Disease progress of apple scab caused by *Venturia inaequalis* in environmentally friendly growing systems

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Key words: *Venturia inaequalis*, cumulative disease progress curves, regression analysis, AUDPC, integrated fruit production, organic apple production

Abbreviations: AUDPC = area under disease progress curve, cv. = cultivar

Summary: Progression of apple scab epidemic in six apple cultivars, including two current and susceptible (Gala Must, Elstar), two old (Egri Piros, Darusóvári), and two resistant cultivars (Relinda, Releika), were described and analysed in a two-year-study, in two environmental-friendly growing systems (organic and integrated). Curves of disease progress, linear regression analysis of transformed disease incidence data and Area Under Disease Progress Curves (AUDPC) were used to characterise the epidemic processes of the selected cultivars. Cumulative disease progress curves showed continuous but asymmetrical scab development on the moderate or highly susceptible cultivars Gala Must, Elstar and Egri Piros, and on the tolerant or resistant cultivar Darusóvári and Relinda, in both systems. The cultivar Releika showed no symptoms either on fruit or leaf. In linear regression analysis, the best linearisation was given by logistic transformation. Adequate parameters leaf disease incidence rate, of obtained from a regression equation, were higher in the organic system than in the integrated system. Values of AUDPC showed great differences in leaf disease incidences among cultivars and between growing systems. AUDPC gave more differences for comparison of progresses of disease epidemic than growth rate of disease in different systems of disease control. Moreover, the obtained results were compared with similar studies on different pathosystems, and biological interpretations of the analyses are discussed below.

Introduction

Both the susceptibility of a host to diseases and the means of preventing them affect disease progression during a growing season. Moreover, weather conditions also play an important role in the build up of an epidemic. These three factors are the bases of disease processes if the pathogen is virulent.

Apple scab, caused by *Venturia inaequalis* (Cooke) Wint., is the most prevalent disease in apple orchards in most areas where apples are grown (*MacHardy*, 1996). It causes damage to leaf and fruits, which affect yield and fruit quality negatively. Hungarian climatic and weather conditions are favourable for severe epidemics of apple scab fungus in rainy years. Crop losses in Hungary due to apple scab would be about 80%, if no control measures were taken.

As environmental consideration has become increasingly significant, changes have been observed in many European countries in the selection of plant protection chemicals. In environmentally friendly horticulture, many observations have been made, guidelines been established, and control methods applied in integrated plant protection and organic production (*Anonymus*, 1989; *Dickler*, 1992; *Freier*, et al. 1992; *Gonda*, 1993; *Bloomers*, 1994 and *Miklay*, 1995). Moreover, plant-breeding programs in numerous countries have attempted to develop resistant varieties by introgressing apple scab resistance of *Malus* spp.

Many new methods, including microbial preparations, have been evaluated for efficacy against apple scab *in vitro* and under natural conditions (*Simard* et al., 1957; *Burchill & Cook*, 1970; *Gupta* 1979; *Heye & Andrews* 1984; *Miedke &*

Kennel, 1990; *Philion* et al., 1997 and *Benyagoub* et al., 1998). Some new resistant apple cultivars (cvs) are also successful against disease complexes (*Fischer*, 1991). In spite all of these, little practical experience is available about the dynamics of disease progression in environmentally friendly growing systems. Moreover, information is also necessary about resistant hosts under different ecological conditions and control methods, in order to evaluate the stability of host resistance.

The main goal of this study was to analyse progress of apple scab epidemic on susceptible and resistant apple cultivars in two environmentally friendly growing systems.

Material and methods

Orchard site

The study was carried out on an experimental apple orchard at Debrecen-Pallag, Hungary. Observations were made in a one hectare experimental field, divided into 2 experimental blocks. The two blocks corresponded with two growing systems: one according to the Hungarian IFP guidelines (*Inántsy*, 1995); and the second according to Hungarian organic production guidelines (*Seléndy*, 1997). These guidelines have been applied since 1997, when the orchard was first planted. Spraying schedules against apple scab in both production systems are summarized in *Tables 1 and 2*. All sprays were applied with an axial blower spray machine (manufacturer Kertitox) with a ceramic hollow cone at 11–12 bar with a volume of 1000 l ha^{-1}.

