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Segmented linear Model to characterize tolerance to tomato yellow leaf curl virus and tomato leaf curl virus in two tomato cultivars under greenhouse conditions

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ABSTRACT- To investigate the amount of yield losses caused by Iranian isolates of tomato yellow leaf curl virus (TYLCV-[Ab]) and tomato leaf curl Karnataka virus (ToLCKV-IR) in Rio Grande (RG) and Grosse Lisse (GL) tomato cultivars, tomato seedlings were separately agroinoculated with an infectious clone of either virus and disease development was monitored over four time intervals. After the emergence of symptoms, disease severity (DS) was evaluated visually using an ordinal rating scale and the data was converted to a ratio scale. Losses caused by the viruses were estimated by measuring vegetative indices including wet and dry weight and height of the aerial and underground parts of the plants. Initial DS in plants infected by ToLCKV-IR was less than those infected by TYLCV-[Ab]. Descending order of rates of disease increase for the studied pathosystems was as follows: ToLCKV-IR-infected GL>TYLCV-Ab-infected GL> TYLCV-[Ab]-infected GL> ToLCKV-IR-infected RG. With delayed inoculation, DS was reduced in both cultivars inoculated with either of the two viruses. A segmented linear model showed a very good fit to the data of relative biomass and duration of exposure of all pathosystems. The estimated parameters of the model were used to evaluate different aspects of the damage curve. The highest *crop loss disease threshold* was observed for GL cultivar irrespective of the inoculated virus. Similar results were achieved when the *desensitization disease level* was evaluated. *Curvilinear tolerance* or *slope* did not show any significant difference between the infected-GL plants to either virus and also TOLCKV-IR-RG, while TYLCV-[Ab]-RG showed the highest *curvilinear tolerance*. Findings of this study can be applied in screening for tolerant tomato plants.

INTRODUCTION

Geminiviridae is a large and diverse virus family with a wide range of hosts among monocotyledonous and dicotyledonous plants causing significant crop losses. Geminiviruses are characterized by geminate morphology of their capsids and circular single-stranded DNA genomes. *Begomovirus* is one of important genera of the family whose species are transmitted in a persistent circulative manner by the whitefly *Bemisia tabaci* (Varsani et al., 2017).

Tomato leaf curl is among important and destructive diseases of tomato in warm and temperate regions of the world caused by a number of variants of tomato leaf curl virus (ToLCV) and tomato yellow leaf curl virus (TYLCV), belonging to the genus *Begomovirus* Czosnek 2007). TYLCV infection was regarded as an economic problem in the Mediterranean basin since 1966 (Cohen and Nitzany 1966). Since the first report, TYLCV and similar viruses have spread across the Middle East with an alarming speed (Hajimorad et al., 1996; Mansour and Al-Musa 1992). Biological, molecular, and epidemiological characteristics as well as some aspects of the control of these diseases have been investigated in a number of studies which showed these viruses have spread throughout the globe (Pico et al., 1996; Moriones and Navas-Castillo 2000; Czosnek 2007; Czosnek 2008; Glick et al., 2009) including long-distance movements to the Americas and Australia (Mabvakure et al., 2016). Recent spread of these and other begomoviruses has been closely related to the global distribution of the Middle East-Asia Minor (formally referred to as the B biotype) and the Mediterranean (formally referred to as the Q biotype) cryptic species of their vector, *B. tabaci* (Bedford et al.,

1994; De Barro 1995; De Barro et al., 2011). In Iran, a rapid and wide spread of TYLCV across tomato growing regions (Bananej et al., 2004; Behjatnia et al., 2004; Fazeli et al., 2008; Pakniat et al., 2010) has occurred since its first report from this country (Hajimorad et al., 1996). Despite the noticeable economic importance of tomato leaf curl disease, the relationship between disease development and resulting yield losses has not been extensively studied.

An understanding of crop losses using relatively simple models of yield as a main objective in relation to disease severity is useful in determining whether a potential disease control method has an effect on yield (Cooke 2006; Madden et al., 2007). Thus, the present study was designed to model losses caused by TYLCV-[Ab] and ToLCKV-IR, as severe and mild virus species respectively, on two tomato cultivars through evaluation of disease severity, duration of exposure and relative biomass of the inoculated plants under greenhouse conditions.

MATERIALS AND METHODS

Plant Materials

Two tomato cultivars, Rio Grande (RG) and Grosse Lisse (GL), were used in this study to evaluate their responses to a severe (TYLCV-[Ab]) and a mild (ToLCKV-IR) virus species. Seeds of each cultivar were sown in pots containing sterilized field soil, peat moss, and sand (3:2:1), and they were grown at 20-25°C in a greenhouse. Seedlings were transferred from seedling trays to 6-inch pots at the two-leaf stage. Experimental design of the study was based on a randomized complete block design in a factorial arrangement with four replications. The pots were arranged in four blocks, each containing a randomized combination of two cultivars (RG and GL), four duration of exposures as a measure of inoculum pressure (60, 50, 40, and 30 days equivalent to inoculation at 24, 34, 44, and 54 days after sowing date where 30 days after the last inoculation all plants were evaluated) and two virus inoculation treatments (ToLCKV-IR, TYLCV-[Ab]), and mock inoculations as negative controls.

