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Development and evaluation of a model for management of brown rot in organic apple orchards

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Abstract Temporal development of brown rot (*Monilinia fructigena*) on fruits was analysed in two organic apple orchards on three apple cultivars in Eastern Hungary from 2002 to 2006. The three-parameter logistic function gave the best fit to brown rot over four non-linear growth functions in all cultivars, years and orchards. Depending on location, year and cultivar, disease increased continuously from 6 to 8 weeks before harvest up to harvest, reaching 19–37% of disease incidence. Disease variables of Y_f, the final disease incidence; β, relative rate of disease progress; $AUDPC_S$, standardized area under disease progress curve; $T_{1.5}$, the time when disease incidence reaches 1.5% (day), and M, the inflection point were derived from the three-parameter logistic function. The disease variables of Y_f, β, and $AUDPC_S$ were used in a computer simulation for predicting temporal brown rot development, and the disease variables of $T_{1.5}$, M, and Y_f were used to determine threshold values for epidemic intensity. Afterwards these were used to construct a fundamental model for developing a brown rot forecasting and management strategy (BRFMS). The fundamental model contained four parts: i) data insertion and analyses by computer simulation of pathogen submodels, ii) calculation of yield loss threshold levels based on disease incidence, iii) determination of epidemic intensity levels and iv) a decision module with suggestions for disease management practices for each epidemic intensity level. The fundamental model was supplemented with the prediction of occurrence of the first fruit rot symptoms and with the insect injury prediction related to brown rot development in order to complete a BRFMS for organic apple orchards. In a 3-year field evaluation from 2006 to 2008, season-long application of BRFMS treatments reduced the number of sprays against brown rot by 22–33% compared with the treatments of general spray schedules against brown rot.

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Introduction

Monilinia fructigena (Aderh. & Ruhl.) Honey is an important pathogen causing pre- and post-harvest fruit rot in apple orchards (Byrde and Willetts 1977). Losses caused by *M. fructigena* are usually low (between 0 and 9%) in well-treated conventional and integrated apple orchards (Moore 1950; Berrie 1989; Falconi and Mendgen 1994; van Leeuwen et al. 2000, 2002; Xu and Robinson 2000; Holb 2009); however, considerable yield loss may be expected from unsprayed and/or poorly managed organic apple orchards (Burchill and Edney 1972; Holb and Scherm 2007). Burchill and Edney (1972) assessed 36% fruit loss in unsprayed cider apple orchards. Holb and Scherm (2007) and Holb (2009) demonstrated that pre-harvest yield loss caused by *M. fructigena* could reach 46% by harvest in a susceptible apple cultivar in organic orchards. These and earlier studies (e.g. Bertram 1916; Harrison 1933) also emphasized that insects were significantly related to yield loss caused by *M. fructigena*.

Brown rot control is usually associated with apple scab control in organic apple orchards, but no warning systems are used in general for timing spray application against brown rot. A general strategy is to avoid insect injury by sprays of biological products and reduce sporulation of the infected fruits by regular sprays of sulphur and/or copper compounds. Sulphur compounds can suppress populations of several predatory mite species (e.g. Childers et al. 2001; Prischmann et al. 2005), which are essential in biological control against phytophagous mites in organic orchards (Anon. 2000; Weibel and Häseli 2003). In addition, copper sprays can negatively affect soil ecology and earthworm populations (e.g. Van Rhee 1976; Paoletti et al. 1998; Friis et al. 2004). All in all, there is a need for a brown rot management strategy for timing spray applications in order to reduce copper and sulphur sprays against brown rot. However, such a strategy is not known for organic apple orchards.

Most of the PC based forecasting systems are based on the Seem-forecasting model for apple scab, which contains several submodels such as the fundamental pathogen submodel (Seem et al. 1989). The pathogen submodel contains a basic data set of the pathogen epidemiological features (such as spore dispersal, infection, disease development, and their relationship with weather parameters) and specific analysing methods or mathematical tools. Construction of a submodel for certain plant pathogens can directly contribute to practical spray application. In case of brown rot, caused by *M. fructigena,* several studies emphasized that insect and/or bird injuries were significantly related to yield loss caused by *M. fructigena* (Bertram 1916; Harrison 1933; Lack 1989; Van Leeuwen et al. 2000; Xu and Robinson 2000), and the study of Holb and Scherm (2008) quantified the contribution of insect injury to brown rot development using correlation analyses and Jaccard indices. Autoregressive models for seasonal spore dispersal were developed, and the relationship between weather parameters and spore dispersal/ infection as well as between seasonal spore dispersal and corresponding disease incidence data were determined for *M. fructigena* (Xu and Robinson 2000; Holb 2008; Bannon et al. 2009). In addition, one study (Holb and Scherm 2007) demonstrated that the inoculum source of the first symptoms on the tree originated from dropped fruits, which was based on the time lag period of AUDPC determined by the initial point of the fruit incidence on the orchard floor and the initial point of the fruit incidence on the tree. However, no study has analysed the structure of temporal brown rot development in highly infested (e.g. organic) apple orchards on early-season and late-season cultivars. This knowledge is missing yet is needed to be able to complete a basic brown rot forecasting model. A basic forecasting model can be incorporated into a disease management strategy, and thus a practically applicable brown rot forecasting and management strategy (BRFMS) can be reached that may also meet the reduction of fungicide use in organic apple orchards.

The objectives of this study were, firstly, to select the best mathematical function to describe disease progress of brown rot on apple fruit and identify the most important disease variables for brown rot development; secondly, to construct a BRFMS; and, thirdly to evaluate this BRFMS in season-long spray programs for 3 years in organic apple orchards.

Materials and methods

Assessment and analyses of temporal brown rot progress

Orchard site and plant material

A 5-year study (2002–2006) was carried out in two organic apple orchards in Eastern Hungary. The first orchard was located in Nagykálló (47°53′10″N, 21°51′20″E), and the second was in Eperjeske (48°21′30″N, 22°13′10″E), 77 km north of Nagykálló. The orchards consisted of seven and four apple cultivars, with a minimum of 600 and 800 trees of each cultivar. The orchards had 80 and 30 rows, respectively, with a planting distance between rows of 4 m and within rows of 1.5 m. Trees were on rootstocks M9 at Nagykálló and M26 at Eperjeske and pruned to spindle shape in both locations. Hungarian organic production guidelines (Anon 1997) derived from the IFOAM (International Federation of Organic Agriculture Movements) standards (Anon 1989, 2000) had been applied since the orchards were planted in 1996. Spray schedules against fungal diseases during 2002–2006 are given in Table 1. All sprays were applied with a Kertitox 2000 axial fan sprayer (Debreceni Gépgyár B.V., Debrecen, Hungary) with a ceramic hollow cone nozzle at a spray pressure of 1.1–1.2 MPa with a volume of 1000 $l\,ha^{-1}$. Rainfall ($mm\,day^{-1}$), and temperature ($°C\,day^{-1}$) were recorded with a METOS agrometeorological station (Pessl Instrument GmbH, Weiz, Austria) in both orchards from 1 May to 10 October in 2002 and 2003.