The experimental field consists of 40 apple cultivars. The plantation consists of 15 old Hungarian, 15 resistant, 10 current cultivars. The cultivars are planted in randomised blocks with five replicates in the experimental field. Each block consists of seven trees, but observations were only made on the middle five trees of each plot. Single trees were used as observation units. The dwarf trees grafted on M26 rootstock were planted at a distance of 4 x 1.5 m and pruned to a spindle shape. Observations were made on all cultivars but 6 characteristic cultivars (2 old ones: cv. Egri Piros, cv. Darusóvári, 2 resistant ones: cv. Relinda, cv. Releika, 2 current ones: cv. Royal Gala, cv. Elstar) were chosen to analyse disease progress.

Potential infection periods, based on the criteria of *Mills & La Plante* (1951), were recorded from middle of April, until the beginning of October, in both years.

Disease assessment

Disease assessments were made on leaves and fruits in both years. For leaves, 5 leaf sampling units were chosen at random, and for each unit, one-year-old lateral twigs were selected with 50 leaves. The selected leaf unit was tagged at the beginning of May, and the total number of healthy and diseased leaves of each leaf unit was counted on each observation occasion, and recalculated on the basis of 50 leaves per unit. Twenty fruits were chosen at random for each observation tree as fruit sampling units on each observation occasion. Assessments were made for leaf and fruit units separetely. Leaves and fruits were considered to

Table 1 Spraying schedule against apple scab in integrated and organic growing systems, Debecen-Pallag, 1998

Integrated			
Date	Phenological stage	Applied product (active ingredient)	Dosage (%)
(25 March	Bud swelling	Agrol plusz (vaseline oil)	3)
02 April	Green tip	Champion 50 WP (copper hydroxide)	0.3
10 April	Appearence of flower bud	Champion 50 WP (copper hydroxide)	0.3
18, 24 April	Full bloom	Chorus 75 WG (cyprodinil)	0.02
06 May	First petal fall	Score 250 EC (difenoconazole)	0.025
		Efuzin 500 FW (dodine)	0.1
10, 17 May	Last petal fall	Score 250 EC (difenoconazole)	0.025
		Champion 50 WP (copper hydroxide)	0.03
26 May	Fruit setting	Clarinet (pyrimethanil+fluquinconazole)	0.15
3m 17 June	Fruit swelling	Score 250 EC (difenoconazole)	0.06
24 June	Fruit swelling	Clarinet (pyrimethanil+fluquinconazole)	0.15
02 July	Fruit swelling	Score 250 EC (difenoconazole)	0.025
10 July	Fruit swelling	Captan 50 WP (captan)	0.3
17 July	Fruit swelling	Discus DF (krezoxim-metil)	0.02
29 July, 11 August	Fruit swelling	Discus DF (krezoxim-metil)	0.02
Organic			
Date	Phenological stage	Applied product (active ingredient)	Dosage (%)
(25 March	Bud swelling	Agrol plusz (vaseline oil)	3)
02 April	Green tip	Champion 50 WP (copper hydroxide)	0.3
10 April	Appearence of flower bud	Champion 50 WP (copper hydroxide)	0.3
18, 24 April	Full bloom	Thiovit (elementary sulphur)	0.4
06 May	First petal fall	Thiovit (elementary sulphur)	0.4
12, 20 May	Last petal fall	Thiovit (elementary sulphur)	0.4
26 May	Fruit setting	Thiovit (elementary sulphur)	0.4
3, 17 June	Fruit swelling	Sulfur 900 FW (elementary sulphur)	0.4
24 June, 02, 10 July, 17, 29 July, 11 August	Fruit swelling	Thiovit (elementary sulphur)	0.4

Table 2 Spraying schedule against apple scab in integrated and organic growing systems, Debecen-Pallag, 1999

