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Modelling greenhouse climate factors to constrain internal fruit rot (*Fusarium* spp.) in bell pepper

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Abstract

Internal fruit rot in bell pepper is an important fungal disease which results in mycelium growth and/or necrosis on the ovarium and fruit flesh. It is mainly caused by members of the *Fusarium lactis* species complex (FLASC) and emerged as a major threat for bell pepper production worldwide. Infection already starts during anthesis but the symptoms are only visible later on in the production chain. An accurate prediction of the disease incidence in the greenhouse based on environmental parameters is an important step towards a sustainable disease control. Based on a large dataset (2011-2016) a binomial, logistic regression model was developed. This model enables an accurate prediction of internal fruit rot occurrence based on simple and robust input parameters such as temperature and relative humidity during anthesis. Spore density was included as a simplified, practical parameter describing the presence or absence of internal fruit rot one week earlier. The obtained model was validated with an independent dataset of five different commercial bell pepper greenhouses. The chance of internal fruit rot infection increased with temperature and relative humidity. Once a greenhouse is infected, only lower temperatures can reduce future risks. However, the chance of the disease to occur remains very high. This prediction model offers a strong instrument for growers to optimize greenhouse climate conditions to restrain internal fruit rot incidence. In addition, the model can be used to apply accurate biological or chemical treatments to achieve a more sustainable greenhouse control. A guideline table for climate adjustment is presented.

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Key words: *Fusarium lactis* species complex, Internal fruit rot, *Capsicum annuum,* Predictive model

Introduction

The popularity of bell pepper (*Capsicum annuum* L.) for fresh market consumption or ready- to- eat foods has grown significantly during the past decades. Especially coloured bell peppers have attracted consumers because of their flavor, shape, high vitamin concentrations and low calories (Howard et al., 1994). To satisfy the demand for high quality fruit, bell peppers are mostly grown in protected environments such as greenhouses (Jovicich et al., 2005; Cantliffe et al., 2008). Typical greenhouse production areas such as Belgium, The Netherlands, Spain, UK, Israel, Canada, South Korea and USA have been reported to suffer from a disease called internal fruit rot (Utkhede and Mathur, 2003; Yang et al., 2009; Choi et al., 2010; Van Poucke et al., 2012; Kline and Wyenandt, 2014; O'Neill and Mayne, 2015a; Frans et al., 2016a) This disease is mainly caused by members of the *Fusarium lactis* species complex (FLASC) and to a lesser extent by *Fusarium oxysporum* and *Fusarium proliferatum* (Hubert et al., 2003; Yang et al., 2009; Van Poucke et al., 2012). Infection of internal rot starts during anthesis (Yang et al., 2010). *Fusarium* spores deposited on the stigma, grow rapidly through the style resulting in a latent infection of the fruit ovary. During coloring, the fungus can become active and develops a whitish-pink mycelium on the seeds and/or necrosis on the inner fruit wall. At a later stage, when fruits have already progressed to the supply chain towards customers, external symptoms such as sunken lesions can be noticed (Yang et al, 2010; Van Poucke et al., 2012, Frans et al, 2016b). Annual yield losses are estimated at 5% with seasonal peaks up to 37% but can vary greatly between cultivars and production years (Hubert et al., 2003; Utkhede and Mathur, 2003; Frans et al., 2016b; O'Neill and Mayne, 2015a). This high variability in internal fruit rot occurrence during the growing season makes it challenging to achieve an optimal chemical or biological control of the disease. Till now, control measures are insufficient and restricted to some experimental trials of chemical and biological agents aiming on prevention of the initial flower infection (Utkhede and Mathur, 2005; O'Neill and Mayne, 2015a; Frans et al., 2017). Moreover, proper application timing is hard to predict because new flowers open daily on bell pepper plants, which makes a daily application very labor-intensive. Therefore, a more profound understanding how environmental factors influence internal fruit rot in bell pepper is essential to respond in a timely and efficient manner with accurate chemical or biological fungicide applications. Such predictions are considered a key principle in the concept of integrated pest management (IPM) (Stern et al., 1959; Goodell, 2009). More specifically, prediction systems provide an indication or quantification of the disease development, especially when the disease is likely to exceed an economic-injury threshold and thus demands a treatment. For most prediction systems, the relationship between disease development and environmental factors are the key component and often the only component (Van Maanen and Xu, 2003). It is widely agreed that environmental factors such as high relative humidity (RH) and temperature are the driving force in the development of epidemics (Van Maanen and Xu, 2003; Rabbinge and Bastiaans, 1989; Hardwick, 1998). The preceding decades, higher fossil-energy prices forced greenhouse growers to adjust their climate control leading to an overall higher relative humidity in bell pepper greenhouses. It has been postulated that these climate adjustments have been favouring internal fruit rot development in bell pepper (Van Poucke et al., 2012). Therefore, a predictive model for internal fruit rot occurrence based on common environmental conditions in European greenhouses has been developed in this study. This model includes the environmental parameters greenhouse temperature and relative humidity, but also the presence of the disease one week earlier as a simplified parameter describing spore density. As such the model aims to sustain growers in managing greenhouse macroclimate conditions in order to prevent disease outbreak. The presented model is a first step to develop a realistic, robust prediction model for the *Fusarium* internal fruit rot disease in greenhouse-grown bell pepper.