Inoculation and Infectivity Assay

Agroinoculation was carried out using a 1.5 mer infectious clone (pBin20-1.5ToLCKV-IR, Behjatnia et al., 2009) of ToLCKV-IR (GenBank Acc. No. AY297924) and a 1.5 mer infectious clone (pBin20-1.5TYLCV-[Ab], Pakniat et al., 2010) of TYLCV-[Ab] (GenBank Acc. No. FJ355946). *Agrobacterium tumefaciens* cultures carrying infectious clone of TYLCV-[Ab] or ToLCKV-IR were grown separately at 28°C for 36-48 hours and were diluted to give an optical density of 1.0 at 600 nm. Healthy tomato seedlings of each cultivar at four-leaf stage (24 days after seeding) were inoculated at the crown and the several leaf nodes with *A. tumefaciens* cultures carrying the infectious clone of TYLCV-[Ab] or ToLCKV-IR. The next stages of inoculation were 10, 20, and 30 days after the first inoculation (34, 44, and 54 days after seeding). Each seedling was inoculated through injection of 100 µl of proper *Agrobacterium* culture. A few tomato seedlings of each cultivar were also inoculated with free infectious clone-*A. tumefaciens* culture at each inoculation stage, and these seedlings served as the controls. All inoculated seedlings were maintained in the greenhouse under insect-free conditions. Sampling was done from newly emerged leaves 30 days post-inoculation (dpi) from inoculated plants, and total DNA was extracted from each sample using a modified CTAB (hexadecyltrimethyl ammonium bromide) DNA extraction procedure (Gawel and Jarret 1991). Presence of TYLCV-[Ab] and ToLCKV-IR was verified by PCR using TYLCV- [Ab]p2613^{c}/1543^{v} and ToLCKV-IRP1540^{c}/419^{v} specific primer pairs, respectively (Table 1). After ensuring regarding infection of virus-inoculated plants with either TYLCV-[Ab] or ToLCKV, DS and plant growth parameters were recorded as described below.

Polymerase Chain Reaction (PCR)

PCR was carried out in a 20 µl reaction mixture containing 10-15 ng of DNA template, 1 µM of each primer, 0.2 mM of each dNTP, 1.5 mM of MgCl2 and 1.25 U of *Taq* DNA polymerase (Cinagen, Iran) in the reaction buffer provided by the same source. The mixture was heated for 5 min at 94°C and was subjected to a 30 cycle-PCR program of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 3 min. The final cycle was followed by 10 min incubation at 72°C. Then, the reaction mixture was electrophoresed in a 1% agarose gel, and was stained with ethidium bromide and was visualized by UV light.

Measurements of Disease Severity (Ds) and Plant Growth Parameters

DS evaluations were made at four 10-day time intervals respectively at 30, 40, 50 and 60 days after seeding. DS was assessed indirectly using an ordinal rating scale with 5 symptom scale codes including 0=symptomless, 1= mild leaf curling and reduction in leaf size, 2 = moderate leaf curling and reduction in leaf size, 3 = relatively severe leaf curling and yellowing as well as reduction in leaf size, and 4 = severe leaf curling and yellowing as well as reduction in leaf size. DS measurement scale codes were converted from original ordinal scale to a ratio scale normalized between 0-1 using the following equation (Madden et al., 2007):

$$DS = \frac{\sum[(0 \times a) + (1 \times b) + (2 \times c) + (3 \times d) + (4 \times e)]}{(a + b + c + d + e) \times 4} \quad (1)$$

where a, b, c, d, and e are number of infected tomato plants scored between 0 to 4, respectively, according to their level of disease severity.

Thirty days after the last inoculum injection, fresh weight and height of the virus-inoculated and control plants of both cultivars were measured. Relative shoot fresh weight (the ratio of weight of the plants to a mean weight of their controls) were measured for all replicates.

Table 1. Details related to specific primers used in this study.

Primer	Nucleotide position*	Size (nt)	Sequence (5′-3′)
ToLCKV-IRP1540[c]	1515-1540	26	CGTCGACGAGTTGATCTACCGTGTGG
ToLCKV-IRP419[v]	419-443	25	CAAGGCAAAGGCATGGGCGAACAGG
TYLCV-[Ab]p2613[c]	2581-2613	33	CCTCGTCTATTTAAAATATATGCCAAAAATTAT
TYLCV-[Ab]p1543[v]	1543-1575	33	TTACGTCTTATTGTTTTCTTCTTGGATATCTTG

* Nucleotide positions of Iranian isolate of tomato yellow leaf curl virus (TYLCV-[Ab]) and tomato leaf curl Karnataka virus (ToLCKV-IR) were obtained from the GenBank database (accession numbers of FJ355946 and AY297924, respectively).
[C] Complementary-sense strand primer [V] Virion-sense strand primer

Statistical Analysis

To investigate the effect of the virus, host, exposure duration (a measure of inoculum pressure) and their interaction on host biomass, factorial analysis of variance was performed using SAS software Ver.9.1 (SAS institute 1996) and in case of a significant effect, treatment means were compared by Duncan's multiple range test (DMRT).

Disease severity was modeled as a function of exposure duration. DS was related to exposure duration (a measure of inoculum pressure) using logistic model (Madden et al., 2007) with two parameters (Eq. 2).

$$DS = \frac{1}{1+be^{-kd}} \quad (2)$$

where *DS* and *d*, are disease severity and exposure duration, respectively, *b* ln[y0/(1-y0)] and *k* are intercept (initial disease level (y0) in logit scale]) and slope (rate of increase in DS) of the logistic model, respectively. Initial disease levels and rates of increase in DS of the two cultivars (challenged with TYLCV-[Ab] or ToLCKV-IR-IR) were compared using t-tests by testing the null hypotheses stating no difference between the parameters. Eqs. 3 and 4 were used for testing the equality of parameters for the null hypotheses related to i^{th} (RG) and j^{th} (GL) cultivars and/or viruses (Madden et al., 2007):

$$t = \frac{\hat{b}_i - \hat{b}_j}{S(\hat{b}_i - \hat{b}_j)} > t_{\alpha/2,df} => RH_0: b_i = \quad (3)$$

$$t = \frac{\hat{k}_i - \hat{k}_j}{S(\hat{k}_i - \hat{k}_j)} > t_{\alpha/2,df} => RH_0: k_i = k_j \quad (4)$$

where, the denominators are standard errors of the differences between two i^{th} and j^{th} estimated slopes (or intercepts) estimated as:

$$S(\hat{b}_i - \hat{b}_j) = \sqrt{s^2(\hat{b}_i) + s^2(\hat{b}_j)} \quad \text{and} \quad (5)$$

$$S(\hat{k}_i - \hat{k}_j) = \sqrt{s^2(\hat{k}_i) + s^2(\hat{k}_j)}. \quad (6)$$

Models were fitted to the data transformed to logit, and the parameters were estimated using Proc REG of SAS program (SAS institute Inc., 1999).