Integrated			
Date	Phenological stage	Applied product (active ingredient)	Dosage (%)
(25 March	Bud swelling	Agrol plusz (vaseline oil)	3)
05 April	Green tip	Cuproxat FW (copper sulphate)	0.5
10 April	Appearence of flower bud	Cuproxat FW (copper sulphate)	0.5
17, 25 April	Appearence of flower bud	Thiovit (elementary sulphur)	0.4
		Discus DF (krezoxim-metil)	0.02
30 April	Full bloom	Thiovit (elementary sulphur)	0.4
		Discus DF (krezoxim-metil)	0.02
04 May	First petal fall	Bayleton 25 WP (triadimefon)	0.03
		Chorus 75 WG (cyprodinil)	0.02
10 May	Last petal fall	Bayleton 25 WP (triadimefon)	0.0
		Efuzin 500 FW (dodine)	0.03
19 May	Last petal fall	Discus DF (krezoxim-metil)	0.02
		Efuzin 500 FW (dodine)	0.2
8 June	Fruit swelling	Mythos 30 SC (pyrimethanil)	0.15
15 June	Fruit swelling	Clarinet (pyrimethanil+fluquinconazole)	0.15
25 June	Fruit swelling	Clarinet (pyrimethanil+fluquinconazole)	0.15
08 July	Fruit swelling	Captan 50 WP (captan)	0.3
		Efuzin 500 FW (dodine)	0.2
16 July	Fruit swelling	Mythos 30 SC (pyrimethanil)	0.15
		Captan 50 WP (captan)	0.3
28 July, 12 August	Fruit swelling	Captan 50 WP (captan)	0.3
Organic			
Date	Phenological stage	Applied product (active ingredient)	Dosage (%)
(25 March	Bud swelling	Agrol plusz (vaseline oil)	3)
05 April	Green tip	Cuproxat FW (copper sulphate)	0.5
10 April	Appearence of flower bud	Cuproxat FW (copper sulphate)	0.5
17, 25 April	Appearence of flower bud	Sulfur 900 FW (elementary sulphur)	0.4
30 April	Full bloom	Sulfur 900 FW (elementary sulphur)	0.4
04 May	First petal fall	Sulfur 900 FW (elementary sulphur)	0.4
10 May	Last petal fall	Sulfur 900 FW (elementary sulphur)	0.4
19 May	Last petal fall	Sulfur 900 FW (elementary sulphur)	0.4
27 May	Fruit setting	Sulfur 900 FW (elementary sulphur)	0.3
8 June	Fruit swelling	Sulfur 900 FW (elementary sulphur)	0.4
15 June	Fruit swelling	Sulfur 900 FW (elementary sulphur)	0.4
25 June, 08, 14, 28 July, 12 August	Fruit swelling	Sulfur 900 FW (elementary sulphur)	0.4

be infected if at least one visible scab lesion was present on the sampling unit.

Data analysis

Disease progress curves. The proportion of diseased leaves and fruits (disease incidence, I) was calculated separately as the number of diseased leaves or fruits divided by the total number of leaves and fruits. At every observation, data from each system were averaged to give single values for disease incidence on leaf or fruit in both years. In order to obtain information on disease epidemics, disease progress curves were constructed by plotting percentages of disease incidence against time in days after the first assessment date (7 May).

Regression analysis. Progress curves of indices data (dependent variable correspond to 'y' axis) were linearised based on transformation equations of *Hau & Kranz* (1977). The transformation equations are: logarithmic (10 based): $z = \log(x)$, exponential: $z = \ln(x)$, Gompertz: $z = -\ln(-\ln(x))$, logistic: $z = \ln\ (x/(1-x))$, monomolecular $z = \ln(1/(1-(x))$. Time (independent variable correspond to 'x' axis) was used without transformation. Linear regression analyses were performed for all linearised dependent variables against non-transformed independent variables. The best regression equations were selected by the following criteria:

- constants and coefficients with reasonably small standard error;
- P-value < 0.1;
- as high R^2 (coefficient of determination) as possible.

For the following explanation of disease dynamics, only one type of transformation was selected for all epidemic curves, which gave the best result of general criteria. Obtained linear regression equations were used to quantify the disease growth rate parameters (k). Rate parameters were obtained from slope values of linear regression of transformed disease proportions over time (*Berger*, 1981).