Material and Methods

Model input

A large dataset for internal fruit rot occurrence in bell pepper has been collected at Research Centre Hoogstraten. Between 2011 and 2016, the presence of internal fruit rot of bell peppers was determined every week in different greenhouse compartments. Each week 102 ± 5 bell peppers were visually examined on internal fruit rot symptoms. As such, a thorough dataset of 390 weekly internal fruit rot observations was created (Table 1). Temperature (°C) and relative humidity (%) were automatically logged in each compartment and registered by means of a Priva Electronic Measuring Box (Priva, The Netherlands). A useful model requires realistic data input values including some extreme values. Therefore, the range and frequency distribution of these parameters and the relation between temperature and relative humidity was checked.

Bell pepper plants were grown in rockwool bags (Cultilene, The Netherlands) under commercial greenhouse climate conditions. Average greenhouse temperature was 22 °C (25/18 °C day/night) and relative air humidity was about 75%. Plants were sown at the end of October and transplanted into the PCH greenhouse at the beginning of December. Specific plant dates for each year are presented in Table 1. Plant density was 7.1 stems per m². Plants were fertilized comparable to those in commercial greenhouses conditions.

Table 1: Input data used in the model of all production years. Every production year, different compartments were followed for several weeks (n). The bell pepper cultivar was meanly 'Viper' but changed in 2016 due to the unavailability of this rather sensitive cultivar towards internal fruit rot.

Year	Transplantation date	Cultivar	Compartment	Surface (m²)	n
2011	2 Feb	Viper	A	500	27
			B	500	27
			C	500	26
			D	500	23
2012	19 Dec	Viper	E	300	25
			B	500	28
			C	500	26
			D	500	26
2014	18 Dec	Viper	B	500	32
			D	500	32
2015	8 Dec	Viper	B	500	26
			D	500	23
2016	6 Dec	Redline	E	300	23
			C	500	23
			D	500	23

Model statistics

A predictive model was built to estimate the chances of internal fruit rot to occur under certain climate conditions. Therefore, the weekly percentages of internal fruit rot were converted into binomial absence (0) or presence (1) data. Simplifying the dataset overcomes variability between cultivars and creates more robust general predictions.

Three logic parameters were selected to predict the occurrence of internal fruit rot. The first two selected parameters are weekly mean temperature in degrees Celsius and mean relative humidity (%) during anthesis. The occurrence of internal fruit rot one week prior observations was taken as a third parameter. These parameters are referred to as T°, RH and I respectively throughout the text.

The relation between the presence of internal fruit rot and these three parameters was checked in a binomial, logistic regression model (generalized linear model). Model selection started with a full interactive model and non-significant interactions or parameters were subsequently removed. All statistics were carried out in R Studio version 3.2.5. Predicted probabilities from this generalized linear logistic regression model were reconverted to its original state using the formulae as $e^{model}/(1+e^{model})$.