Experimental design and disease assessment

At both locations, the same three apple cultivars, an early-season cultivar (Prima) and two late-season cultivars (Idared and Mutsu), were selected for disease assessment from 2002 to 2006. Differences in susceptibility to brown rot for the three cultivars have not been indicated in previous studies. Fruit size in month-periods are given in Table 2 for each cultivar, as they differed among early- and late-season cultivars in calendar time, and later fruit development stages of all cultivars are considered to be more susceptible to brown rot (van Leeuwen et al. 2000; Xu et al. 2001). Four experimental plots (i.e. four replicates) were prepared in both orchards. Each experimental plot consisted of rows with a minimum of 150 trees for each cultivar. Twenty trees were selected randomly (as subsamples) within each experimental plot on each assessment date, i.e. new groups of twenty trees were always selected for the following assessment date. On each selected tree, disease assessments were made on all fruits (approx. 70–120 fruit per tree) on a weekly basis (7–9 days) from 20 May until 30 August for cv. Prima and until 10 October for cvs. Idared and Mutsu. Fruits were harvested on 30 and 31 August for cv. Prima, on 3 and 5 October for cv. Idared and on 11 and 14 October for cv. Mutsu in 2002 and 2003, respectively. All fruits, including healthy and diseased, were counted on each selected tree at each assessment date. A fruit was considered to be diseased if it showed typical sporulation of *M. fructigena*. Brown rot incidence was calculated as the percentage of diseased fruits.

Curve fitting and disease variables

For each experimental plot, data of each subsample were taken at each assessment date. As the field design consisted of a specific effect of experimental plots with subsamples (a repeated measures design with additional random effects), a nonlinear mixed-effect modelling approach was used according to Pinheiro and Bates (2000). For our study we fitted models based on the three-parameter logistic function, $Y_t = \frac{Y_f}{1+e^{-\beta(t-M)}}$, which is suggested in several scientific works (see e.g. Campbell and Madden 1990; Ngugi et al. 2000; Holb et al. 2005). For the discussion on the choice of this particular family of curves see Holb et al. (2005). Models were fitted using R version 2.8.1 (Anon 2008) with the statistical package 'nlme' (nonlinear mixed-effect) (Pinheiro et al. 2008). For selecting the best fitted models, both the Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC) were used (Burnham and Anderson 2002). Furthermore, likelihood ratio test was also applied for the comparison of the fitted models. Besides the above-mentioned model selection methods, an important purpose of the curve fitting was to analyse the impact of several factors—year, location, cultivar—hence they were all tested through certain fixed and random effects in order to obtain the most appropriate model and to understand the 'type' of the

Table 1 Spray schedules against fungal diseases in two Hungarian organic apple orchards (2002–2006, Nagykálló and Eperjeske)

Nagykálló				Eperjeske			
Date	Phenolog. stage[a]	Trade name[b]	Dosage	Date	Phenolog. stage	Trade name	Dosage
2002				2002			
1 April	Bud break	Rézoxiklorid 50WP	2.0 kg ha^{-1}	26 March	Bud break	Cuproxat FW	1.0 kg ha^{-1}
14 April	Green tip	Cuproxat FW	1.0 l ha^{-1}	5 April	Green tip	Cuproxat FW	1.0 kg ha^{-1}
22, 28 April, 4, 14, 21, 29 May, 3, 12, 15 June, 11, 25 July, 15, 31 August, 5, 14 Sept	After green tip	Tiosol	10.0 l ha^{-1}	13, 24 April, 2, 9, 18, 27 May, 2, 12, 24 June, 1, 8, 22 July, 8, 15, 27 August, 5, 14 25 Sept	After green tip	Szulfur 900FW	4.0 kg ha^{-1}
2003				2003			
29 March	Bud break	Rézoxiklorid 50WP	2.0 kg ha^{-1}	27 March	Bud break	Cuproxat FW	1.0 kg ha^{-1}
6 April	Green tip	Rézoxiklorid 50WP	1.0 kg ha^{-1}	4 April	Green tip	Cuproxat FW	1.0 kg ha^{-1}
15, 22, 28 April, 5, 17, 25 May, 1, 14, 27 June, 14, 26 July 4, 21, 31 August, 5, 14, 21 Sept	After green tip	Kumulus S	4.0 kg ha^{-1}	13, 18, 27 April, 9, 14, 26 May 6, 17, 29 June, 11, 26 July, 8, 19, 30 August, 2, 11, 21 Sept	After green tip	Szulfur 900FW	4.0 kg ha^{-1}
2004				2004			
1 April	Bud break	Rézoxiklorid 50WP	1.0 kg ha^{-1}	29 March	Bud break	Cuproxat FW	1.0 kg ha^{-1}
15 April	Green tip	Cuproxat FW	1.0 l ha^{-1}	7 April	Green tip	Rézoxiklorid 50WP	2.0 kg ha^{-1}
23, 27 April, 5, 13, 20, 29 May, 2, 11, 14 June, 18, 27 July 7, 24 August, 15, 27 Sept	After green tip	Tiosol	10.0 l ha^{-1}	10, 23 April, 5, 11, 19, 26 May, 4, 17, 28 June, 6, 20 July, 2, 15, 31 August, 9, 21 Sept	After green tip	Tiosol	10.0 kg ha^{-1}
2005				2005			
28 March	Bud break	Rézoxiklorid 50WP	2.0 kg ha^{-1}	31 March	Bud break	Cuproxat FW	1.0 kg ha^{-1}
7 April	Green tip	Rézoxiklorid 50WP	1.0 kg ha^{-1}	8 April	Green tip	Cuproxat FW	1.0 kg ha^{-1}
17, 21, 30 April, 6, 16, 24 May, 1, 13, 26 June, 17, 25 July, 10, 16, 31 August, 3, 11, 23 Sept	After green tip	Kumulus S	5.0 kg ha^{-1}	9, 15, 23 April, 2, 10, 18, 26 May 5, 18, 27 June, 9, 21 July 7, 17, 31 August, 3, 11, 24 Sept	After green tip	Szulfur 900FW	5.0 kg ha^{-1}
2006				2006			
2 April	Bud break	Cuproxat FW	1.0 l ha^{-1}	31 March	Bud break	Rézoxiklorid 50WP	2.0 kg ha^{-1}
13 April	Green tip	Rézoxiklorid 50WP	1.0 kg ha^{-1}	6 April	Green tip	Cuproxat FW	1.0 kg ha^{-1}
20, 26 April, 3, 11, 20, 25 May, 1, 9, 20 June, 10, 18, 28 July 5, 20, 31 August, 11, 28 Sept	After green tip	Tiosol	11.0 l ha^{-1}	14, 21 April, 1, 8, 15, 24 May, 1, 10, 23 June, 1, 10, 21 July, 5, 14, 25 August, 2, 9, 17 Sept	After green tip	Kumulus S	5.0 kg ha^{-1}

[a] Bud break: when dormant bud is open. Green tip: when first green tissue appears after bud break. After green tip: all phenological stages from early tight cluster until harvest.

