	97.4	6.05	12.57	2.43	9.70

I = inoculated
S = sprayed
P_0, P_1, P_2 = parameters of equations of fitted curves
R^2 = coefficient of determination
Y_{MAX} = maximum value of ADM
AGR_{AVG} = average increase in absolute ADM accumulation
AGR_{MAX} = maximal increase in ADM accumulation
X_{AGRMAX} = point of maximal speed of growth (according to code of sampling)
RGR_{AVG} = average increase in relative ADM accumulation
ADM = achene dry matter
* = value expressed as the number of samplings

of genotypes (significant linear and quadratic component). The parameters of the functions, the calculated growth characteristics, can be seen in *Table 2. Figures 1–5* illustrate the estimated growth curves of each hybrid in the infected and sprayed treatments. The differences in ADM accumulation as the result of the two treatments can be clearly seen on the figures. For all the genotypes the curves obtained for plants inoculated with *D. helianthi* were flatter, indicating a smaller speed of dry matter accumulation, than those of plants sprayed with fungicide. The difference between the two curves was smallest for hybrid NA/2, while the curves deviated considerably from each other with time in hybrid NA/6.

Analysis of variance on the characteristics for sprayed hybrids and those inoculated with *Phomopsis helianthi* mycelium demonstrated what changes occurred in the accumulation process as the result of artificial inoculation.

The value of the Y_{MAX} characteristic, which gives a good reflection of the yield, was significantly lower in infected hybrids than in sprayed plants *(Table 3).* However, there was no significant difference between the genotypes in the magnitude of the reduction in Y_{MAX} (difference, d) taking place due to infection with the fungus. If the treatment sprayed with fungicide was taken as the basis, the hybrid NA/5 had the greatest yield potential.

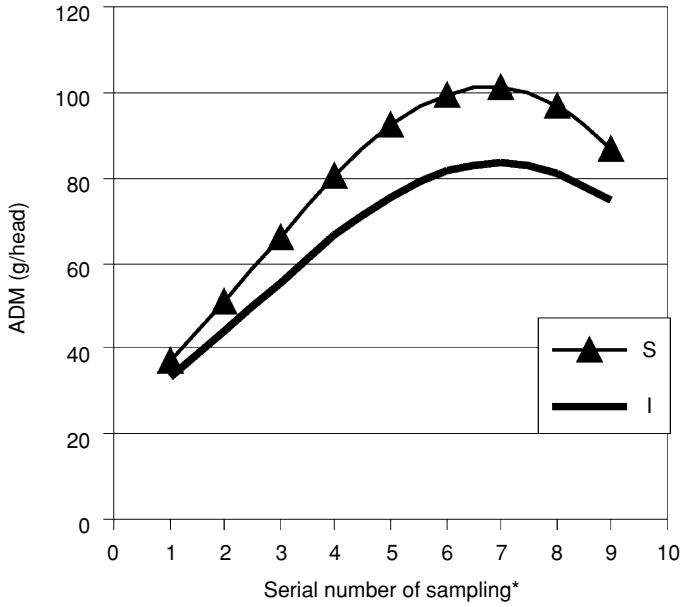

Fig. 1. Trend of ADM accumulation in hybrid NA/2 under sprayed (S) and infected (I) conditions
ADM = achene dry matter
* = first sampling: 7 days after the end of flowering