Validation

The model was validated with an independent dataset collected at five different commercial bell pepper greenhouses in Belgium and The Netherlands. Internal fruit rot occurrence was determined on 20 fruits at a vegetables and fruit auction (Coöperatie Hoogstraten, Belgium). Climate data were obtained from the climate computers of these different growers. In accordance to the model input dataset, the range and frequency distribution of these parameters and the relation between temperature and relative humidity was checked. In total, a dataset of 443 weekly internal fruit rot observations was available for validation. This dataset was collected between 2012 and 2015 and is a mix of many different bell pepper cultivars from different growers, compartments and years (Table 2).

Table 2: Validation data Belgian and Dutch bell pepper growers

Nursery location	Coordinates	Year	Cultivar	n
Beerse, Belgium	N 51.3125, E 4,8076	2012	Divers	48
		2013	Divers	51
		2014	Divers	31

		2015	Divers	17
Merksplas, Belgium	N 51.3670, E 4.8994	2012	Divers	22
		2013	Divers	33
		2014	Divers	16
		2015	Divers	6
Rijkevorsel, Belgium	N 51.3377, E 4.7431	2012	Divers	25
		2013	Divers	38
		2014	Divers	16
		2015	Divers	13
Dongen, The Netherlands	N 51.6304, E 4.9828	2012	Divers	32
		2013	Divers	13
		2014	Divers	18
		2015	Divers	3
Wuustwezel, Belgium	N 51.3779, E 4.6750	2012	Divers	34
		2013	Divers	39

The logistic regression model was used to estimate the chance of internal fruit rot occurrence for each weekly observation. To validate the model, predictions were compared with the observed proportion of internal fruit rot observations. A problem arises as the model output is a chance of internal fruit rot, while the observed value is 0 or 1 (absence or presence). Therefore, the model output was rounded to one decimal place and grouped together according to the following categories: (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0). For each category, the presence of internal fruit rot was checked in the observed dataset and the proportion of observations with infection determined. This way we can compare the model output, which is a chance of internal fruit rot with the actual, observed proportion of infected plots. Finally, the predictive value of the model was tested by comparing mean square errors (MSE) of the validated and original datasets with F statistic ($\alpha = 0.05$).

Parameter simulation

The validated model was used to simulate the chance of internal fruit rot to occur under different greenhouse climatological conditions in both the attendance or absence of the disease one week earlier. Simulations were performed within the realistic ranges of temperature and relative humidity observed in Belgian and Dutch greenhouses.

Results

Model input: greenhouse temperature and relative humidity

Weekly mean temperature of the model input and validation dataset ranged between 18 and 26°C with a mean of respectively 21.8 ± 0.1 °C and 21.9 ± 0.1 °C . The frequency distribution of this parameter is for both datasets comparable and covers a realistic temperature range implemented in Belgian and Dutch greenhouses (Fig. 1).