Plant biomass (measured as plant fresh weight) as a function of DS was modelled. Biomass was related to DS using a segmented model (Madden et al., 2007) with four parameters (Eqs. 5, Fig. 1).

$$w = \begin{cases} \beta_0 & if \quad y < \alpha_0 \\ \beta_0 - (\beta_1(y - \alpha_0)) & if \quad \alpha_0 \le y < \alpha_1 \\ \beta_0 - (\beta_1(\alpha_1 - \alpha_0)) & if \quad y > \alpha_1 \end{cases} \quad (5)$$

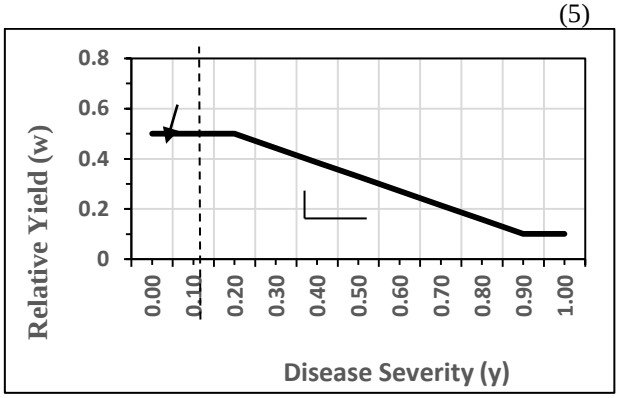


Fig. 1. Segmented model to characterize theoretical relationship between relative yield (w) and disease severity (y) related to disease/inoculum pressure. Parameters are β_0= *initial tolerance*, β_1= *curvilinear tolerance or slope* (a measure of steepness of yield loss curve), α_0= *crop loss disease threshold* (a value of disease severity below which no significant loss occurs), α_1= *desensitization disease level* which is the highest possible disease severity where minimum relative yield (w_{min}) is reached above which no further yield loss occurs. $w_{min} = \beta_0 - (\beta_1(\alpha_1 - \alpha_0))$ is the maximum possible loss that is reached or approached at the *desensitization disease level* where *w* = relative yield = W/W_0 .

where, relative yield equals $w = W/W_0$ (W *and* W_0 are absolute biomass of virus and mock inoculated experimental units in grams), and β_0, β_1, α_0, and α_1 are, respectively, *initial tolerance* (non-economic losses occurring under crop loss disease threshold), *curvilinear tolerance or slope* (a measure of steepness of yield loss curve), *crop loss disease threshold* (a value of disease severity below which no significant loss occurs), and *desensitization disease level* which is the highest possible disease intensity where maximum possible loss equals to minimum relative yield (w_{min}) that is reached or approached and above which no further yield loss occurs.

In cases of no *crop loss disease threshold*, a disease threshold close to zero ($\alpha_0 = 0$) indicates that the crop cannot tolerate DS levels higher than zero. When there is evidence of no crop loss disease threshold and w_{min} =

0, Eq. (5) reduces to linear model ($w = \beta_0 - \beta_1 y$) with two parameters (β_0 and β_1).

The least square program for nonlinear models (proc nlin) of the Statistical Analysis System was employed to fit segmented model to the data and estimate model parameters (and their standard errors) and diagnostics. Any significant difference between parameters measuring different aspects of tolerance of tomato cultivars (infected to either of the viruses) was investigated by t-tests as described above.

RESULTS AND DISCUSSION

Infectivity of Agroinoculated Plants

DNA extracts obtained from plants agroinoculated with infectious clones of TYLCV-[Ab] and ToLCKV-IR were analyzed by PCR for the presence of TYLCV-[Ab] and ToLCKV-IR at 30 dpi, using the corresponding viral specific primer pairs (Table 1). DNA fragments of the expected sizes (1071 bp for TYLCV-[Ab] and 1122 bp for ToLCKV-IR) were amplified from all symptomatic plants 30 dpi (data not shown), indicating a 100% infectivity of the viruses.

Effect of Exposure Time, Cultivar and Virus on Plant Growth and Disease Severity

Results showed that main effects of cultivar, virus, and exposure time had significant effect on disease severity (*P=0.04*; *P<0.0001*; and *P<0.0001*, respectively) and plant growth measured by shoot fresh weight (*P<0.0001*; *P<0.0001*; and *P<0.0001* respectively), all measured by type 3 SS analysis. There was a significant interaction between cultivar, virus, and exposure time (*P= 0. 0005* and *P= 0. 05* for DS and Shoot Fresh Weight, respectively). There was significant increase in disease severity and simultaneously significant reduction in shoot fresh weight with an increase in exposure time. TYLCV-[Ab] caused significantly higher levels of disease severity on both cultivars at all exposure times (Fig. 2) but, plant growth losses caused by both viruses did not show significant differences irrespective of the cultivar and exposure time (Fig. 2).

Modeling Disease Progress

The least square program for nonlinear models (proc nlin) of the Statistical Analysis System (SAS institute Inc., 1999) was primarily used to evaluate the overall fit of exponential family equations (particularly logistic and Gompertz models) to the data. Different patterns of increment in DS in relation to exposure duration were observed for four pathosystems including GL-TYLCV-[Ab]; GL-ToLCKV-IR; RG-TYLCV-[Ab], and RG-ToLCKV-IR. DS showed a classical sigmoid pattern and started with a different initial disease level for each pathosystem, continued with a subsequent exponential increase in short exposure duration times followed by a significant decrease in the rate of increase for both RG and GL cultivars infected with the viruses used in this study. Comparison of the goodness of fit for four disease progress curves of the two models showed that, logistic model provided a more satisfactory statistical fit than other models used to describe the DS progress curves of all four pathosystems.

For simplicity of presentation, the least square program of SAS for regression analysis (proc reg) was employed to fit simple linear regression technique using the logit transformed observed data, estimate linearized logistic model parameters and, diagnostics. Observed and theoretical DS progress curves are shown in Fig. 3. Model diagnostics including F-test (goodness of fit test), R^2, and their estimated parameters (with their standard errors) are summarized in Table 2. Initial disease levels and rate of disease increases (measured by intercept and slope parameters of the linearized logistic models) of different epidemics resulting from infection of the two tomato cultivars with ToLCKV-IR or TYLCV-[Ab] were used to compare the pathosystems.

