

Assessment of Selected Pea Genotypes Reaction to *Ascochyta* Blight under Field Conditions and the Impact of Disease Severity on Yield Components

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Abstract

The response of pea (*Pisum sativum*) genotypes to ascochyta blight disease and the effect of disease severity on yield components were evaluated in a 4-year trial under field conditions. Peas were inoculated with *Ascochyta pinodes*, the anamorph of *Mycosphaerella pinodes*, or with *Phoma pinodella* separately and with a mixture of both species. Mean infection ratings of all inoculation treatments were significantly higher compared to controls, with the highest infection for inoculation with *A. pinodes*. Pea genotypes significantly differed for ascochyta blight severity. The differences in extent of infection of the least and the most infected genotypes were quite consistent throughout the study. Frequencies of *A. pinodes* and *P. pinodella* isolated from diseased leaf fragments of ten pea genotypes depended on growing season. Deleterious effects of the inoculation with *Ascochyta*, *Phoma* and *Ascochyta+Phoma* treatments on all evaluated agronomic parameters were observed. Greater lodging of plants in all fungi-inoculated treatments was noticed. Inoculated plants revealed decreased seed yield components as follows: number of pods and seed per plant was reduced by 12% - 19% and 18,5 - 22%, respectively. Seed weight per plant decreased by 18% - 27,7% in comparison with controls. There were also quite large differences between pea genotypes in reduction of all yield components.

Keywords: *Ascochyta* blight severity, *Mycosphaerella pinodes*, *Pisum sativum*, *Phoma pinodella*, Yield components

1. Introduction

Three fungal species: *Ascochyta pisi* Lib., *Mycosphaerella pinodes* (Berk. & Blox.) Vesterg. [teleomorph of *Ascochyta pinodes* (Berk. & Blox.) Jones] and *Phoma medicaginis* var. *pinodella* (L.K. Jones) Boerema, are responsible for ascochyta blight disease of field pea (*Pisum sativum* L.). They cause lesions on leaves, stems and pods while the last two species also infect stem bases. All species can be seed-borne. Infected seeds show varying degrees of shrivelling and discoloration, while other infected seeds remain symptomless. Planting of infected seeds reduces number or vigor of emerging plants.

Ascochyta blight is a very important foliar disease in field peas worldwide. The disease is particularly destructive in the temperate zones of Europe, North America, Australia and New Zealand (Waller, 1974; Bretag *et al.*, 1995; Garry *et al.*, 1998; Bretag & Ramsey 2001). According to Marcinkowska (1996b, 2002) *M. pinodes* was prevalent on pea in several regions of Poland. The disease is apparent as a severe foliar blight and foot rot, causing yield losses. The yield losses in commercial pea fields were estimated from 10% to 20%, but in some trials were also over 50% (Xue *et al.*, 1997; Xue & Warkentin, 2001; Boros & Wawer, 2007).

Infection level with ascochyta-complex fungi fluctuates from year to year and region to region depending on local climatic conditions (Bretag *et al.*, 1995, Marcinkowska, 1996a, 1996b). Therefore evaluation of reaction of different genotypes or selection for resistance can be properly done only in years of disease epidemic (Tivoli & Banniza, 2007). To overcome some of the potential problems of testing in fields with natural disease pressure,

incorporation of laboratory prepared inoculum to increase the pathogen population is practiced (Ali *et al.*, 1978; Tivoli *et al.*, 1996; Xue *et al.*, 1997; Boros & Wiewióra, 2004; Tivoli *et al.*, 2006; Wang *et al.*, 2006).

This study was undertaken to evaluate responses of selected field pea genotypes inoculated with *M. pinodes* or *P. pinodella* to these fungi under field conditions. Effect of ascochyta blight disease severity on yield components was also examined.

2. Materials and methods

2.1 Experiment layout and growth of plants

Field studies were conducted at the Plant Breeding and Acclimatization Institute, Radzików (PBAI), in 1998-2001. Ten field pea genotypes (RAH 796, RAH 897, RAH 997, 1166/96, 1528/96, 344/87/3, Miko, Rubin, Kestor and Agra) were used for these tests. Experiments were carried out in split-plot design with four treatments as a main plot and genotypes as the subplots. Peas were grown on one-row plots, 1,5 m long with 50 plants per plot and 50 cm row spacing with four replications each year. Plots were seeded with a precision plot drill on 10, 12, 13 and 15th April in consecutive years of testing. Lodging score was determined by visually rating each plot at maturity stage July/August on scale of 1 to 9 (1=completely flat to 9 = erect). Then 10 plants per plot were randomly taken for evaluation of the following traits: plant height, number of pods, seeds and seed yield per plant, 1000 seed weight (TSW) and protein content using INFRATEC Food & Feed 1255 analyzer. Percentage losses were calculated based on differences between the control and fungi-inoculated treatments for a given trait using the formula: (trait-control – trait fungi-inoc)/trait-control*100.