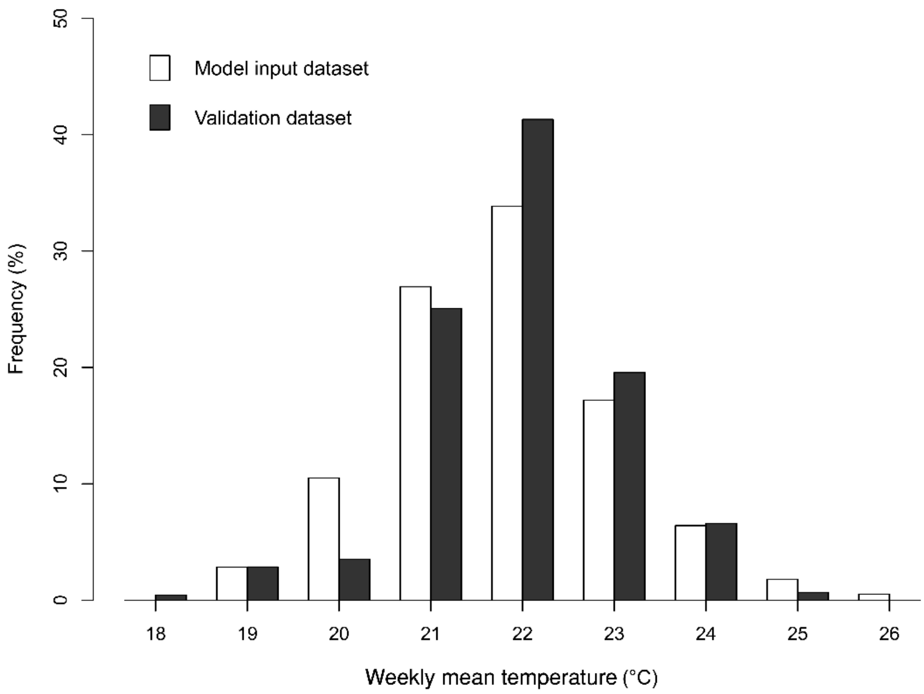

Fig. 1: Frequency distribution of the weekly mean temperature (°C) parameter values used as input variables in the model and validation datasets

Mean relative humidity values of the model input and validation dataset ranged between ± 65 and 90 % with a mean of respectively 79.7 ± 0.2 % and 78.4 ± 0.2 %. The frequency distribution is illustrated in Fig. 2. Analogously to the temperature values, this parameter contains the realistic range of humidity values found in Belgian and Dutch greenhouses for both datasets.

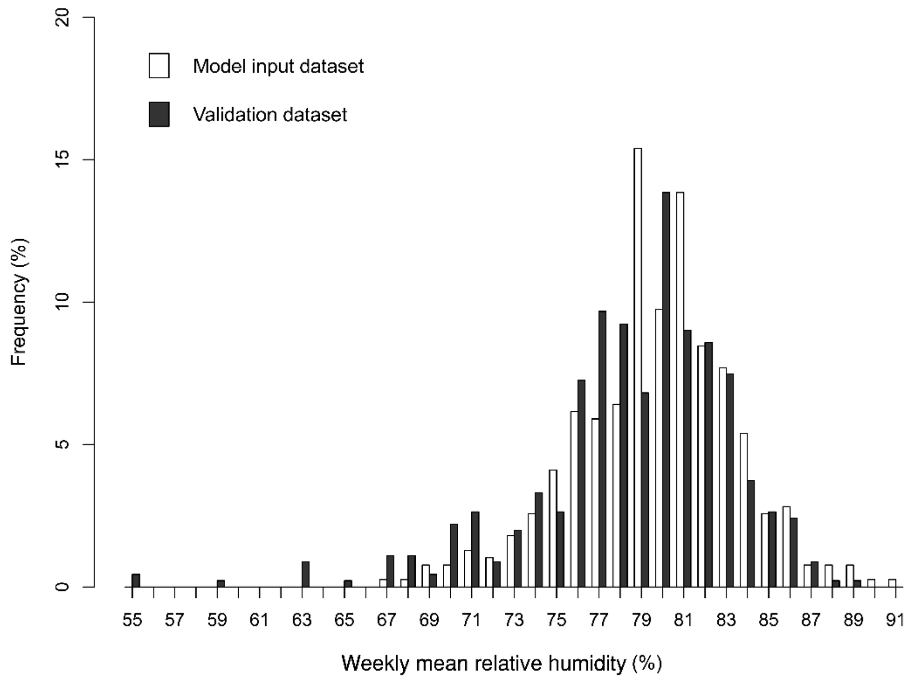

Fig. 2: Frequency distribution of the weekly mean relative humidity (%) parameter values used as input variables in the model and validation datasets

Data distribution of the weekly mean temperature and relative humidity data for the model input (A) and validation dataset (B) showed no correlation between both parameters and thus includes different combinations of high/low temperatures and relative humidity values (Fig. 3).