Significantly lower initial disease levels were observed for ToLCKV-IR infected tomato plants of either cultivar and between the two cultivars for GL ToLCKV-IR-infected plants, the lowest initial disease level was observed on GL. While ToLCKV-IR-infected plants of GL cultivar showed the lowest initial disease level, they had the highest rate of disease increase, which was significantly different from the other pathosystems. Rate of disease increase in TYLCV-[Ab]-tomato pathosystems did not differ significantly.

Segmented Linear Model to Describe Damage Curve

The segmented linear model showed a very good fit to the data of relative biomass and exposure duration of all pathosystems. Estimated parameters, their standard errors, model diagnostics including F-test and R^2 as well as mean comparison of the parameters for the pathosystems are shown in Table 3. Observed and predicted yields or biomass of TYLCV-[Ab]/ToLCKV-IR-infected GL/RG tomato plants measured as a proportion of maximum biomass in control (mock inoculated disease free plants) are plotted against exposure duration (used as a measure of inoculum pressure) as shown in Fig. 4.

The highest crop loss disease threshold (α0) was observed for tomato plants infected with TYLCV-[Ab] of both cultivars with GL-TYLCV-[Ab] and RG-TYLCV-[Ab] 0.3501 [95% CI: 0.3479-0.3522] and 0.3140 [95% CI: 0.3041-0.3240], respectively. No significant difference was observed in crop loss disease threshold (α0) between GL-ToLCKV-IR [95% CI: 0.1513-0.1974] and RG-ToLCKV-IR [95% CI: 0.1561-0.1815]. (Table 3).

Similar results were achieved when the *desensitization disease level* (α_1) which is the highest possible DS where minimum relative yield wmin is reached above which no further yield loss occurs, was evaluated. Both GL and RG cultivar plants infected with TYLCV-[Ab] reached their *desensitization disease level* (α_1) at DS levels slightly greater than 0.6 while the parameter occurred at DSs close to 0.4 for ToLCKV-IR infected plants of both cultivars (Table 3). *Initial tolerance* (β_0) of both cultivars was greater than 95 % as much as the disease free yield (measured as biomass). The highest *initial tolerance* was observed for GL

tomato plants infected with TYLCV-[Ab] which was significantly higher than those of three other pathosystems (with no significant difference to each other) (Table 3).

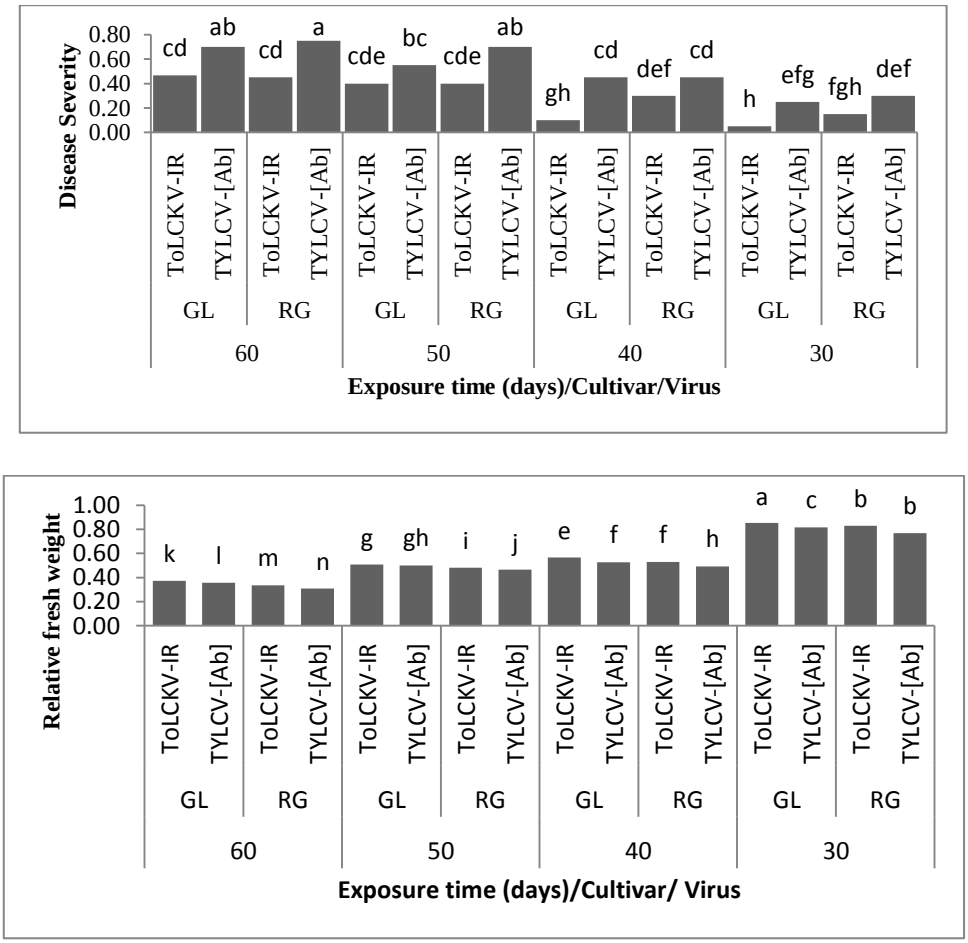


Fig. 2. Mean comparison of disease severity (a) and relative shoot fresh weight (b) of two tomato cultivars (Grosse Lisse =GL and Rio Grande =RG) inoculated with ToLCKV-IR and TYLCV-[Ab] at four different exposure times (1=60, 2=40, 3=40, and 4=30 days) using Duncan's Multiple Range Test. Bars with at least one letter in common are not significantly different at a significance level of 0.05 (for details see the text).

