Disease Progress Curves of Sclerotinia Stem Rot of Canola Epidemics in Golestan Province, Iran

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ABSTRACT

Sclerotinia Stem Rot (SSR), caused by *Sclerotinia sclerotiorum*, is believed as the most important disease of canola (*Brassica napus*) in Iran. Temporal analysis of the disease epidemics was carried out by evaluating SSR in 80 fields in four locations of: Gorgan, AliAbad, Kalaleh and Gonbad in Golestan Province during 2006 and 2007. Scouting of the fields to record disease incidence (I) and disease severity (S) was started before the end of flowering and continued weekly up to harvest time. Disease Progress Curves (DPCs) were studied using mathematical growth models and their goodness of fit determined based on such statistics as coefficient of determination (\mathbb{R}^2), standard error of estimates (SEE) and residual plots. Gompertz model with a mean \mathbb{R}^2 of 94.69% was selected as the most appropriate model for describing SSR progress in field conditions of Golestan Province. Rates of increase (\mathbf{r}_G) per unit of disease in the canola fields were 0.003 to 0.077 (with an average of 0.03). This is the first temporal study of canola SSR in Iran.

Keywords: Canola (*Brassica napus*), Disease progress curve, Epidemiology, *Sclerotinia sclerotiorum*, Temporal analysis.

INTRODUCTION

Sclerotinia stem rot (SSR) caused by Sclerotinia sclerotiorum deBary, is one of the most important diseases of canola (*Brassica napus*). SSR is reported from most of the canola-producing areas of the world including India, China, Canada, USA, Brazil, France, Germany, England, Italy, Sweden, Finland and Denmark (Anonymous, 2005; Bhowmik, 2003; Natti, 1971).

In Iran, SSR is observed mostly in canolagrowing areas of the northern provinces, especially Mazandaran and Golestan. Few studies have been carried out on characterizing the real situation regarding canola SSR in Iran. Barari *et al.* (2000) surveyed the canola-growing areas of Mazandaran Province, found the disease in all these areas, and reported an average incidence of 12.28 to 54.4 percent. Golestan Province is the most important region of canola cultivation in the country. SSR is a major yield limiting factor of the crop in the province. Aghajani *et al.* (2008) have reported an SSR incidence of 1 to 81.5 percent in this province.

Disease development through time is a dynamic process. To compare and predict disease development, it is necessary to quantify, mathematically model changes in disease development through time (Bowen, 1997). Disease progress curves over time have been referred to as the "*signature*" of the epidemic and represent an integration of all host, pathogen and environmental effects occurring during the epidemic (Bowers and Kinkel, 1997; Campbell and Madden, 1990).

Growth curve models were adapted by Vanderplank (1963) to quantitatively

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describe Disease Progress Curves (DPCs). The most common models for fitting DPCs are the monomolecular, logistic and Gompertz models (Campbell and Madden, 1990). Simulation analyses, using these models, may give further insight into the mechanisms of the epidemic or certain management strategies (resistant varieties and fungicides) that could be adopted with no need for any expense and resources that would otherwise be required for welldesigned field trials.

Nelson and his colleagues (1989) analyzed disease progress curves in sunflower sclerotinia wilt through three mathematical models and concluded that the Weibull model best fitted the disease data. There are many studies carried out about the epidemiology and forecasting of sclerotinia disease in such different crops as soybean (Boland and Hall, 1988; Mila et al., 2003), carrot (Kora et al., 2003; 2005), lettuce (Clarkson et al., 2007), bean (Natti, 1971), peanut (Smith and Hollowell, 2006), and sunflower (Nelson et al., 1989). Unfortunately in any of these studies, DPCs of sclerotinia disease (especially on canola) were not described in detail, and the most convenient models not introduced. Few studies that referred to DPC, used it as Area Under Disease Progress Curve (AUDPC) for a screening of cultivars or studying the effectiveness of different control measures.