Area Under Disease Progress Curve. Area under disease progress curve(s) (AUDPC) was also calculated based on *Naragajan & Muralidharan* (1995).

$$\text{AUDPC} = \sum_{i=n}^{n} 0.5 * (x_i + x_{i-f}) * d$$

where x_i = disease incidence of fruit and leaf at the end of the week 'i', f = the number of successive evaluations of disease, d = interval between two evaluations.

Results

Weather conditions

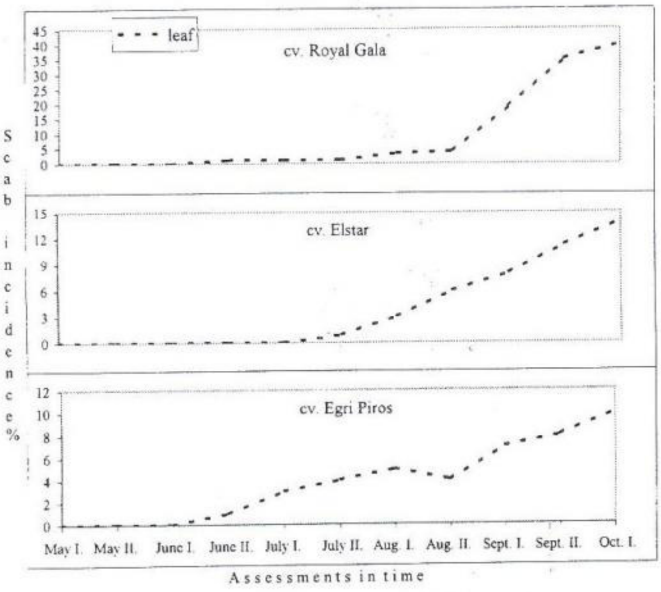
A total of 39 and 41 infection periods were recorded from green tip through to harvest in 1998 and 1999, respectively. Mills infection periods were heavy 12 and 18 times, moderate 17 and 9 times and light 10 and 13 times, in 1998 and 1999. The spring of both years was relatively wet. Mean daily temperatures ranged from 8 to 29 °C, but temperatures were between 17 and 23 °C during most of the period. In conclusion, weather parameters were favourable for continuous disease progression during both growing seasons.

Cumulative disease progress curves

Figures 1 and *2* show the average scab disease progression on selected apple cultivars in 1998 and 1999.

No fruit scab was observed on the selected six cultivars in integrated production (disease progress curves not shown). Leaf scab was observed on 3 out of 6 selected cultivars (cv. Gala Must, cv. Elstar, cv. Egri Piros). Considerable increases in cumulative disease progression can be observed from the middle of August, when spraying was stopped *(Figure 1)*.

In the organic growing system, five out of the six cultivars were infected *(Figure 2)*. Only cv. Releika showed no symptoms on either fruit or leaf (disease progress curves not shown). The scab resistant cv. Relinda revealed low disease incidence both on leaf and fruit. Increase in leaf scab could be seen only after the middle of August. No fruit symptoms were observed on cv. Darusóvári, and less than four percent of fruit scab were on cv. Egri Piros.



* data of fruit scab incidence were zero, therefore the data curves of fruit disease incidence are not shown.

Figure 1 Disease progress curves of leaf incidence of apple scab on 3 apple cultivars in integrated fruit production in the average data of 1998 and 1999, Debecen–Pallag*