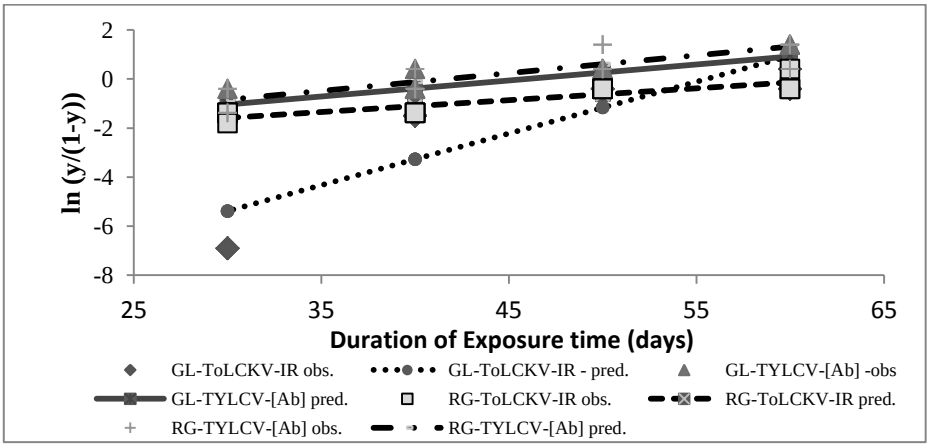


Fig. 3. Observed and theoretical disease progress curves against duration of exposure to the pathogen (a measure of inoculum pressure) for cultivars Rio Grande (RG) and Grosse Lisse (GL) infected to ToLCKV-IR or TYLCV-[Ab]. Best fit model for all pathosystems was logistic model [y = a/(1+b*exp(-kt))] and linearized form of the model was fitted using logit transformation of disease severity data [ln(y/(1-y))]. For details on model diagnostics and parameter estimates and t-tests used for testing parameter equality null hypotheses see Tables 2 & 3

Table 2. Model ANOVA, R^2, and estimated parameters (and their standard errors) for logistic linearized disease progress model for tomato cultivars Grosse Lisse and Rio Grande (infected to ToLCKV-IR and TYLCV-[Ab]).

Model Parameters (diagnostics)	Grosse Lisse		Rio Grande	
	ToLCKV-IR	TYLCV-[Ab]	ToLCKV-IR	TYLCV-[Ab]
intercept	-6.0716	-1.2450	-1.7503	-1.0761
SE (intercept)	0.7197	0.21641	0.1390	0.2445
slope	0.0570	0.0176	0.0131	0.0195
SE (slope)	0.0091	0.0027	0.0018	0.0031
Pr> F	< 0.0001	< 0.0001	< 0.0001	< 0.0001
R^2	0.719	0.729	0.798	0.721

Curvilinear tolerance or slope (β_1 = a measure of steepness of yield loss curve) did not show any significant difference between the GL plants infected with either virus and also RG plants infected with ToLCKV-IR while TYLCV-[Ab]-infected plants of RG cultivar recorded the highest *curvilinear tolerance* (or the lowest slope) (Table 3 and Fig. 4).

Epidemics have many direct and indirect effects on crops including the reduction of yield. Crop losses caused by plant diseases evidently represent a massive challenge to food security and food safety which cannot be overlooked. TYLCV is an economically important virus causing losses to tomato plants world-wide and occurs across most Mediterranean countries, parts of sub-Saharan Africa, Asia, Japan, Australia, the Caribbean islands, and the USA (Polston et al., 1999). As in a greenhouse study, mean total yield per plant reduced in TYLCV infected tomato plants by 63% compared to their healthy controls (Makkouk et al 1979). TYLCV infection may cause yield losses ranging from 28% up to 92% and even as high as 100% making tomato production unprofitable (Nakhla and Maxwell 1998; Moriones and Navas-Castillo 2000). The effect of TYLCV infection on nutritional components of fruits and chlorophyll content in the leaves of tomato was studied in a field experiment (Bhyan et al., 2007). The researchers showed that TYLCV infection in tomato plants had a negative effect on fruit nutrition. Vitamin C, nitrogen, phosphorus, potassium, iron, and chlorophyll A contents in tomato leaves could be modeled as a negative linear regression of TYLCV infection percentage (Bhyan et al., 2007). ToLCV is another economically important tomato virus causing considerable losses to yield of the tomato products. It has been reported from a wide range of geographical regions including Phillipines (Retuerma et al., 1971), Africa (Nour Eldin et al., 1969), Middle East (Makkouk et al., 1979), Taiwan (Green et al., 1987), and India (Chatchawankanphanich et al., 1993). Losses ranging from 50 to 75% in the yield of tomato due to ToLCV and even as high as up to 97% in autumn season have also been reported (Ajlan et al., 2007). To our knowledge, there are no investigations modelled yield or yield correlates such as biomass as a function of disease severity in case of these two important viruses.

Analysis and modeling of crop losses is fundamental to plant disease management. Some plant disease epidemiologists believe that, it is not possible to conduct any study in epidemiology, and also no plant disease survey and their applications would be conducted if crop losses are not measured (Madden et al., 2007; Savary et al., 2006; Zadoks 1985; Zadoks and Schein 1979). Modeling approach has been used for evaluating the losses caused by a number of crop diseases including but not limited to the groundnut rust and leaf spots (Savary et al., 1990; Savary and Zadoks 1992), rice leaf blast (Bastiaans 1993), and virus diseases (Madden et al., 2000).

Tolerance is defined as the ability of a host to cope with pathogen infection across a range of pathogen loads. Unlike abundant literature published on resistance (host's ability to limit pathogen multiplication), tolerance is comparatively less studied and understood (Pegán and García-Arenal 2018). Tolerance of plants to fungi, oomycetes, and viruses has received considerable attention (Politowski and Browning 1978; Rubio et al 2003; Pilowsky and Cohen 1990). While tolerance to virus infection has been widely analyzed in crops, there are very few studies quantitatively investigated tolerance although none of them utilized the empirical modeling approach to study tolerance quantitatively.