2.2 Inoculum preparation and field inoculation

A mixture of the pathogenic isolates was used for inoculation (11 of *A. pinodes*, tested by Marcinkowska & Witkowska (1996) and 5 isolates of *P. pinodella*). Inoculum was prepared from cultures grown at 20 °C for 14 days on Coon's (CN) medium (Ali *et al.* 1976) with 14-h photoperiod. Conidial suspension was prepared by flooding the surface of cultures with sterile distilled water, gently scraping the colony and then removing hyphae via filtration. The concentration of conidia was determined with a haemocytometer.

Plants of three out of four main plots were inoculated with *A. pinodes* (Aps), with *P. pinodella* (Pmp) separately or with mixture of the both species (Aps+Pmp). Inoculation was done with a conidial suspension of 2×10^6 spores in one ml with 0.01% Tween 20 on 24 May in 1998, 27 May in 1999, 23 May in 2000 and 2001. The inoculum was applied to pea plants with a compressed air sprayer with a single nozzle. Application of inoculum was done to run-off in late evening, then plants, including controls, were covered with polypropylene tents to assure 100% relative humidity for infection and disease development. Control plots were sprayed with fungicide Bravo 500 at rate of $2 \text{ l} \times \text{ha}^{-1}$ at the time of inoculation and then at the mid-flowering stage of plant growth.

2.3 Weather conditions

Weather conditions during the growing seasons of 1998 to 2001 differed substantially, mainly from the time of inoculation to plants maturity (Fig 1.). Mean temperatures in late May until the pea crop maturity were higher than long term data for three out of four growing seasons with the highest in 1999. Similarly, rainfall distribution was quite different in individual years. In 1998 and 1999 the amount of rainfall starting from time of the inoculation to the harvest was similar but in 1999 was more uniformly distributed. In 2000 temperature from the end of May to the end of July was also higher compared to long term data and this period was very dry, causing growing conditions that were unfavorable for the pea crop disease development. The temperatures in 2001 were nearer to average of long term data, only July had significantly higher temperatures. The amount of precipitation from the end of May to the end of July was close to that of 1998 and 1999 with the highest amount in late July.

2.4 Disease assessment and statistical analysis

Disease intensity was assessed three times during the growing season using a 0-5 scale (Tivoli *et al.*, 1996) where 0- no lesion; 1- a few scattered flecks; 2- numerous flecks; 3, 25-50% plant parts covered by small coalesced lesions; 4, 50-75% plant parts covered; 5, 75-100% plant parts covered by lesions. First assessment was done two weeks after inoculation and the next at two weeks intervals. The increase of disease with time was calculated using Van der Plank (1960) equation:

$$\text{RIR} = (230 / (t_2 - t_1)) * \log(m x_2 * (1 - x_1) / (x_1 * (1 - x_2)))$$

where:

RIR – relative infection rate over time period between t_1 and t_2

m = coefficient of susceptible tissue increase

x_1 - disease severity at the time (t_1)

x_2 - disease severity at the time (t_2)

Disease severity exhibited a mean percentage of infection area of plant expressed as decimal fraction.

In 1999 and 2000 leaves showing the disease symptoms were collected from one replication of the experiment for all genotypes and inoculation treatments. Lesions on leaves were cut into small pieces and disinfected in 1% NaOCl for isolation on CN medium in 7 cm diameter plates. Fungal cultures growing from the pieces were identified and counted after eight days of incubation. Identification of *Ascochyta* species was based on CMI descriptions (Punithalingam and Holliday, 1972). *P. pinodella* was identified according to Noordeoos *et al.* (1993).

Analysis of variance was conducted with the General Linear Model procedure in SAS (SAS Institute, Inc.2004). Tukey's honestly significant difference (HSD) was used for all mean comparison.