[b] Cuproxat FW: 350 g l^{-1} copper sulphate, NuFarm Ltd., Linz, Austria; Szulfur 900 FW: 900 g l^{-1} elementary sulphur, Budapesti Vegyiművek GmbH, Budapest, Hungary; Rézoxiklorid 50 WP: 50% copper oxychloride, Agroterm Ltd., Peremarton, Hungary; Kumulus S: 80% elementary sulphur, BASF Hungaria Ltd., Budapest, Hungary; Tiosol: 29% calcium polysulphides, Tiosol Ltd., Kistelek, Hungary.

Table 2 Mean fruit size in diameter (mm) from mid-May to early October for three apple cultivars over a 5-year period (2002–2006, Nagykálló and Eperjeske, Hungary)

Month	Prima	Idared	Mutsu
mid-May	8.0[a]±0.4[b]	5.1±0.5	5.3±0.3
mid-June	36.1±1.2	34.1±1.3	37.6±0.6
mid-July	61.2±1.8	52.1±1.7	62.7±1.3
mid-August	70.1±2.6	65.6±2.0	73.2±1.7
mid-September	–	73.9±2.4	80.7±2.1
early October	–	76.3±3.2	82.3±3.3

[a] Mean data of 2 years and two locations.

[b] Standard deviation of means.

impact (if any) of these factors. Based on the t tests, the significance of such effects was also tested.

For an easier interpretation, only the mean values of incidence were plotted against time for each cultivar, location and year together with the best model in order to make visible the goodness-of-fit of a single case. However, it is noted that mean values were only used for the graphical plots, the models were all fitted to the original data sets.

Given the estimates of the parameters (Y_f, the final disease incidence; β, the relative rate of disease progress; and M, the inflection point at which disease progress is fastest), one can derive some further parameters (disease variables), which are often used for the description of the disease progress in the literature. For the three cultivars analysed we show the values of some indicators, namely Y_t, an estimate for disease at different time points t; θ, the absolute rate calculated as $Y_f*\beta/4$; $T_{1.5}$, the time when disease incidence reaches 1.5% (this variable provides an estimate of the time at which disease could be first observed in the field); the standardised area under the disease progress curve ($AUDPC_S$).

Development of brown rot forecasting and management strategy (BRFMS)

The BRFMS was developed in three steps: firstly a fundamental model was created, secondly occurrence of first fruit rot symptoms was predicted, and thirdly insect injury prediction related to brown rot development were prepared and attached to the fundamental model in order to complete a BRFMS for organic apple orchards (Fig. 1). The fundamental model contained four parts: i) data insertion and analyses by computer simulation of pathogen submodels, ii) calculation of yield loss threshold levels based on disease incidence, iii) determination of epidemic intensity levels, and iv) a decision module with suggestions of disease management practices for each epidemic intensity level (Fig. 1).

The fundamental model

In the fundamental model, data insertion and analysis by computer simulation of pathogen submodels were based on four epidemiological features of the fungus assessed under field conditions: i) seasonal spore dispersal, ii) relationship between seasonal spore dispersal and weather parameters, iii) relationship between seasonal spore dispersal and corresponding disease incidence data, and iv) features of temporal disease progress curves. Forecasting in the pathogen submodel was based on certain mathematical functions, correlation analyses and/or on some derived parameters from the above steps as follows:

i) Seasonal spore dispersal was predicted using autoregressive (AR) models with 6 autoregressive parameters (Φ_i) as a function of $y_t = \sum_{i=1}^{n} \Phi_i y_{t-1} + a_t$ developed for *M. fructigena* by Holb (2008). $y_t = x_t - x_{t-24}$ is the difference between the current number of conidia, x_t, and the number in the

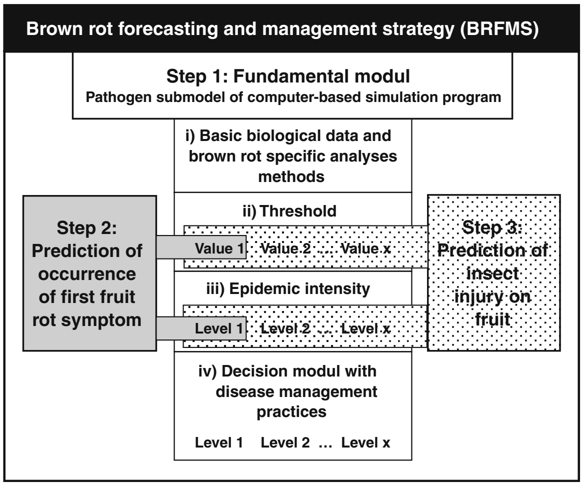

Fig. 1 Basic elements for developing a brown rot forecasting and management strategy (BRFMS) against *Monilinia fructigena* in organic apple orchards

equivalent number of conidia the previous day, x_{t-24}, (this difference is used to remove diurnal periodicity); parameter Φ_i represents autoregressive parameters from 1 to n; and a_t is a white noise with a variance of δa^2.

ii) Using correlation coefficients determined by Holb (2008) on 3-year data of temperature, relative humidity and wind speed related to data of seasonal spore dispersal were incorporated into the fundamental model.

iii) The three-parameter Gompertz function of $y = 21.709 - 30.74\exp(-\exp(0.00004(x - 29455)))$ developed on a 3-year data set by Holb (2008) was used for describing the relationship between seasonal spore dispersal and corresponding disease incidence data in the fundamental model.

iv) The three-parameter logistic functions for temporal brown rot progress developed in this study were used to generate the disease progression of the predictive season.

For calculation of yield loss threshold levels and determination of epidemic intensity levels in the fundamental model, threshold values of brown rot development were determined by the maximum values of the parameters $AUDPC_S$, Y_f, and β derived from the logistic functions of brown rot disease progress curves, and furthermore epidemic intensity levels were developed from the derived parameters T_0, M, and Y_f.

Prediction of first symptom occurrence and prediction of insect injury

Prediction of first symptom occurrence (T_0) in the first level of epidemic intensity was based on using the method of time lag period of *AUDPC* between fruit rot incidence on the orchard floor and on the tree developed by Holb and Scherm (2007) in a 4-year brown rot study.

A majority of insect injury was assumed to be caused by codling moth larvae according to the study of Holb and Scherm (2008). Therefore, regression equations between codling moth trapping and insect injury as well as between insect injury and brown rot incidence developed by Holb and Scherm (2008) in a 4-year brown rot study were applied to predict the insect injury in the further levels of threshold and epidemic intensity parts of the disease forecasting management strategy.