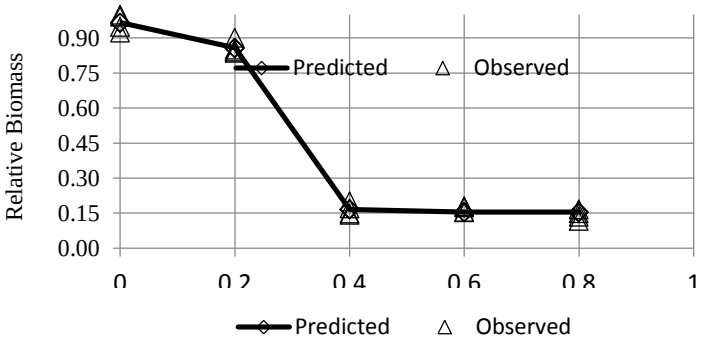
Herein, modeling approach was applied to evaluate the effect of TYLCV and ToLCV on correlates of tomato yield under greenhouse conditions. In our approach univariate ANOVA was first used to find any significant main effect and/or their interactions on disease and plant growth responses; however this modeling approach while being competent enough to find significant main effects and their interactions, was unable to explain why despite significant differences in levels of disease severity between the two viruses, the shoot fresh weight differences were non-significant (Fig. 2). Alternatively, a different modeling approach was employed to translate inoculum pressure into parameters of disease progress curves and define plant growth as a function of disease severity to further clarify the situation. Inoculation of tomato plants in four time intervals used herein to study losses caused by viruses was recommended as an appropriate approach for systemic diseases of plants, such as those caused by viruses, phytoplasmas, spiroplasmas, and some soil-borne pathogens (Madden et al., 2007). Time of infection is often a good predictor of final yield (or yield loss) for these types of diseases (Madden et al., 2000). For instance, with controlled inoculations of plants, the effect of infection on yield often declines by increasing the time during the season when plants are inoculated.

Table 3. Model diagnostics (ANOVA (Pr>F), R^2) and estimated parameters (and their standard errors) for segmented/linear crop loss models with relative biomass as the response variable and four parameters for two tomato cultivars (Grosse Lisse and Rio Grande) infected to ToLCKV-IR and/or TYLCV-[Ab].

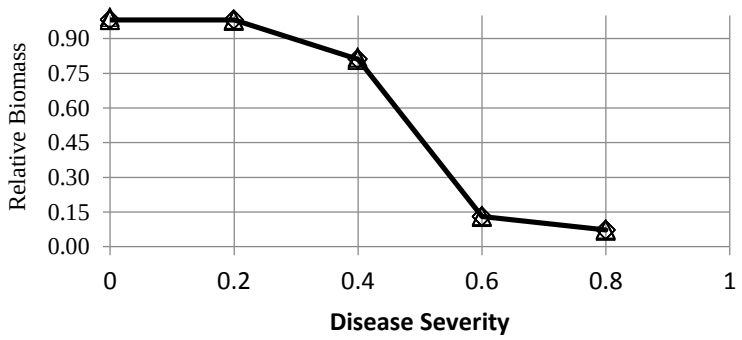
Model Parameters* (& diagnostics)	Grosse Lisse		Rio Grande	
	ToLCKV-IR	TYLCV-[Ab]	ToLCKV-IR	TYLCV-[Ab]
$\hat{\alpha}_0$	0.174(c)	0.350(a)	0.169(c)	0.314(b)
SE($\hat{\alpha}_0$)	0.0109	0.0010	0.0060	0.0047
$\hat{\alpha}_1$	0.401(c)	0.617(a)	0.403(c)	0.602(b)
SE($\hat{\alpha}_1$)	0.0088	0.0010	0.0048	0.0041
$\hat{\beta}_0$	0.965(b)	0.981(a)	0.966(b)	0.955(b)
SE($\hat{\beta}_0$)	0.0267	0.0017	0.0135	0.0059
$\hat{\beta}_1$	3.720(a)	3.403(a)	3.460(a)	2.928(b)
SE($\hat{\beta}_1$)	0.1889	0.0168	0.0955	0.0590
w_{min}	0.124	0.072	0.154	0.111
Pr> F	<0.0001	<0.0001	<0.0001	<0.0001
R^2	0.9851	0.9998	0.9957	0.9984

*β_0, β_1, α_0, α_1 and w_{min} are respectively *initial tolerance*, *curvilinear tolerance or slope* (a measure of steepness of yield loss curve), *crop loss disease threshold* (a value of disease severity below which no significant loss occurs), *desensitization disease* and $w_{min} = \beta_0 - (\beta_1(\alpha_1 - \alpha_0))$ which is the maximum possible loss that is reached when desensitization disease level is approached where w=relative yield=W/W_0.