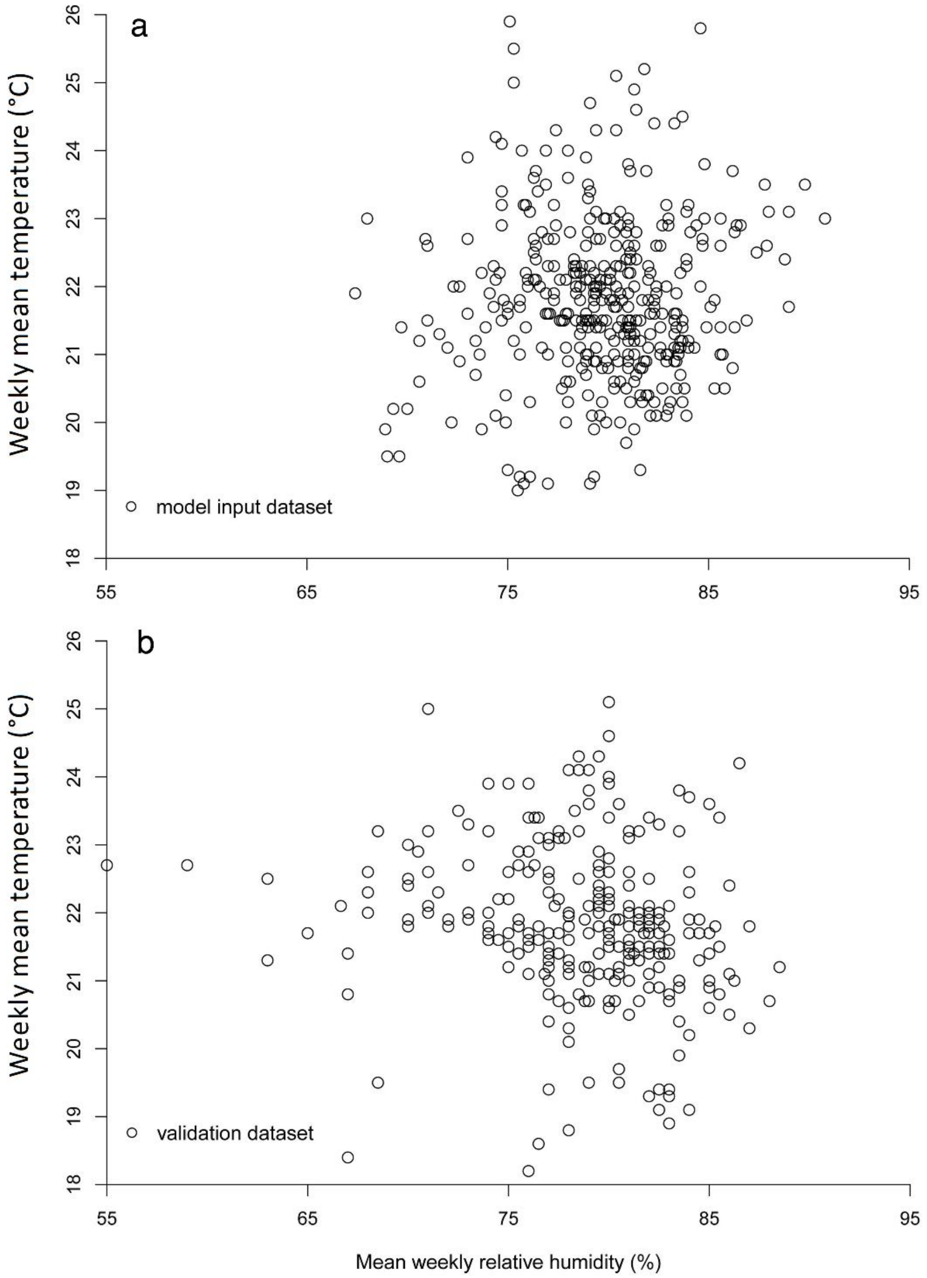

Fig. 3: Distribution of weekly mean temperature (°C) in relation to weekly mean relative humidity (%) for the model input and validation dataset

Model statistics

The full interactive model including all three parameters, i.e. T°, RH and I, tested non-significant for all variables and interactions (P > 0.05). Removing all non-significant interactions resulted in a highly significant model containing all three parameters. This model includes an additive effect of I, T° and RH and an interaction between I and RH (Equation 1). Statistical details and coefficient values (intercept, a, b, c and d) are provided in Table 3.

$$\text{Chance of internal fruit rot} = \text{intercept} + a*I + b*T° + c*RH + d*I*RH$$