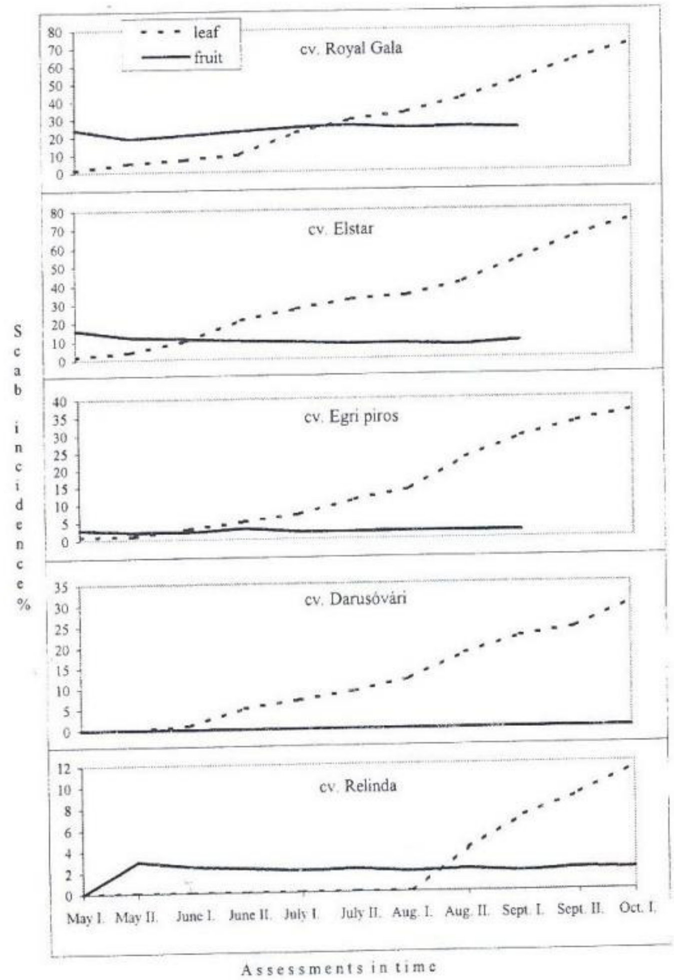


Figure 2 Disease progress curves of leaf and fruit incidence of apple scab on 5 apple cultivars in organic fruit production in the average data of 1998 and 1999, Debrecen–Pallag

The first lesions of leaf scab appeared at the middle of May on both cultivars and cumulative leaf disease progression increased continuously until reaching about 35 percent. Susceptible cultivars (cv. Gala Must and cv. Elstar) showed high values of fruit disease incidence, but fruit scab decreased slowly during both seasons. The first leaf scab symptoms were observed at the end of April on both susceptible cultivars (data not shown), and severe leaf scab epidemics developed by the middle of October.

Analysis of curves

Regression analysis. Data of all disease progress curves were linearised by all transformation equations. Generally, the best linearisation was given by logistic transformation. Values of intercept, slope, standard errors (SE), coefficient of determination (R^2) and mean standard errors (MSE) of obtained linear regression equations can be seen in *Table 3*. The criteria of linear regression are fulfilled, and R^2 shows generally 0.8 values, standard errors are reasonably small in the case of leaf incidence. Disease growth rate parameters (k) obtained from the slope of linear regression equations

Table 3 Linear regression analyses of disease progress curves of leaf and fruit scab incidences on 5 apple cultivars in organic and integrated fruit growing systems, Debrecen–Pallag, 1998–1999[a]

Cultivars	System[b]		MSE[c]	R^2 [d]	Intercept[e]	Se_i [f]	Slope[g]	Se_s [h]	Equations	F-test[i]
Gala Must	ORG	fruit	0.012	30.2	-1.288	0.069	0.002	0.001	y = 0.002 x – 1.288	*
Gala Must	ORG	leaf	0.144	94.6	–3.684	0.215	0.032	0.002	y = 0.032 x – 3.684	***
Gala Must	INT	leaf	0.306	89.6	–7.116	0.588	0.044	0.005	y = 0.044 x – 7.116	***
Elstar	ORG	fruit	0.022	71.7	–1.865	0.092	–0.005	0.001	y = 0.006 x – 1.865	**
Elstar	ORG	leaf	0.154	93.4	–3.292	0.222	0.029	0.002	y = 0.029 x – 3.292	***
Elstar	INT	leaf	0.134	88.2	–6.938	0.673	0.036	0.005	y = 0.036 x – 6.938	**
Egri Piros	ORG	fruit	0.024	20.3	–3.619	0.097	–0.002	0.001	y = –0.002 x – 3.619	*
Egri Piros	ORG	leaf	0.082	96.1	–4.491	0.163	0.028	0.001	y = 0.028 x – 4.491	***
Egri Piros	INT	leaf	0.105	88.2	–4.893	0.346	0.018	0.003	y = 0.186 x – 4.893	***
Relinda	ORG	fruit	0.006	76.1	–3.520	0.063	–0.004	0.001	y = –0.004 x – 3.520	**
Relinda	ORG	leaf	0.015	93.6	–5.662	0.473	0.024	0.003	y = 0.024 x – 5.662	**
Darusóvári	ORG	leaf	0.182	86.4	–4.516	0.361	0.026	0.003	y = 0.026 x – 4.516	***