3. Results

The year of testing had a significant effect on all agronomic traits of field pea (table 1). Overall the best pea crop performance was in growing season 2001. Significant differences were observed among field pea genotypes for all measurements (table 2.). Six of tested genotypes were semi-leafless and four of them, Rubin and Kwestor, line 1696/96 and 344/87//3 were normal leaved. The line 344/87/3 showed the longest vegetation period, the tallest plants, the highest number of pods and seed per plant but had lowest 1000 seed weight (TSW). This line was the most susceptible to lodging among tested pea accessions. Differences between remaining genotypes for all parameters were smaller.

The first lesions appeared in 3 to 4 days after inoculation. These initial lesions were very small, and appeared in the form of numerous flecks. The spreading of the disease to higher parts of plants depended on weather conditions of growing season as well as on cultivar susceptibility. Disease control in fungicide sprayed combinations was not completely effective. Five or six genotypes depending on treatment combinations showed lower infection rates than the respective treatments means (table 3). Response of genotypes to inoculation treatments was consistent. Through the study, lines 344/87/3, Miko then RAH 897 and line 1528/96 showed the lowest intensity of aschochyta blight while Rubin, line 1166/96 followed by Kewstor and RAH 796 were the most infected. Mean infection ratings of all inoculation treatments were significantly higher comparing to control treatment with the highest infection for inoculation with *A. pinodes*. Pea genotypes with the lowest intensity of aschochyta blight also showed the slowest progress of the disease as expressed with the relative infection rate (RIR) while the most infected showed the fastest progress of the disease among genotypes (table 4).

There was a significant effect for year on mean infection of pea plants. The highest aschochyta blight infection was in 1998 and 1999 and the lowest in 2000 (table 5). During the study, the highest mean disease severity was for inoculation with *A. pinodes*, followed by combined inoculation with the mixture of *A. pinodes* and *P. pinodella*, then *P. pinodella*. The control (protected with fungicide) showed weak infections with aschochyta blight fungi.

When the impact of inoculation treatments was considered independently on cultivars and years, deleterious effects of Aps, Pmp and Aps+Pmp in comparison to the control were observed for all evaluated agronomic parameters (table 6). Greater lodging of plants of all fungi-inoculated treatments was observed mainly in both Aps and Aps+Pmp treatments. Reduction of seed yield components was as follows: number of pods per plant was reduced by 12% - 19%, number of seeds per plant by 18,5 - 22% and seed weight per plant by 18% - 27,7 % for fungi-inoculated combinations in comparison with respective controls. There were also quite large differences between pea genotypes in reduction of all yield components. The average seed yield per plant losses among pea genotypes ranged from 17,3 % and 17,6 % on RAH 796 and RAH 897 to 29% and 31% on Rubin and line 344/87/3, respectively. Some pea genotypes (RAH 796; RAH 896 and line 1167/96) revealed relatively low percentage loss in their yield despite having high disease ratings. Conversely, Rubin, line 344/87/3, line 1528/96 and Miko suffered high losses in their potential yield. The last three genotypes showed the highest resistance among those tested to aschochyta blight fungi.

Isolation of fungi from diseased leaves fragments of ten pea genotypes revealed that in 1999 *A. pinodes* was more frequently responsible for leaf spots than *P. pinodella*, irrespective of inoculation treatments. But in the next growing season (2000), *P. pinodella* dominated (table 7.). *A. pisi* was isolated only sporadically.

4. Discussion

Aschochyta blight is an important constraint on field pea production worldwide (Bretag and Ramsey, 2001). Infection and disease development depends on primary inoculum and on weather conditions. In this study we

assessed responses of selected field pea genotypes to ascochyta blight fungi under field conditions after inoculation with *A. pinodes* or *P. pinodella* separately and with a mixture of the both species. On the non-inoculated plots, ascochyta blight developed naturally from either the inoculum present in the environment or on infected seed, but the spray applications with Bravo generally prevented a disease buildup. Significant differences of ascochyta blight severity were observed among cultivars. The high significant correlation coefficients among mean infection scores of each pea genotype in following years of the evaluation (from $r = 0.78$ to 0.85) indicating stable genotypes ranking throughout the study. The infection degrees of the least and most infected cultivars were generally quite consistent over four years. Among tested genotypes, none were found to possess a high level of resistance to ascochyta blight, similarly to other reports (Ali *et al.*, 1978; Kraft *et al.*, 1998; Xue & Warkentin, 2001; Boros & Wawer, 2007). However, some genotypes moderately susceptible were identified. Pea genotypes of the lowest infection with aschochyta blight fungi also showed the slowest progress of the disease expressed as relative infection rate.