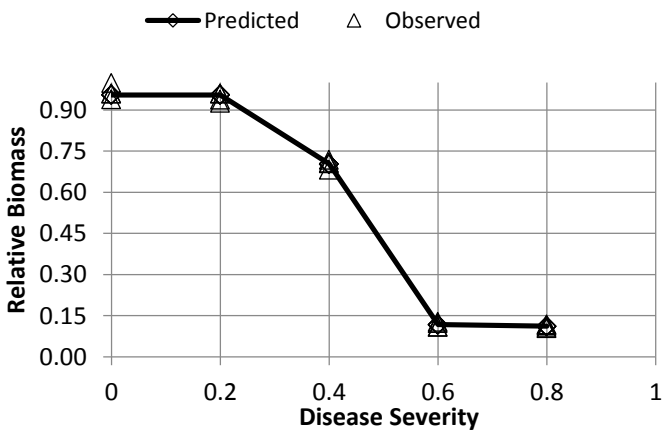
ToLCKV-IR - Rio Grande



ToLCKV-IR - Grosse Lisse



TYLCV-[Ab] - Rio Grande



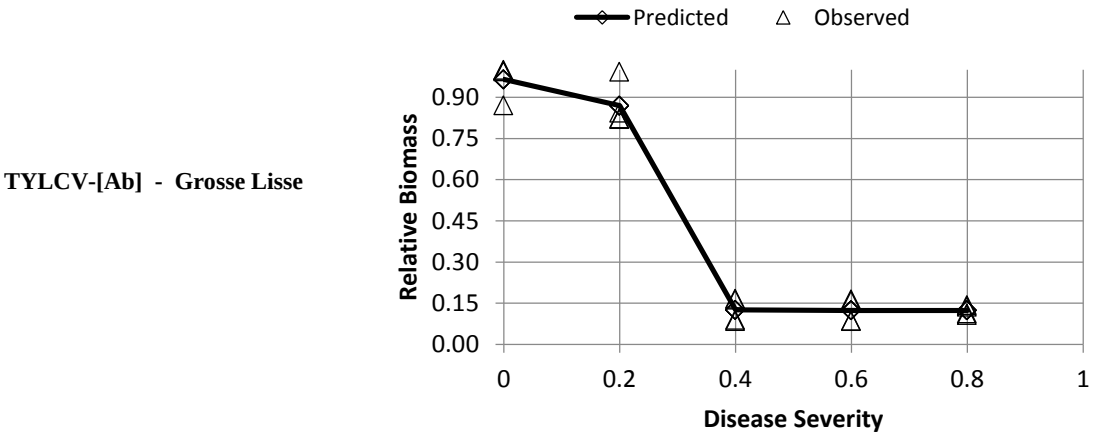


Fig. 4. Relative yield determined as fraction of maximum yield in control (disease free pots). Empty triangles represent observed relative yields and there are four observations in each disease severity and segmented linear curves are predicted values based on the best-fit models. For details on models diagnostics and parameter estimates see Table 3.

While plants infected early in the season may have a very low yield, plants infected late may have approximately the same yield as non-infected plants (Fargette and Vié 1995; Madden and Nutter 1995).

Disease progress curves of two tomato cultivars (GL and RG), each agroinoculated with TYLCV-[Ab] or ToLCKV-IR, were analyzed by fitting appropriate models from the exponential model family. Exposure duration to the disease was used as a measure of inoculum/disease pressure. Logistic model described the progress of DS as function of exposure duration and hence parameters of linearized logistic model were estimated for all four pathosystems (GL-TYLCV-[Ab]; GL-ToLCKV-IR; RG-TYLCV-[Ab]; and RG-ToLCKV-IR) and were used for comparisons of the pathosystems. The intercept and slope parameters on logit line could be of value in determination of initial inoculum and rate of increment in DS. Lower intercept values may be an indication of smaller amount of initial inoculum and/or higher disease tolerance or generally speaking higher relative initial tolerance. Initial disease level of ToLCKV-IR on both cultivars was significantly lower than those of TYLCV-[Ab] with the lowest initial disease level recorded for GL-ToLCKV-IR. Rate of DS increase for TYLCV-[Ab] on both cultivars was moderate while GL-ToLCKV-IR and RG-ToLCKV-IR recorded the highest and lowest values for disease increase, respectively, among the four pathosystems. Interestingly, the pathosystems with the highest and lowest initial disease levels had the lowest and highest rates of disease increase. Although a strong negative correlation (r = - 0.84) was observed between initial disease level and rate of disease increase, statistical significance of the (-) correlation could not be verified due to the low number of replicates. Rees et al., (1979) reported strong correlation between low intercept values and high r for stem and leaf rust resistance in wheat.

To our knowledge there are no studies investigated the effect of these two viruses on damage curve of tomato cultivars. Our results indicated evident increased yield losses with longer duration of exposure equivalent to higher inoculum pressure in an inverse sigmoid pattern, when relative biomass was plotted against exposure duration. There was strong correlation between initial disease level and crop loss threshold ($\hat{\alpha}_0$) suggesting that, lower initial disease level resulted to lower disease level. In this case, the plant can tolerate the negative effect regarding presence of the pathogen on its yield. However, there was no correlation between initial disease level and initial tolerance ($\hat{\beta}_0$). Actually initial tolerance for all pathosystems was higher than 95% as much as the biomass produced by the disease free plants.

A relatively strong positive correlation (r=+0.61) was found between curvilinear tolerance ($\hat{\beta}_1$) and rate of disease increase and also a relatively strong negative correlation (-0.81) was observed between $\hat{\beta}_1$ and initial disease level, indicating that curvilinear tolerance is a function of rate of disease increase and more important than tolerance to initial disease level (Table 3 and Fig 4). Strong negative correlation between *desensitization* disease level ($\hat{\alpha}_1$) and curvilinear tolerance ($\hat{\beta}_1$) also showed that, curvilinear tolerance determines how fast a pathosystem approaches its maximum possible loss level and also a disease level in which maximum possible loss occurs (Table 3 and Fig. 3).

Despite of high values of initial tolerance (β_0) for the pathosystems related to all tomato virus combinations, it did not have any correlation with maximum possible loss that is reached when *desensitization disease level* is approached (w_{min}). Both *crop loss disease threshold* (a value of DS below which no significant loss occurs or α_0), and *desensitization disease level* (α_1) which is the highest possible DS where minimum relative yield (w_{min}) is reached are pathogen specific with higher levels for both parameters in TYLCV-[Ab] infected tomato plants of both cultivars. These two important parameters of segmented linear crop loss model fitted to the data are of paramount importance with curvilinear tolerance in determination

of the w_{min} or maximum possible loss that is reached when *desensitization disease level* is approached. These findings underline implication of inclusion of important destructive plant viruses in experiments designed to screen virus tolerant plant cultivars. A negative correlation found between curvilinear tolerance ($\hat{\beta}_1$) and *desensitization disease level* (α_1) (r=-0.72) also indicates that if the plant has a lower curvilinear tolerance, the *desensitization* disease level will be reached at significantly shorter durations of exposure to the virus.