The objective of this study was to determine the goodness of fit of mathematical models with field disease data for canola SSR in Iran, for describing the disease epidemics in Golestan Province in the northern part of the country.

MATERIALS AND METHODS

Disease Progress Data

Field studies were conducted at four locations (Gorgan, Ali Abad, Kalaleh and Gonbad) in different parts of Golestan Province for two successive crop seasons (2006-2007). Ten canola fields (hybrid Hyola 401) were yearly selected for each location (40 fields each year). Following flowering (during March), the fields were weekly scouted and approximately 500-600 plants per field randomly selected and assessed for recording SSR intensity. Disease incidence (I) was determined using the formula $I = \sum [x/N]$ where x is the number of diseased plants and N the total number of evaluated plants (Cardoso et al., 2004). Disease severity (S), which is sometimes known as disease severity index (McRoberts et al., 2003), was estimated as $S = \sum (x_i n_i)/5N$ (Cardoso *et al.*, 2004), in which x_i represents disease severity grade based on a descriptive scale (0: No disease, 1: Small branch infected, 2: Large branch infected, 3: Stem at least 50% girdled, 4: Plant dead, but some yield obtained, 5: Plant dead, poor yield) (Bradley *et al.*, 2006), and n_i indicating the number of diseased plants on the *i*th grade of the disease scale (Cardoso et al., 2004). Almost all the surveyed fields were sprayed with teboconazole (Folicur, 1 lit ha⁻¹) by the farmers for control of the disease and at 20-30% flowering stage.

Analysis of Disease Progress Curves Using Linear Models

To construct DPCs, S values for each field were plotted against (sampling) time. DPCs were fitted to four growth models using linear regression analysis (Berger, 1981). Equations with linear parameters from each of four models of Richard's family of growth curves, i.e. monomolecular (ln [1/(1 $y)] = ln [1/(1-y_o)] + r_M t)$, logistic (ln [y/(1y]= $ln [y_o/(1-y_o)]+r_I t$, log-logistic (ln $[y/(1-y)] = ln [y_o/(1-y_o)] + r_{LL}[ln (t)])$, and Gompertz $(-ln [-ln(y)] = -ln [-ln(y_o)] + r_G t)$, were employed as predicted equations to statistically compare linearly transformed data (Bowen, 1997; Campbell and Madden, 1990; Nutter and Parker, 1997; Soto-Estrada and Adaskaveg, 2004). Variables of the models were: y= Mean severity of disease (S) as a proportion from 0 to 1 at time t, $y_0 =$

The initial disease level, and $r^* = \text{Rate}$ of disease increase for each model.

After the regression analysis, goodness-offit of the models was determined by examining the coefficient of determination (\mathbf{R}^2) , which is the proportion of the variation in the data accounted for by the regression model, standard error of estimates (SEE_y), and the plot of the standardized residuals versus the predicted values. An $R^2 \ge 80\%$ is desirable; if $R^2 \le 50\%$, the model fits the data poorly. In examining the graph or plot of residuals plotted against the predicted values, a random scatter of points suggests an appropriate fit of the chosen model. A scatter of points forming a "U" or "~" shape suggested a model whose fit departs symmetrically from the data. Such a symmetrical pattern of standardized residual values is undesirable (Campbell and Madden, 1990; Neher et al., 1997). To compare models using different transformations of the dependant variables for goodness-of-fit, predicted transformed y was back-transformed and the coefficient of determination calculated based on these values (R^{*2}) (Campbell and Madden, 1990). Having selected the most suitable models, regression analysis was performed between observed and back-transformed dependent variables. Analysis of variance (ANOVA) was used to reveal any significant difference between the regions in rate parameter. Mathematical and statistical analyses were performed through software StatGraphics Centurion XV Version 15.2.05 (StatPoint Inc., Herdon, VA, USA).