Practical evaluation of BRFMS in season-long spray programs

Season-long spray schedules were performed for practical evaluations of the newly developed BRFMS in the same two apple orchards at Eperjeske and Nagykálló, as used for the disease development study. Three equally sized treatment blocks were established with cv. Idared in both orchards from 2006 to 2008. In block 1, brown rot management was applied based on the newly developed BRFMS (according to Fig. 4, a delay in the onset of sprays compared to the IFOAM standard), in block 2, general brown rot management was applied according to IFOAM standards (according to tree phenological stages preventive fungicide spray schedules applied at 10–14 day intervals), and in block 3, no brown rot and insect management was applied. All blocks were set up in 2006 and were re-randomised in each year. All blocks were replicated four times and each consisted of a minimum of 30 trees per cultivar. Treatment blocks were prepared on 15 February 2006, 19 February 2007, and 11 February 2008, and one spray was applied with Funguran-OH 50 WP (77% copper hydroxide, Spiess-Urania Chemicals GmbH, Hamburg, Germany) at 1 kg ha^{-1} at the same dates in blocks 1 and 2. Other sprays in both blocks were applied from 5 weeks after fruit set until harvest with Kumulus S (80% wettable sulfur, BASF Hungaria Ltd., Budapest, Hungary) at 4–7 kg ha^{-1} against brown rot and Dipel ES (3.2%, *Bacillus thuringiensis*, Valent Biosciences, USA) to avoid fruit injury caused by moth larvae. In block 1, sprays were applied according to the prediction of the new BRFMS; while in block 2, sprays were applied according IFOAM standards at 10–14-day intervals. In each year and location, brown rot incidence and the percentage of injury incidence were assessed at harvest according to Holb and Scherm (2008).

Data consisting of number of sprays, brown rot incidence and the percentage of injury incidence were subjected to analyses of variance (ANOVA) in order to determine the effect of spray treatment, location and year. Brown rot incidence and the percent of injury incidence data were transformed to *angular* (Y=arcsine $[\%]^{1/2}$) before the analysis. For all the three measures, F-tests ($P<0.05$) were followed by an unprotected Least Significance Difference (LSD)-test for comparison of the means of the three spray treatments' data using $LSD_{0.05}$ values.

Results

Weather conditions

Monthly mean temperature and rainfall amounts during the growing seasons were given in Table 3 for both locations from 2002 to 2008, respectively. The year 2003 was favourable for codling moth damage and especially years of 2005, 2006, 2007, and 2008 were conducive for fruit rot.

Analyses of temporal brown rot progress

In cv. Prima, disease progress started at the end of June or at the beginning of July in all years and both locations (Fig. 2). For cvs. Idared and Mutsu, disease progress started only in late July or early August. Disease increased continuously from 6 to 8 weeks before harvest up to harvest depending on the year, cultivar and location (Fig. 2).

Based on the role of the factor (grouping) variables—cultivar, location, and year—several models were tested, where the factors either had no particular separate effect on the model parameters or had some fixed or random types. Main results on the comparison of the best five models are summarised in Table 4. In the first model, all parameters were only associated with a single fixed effect, i.e. the grouping variables did not have a separate effect on the variables (Model 1). The parameters were significant ($P<0.05$; i.e. the parameters differed from zero); however, the structure of the estimates of the random effects suggested some hidden structure, i.e. a possible relationship of the factors with the parameters. This is demonstrated in Fig. 3, where for instance the impact of the cultivar variable, as well as the others, can easily be seen. Hence, further fixed effects of the factors (cultivar, location and/or year) were added to the model: first, only the effects of the factor cultivar for all parameters (Model 2); secondly, the factors cultivar and location for all parameters (Model 3); and thirdly, the previous model extended with the third factor, i.e. the year effects for all parameters of the curve (Model 4). The *t*-tests showed in all cases that the effects of cultivar were significant for all parameters in these models. However, both in Models 3 and 4, the location did not play any significant role (Table 5). Hence, in the next model (Model 5) we omitted the location as a factor. Table 4 shows the comparison of the five models. Based on the information criteria (AIC, BIC) the last two models seemed to be the best candidates; however, BIC and the likelihood ratio test clearly showed that Model 5 was the best. We concluded that Model 5 (which is a simplification of Model 4) was a reasonable choice for our purpose. Note furthermore, that the size of the random effect SD parameters in Model 5 relative to the parameter values is large (1.93, 10–24%) for the rate parameter β, slightly smaller (5.46, 7–15%) for the inflection point M, implying a few days of uncertainty, and not too large (2.85, 4–7%) in the case of the asymptote parameter. Table 5 shows the parameter estimates of the selected model (Model 5). The estimates clearly show the difference in the properties of the disease progress on early and late ripening cultivars (rate of the increase, the time corresponding to the inflection point).

Table 3 Monthly mean temperature (°C) and rainfall amounts (mm) during the growing seasons (from 1 May until 10 October) over a 7-year period (2002–2008, Nagykálló and Eperjeske, Hungary)

Year	Mean temperature		Rainfall	
	Nagykálló	Eperjeske	Nagykálló	Eperjeske
2002	11.2, 23.3 [a]	10.2, 22.1	277.3	298.7
2003	10.9, 22.6	10.6, 21.5	185.2	202.5
2004	10.6, 21.9	10.1, 22.1	287.3	278.2
2005	11.4, 23.1	10.1, 20.9	300.2	303.2
2006	10.1, 21.9	10.8, 20.7	321.1	311.1
2007	10.9, 23.3	10.9, 22.0	314.1	298.1
2008	10.1, 22.0	10.4, 21.9	345.1	333.2

[a] Miminum and maximum values of monthly mean temperature data during the period between 1 May and 10 October.

Development of brown rot forecasting and management strategy (BRFMS)

The BRFMS is provided in Fig. 4. The centre of the model is the computer simulated pathogen submodel, which requires insertion of field data and methods of data analysis for prediction purposes. Collected data from the field of a prediction year (weekly assessed fruit brown rot incidence, weather parameters) and