The frequency of occurrence for *A. pinodes* and *P. pinodella* isolated from diseased leaf fragments of ten pea genotypes was growing season depended. Our results of inoculation done with a mixture of both species did not indicate an *A. pinodes* and *P. pinodella* interaction in plant infection score, the phenomenon recently studied by Le May *et al.* (2006). Differences in frequency of both fungi in two growing seasons might partially be associated with differences in weather conditions as previously reported by Roger *et al.* (1999). Weather conditions in 1999, warm and rainy, were favorable for *A. pinodes* whereas warm and dry weather of 2000 prevented to buildup *A. pinodes*, perhaps permitting *P. pinodella* to be isolated more frequently. Probably there were differences in primary inoculum between years of study. In 2000 the experiment plots were settled close to a field where pea was the previous crop and initial level of inoculum might have been higher.

Observations indicating that higher ascochyta blight rating was significantly ($P < 0.05$) associated with higher lodging. Overall correlation coefficient between these two parameters was $r = -0,32$ and for the individual genotype from $r = -0,44$ to $r = -0,70$. This might indicate that higher lodging created more favorable conditions for ascochyta blight development, probably because high relative humidity under lodged plant promoted infection and further disease development. On the other hand infection was able to weaken stems, making them more susceptible to lodging. Results of this study documented our earlier observation that cultivars that were not prone to lodging generally had lower ratings of blight caused by *A. pinodes*, recently confirmed by Wang *et al.* (2006). In other studies, resistance to *A. pinodes* was found to be positively correlated with lodging resistance, and both lodging and the blight were negatively correlated with the proportion of xylem, lignin and fiber content of pea stems (Banniza *et al.* 2005). Muehlbauer and Kaiser (1994) stated that a semi-leafless trait could have a beneficial effect on mycosphaerella (ascochyta) blight because of reducing lodging and promoting air movement through the canopy. However, the semi-leafless trait was not always associated with lower infection with ascochyta. Line 344/87/3 had disease rating lower than semi-leafless genotypes, and semi-leafless RAH 796 had a blight rating comparable to normal-leaf type genotypes Kwestor and line 1166/96.

Clear reductions of seed yield components were observed after inoculation of pea plants with *A. pinodes* (Aps) and *P. pinodella* (Pmp) separately, and in mixture (Aps+Pmp). Our results concerning reduction of yield components are consistent with those previously found by Tivoli *et al.* (1996), Garry *et al.* (1998) and Beasse *et al.* (1999) who found that mycosphaerella blight caused a decrease of number of reproductive nodes per stem, number of pods per stem, as well as number of seed per pod, per stem, and reduction in seed size. The highest decrease of yield components was found for treatments with *A. pinodes* and the lowest for treatments with *P. pinodella*. These results indicated that infection with *P. pinodella* was less destructive for field grown pea crops. Three pea genotypes among those tested revealed relatively low percentage loss in their seed yield per plant, despite having high disease ratings. This observation supports an earlier report that some cultivars yielded reasonably well under severe disease pressure, while others poorly (Xue, 2003; Boros & Wawer, 2007). This illustrates the need to assess the impact of ascochyta blight on yield in order to identify cultivars that are tolerant to ascochyta blight. The differences in tolerance may be of practical value.

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Table 1. Mean agronomic performance of field pea, calculated across cultivars and inoculation treatments

Parameters	Growing seasons			
	1998	1999	2000	2001
Days to maturity	93.5 c	99.9 a	94.0 c	95.8 b
Lodging (scale 9-1)	5.3 bc	5.0 c	5.9 ab	6.5 a
Plant height (cm)	na	85.6 a	53.5 b	80.1 a
Number of pods/plant	na	7.6 b	3.5 c	8.7 a
Number of seeds/plant	na	23.8 b	12.0 c	30.4 a
Weight of seeds/plant	na	4.7 b	2.6 c	6.1 a
TSW (g)	203.2 bc	195.4 c	213.3 a	211.2 ab
Protein content (% d.m.)	24.5 b	26.7 a	26.7 a	24.8 b

“a-c “ Means followed with the same letters in each row are not significantly different according to Duncan’s New Multiple Range Test ($P \leq 0.05$).