RESULTS

Results of linear regression analyses in the evaluation of four growth curve models for describing disease progress of Sclerotinia stem rot of canola during 2006 and 2007 revealed that all models could describe disease progress to an acceptable level (Table 1 and Figure 1), but as based on regression statistics, especially residual plots, the best fit model was chosen for each field (Tables 2 and 3). Based on these criteria, the Gompertz model was chosen as the best fit model for describing disease progress of canola SSR. This model (mean R^2 = 96.8%) described disease progress data in some 40 percent of the studied fields (Table 2). In addition to these fields, this model described acceptably (minimum R^2 = 91%) the epidemics of the other fields (Table 1). The logistic and monomolecular models presented the best fit with disease progress data (27.5 and 21.25 percent of the fields, respectively).

Regression parameters for the 10 fields in each location (during two years) are summarized in Table 3. It can be noticed that the Gompertz model was the best fitting model in 2007 (for 70 percent of the fields), but, as for 2006, the monomolecular and logistic (in the case of 40 and 37.5 percent of the fields, respectively) presented the best fit (Table 2). Monomolecular model appropriately described the temporal progress of epidemics with relatively low final intensity (except for H-2007-10 field with a final intensity of 0.27). These results of monomolecular model were similar to those of the linear model (data not shown).

There was a significant difference observed among the rate parameters of Gompertz model (r_G) in sampling regions (P= 0.005). Ali Abad had the highest r_G (Mean= 0.037 per unit per day), while this parameter was lowest in Gonbad region (Mean= 0.023) (Figure 2).

DISCUSSION

Overall results of linear regression analyses of transformed disease intensity data of 80 fields of four different locations in Golestan Province during two years (2006 and 2007) showed that Gompertz model could probably best describe canola SSR epidemics with respect to time. Efficacy of this model had already been proved for appropriately describing such plant disease epidemics as peach rusty spot (Furman *et al.*, 2003), citrus canker (Gottwald *et al.*,

| Location | Year | No. fields | Model | $R^{2}(\%)$ | SEEy | $R^{*2}(\%)$ | SEEy* | Residuals |
|----------|------|------------|---------------|-------------|-------|--------------|-------|-----------|
| Gorgan | 2006 | 10 | Monomolecular | 93.9 | 0.004 | 93.9 | 0.004 | Not OK |
| | | | Logistic | 96.1 | 0.145 | 96.2 | 0.002 | Not OK |
| | | | Log-logistic | 96.2 | 0.146 | 96.3 | 0.002 | OK? |
| | | | Gompertz | 96.1 | 0.032 | 96.3 | 0.002 | OK? |
| | 2007 | 10 | Monomolecular | 90.3 | 0.028 | 90.3 | 0.024 | OK? |
| | | | Logistic | 92.2 | 0.520 | 94.1 | 0.021 | Not OK |
| | | | Log-logistic | 92.9 | 0.495 | 94.8 | 0.019 | Not OK |
| | | | Gompertz | 95.6 | 0.095 | 97.5 | 0.012 | OK |
| Ali Abad | 2006 | 10 | Monomolecular | 84.2 | 0.045 | 83.8 | 0.027 | Not OK |
| | | | Logistic | 95.7 | 0.217 | 93.9 | 0.012 | OK |
| | | | Log-logistic | 92.7 | 0.259 | 95.5 | 0.013 | OK? |
| | | | Gompertz | 94.9 | 0.077 | 94.6 | 0.015 | Not OK |
| | 2007 | 10 | Monomolecular | 85.0 | 0.071 | 90.8 | 0.041 | OK |
| | | | Logistic | 90.7 | 0.562 | 94.0 | 0.030 | OK? |
| | | | Log-logistic | 90.7 | 0.565 | 94.1 | 0.028 | OK? |
| | | | Gompertz | 92.5 | 0.166 | 95.6 | 0.015 | Not OK |
| Kalaleh | 2006 | 10 | Monomolecular | 90.8 | 0.017 | 90.8 | 0.014 | OK |
| | | | Logistic | 90.8 | 0.453 | 91.4 | 0.020 | OK |
| | | | Log-logistic | 90.7 | 0.454 | 91.4 | 0.020 | OK |
| | | | Gompertz | 91.0 | 0.108 | 92.3 | 0.017 | Not OK |
| | 2007 | 10 | Monomolecular | 88.6 | 0.022 | 87.6 | 0.026 | Not OK |
| | | | Logistic | 96.7 | 0.281 | 97.0 | 0.032 | Not OK |
| | | | Log-logistic | 97.0 | 0.271 | 97.2 | 0.031 | Not OK |
| | | | Gompertz | 97.7 | 0.070 | 97.2 | 0.024 | OK |
| Gonbad | 2006 | 10 | Monomolecular | 95.8 | 0.020 | 95.8 | 0.017 | Not OK |
| | | | Logistic | 97.1 | 0.230 | 95.5 | 0.005 | OK |
| | | | Log-logistic | 97.4 | 0.217 | 95.2 | 0.006 | OK |
| | | | Gompertz | 97.9 | 0.045 | 96.7 | 0.005 | OK |
| | 2007 | 10 | Monomolecular | 83.4 | 0.008 | 83.1 | 0.007 | OK |
| | | | Logistic | 92.8 | 0.322 | 88.4 | 0.005 | OK |
| | | | Log-logistic | 89.4 | 0.399 | 92.0 | 0.005 | OK |
| | | | Gompertz | 91.8 | 0.071 | 92.1 | 0.007 | OK |