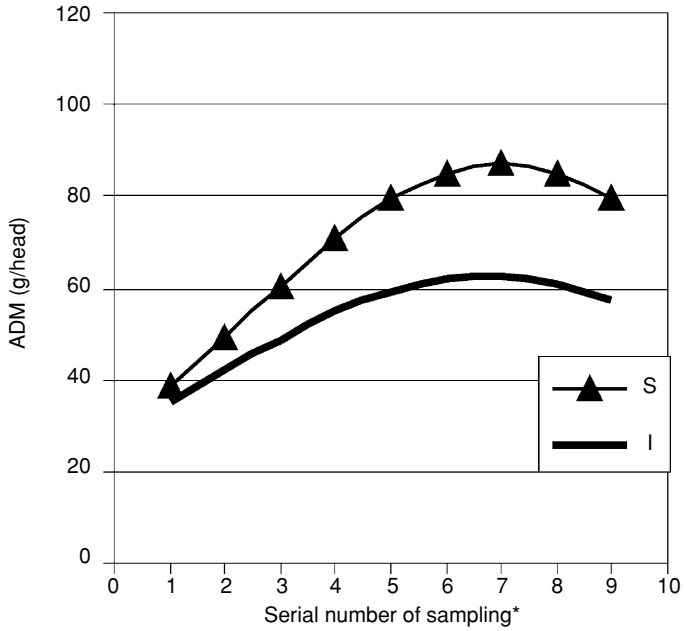

Fig. 2. Trend of ADM accumulation in hybrid NA/3 under sprayed (S) and infected (I) conditions
ADM = achene dry matter
* = first sampling: 7 days after the end of flowering

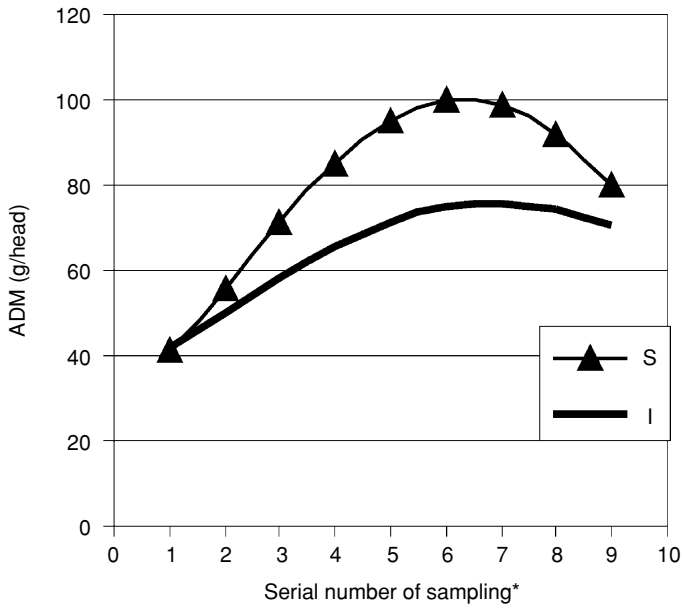

Fig. 3. Trend of ADM accumulation in hybrid NA/4 under sprayed (S) and infected (I) conditions
ADM = achene dry matter
* = first sampling: 9 days after the end of flowering

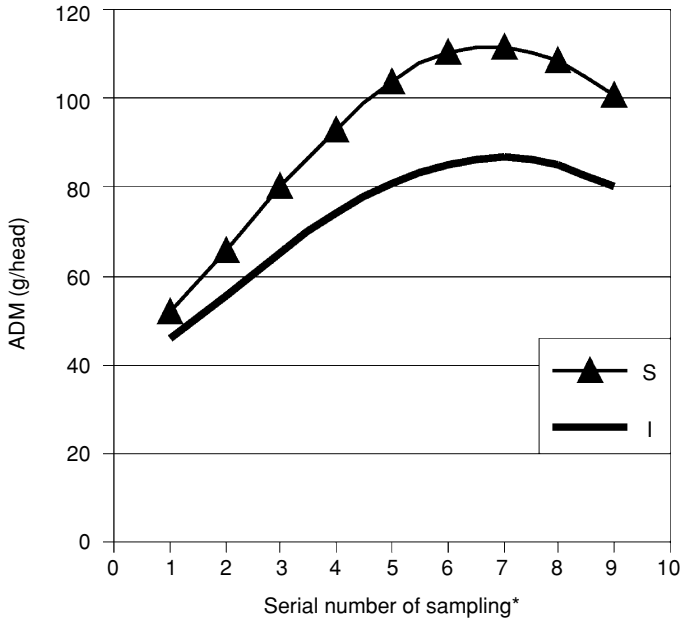

Fig. 4. Trend of ADM accumulation in hybrid NA/5 under sprayed (S) and infected (I) conditions
ADM = achene dry matter
* = first sampling: 9 days after the end of flowering