Yield loss (50) or YL_{50} is a critical value used to compare pathosystems representing relative loss at 50% disease level. Expected YL_{50} values for GL-TYLCV-[Ab], GL-ToLCKV-IR, RG-TYLCV-[Ab], and RG-ToLCKV-IR pathosystems were equal to 0.53, 0.88, 0.59, and 0.85, respectively. Although, initial tolerance of these pathosystems was more or less ≥ 95% of the control non-infected plants (0.98, 0.97, 0.95, and 0.97, respectively) they all reached almost total loss when they were given sufficient exposure times (w_{min} = 0.072, 0.124, 0.111, and 0.154). It can be postulated that, exposure duration of the host plant to a certain virus disease mainly determines the maximum crop loss of a pathosystem, and if the pathosystems have sufficient time, they will all finally approach a substantial maximum possible loss irrespective of tolerance of the host measured by magnitude of damage curve parameters such as initial tolerance, crop loss disease threshold, curvilinear tolerance and *desensitization* disease level (Fig. 5). It seems that, no matter how high the cultivar tolerance, it cannot guarantee the reduction of losses caused by the viral pathogens to an acceptable level if the host plants are exposed to the viruses for a long time. This finding highlights the importance of implementation of plant disease management strategies to avoid long exposure times of the host plant to the diseases and subsequent high yield losses. Findings of the present study on tomato TYLCV-[Ab]/ToLCKV-IR pathosystems suggest a novel tool for measuring different aspects of plant tolerance to the plant virologists and breeders while screening the plants for tolerance and resistance.

CONCLUSIONS

Botanical epidemics are studied partly because of the costs of yield losses they cause. Crop loss assessment is usually used for studying the relationship between disease severity and resulting yield loss of crops. Using a semi-empirical approach, a segmented linear model was fitted to the relative biomass of two tomato cultivars exposed to a severe (TYLCV-[Ab]) and a mild (ToLCKV-IR-IR) virus species for different lengths of times. Model parameters measured different aspects of tomato tolerance to the viruses and the findings can help tomato breeders in evaluation and screening of tomato germplasms.

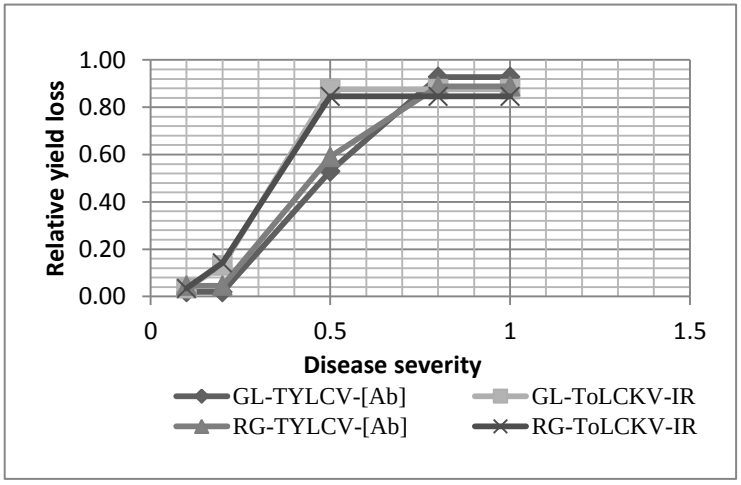


Fig. 5. Relative yield loss (1-relative biomass) plotted against disease severity for the four pathosystems: GL-TYLCV-[Ab]= Grosse Lisse– TYLCV-[Ab]; GL-ToLCKV-IR= Grosse Lisse –ToLCKV-IR; RG-TYLCV-[Ab]= Rio Grande–TYLCV-[Ab]; RG-ToLCKV-IR= Rio Grande- ToLCKV-IR. For details see the text.

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مقاله علمی- پژوهشی

مدل تجربی خطی تکه ای برای توصیف جنبه های مختلف تحمل به ویروس پیچیدگی برگ زرد گوجه فرنگی و ویروس پیچیدگی برگ گوجه فرنگی در دو رقم گوجه فرنگی در شرایط گلخانه ای

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ویروس پیچیدگی برگ زرد گوجه فرنگی

چکیده- به منظور بررسی میزان خسارت محصول بوسیله جدایه‌های ایرانی ویروس پیچیدگی برگ زرد گوجه فرنگی (TYLCV-[Ab]) و ویروس کارناتاکای پیچیدگی برگ گوجه فرنگی (ToLCKV-IR) بر روی ارقام گوجه فرنگی ریوگراند (RG) و گروس لیزی (GL)، گیاهچه‌های گوجه فرنگی با همسانه‌های عفونت زای هر ویروس بطور جداگانه با روش آگروواینوکولیشن مایه زنی شدند و پیشرفت بیماری در چهار فاصله زمانی پایش گردید. پس از ظهور علائم شدت بیماری بصورت چشمی و با استفاده از یک مقیاس نمره دهی ترتیبی ارزیابی شد و داده ها به مقیاس نسبی تبدیل شدند. خسارات ویروس ها با اندازه گیری شاخص های رشد شامل وزن تر و خشک و ارتفاع بخش های هوائی و زیرزمینی گیاهان برآورد شد. شدت اولیه بیماری در گیاهان آلوده به ToLCKV-IR کمتر از گیاهان آلوده به TYLCV-[Ab] بود. حداکثر سرعت افزایش بیماری در گیاهان رقم GL آلوده به ToLCKV-IR مشاهده شد و بعد از آن به ترتیب سرعت افزایش بیماری گیاهان آلوده به TYLCV-[Ab] از هر دو رقم قرار گرفتند و کمترین سرعت افزایش بیماری در گیاهان GL آلوده به ToLCKV-IR مشاهده شد. با تأخیر در زمان مایه زنی، شدت بیماری در هر دو رقم آلوده به هر یک از دو ویروس کاهش یافت. در همه پاتوسیستم ها، مدل خطی تکه ای برازش خیلی خوبی به زیست توده نسبی و مدت تماس نشان داد. پارامترهای برآورد شده مدل برای ارزیابی جنبه های مختلف نمودار خسارت استفاده شد. صرفنظر از ویروس مایه زنی شده به گیاه بالاترین *آستانه خسارت بیماری* در رقم GL مشاهده شد. وقتی *سطح حساسیت زدائی بیماری* ارزیابی شد، نتایج مشابهی حاصل شد. *تحمل منحنی- خطی* یا شیب بین گیاهان GL آلوده به هر یک از دو ویروس و گیاهان RG آلوده به ToLCKV-IR تفاوت معنی داری نشان نداد، ولی رقم RG آلوده به TYLCV-[Ab] بالاترین *تحمل منحنی- خطی* را نشان داد. نتایج این پژوهش می تواند در غربال گری برای گیاهان گوجه فرنگی متحمل استفاده شود.