Lodging score 9-1 where 9 = no lodging, erect plants and 1 = totally lodged

“na“ not analysed

Table 2. Agronomic performance of ten field pea genotypes, calculated across four treatments and years

Genotypes	Days to maturity	Lodging (9-1)	Plant height (cm)	Number of pods per plant	Number of seeds per plant	Weight of seeds per plant (g)	1000 seed weight (g)	Protein content (% d.m.)
Rubin	96.1 c	4.3 d	63.3 ef	5.7 d	19.5 cd	4.3 b	223.8 ab	27.1 a
RAH 997	93.8 f	6.7 a	72.4 bc	6.4 bcd	20.9 bcd	4.8 ab	231.9 a	25.1 cd
1528/96	94.6 de	6.7 a	74.6 b	7.0 bc	21.5 bcd	4.5 ab	207.4 cd	25.4 cb
1166/96	93.6 f	5.4 c	60.0 f	7.6 b	20.6 bcd	4.4 ab	209.9 cd	25.6 cb
RAH 897	93.4 f	6.4 ab	68.8 cd	5.7 d	18.0 d	4.1 bc	227.4 ab	25.9 b
Agra	97.2 b	5.9 bc	62.6 ef	5.8 cd	24.5 b	4.8 ab	200.0 d	25.7 cb
Kwestor	94.0 ef	5.9 bc	74.1 b	6.6 bcd	24.1 bc	5.3 a	223.2 ab	25.3 bcd
Miko	93.9 f	5.8 c	71.3 bc	6.0 cd	21.9 bcd	4.9 ab	217.8 bc	24.8 d
344/87/3	106.8 a	3.3 e	117.4 a	9.2 a	30.5 a	3.2 c	105.4 e	26.9 a
RAH 796	94.8 d	6.5 ab	65.9 de	5.9 cd	19.4 d	4.1 bc	211.0 c	25.0 cd

“a-f“ Means followed with the same letters in each column are not significantly different according to Duncan’s New Multiple Range Test ($P \leq 0.05$).

Table 3. Final degree of pea plant infection in relation to inoculation treatments (average over years)

Genotypes	Inoculation treatments				Average
	Control	Aps	Pmp	Aps+Pmp	
Rubin	2.30	4.00	3.35	3.25	3.29 a
RAH 997	1.48	2.65	2.30	2.50	2.23 de
1528/96	1.45	2.58	2.23	2.28	2.11 de
1166/96	1.75	3.23	2.73	2.63	2.58 b
RAH 897	1.45	2.58	2.18	2.28	2.10 e
Agra	1.58	2.93	2.30	2.38	2.28 cd
Kwestor	1.80	2.95	2.68	2.50	2.47 b
Miko	1.38	2.48	1.78	2.03	1.90 f
344/87/3	1.20	1.85	1.48	1.73	1.56 g
RAH 796	1.50	3.03	2.53	2.70	2.43 bc
Average	1.58 c	2.83 a	2.34 b	2.44 b	

“a-f” Average for inoculation treatments and cultivars followed with the same letters are not significantly different according to Duncan’s New Multiple Range Test ($P \leq 0.05$).

Table 4. Ascochyta blight disease progress in plant of ten pea genotypes (average over inoculation treatments and years)

Genotypes	Degrees of infections (0-5)			RIR
	1 st term	2 nd term	3 rd term	
Rubin	2.13 a	2.78 a	3.29 a	0.16 ab
RAH 997	1.57 bcd	1.87 d	2.23 de	0.14 ac
1528/96	1.49 cd	1.79 de	2.11 de	0.13 bc
1166/96	1.60 bcd	2.12 b	2.58 b	0.17 a
RAH 897	1.51 bcd	1.83 de	2.10 e	0.14 bc
Agra	1.56 bcd	1.90 cd	2.28 cd	0.15 abc
Kwestor	1.67 b	2.08 b	2.47 b	0.16 ab
Miko	1.44 d	1.68 e	1.90 f	0.11 cd
344/87/3	1.24 e	1.49 f	1.56 g	0.09 d
RAH 796	1.65 bc	2.06 bc	2.43 bc	0.15 ab

“a-f” Means followed with the same letters in each column are not significantly different according to Duncan’s New Multiple Range Test ($P \leq 0.05$).

Table 5. Mean infection of field pea in consecutive growing seasons in relation to inoculation treatments (average for ten genotypes)

Inoculation treatments	Growing seasons			
	1998	1999	2000	2001
Control	1.92	1.31	1.19	1.93
Ascochyta (Aps)	3.24	3.18	2.34	2.58
Phoma (Pmp)	2.63	3.2	1.39	2.19
Ascochyta + Phoma (Aps+Pmp)	3.0	2.67	1.98	2.16
Average	2.70 a	2.59 a	1.73 c	2.22 b

“a-c” Means for growing seasons with the same letters are not significantly different according to Duncan’s New Multiple Range Test ($P \leq 0.05$).