[a] Used data set of fruit and leaf incidences are linearised by logistic transformation.
[b] ORG fruit = fruit incidence data in organic growing system, ORG leaf = leaf incidence data in organic growing system, INT fruit = fruit incidence data in integrated growing system, INT leaf = leaf incidence data in integrated growing system.
[c] MSE = mean standard error.
[d] R^2 = coefficient of determination.
[e] Slope values is the coefficient of linear regression analysis and the disease growth rate (k) of disease progress.
[f] SE_i = standard error of intercept.
[g] Intercept is the constant of linear regression analysis.
[h] SE_s = standard error of slope.
[i] F-test = *** < 0.01, ** 0.01 – 0.05, * 0.05 – 0.1, ns > 0.1.

showed the highest values on leaf disease incidence of cv. Gala Must and cv. Elstar in the organic growing system. Adequate leaf disease incidence rate parameters were higher in the organic system than in the integrated system. Values of rate parameters of fruit incidences were low and with negative values on cv. Elstar, cv. Egri piros and cv. Relinda.

Area Under Disease Progress Curves. In all cases, values of adequate AUDPC were higher in the organic than in the integrated fruit growing system. Values of AUDPC showed great differences in leaf disease incidences among cultivars *(Table 4.)*. Differences in leaf incidences were the largest in the organic growing system. Leaf AUDPC values of susceptible cultivars were two to more than ten times higher compared to old and resistant cultivars, respectively. Great differences can be seen in fruit incidence of AUDPC values in the organic production system. Cultivar differences in AUDPC values were smaller in integrated production. The values of AUDPC also showed significant differences between growing systems *(Table 4)*.

Discussion

The present study describes and analyses epidemic disease procession of apple scab on susceptible and resistant apple cultivars in two environmentally friendly growing systems.

Cumulative disease progress curves showed continuous but asymmetrical scab development on the moderate or highly susceptible cultivars Gala Must, Elstar and Egri Piros, and on the tolerant or resistant cultivars Darusóvári and Relinda, in both systems. In the past, researchers found that apple scab epidemic follows skewed progressive curves, but not symmetric normal distribution (*Analytis & Kranz,*

Table 4 Area under disease progress curves (AUDPC) of leaf and fruit incidence of apple scab on 6 apple cultivars in organic and integrated fruit growing systems, Debrecen-Pallag, 1998–1999[a]

Cultivars	Integrated		Organic	
	leaf	fruit	leaf	fruit
Gala Must	1275.1 ab	0.0	4440.7 a	2991.0 a
Elstar	541.5 b	0.0	4875.1 a	1233.5 b
Egri Piros	555.3 b	0.0	2167.5 b	288.6 cd
Darusóvári	0.0 c	0.0	1695.1 b	0.0 d
Relinda	0.0 c	0.0	386.3 c	314.2 c
Reanda	0.0 c	0.0	0.0 c	0.0 d
F-test[c]	***	–[e]	***	***
SED (df =24)[d]	46.11	–	331.3	111.3
$LSD_{0.05}$	95.16	–	703.6	292.9

Systems	Means of cultivars		Means of systems
	leaf	fruit	friut+leaf
integrated	366.1 a	0.0 a	183.1 a
organic	2260.8 b	804.4 b	1532.6 b
F-test	**	*	***
SED (df =10)	714.3	457.3 (df =22)	518.2
$LSD_{0.05}$	1811.2	802.4 1074.3	

a Used data set of fruit and leaf incidences are mean data from 1998 and 1999.
b Values within columns followed by different letters are significantly different.
[c] F-test = *** < 0.01, ** 0.01 – 0.05, * 0.05 – 0.1, ns > 0.1.
[d] SED = standard errors of differences of mean values, df = degrees of freedom.
[e] Because of zero values, no F-test, SED and LSD values are available.