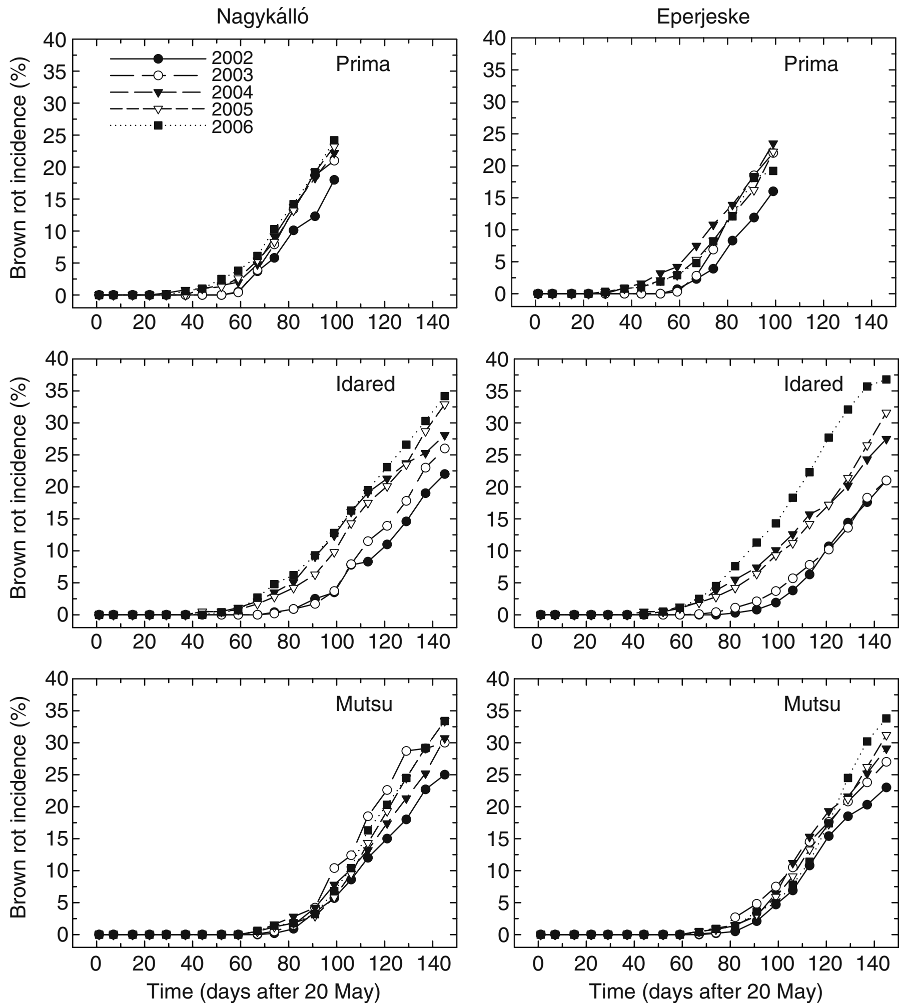

Fig. 2 Disease progress curves of brown rot incidence caused by *Monilinia fructigena* assessed on three apple cultivars (Prima, Idared, and Mutsu) in two organic apple orchards (Nagykálló and Eperjeske) from 2002 to 2006. Points represent mean values of the 14 and 20 incidence data in a year (80×14 and 80×20 data set in total annually) for early- and late-season cultivars, respectively

analyses methods including certain mathematical functions and derived parameters (AR6 model for spore dispersal; correlation coefficients for weather parameters; three-parameter Gompertz function for relationship between spore dispersal and corresponding disease incidence, maximum values of $AUDPC_S$, Y_f, and β derived from temporal brown rot progress curves) were inserted into the computer-based simulation program (Fig. 4A). A preliminary three-parameter logistic function was fitted by the simulation program after the fourth assessment date, and $AUDPC_S$, Y_f and β were calculated from the function for the given date. Then these calculated values of $AUDPC_S$, Y_f and β were compared with the given maximum values inserted into the simulation program at the beginning. If the calculated values reached the given values, then three threshold values of the brown rot development could be calculated: i) the first threshold value was the time when disease incidence reached 1.5% ($T_{1.5}$), ii) the second threshold value was the inflection point (M), and iii) the third threshold value was the final disease incidence (Y_f). Each threshold value corresponded to an epidemic intensity level that ranged between lower asymptote (Y_0) and $T_{1.5}$ for the first level (beginning of the disease development), between $T_{1.5}$ and M for the second level (increasing phase of the disease development), and between M and Y_f for the third level (final phase of the disease development).

An essential threshold and epidemic intensity part of the BRFMS was the timing of the first spray against fruit rot by predicting the occurrence of first infected fruit on the tree (Fig. 4B). In the first step, assessment of the first infected fruit on the orchard

Table 4 Evaluating goodness-of-fit of three parameter logistic models for temporal brown rot development by nonlinear mixed effect modelling approach including fixed and random effect of the factors cultivar (Prima, Idared, and Mutsu), location (Eperjeske and Nagykálló) and/or year (2002–2006)

Model[a]	df[b]	AIC[c]	BIC[d]	likelihood ratio test	comparison	P-value
1	10	5855.8	5912.6	−2917.9		
2	16	5589.7	5680.6	−2778.8	1 vs 2	<0.0001
3	19	5591.8	5699.7	−2776.9	2 vs 3	0.2761
4	31	5391.9	5567.9	−2664.9	3 vs 4	<0.0001
5	28	5395.1	5554.0	−2669.5	4 vs 5	0.0281

[a] Model 1: no fixed effects of the factors were included; Model 2: cultivar was included with fixed effects on each parameter; Model 3: cultivar and location were both included with fixed effects on each parameters; Model 4: cultivar, location and year were included with fixed effects on each parameters; Model 5: cultivar and year were used as fixed effect in the model.

[b] df = degree of freedom.

[c] AIC = Akaike's Information Criterion.

[d] BIC = Bayesian Information Criterion.

floor was inserted into the pathogen submodel according to Holb and Scherm (2007), which was based on time lag period of AUDPC determined by the initial point of the fruit rot incidence on the orchard floor and by the point of the appearance of fruit incidence on the tree. Hence the method was applied to predict the first symptom appearance (T_0) on the tree. The predicted value of T_0 was used in the first level of epidemic intensity and to time the first fungicide spray against brown rot. With this approach, 5–10 days prediction of appearance of first symptoms was achieved.

The success of BRFMS in the three epidemic intensity levels was largely dependent upon the prediction of insect injury (Fig. 4C). In our model, counts of codling moth adults from pheromone traps were used as the basic data sets. The incidence value of fruits injured by insects were predicted based on the regression models (see Holb and Scherm 2008) in which relationship between trapped moth, injured fruit and actual brown rot incidence was explained. With this approach, 7–14 days earlier prediction of a