Table 6. Impact of inoculation treatments on agronomic performance of pea plants, calculated across cultivars and years of testing

Parameters	Inoculation treatments			
	Control	Aps	Pmp	Aps + Pmp
Days to maturity	97.0 a	95.1 c	95.8 b	95.3 bc
Lodging (9-1 scale)	6.5 a	5.2 c	6.1 b	5.0 c
Plant height (cm)	78.5 a	70.8 b	71.2 b	71.6 b
Number of pods/plant	7.5 a	6.2 b	6.6 b	6.1 b
Number of seeds/plant	25.9 a	20.3 b	21.7 b	20.3 b
Weight of seeds/plant	5.4 a	3.9 b	4.4 b	4.1 b
TSW (g)	214.8 a	194.6 c	210.6 ab	203.0 b
Protein content (% d.m.)	25.3 b	26.0 a	25.1 b	26.3 a

“a-c” Means followed with the same letters in each row are not significantly different according to Duncan’s New Multiple Range Test ($P \leq 0.05$).

Table 7. Frequency of *Mycosphaerella pinodes* (Aps) and *Phoma pinodella* (Pmp) isolated from infected fragments of pea plant in 1999 and 2000 growing seasons (average for 10 genotypes)

Parameters	Inoculation treatments							
	Aps		Pmp		Aps+Pmp		Control	
Isolated fungi	Aps	Pmp	Aps	Pmp	Aps	Pmp	Aps	Pmp
<i>Growing season 1999</i>								
Number of cultures	176	6	79	73	98	65	81	27
Number of strips	182		152		163		108	
<i>Growing season 2000</i>								
Number of cultures	54	55	0	132	21	97	6	3
Number of strips	109		132		118		96	

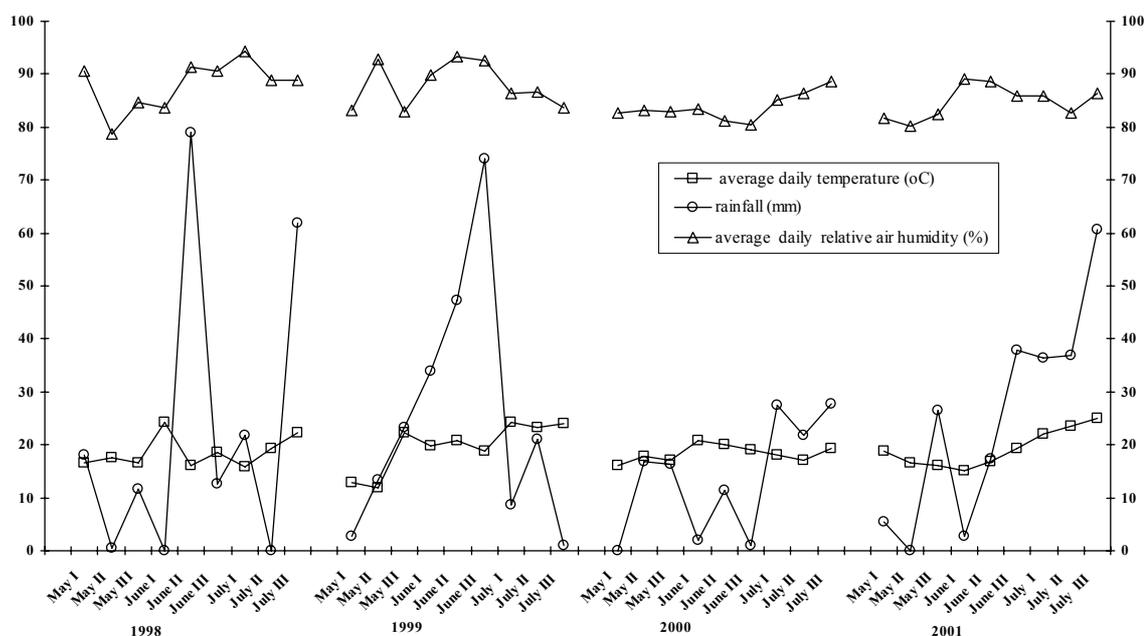


Figure 1. Weather conditions during growing seasons of 1998-2001 at Radzików(I, II, III – decades)