Table 1. Summary of linear regression statistics (mean) for evaluation of four growth curve models to describe disease progress of sclerotinia stem rot of canola at four regions in Golestan Province, Iran.

1989), Grapevine Leafroll-Associated Virus 3 (Habili and Nutter, 1997), and powdery mildew in pea (Viljanen-Rollinson *et. al.*, 1998). It presented the best fit with some 40 percent of the studied field epidemics. The Log-logistic model (11.25% of the fields) was not comparable with the Gompertz for describing SSR epidemics, but the Logistic and Monomolecular models offered good fits in nearly 27.5 and 21.25 percent of the epidemics, respectively (Table 2).

The slope of the Gompertz transformed (Gompit) line (Gompertz rate= k or r_G) is very different for plant disease epidemics. Many leaf spot diseases have a Gompertz rate of k < 0.1 (Berger, 1981), but Comway *et al.* (1987) reported a rate of 0.961 for Cercospora blight in asparagus. The lower value of r_G (0.008) had been reported for

Dutch elm disease and yucca rusts (Berger, 1981). In this study, $r_{\rm G}$ ranged from 0.003 to 0.077. It is evident that canola SSR epidemic spreads of a relatively slow rate as compared with the other epidemics. Based on Figure 2, Ali Abad and Gonbad regions had the maximum and minimum values of $r_{\rm G}$, respectively which could be attributed to diverse climatic conditions in these regions. While Ali Abad experienced a more humid climate (T= 16°C, Precipitation= 121 mm, RH= 82.5%), the Gonbad region was less humid and warmer (T= 18°C, Precipitation= 67.5 mm, RH = 76%) during the disease spread and progress period (April and March). The weather condition in Kalaleh Gorgan regions and was somewhere between these two extremes.

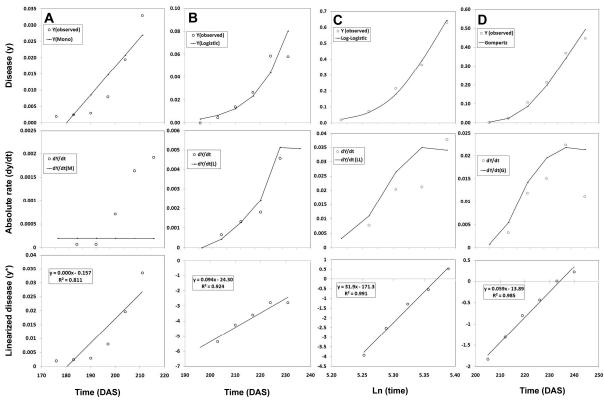


Figure 1. Disease progress curves (y vs. t), change in rate (dy/dt vs. t) or absolute rate of increase, and linearized form (y^* vs. t) of disease amount graphs of canola SSR in Golestan province, Iran. (A) Monomolecular (field G6-07), (B) Logistic (field A5-07), (C) Log-logistic (field A3-07), and (D) Gompertz (field A2-07). DAS: Days after sowing.