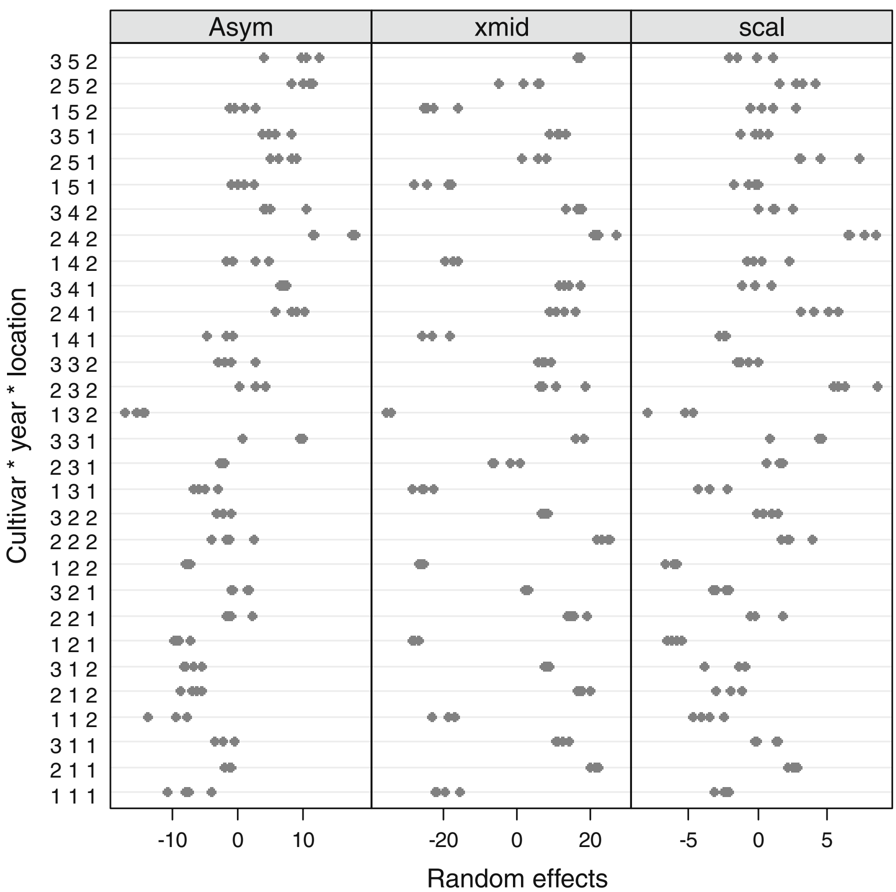

Fig. 3 Random effect patterns of Y_f (final disease incidence, referred as 'Asym'), M (inflection point, referred as 'xmid'), and β (relative rate variable, referred as 'scal') of the three parameter logistic model for combination of factors cultivar (with values 1, 2, 3), location (1, 2) and year (1, 2, 3, 4, 5). In model 1, cultivar, location and year were not fixed in the mixed effect modelling approach. Note: cultivar 1: Prima, cultivar 2: Idared, and cultivar 3: Mutsu; location 1: Nagykálló and location 2: Eperjeske; and year 1: 2002, year 2: 2003, year 3: 2004, year 4: 2005, and year 5: 2006

Table 5 Estimates of the coefficients and the significance levels of three-parameter logistic Model 3 and Model 5 for temporal brown rot development in 5 years (2002–2006) on three apple cultivars (Prima, Idared, and Mutsu) and at two locations (Eperjeske and Nagykálló). Cultivar and location were included with fixed effects in Model 3, and cultivar and year were included with fixed effects in Model 5

Model parameters	Value	SE [a]	df [b]	*t*-value	*P*-value
Model 3 [c]					
Y_f (intercept)[d]	25.055	1.148	2029	21.825	< 0.001
Y_f (Eperjeske)	−0.975	1.137	2029	−0.857	0.391
M (intercept)	81.107	1.229	2029	65.955	< 0.001
M (Eperjeske)	1.451	1.225	2029	1.184	0.236
β (Intercept)	9.894	0.496	2029	19.937	< 0.001
β (Eperjeske)	0.080	0.493	2029	0.162	0.871
Model 5					
Y_f (intercept)	18.323	0.846	2020	21.645	< 0.001
Y_f (Idared)	8.717	0.799	2020	10.902	< 0.001
Y_f (Mutsu)	7.990	0.764	2020	10.453	< 0.001
Y_f (2003)	3.432	0.960	2020	3.572	0.001
Y_f (2004)	3.853	0.955	2020	4.034	< 0.001
Y_f (2005)	13.299	1.071	2020	12.416	< 0.001
Y_f (2006)	12.873	1.001	2020	12.866	< 0.001
M (intercept)	84.584	1.451	2020	58.259	< 0.001
M (Idared)	37.027	1.342	2020	27.576	< 0.001
M (Mutsu)	35.721	1.305	2020	27.362	< 0.001
M (2003)	−3.277	1.699	2020	−1.928	0.053
M (2004)	−7.537	1.701	2020	−4.429	< 0.001
M (2005)	2.387	1.748	2020	1.365	0.172
M (2006)	−3.255	1.711	2020	−1.902	0.057
β (Intercept)	8.488	0.551	2020	15.378	< 0.001
M (Idared)	6.198	0.500	2020	12.389	< 0.001
M (Mutsu)	2.844	0.486	2020	5.847	< 0.001
M (2003)	−0.450	0.640	2020	−0.703	0.481
M (2004)	1.723	0.645	2020	2.669	0.007
M (2005)	3.676	0.651	2020	5.643	< 0.001
M (2006)	2.935	0.641	2020	4.575	< 0.001

[a] SE = standard error.

[b] df = degree of freedom.

[c] In case of Model 3 only the intercepts and the location factor related parameters are shown.

[d] Intercept covers cultivar Prima in 2002 at Nagykálló.

newly infected, injured fruit could be achieved in all epidemic intensity levels.

Then, in the decision module, brown rot management practices were suggested throughout the three epidemic intensity levels (Fig. 4D). In the period of the first epidemic intensity level, timing of the first fungicide spray, preventive insect spray programs based on injury prediction as well as the removal of dropped fruits according to the study of Holb and Scherm (2008) were applied. In the period of the second epidemic intensity level, fungicide sprays based on model prediction, insect spray programs based on injury prediction, and weekly removal of infected fruits from the tree and from the orchard floor were needed. In the period of the third epidemic intensity level, continuous insect spray programs based on injury prediction, intensive brown rot spray programs to reduce fruit rot and the removal of infected fruits were needed.

Practical evaluation of BRFMS in the general spray program

Analysis of variance of the number of sprays, brown rot incidence, and injury incidence indicated significant differences (*P* values ranged from 0.013 to 0.044) between the two locations, among three spray treatments, and 3 years (*data not shown*).

The number of sprays against brown rot was between 8 and 12 in the general spray schedules while the number of sprays was significantly (P values ranged form 0.021 to 0.046) reduced to 6–8 in the new disease management strategy in both locations and in the 3 years (Table 6). In general, the number of sprays against brown rot was reduced by 22.2–33.3% in the new management strategy compared with the IFOAM general spray schedules. Despite the spray reduction in the BRFMS treatments, both incidence types were not significantly different from each other in any years and locations in the two spray programs (BRFMS and general BRDM). In the 3-year field test, brown rot incidence and percent insect injury incidence on fruit were significantly ($P<0.05$) lower in both spray treatments in the two organic apple orchards compared to untreated control plots (Table 6).