Equation 1

Table 3: Statistics of the logistic regression model

Parameter	Estimate	SE	z-value	P-value
Intercept (i)	-18.367	4.321	-4.25	< 0.001
I	12.894	5.281	2.442	0.0146
Temp	0.342	0.11	3.093	0.0019
RH	0.134	0.045	2.942	0.0033
I*RH	-0.144	0.066	-2.178	0.0294

Validation

Predicted probabilities were calculated and subsequently fitted and validated with the independent dataset. Comparison of Mean Square Error (MSE) values of both the original and validation data set by F statistic confirmed the predictive value of the model (P > 0.05). Fig. 4 illustrates the predicted and observed values with numbers between brackets indicating the amount of observations per prediction category.

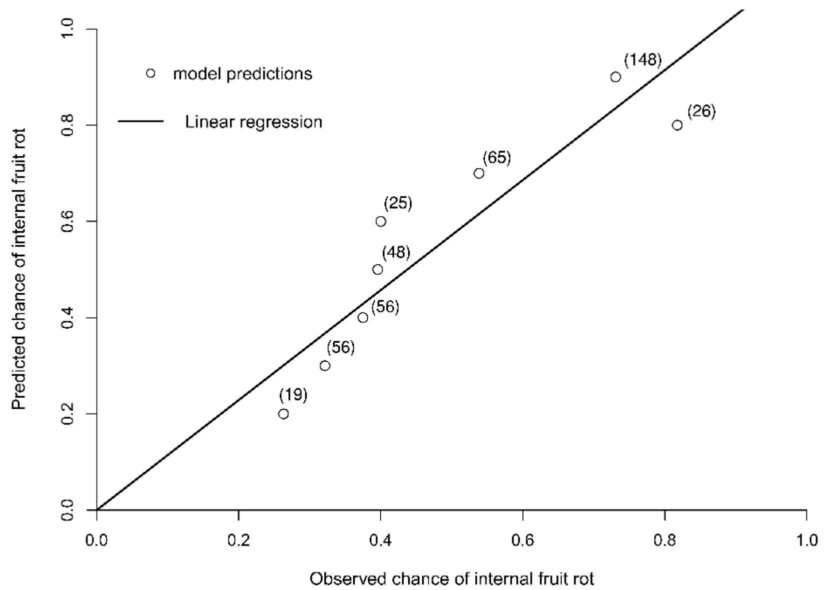

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Fig. 4: Validation of the model based on field data of bell pepper growers. Numbers between brackets indicate the number of observations for each prediction category (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0).

Parameter simulation

Temperature during anthesis has a beneficial influence on the occurrence of internal fruit rot (Fig. 5). Higher temperatures lead to greater chances of internal fruit rot . This effect is visible in situations when internal fruit was either absent (I = 0) or present (I = 1) in the greenhouse one week earlier. Clearly, the presence of internal fruit rot one week earlier further increases the chance of subsequent infections.

Relative humidity strongly influences the chance of primary internal fruit rot infections. Once the infection established in the greenhouse (I = 1), the effect of humidity is negligible. Then, only lower temperatures seem to reduce the chance of internal fruit rot infections to manifest (Fig. 5).

I	Temperature	Relative humidity (%)										
	(°C)	50	55	60	65	70	75	80	85	90	95	100
0	16	0	0	0	0	0	0,1	0,1	0,2	0,3	0,5	0,6
	17	0	0	0	0	0	0,1	0,1	0,2	0,4	0,5	0,7
	18	0	0	0	0	0,1	0,1	0,2	0,3	0,5	0,6	0,8
	19	0	0	0	0	0,1	0,1	0,2	0,4	0,5	0,7	0,8
	20	0	0	0	0,1	0,1	0,2	0,3	0,5	0,6	0,8	0,9
	21	0	0	0	0,1	0,1	0,2	0,4	0,5	0,7	0,8	0,9
	22	0	0	0,1	0,1	0,2	0,3	0,5	0,6	0,8	0,9	0,9
	23	0	0	0,1	0,1	0,2	0,4	0,6	0,7	0,8	0,9	0,9
	24	0	0,1	0,1	0,2	0,3	0,5	0,6	0,8	0,9	0,9	1
	25	0	0,1	0,1	0,2	0,4	0,6	0,7	0,8	0,9	0,9	1
1	16	0,4	0,4	0,4	0,3	0,3	0,3	0,3	0,3	0,3	0,3	0,3
	17	0,5	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,3
	18	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,4	0,4	0,4
	19	0,6	0,6	0,6	0,6	0,6	0,6	0,6	0,5	0,5	0,5	0,5
	20	0,7	0,7	0,7	0,7	0,7	0,6	0,6	0,6	0,6	0,6	0,6
	21	0,8	0,8	0,8	0,7	0,7	0,7	0,7	0,7	0,7	0,7	0,7
	22	0,8	0,8	0,8	0,8	0,8	0,8	0,8	0,8	0,8	0,8	0,7
	23	0,9	0,9	0,9	0,9	0,8	0,8	0,8	0,8	0,8	0,8	0,8
	24	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9
	25	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9