Table 2. Best fitting growth models for describing disease progress of Sclerotinia stem rot of canola at four regions in Golestan Province.

| | Gorgan | | AliAbad | | Kalaleh | | Gonbad | | Total (%) | | |
|---------------|--------|------|---------|------|---------|------|--------|------|-----------|------|-------|
| Model | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | All |
| Monomolecular | 5 | 0 | 1 | 0 | 4 | 0 | 6 | 1 | 40 | 2.5 | 21.25 |
| Logistic | 3 | 1 | 6 | 1 | 5 | 2 | 1 | 3 | 37.5 | 17.5 | 27.5 |
| Log-logistic | 2 | 1 | 1 | 1 | 0 | 2 | 2 | 0 | 12.5 | 10 | 11.25 |
| Gompertz | 0 | 8 | 2 | 8 | 1 | 6 | 1 | 6 | 10 | 70 | 40 |

Table 3. Linear equation parameters (range) describing disease progress of sclerotinia stem rot of canola in Golestan Province, Iran.

| Location | Year | No. fields | Best fitting model | Slope (r) | Intercept (C) |
|----------|------|------------|--------------------|---------------|--------------------|
| Gorgan | 2006 | 10 | Monomolecular | 0.000 - 0.003 | (-0.43) – (-0.01) |
| | 2007 | 10 | Gompertz | 0.021 - 0.041 | (-9.73) – (-5.78) |
| Ali Abad | 2006 | 10 | Logistic | 0.073 - 0.280 | (-56.59) – (-3.79) |
| | 2007 | 10 | Gompertz | 0.004 - 0.075 | (-15.77) – (-1.67) |
| Kalaleh | 2006 | 10 | Logistic | 0.040 - 0.156 | (-31.97) – (-9.82) |
| | 2007 | 10 | Gompertz | 0.023 - 0.061 | (-12.94) – (-6.16) |
| Gonbad | 2006 | 10 | Monomolecular | 0.014 - 0.058 | (-9.13) – (-3.79) |
| | 2007 | 10 | Gompertz | 0.011 - 0.029 | (-6.91) – (-1.91) |



The progress of SSR type diseases depends mainly on growth stage of host plant, i.e. it cannot develop and progress at any time during the growing season and is limited to a short period of time during plant growth. These diseases are considered as monocyclic ones whereas they may behave like polycyclic diseases (Heffer Link and Johnson, 2007). Fusarium Head Blight (FHB) of wheat is an important disease that follows this pattern. Fungus ascospores are present in the air above the wheat field for a long time, but they are able to infect the wheat heads only in a certain short period namely during the anthesis stage (Wiese, 1991). A study of FHB epidemic revealed that it fits such polycyclic disease models as Logistic and Gompertz, instead of a monocyclic model (Monomolecular) (Taliey et al., 2006).

S. sclerotiorum releases ascospores produced in apothecia for a long duration of time. Apothecia were observed in the canola fields from January to late March in Golestan, but they can infect the plant only after the petals fall, which occurs within a short period of time (10-14 days) during March. The secondary spread of disease in a field occurs via plant contacts. This problem is more severe in fields with a dense canopy.