Discussion

Temporal disease progress and disease variables

This study indicated that brown rot development on apple fruit could be described by a three-parameter

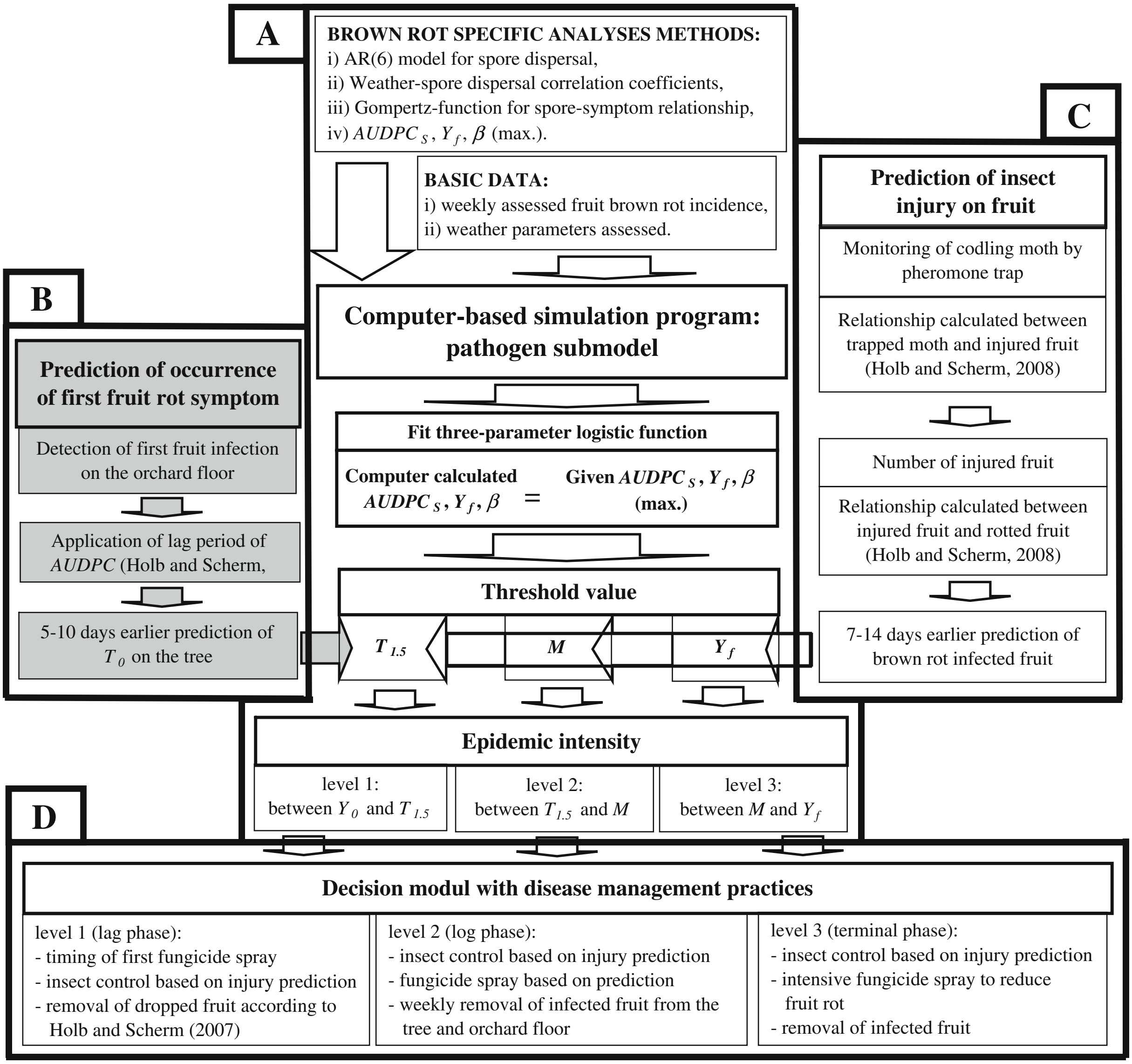

Fig. 4 A brown rot forecasting and management strategy (BRFMS) against *Monilinia fructigena* for organic apple orchards. Explanations: AR6 model: autoregressive model with six autoregressive parameters, *AUDPC*: area under the disease progress curve (%-days) $AUDPC_S$: standardized area under the disease progress curve (%-days), Y_f: is the final disease incidence (%), β: is the relative rate variable (day^{-1}), T_0: is the time when the first fruit symptoms occur on the tree (day), $T_{1.5}$: is the time when disease incidence reaches 1.5% (day), and *M*: is the inflection point, measured in days

logistic function giving the best fit over four non-linear growth functions. Logistic functions on temporal disease development were successfully used for other plant diseases including e.g. sorghum anthracnose (Ngugi et al. 2000), sorghum leaf blight (Ngugi et al. 2000) and apple scab (Holb et al. 2005). However, no attempt has been made before to justify this phenomenon for brown rot on apple fruits mainly due to low brown rot incidence values of most earlier studies (see Berrie 1989; van Leeuwen et al. 2000; Xu and Robinson 2000; Xu et al. 2001).

The goodness of fit of the model clearly shows that the three parameter logistic function was appropriate for describing the development of brown rot. Due to the complex non-linear mixed modelling approach, rather than fitting separate curves for each values of the factor variables as it is often the case in previous

Table 6 Number of sprays, final brown rot incidence on fruit and percent injury incidence at harvest (%) in three season-long spray programs in two organic apple orchards (Nagykálló and Eperjeske, 2006–2008)

	Number of sprays			Brown rot and injury incidence (%)[a]		
Treatments[b]	2006	2007	2008	2006	2007	2008
Nagykálló						
BRFMS	8.3[c] b[d]	7.5 a	7.5 b	26.1 (30.3) a	28.6 (33.1) a	23.3 (20.3) a
General BRDM	12.0 b	9.3 b	10.5 b	23.3 (28.6) a	27.1 (30.2) a	26.4 (22.2) a
Control	–[e]	–	–	52.5 (58.9) b	58.4 (55.5) b	45.1 (41.8) b
$LSD_{0.05}$[f]	2.6	1.4	2.0	10.7 (11.2)	12.1 (10.1)	8.9 (9.6)
Eperjeske						
BRFMS	7.3 a	6.5 a	8.3 a	24.2 (28.3) a	22.6 (27.2) a	25.2 (20.4) a
General BRDM	9.5 b	8.8 b	11.8 b	26.5 (32.1) a	26.4 (30.1) a	24.4 (21.2) a
Control	–	–	–	52.6 (61.4) b	50.8 (58.7) b	57.1 (48.2) b
$LSD_{0.05}$	1.6	1.4	2.8	14.2 (15.1)	13.1 (9.9)	16.1 (11.9)

[a] Percent insect injury incidence on fruits at harvest are in brackets.

[b] *BRFMS* sprays applied according to brown rot forecasting and management strategy; *General BRDM* general brown rot disease management according to Fig. 4, a delay in the onset of sprays compared to IFOAM standard, and Control: no brown rot and insect management.

[c] Data are means of four replications.

[d] Values within columns and location followed by different letters are significantly different.

[e] No spray was applied. Control is not included in the statistical analyses.