1972; *Analytis*, 1973; *Analytis*, 1979 and *MacHardy*, 1996). Investigations showed that asymmetric disease progression required curve-linear transformation for epidemic analysis (*Analytis & Kranz*, 1972). Many scientists found that the logistic and Gompertz models are the most appropriate for the description of curve-linear increase of disease incidence in time (*Jeger*, 1982; *Pataky*, 1998). *Berger* (1981) compared the widely used logistic model (*Van der Plank*, 1963) to the Gompertz model by linearised 113 selected disease curves. He concluded that the Gompertz model was superior to the logistic model in the classification of disease epidemic. In this study, the logistic function gave the highest correlations in both growing systems, in agreement with statements of *Van der Plank* (1963), *Jeger* (1982) and *Pataky* (1988). Moreover, *Analytis* (1973), who studied one type of control system, also claimed that the logistic function was one of the best models for classifying apple scab epidemics. He found good results with Gompertz and Mitscherlich and Bertalanffy transformations (n=2 and 3), but this could not be proved in this study.

The disease growth rate *(k)* obtained from slope values of linear regression equations were variable depending on the growing system, the cultivars and the plant organ *(Table 3)*. *Analytis* (1973), in his epidemiological investigations on apple scab, observed that the rate of disease increase varied from 0.1 to 0.34 in the number and diameter of scab lesion of individual leaves. Values of leaf disease incidence rates ranged from 0.018 to 0.044 in this study. Values of disease growth rate were similar in both systems (IFP and organic) after the middle of August. This was due to the termination of sprayings in both systems. This could provide for a quick disease spread on young, susceptible apple foliage, though the inoculum source was at low level. Consequently, the result of this fast disease spread was that the average disease growth rates in the IFP system were almost as high as in the not so efficient, organic control system. There is no scientific information about disease growth rate of apple scab under different fungicide treatments, but results of investigations of *Plaut & Berger* (1981), *Gregory* et al. (1981) and *Rouse* et al. (1981) supported our findings on other diseases. They found that if epidemics were begun from ever lower levels of initial disease, then early disease progression was increasingly faster or the disease growth rate was increasingly higher. In this work, leaf disease growth rate parameters provide permanent disease progression with a high correlation in the average of both years. In contrast, fruit disease growth rate parameters support a slow disease increase or, in some cases, slow disease decrease in fruit scab epidemic progression. The reason for this is that some of the early-infested fruits had fallen and the ontogenic resistance of fruits steadily increased during the growing season. Consequently, the percentage of diseased fruits was somewhat lower through the summer and into early autumn, compared to the spring disease level.

Although the disease growth rates were similar on plant organs separately in the two growing systems, the "area under disease progress curves" (AUDPC) showed great differences in both control systems. *Van der Plank* (1963) and *Kranz* (1974) found close correlation between the apparent infection rate and the "area under disease progress curves" in some diseases. In contrast, AUDPC was closely related with cumulative disease progress curves and not with disease growth rate in this work. Values of AUDPC referred to the effectiveness of fungicides and level of epidemic in both growing systems. Moreover, AUDPC gave more differences for comparison of disease epidemic progresses than disease growth rate in different disease control systems.

In summary, the results of this study proved that all selected apple cultivars can be grown widely in integrated fruit production, but scab resistant cultivars should be given preference in organic growing. However, scab resistant cultivars have some disadvantageous fruit quality parameters, which can delay the widely spread of these cultivars. *Fisher* (1991), who purified by breeding apple cultivars (cv. Releika, cv. Reanda, cv. Remo cv. Relinda ...etc.), tried to explain this contrast by using complex disease resistance. These cultivars are successful abroad, but here in Hungary, some of these cultivars, such as Relinda, showed low disease incidence. This result shows that different ecological conditions and different strains of apple scab fungus might "break" the resistance of some cultivars. The genes of scab resistance have not been known in most of the old Hungarian apple cultivars, but their no fruit and low leaf disease incidences provide that some old Hungarian cultivars can serve as a good genetic basis for apple breeding; this, in order to improve the scab resistance of current cultivars. Current cultivars can be grown widely in integrated fruit production, but the disease control methods of organic growing are not sufficient enough for growing susceptible apple cultivars. To solve this problem, more efficient disease control methods or biological control agents are necessary in ecologically based growing systems.

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