Fig. 5: Guideline table with predicted probabilities of internal fruit rot in bell pepper in the greenhouse at certain mean weekly temperatures and relative humidity in the absence or presence of internal fruit rot one week earlier (I = 0/1)

Discussion

This research demonstrates a model that predicts the chances of internal fruit rot infections in Belgian and Dutch greenhouses using mean weekly temperature and mean relative humidity during anthesis as input parameters. The presence or absence of the disease one week earlier, as a simplified measurement of spore density, appeared as an additional important parameter in the model. This robust model included data of different cultivars as well as different production years (from 2011-2016) and was validated using an independent dataset obtained from different pepper growers (Fig. 4). The climate input datasets had a realistic range of values common in Belgian and Dutch greenhouses. This is the first time that a prediction model based on climate data is developed for internal fruit rot in greenhouse grown bell peppers.

A warmer and more humid climate in the greenhouse results in higher chances of primary internal fruit rot infections (Fig. 5). At the start of the growing season, plants are small and evaporation is limited, thus greenhouse RH is relatively low. This drier climate combined with cooler temperatures in winter could explain why infections are rarely noticed early in the growing season. However, to reduce energy and heat losses, greenhouse vents are closed during the winter months. Additionally, energy screens are used to further reduce heat losses. This strategy, combined with an increasing crop canopy, results in more humid conditions and thus a higher chance of internal fruit rot infections during progression of the growth season. The summer period is characterized by higher temperatures. At this stage the crop has reached a reasonable stage and evaporation is significant. To avoid an undesired augmentation of RH, growers tend to open the greenhouse vents to cool down and reduce humidity. Both temperature and RH are important factors for crop management. Finding a trade-off to control internal fruit rot is challenging. It is very important for growers to prevent initial infections by cultivating in both dryer and cooler greenhouse conditions. Of course, this advice is restricted within the climate boundaries which are physically possible and necessary for pepper cultivation.

Once infections are established in the greenhouse, subsequently higher temperatures further increase the risk of more infections during the growing season. (Fig. 5). At this stage, RH has a negligible effect, especially at temperatures above 22 °C where chances of internal fruit were high at any given RH (Fig. 5). These higher temperatures are beneficial for growth of FLASC which has previously been reported to show maximal growth *in vitro* at 25 °C (Frans et al., 2017). This negligible effect of RH above 22 °C could also clarify why O'Neill and Mayne (2014; 2015b) could not find a correlation between high RH and internal fruit rot occurrence in different monitored commercial greenhouses were temperature varies around 22 °C. The predicted probabilities, based on robust input parameters (Fig. 5) can be attributed as a guideline to improve climate control and disease management. As a consequence, the model has also economical and sustainable advantages as chemical or biological fungicide applications can be timed more accurately (Utkhede and Mathur, 2005; O'Neill and Mayne, 2015a; Frans et al., 2017). Innovative, technological systems such as multispectral cameras (Polder et al., 2014) or detection of plant volatiles with electronic noses (Wilson, 2013) in combination with environmental conditions can further improve the pathogen forecast model in the future.

Compliance with ethical standards

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Conflicts of interest: The authors declare that they have no conflict of interest.

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