[f] Means in each spray treatment were compared using Least Significance Difference (LSD)-test at P=0.05.

reports (Ngugi et al. 2000; Holb et al. 2005), we obtained a more reliable model, where among others, the parameter estimates have better statistical properties, e.g. the values of the standard errors of the parameters are certainly better than in case of an estimation based on a 'single' time series (Table 5). Furthermore, in such a way the role of the factors (cultivar, location and year) and the corresponding effects (fixed and/or random) on the parameters can all be estimated and evaluated jointly.

This study also demonstrated that although the three-parameter logistic function described the brown rot development excellently by location and year, calculated disease variables showed different features of disease development for the different cultivars. On early-season cv. Prima, brown rot incidence was less than 1% 7 weeks before harvest at day 55 (Y_{55}); and the date when brown rot incidence reached 1.5% (for example $T_{1.5}$=58.6 and 63.9 in 2002 and $T_{1.5}$=60.5 and 62.1 in 2003) was close to values of Y_{55} for cv. Prima (Tables 4 and 5). These findings clearly demonstrated that, in agreement with the study of Xu et al. (2001) and of Van Leeuwen et al. (2000), brown rot development starts 6–8 weeks before harvest. The increasing brown rot incidence around harvest may be due to increasing insect and bird damages (Lack 1989; Van Leeuwen et al. 2000, 2002; Xu et al. 2001; Holb and Scherm 2008) and/or a negative correlation between fruit acidity and brown rot infection (Kaul 1984; Sharma and Kaul 1988). On the other hand, the first diseased fruits were already observed 8–10 weeks before harvest for cvs. Idared and Mutsu. Fruits of these late-season cultivars developed slower and they had a 2–4-week-delay in their developmental stages by the second half of the season compared with fruits of the early-season cultivar Prima. Earlier disease development of late-season cultivars was also demonstrated by disease variables of Y_{95} (brown rot incidence 7 weeks before harvest on late-season cv. Mutsu), which reached 1.5–6.3% on cv. Idared and 1.1–3.4% on cv. Mutsu (Fig. 2). At this time, cv. Prima was ripening, and around 20% of its fruits had brown rot (Fig. 2). The higher disease pressure from the early-season cv. Prima may have increased the probability of infection on the two late-season cultivars, which enhanced an earlier start of a brown rot epidemic than reported in previous studies (Van Leeuwen et al. 2000; Xu et al. 2001).

Brown rot management perspectives

This study demonstrated that the relative rate of disease progress (β) and the final disease incidence (Y_f) derived form the logistic model as well as the standardised area under the disease progress curve ($AUDPC_S$) calculated from the observed data can be interpreted as a general descriptor of brown rot development in an organic apple orchard, irrespective of the date of cultivar harvest, location and year. For other plant diseases, Kranz (1968) proposed two disease variables to compare the pattern of disease epidemics, one for determining the curve pattern (i.e. θ), or for defining a position variable (i.e. Y_0 or Y_f) and another one for measuring epidemic intensity (i.e. *AUDPC*). However, Mora-Aguilera et al. (1996) for Papaya ringspot and Holb et al. (2005) for apple scab in organic apple orchards suggested that fewer than three variables gave a significant reduction in the variance explained. Therefore, according to Mora-Aguilera et al. (1996) and Holb et al. (2005), three disease variables (β, $AUDPC_S$ and Y_f) are recommended to characterise fruit brown rot development in apple orchards under high disease pressure.

Campbell et al. (1980); Kranz (1968) and Mora-Aguilera et al. (1996) demonstrated that the disease variables which characterize the entire pattern of a disease development can be used to improve the disease management and/or in breeding for disease resistance. Moreover, Zadoks and Schein (1979); Campbell and Madden (1990) and Berrie and Xu (2003) pointed out that basic information on the amount of disease and inoculum levels is necessary for producing an up-to-date disease forecast which then can be used successfully for improving disease management. In this study, we developed such a fundamental model, which improved brown rot management for organic apple orchards (Fig. 2). Based on this fundamental model, our study was the first to construct a forecasting based disease management strategy against brown rot of apple (BRFMS). Previous brown rot management strategies have been based on reducing insect injury by chemical sprays and reducing brown rot development by regular application of fungicides against other diseases (Lack 1989; Holb and Scherm 2008). Therefore, previous brown rot controls focused on chemical sprays against codling moth and apple scab and these sprays also showed a certain level of efficacy against brown rot of fruit. However, these strategies did not use the prediction of brown rot occurrence and had no ability to time the spray application against brown rot in order to reduce the number of spray applications.

Our BRFMS uniquely harmonised disease and fruit insect injury development, disease threshold values and epidemic intensity in one strategy, which had the ability to reduce the number of sprays during the season. The fundamental model of our BRFMS relies on three disease variables (β, $AUDPC_S$ and Y_f) derived from the logistic function of disease development. Only one previous study made an attempt to use disease variables as predictors of disease development (Holb et al. 2005). Holb et al. (2005) used the same three disease variables derived from logistic functions to predict apple scab development and time fungicide applications in integrated orchards. In our BRFMS, the fundamental model (based on the three disease variables derived from the logistic function) was supplemented with three epidemic intensity level and threshold values, characterizing the different epidemic features of the season. These then were used to select disease management tools. To our best knowledge these options were not considered in previous PC-based disease management strategies in order to reduce sprays against plant pathogens.

In the fundamental model we had no direct consideration of the amount of overwintered plant parts and the level of the previous year's inoculum. These sources are incorporated in the model throughout the dropped infected fruit occurring in late May and June. Infected twigs and mummified fruits overwinter and they sporulate in spring in April and May (Xu and Robinson 2000), which infect dropped fruits in late May and June (Holb and Scherm 2007) and finally dropped sporulating fruits infect fruits on the tree in late June and July. In sum, infection from mummified fruits in our BRFMS model is incorporated through the dropped infected fruits.

In our BRFMS, fruit injury was based on the injured fruit caused by the codling moth. This prediction was based on our previous findings, where codling moth caused 70–90% of the injury on the fruit, and all other injury factors were negligible (Holb and Scherm 2008). In other regions, other insects or hail or bird damage can be a more important factor of fruit injury than in our BRFMS, which therefore may need to be incorporated in BRFMSs developed for non-Hungarian conditions.

Field application of the BRFMS reduced number of sprays by up to one third, which meant 2–4 fewer applications during summer. Summer spray reduction against brown rot coincides with possibilities for omission of sprays against apple scab as ontogenetic resistance of fruit to scab occurs at the second half of the season (Schwabe et al. 1984). However, spray applications could not be omitted in the 3-week period preceding the harvest as fruit became more and more susceptible to brown rot because natural cracks appeared close to harvest. Furthermore, fruit injury by codling moth increases over time. In addition, our practical trial of BRFMS in organic orchards also indicated that further reduction of sprays against brown rot can be achieved by using less susceptible cultivars to insect injury and/or by using more effective products against both the insects and the diseases.

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