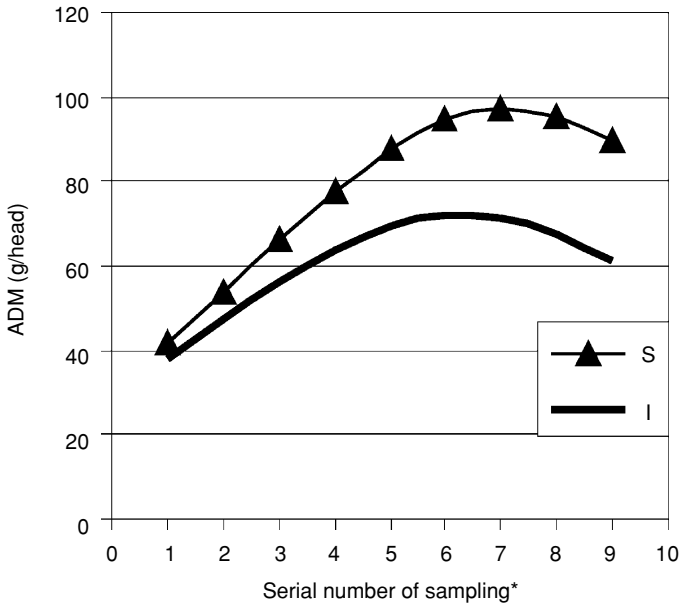

Fig. 5. Trend of ADM accumulation in hybrid NA/6 under sprayed (S) and infected (I) conditions
ADM = achene dry matter
* = first sampling: 7 days after the end of flowering

Table 3

Values of Y_{MAX} (g/head) for each hybrid in the inoculated and sprayed treatments and ANOVA

Hybrid	Sprayed	Inoculated	Decrease(d)
NA/2	101.6	84.6	17.1
NA/3	87.4	63.0	24.3
NA/4	100.8	78.1	22.7
NA/5	112.6	87.8	24.8
NA/6	97.4	72.4	25.0
$LSD_{5\%}$		6.73	9.52
Least significant value for "d"			6.73

Source	df	MS	p
Hybrid	4	695.31	***
Treatment	1	5186.07	***
H × T interaction	4	22.12	ns
Error	27	21.57	

***P = 0.1%
ns = non significant

Table 4

Values of AGR_{AGV} (g/6 days) for each hybrid in the inoculated and sprayed treatments and ANOVA

Hybrid	Sprayed	Inoculated	Decrease(d)
NA/2	6.2	5.3	0.9
NA/3	5.2	2.8	2.4
NA/4	4.8	3.7	1.1
NA/5	6.2	4.4	1.8
NA/6	6.1	2.9	3.2
$LSD_{5\%}$		1.37	1.93
Least significant value for "d"			1.37

Source	df	MS	P
Hybrid	4	4.33	**
Treatment	1	35.79	***
H × T interaction	4	1.66	ns
Error	27	0.89	

**P = 1%
***P = 0.1%
ns = non significant

When the average absolute growth rate (AGR_{AVG}) for the hybrids was analysed, dry matter accumulation was found to take place more rapidly when the genotypes were sprayed with fungicide *(Table 4).* For hybrids NA/2 and NA/4 the infection did not cause a significant decline in this characteristic. Genotype NA/6 responded most sensitively to the infection on the basis of AGR_{AVG}, since the reduction in this characteristic due to inoculation was significantly greater in comparison with hybrids NA/2, NA/4 and NA/5.

The maximum growth rate (AGR_{MAX}) was also greater in genotypes sprayed with fungicide *(Table 5).* The difference between the sprayed and inoculated treatments was significant for all the hybrids. In the sprayed treatment hybrids NA/2, NA/4 and NA/5 had significantly greater AGR_{MAX} than NA/3. In the infected treatment NA/2, NA/4, NA/5 and NA/6 did not differ from each other significantly, but the maximum growth rate of hybrid NA/3 was lower. All in all, however, the greatest reduction in the maximum growth rate (difference, d) was observed for genotype NA/4, being significantly greater than that recorded for NA/2 and NA/6.