SSR progress in a canola field occurs in two different directions (vertical and horizontal). In some fields with a dense canopy, SSR can undergo horizontal spread, considered as secondary infection (Morrall and Dueck, 1982). In addition to a dense canopy, some environmental factors like rain or dew that cause an increase in the humidity. can relative increase the probability of the secondary infections. The results of the study indicated that favorable conditions for a second spread of SSR in most fields led to high rates of a second type infection, and this changed the epidemic to appear like a polycyclic disease, and therefore polycyclic models (like Gompertz) fitting the epidemics best.

SSR is a monocyclic disease (Abawi and Grogan, 1979). Plant to plant spread of *S. sclerotiorum* may occur occasionally, but

this is usually a rare occurrence (Heffer Link and Johnson, 2007). The canola field conditions in this study seemed to be different from this opinion. Disease secondary spread occurs via myceliogenic germination of sclerotia on the soil and plant to plant contact between healthy and diseased plants. This means the diseased plants can be considered as the source of secondary infections for adjacent healthy plants. In fact, they are the centers of disease foci (patches) in the fields.

In fitting models to observed data, it is important to select the model based on the known biology of the pathogen rather than simply on the shape of the curve. It is quite possible to have a data set that fits both the monocyclic and polycyclic models equally well or to have a data set from a known monocyclic epidemic that gives a better fit to a polycyclic model and vice versa (Arneson. 2001). This situation was observed in this study, because all the four models could describe the SSR progress to an acceptable level (Table 1).

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بررسی منحنیهای پیشرفت زمانی بیماری پوسیدگی اسکلروتینیایی ساقه کلزا در استان گلستان، ایران

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Sclerotinia پوسیدگی اسکلروتینیایی ساقه، مهمترین بیماری کلزا در دنیاست که به وسیله قارچ Sclerotinia ملی دو سال زراعی ۲۰۵۹ و ۲۸۶ م ۲۰۸ مزرعه در چهار شهرستان گرگان، علی آباد، کلاله و گنبد مورد ارزیابی قرار گرفت. قبل از پایان دوره گلدهی کلزا، یادداشت برداری از وضعیت بیماری (شدت متوسط بیماری در مزرعه) آغاز شد و تا قبل از برداشت محصول، به صورت هفتگی ادامه یافت. منحنی متوسط بیماری در مزرعه) آغاز شد و تا قبل از برداشت محصول، به صورت هفتگی ادامه یافت. منحنی برازش مدلها بر اساس آماره هایی نظیر ضریب تبیین (R²)، انحراف معیار محاسبات (SEE) و پلات باقیمانده ها تعیین گردید. نتایج نشان داد که چهار مدل تک مولکولی، لجستیک، لاگ لجستیک و گومپرتز، به ترتیب با ۲۰/۲۵، ۲۰/۲۱، دار و ۴۰ درصد ایدمیهای مطالعه شده برازش دارند. بر این اساس، مدل گومپرتز، به ترتیب با ۲۰/۲۵، ۲۰/۲۵، دار او ۴۰ درصد ایدمیهای مطالعه شده برازش دارند. بر این اساس، مدل گومپرتز، به ترتیب با ۲۰/۲۵، ۲۰/۲۵، او ۴۰ درصد ایدمیهای مطالعه شده برازش دارند. بر این اساس، مدل گومپرتز، به ترتیب با ۲۰/۲۵، ۲۵/۲۰ او ۴۰ درصد ایدمیهای مطالعه شده برازش دارند. بر این اساس، مدل گومپرتز، به ترتیب با ۲۰/۲۵، ۲۰/۲۰ از ۲۰۰ درصد، به عنوان مناسبترین مدل جهت توصیف روند پیشرفت بیماری در شرایط مزارع استان گلستان انتخاب گردید. با در نظر گرفتن این نتیجه، نرخ سرانه افزایش بیماری (rG) در مزارع کلزای استان ۲۰۰۰، تا ۲۰/۰، (با میانگین ۲۰۰۰) بوده است. این نخستین بررسی تغیرات زمانی بیماری پوسیدگی اسکلروتینیایی ساقه کلزا در دنیا می باشد.