As the result of inoculation a significant reduction occurred in the maximum point of absolute growth rate (X_{AGRMAX}), as expressed by the serial number of the sample, for hybrids NA/3, NA/4 and NA/6 *(Table 6).* In hybrids NA/2 and NA/5 this characteristic did not change significantly due to the inoculation.

A significant reduction in the average of relative growth rate (RGR_{AVG}) was recorded for genotypes NA/3 and NA/6 as the result of inoculation *(Table 7).* Hybrid NA/2 exhibited a significantly higher RGR_{AVG} value in the inoculated treatment than hybrids NA/3, NA/4 and NA/6.

Table 5

Values of AGR_{MAX} (g/6 days) for each hybrid in the inoculated and sprayed treatments and ANOVA

Hybrid	Sprayed	Inoculated	Decrease(d)
NA/2	15.1	11.6	3.5
NA/3	11.3	7.0	4.2
NA/4	15.3	8.5	6.8
NA/5	14.5	9.9	4.6
NA/6	12.6	9.3	3.2
$LSD_{5\%}$	2.27		3.21
Least significant value for "d"			2.27

Source	df	MS	P
Hybrid	4	20.10	***
Treatment	1	199.74	***
H × T interaction	4	3.97	ns
Error	27	2.46	

***P = 0.1%
ns = non significant

Table 6

Values of X_{AGRMAX} [(1)] for each hybrid in the inoculated and sprayed treatments and ANOVA

Hybrid	Sprayed	Inoculated	Decrease(d)
NA/2	2.7	2.6	0.1
NA/3	2.3	1.4	0.9
NA/4	2.3	1.7	0.6
NA/5	2.1	1.7	0.4
NA/6	2.4	1.6	0.8
$LSD_{5\%}$	0.55		0.78
Least significant value for "d"			0.55

Source	df	MS	P
Hybrid	4	0.81	**
Treatment	1	3.19	***
H × T interaction	4	0.26	ns
Error	27	0.15	

**P = 1%
***P = 0.1%
ns = non significant
(1) = in 6-day units after the end of flowering

Table 7

Values of RGR_{AVG} (%) for each hybrid in the inoculated and sprayed treatments and ANOVA

Hybrid	Sprayed	Inoculated	Decrease(d)
NA/2	10.7	10.2	0.5
NA/3	9.2	6.1	3.0
NA/4	8.3	6.6	1.7
NA/5	8.4	7.0	1.4
NA/6	9.7	6.0	3.7
$LSD_{5\%}$		2.30	3.26
Least significant value for "d"			2.30

Source	df	MS	P
Hybrid	4	0.13	**
Treatment	1	0.43	***
H × T interaction	4	0.03	ns
Error	27	0.03	

**P = 1%
***P = 0.1%
ns = non significant

Discussion

The results indicated differences in dry matter accumulation of hybrids treated with fungicide and those inoculated with *D. helianthi.* As a result of the pathological process induced by the pathogen, the inoculated hybrids had lower values of absolute growth rate and maximum growth rate, while the maximum growth rate occurred at an earlier date. For all the genotypes significant changes were recorded in the values of the Y_{MAX} and AGR_{MAX} characteristics.

The reduction in seed yield as the result of inoculation was manifested differently in various genotypes. While the value of the Y_{MAX} characteristic declined to the same extent in all the hybrids, a greater reduction was observed in AGR_{AVG} for some genotypes (NA/3, NA/6), and in AGR_{MAX} for others (NA/4).

It is worth noting the Y_{MAX} values in the sprayed treatment. The significantly highest value obtained for hybrid NA/5 indicates that, of the genotypes tested, this hybrid was the best combination for yield if protected with fungicide.

The experiments confirmed that the growth analysis method could be used to model changes occurring in the achene dry matter accumulation process as the result of infection with the *D. helianthi* pathogen.

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