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Epidemiology and Aerobiology of *Pseudoperonospora cubensis* in northwest Iran

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Abstract. This study was aimed at evaluating climatic variables and concentrations of airborne sporangia of *Pseudoperonospora cubensis*, the causal agent of cucumber downy mildew and its relation with the severity of the disease. Monitoring was conducted in a cucumber field located in Marand, Iran during 2014-2015. The aerial concentration of sporangia was evaluated by a whirling arm trap and the weather parameters were monitored using a local meteorological station. Statistical analysis indicated a significant correlation between disease severity and some climatic factors (P≤0.05). The results showed that evaluation of airborne sporangia and the use of forecasting models could reduce the risk of disease in the northwest of Iran. The factorial analysis of the climate variables resulted in the development of two factors, average humidity and rain, that could be used as predictor variables in linear regression models for the downy mildew.

Key words. Cucumber, downy mildew, meteorological variables, sporangia dispersal.

INTRODUCTION

Cucumber downy mildew (CDM), caused by *Pseudoperonospora cubensis* [(Berk.& M.A. Curtis) Rostovzev], is a wide spread and devastating disease of cucurbit crops, including cucumber, squash, pumpkin, melon in the open field and in greenhouse productions (Lebeda and Cohen, 2011). Disease occurrence may lead to tremendous yield losses (up to 100%) in all production areas (Call *et al*., 2012, Cohen, 2015).

Disease epidemics are driven by the production of asexually derived sporangia. Environmental and ecological conditions significantly contribute to the progression of downy mildew disease as sporangia are mainly produced during warm (20–25°C) and humid (>85% RH) nights (low light intensity). Sporangia bearing structures (sporangiophores) can be seen arising from stomatal openings and are reliant on wind for dispersal of attached sporangia. Upon contact with susceptible host tissue, sporangia release zoospores which require sufficient leaf wetness/access to free water to swim to and successfully penetrate stomata. Post penetration, intercellular mycelium is produced and the disease cycle continues (Lebeda and Cohen, 2011).

Because of the favorable climatic conditions in Iran, cucurbits can be grown year-round in open fields or greenhouses. This production system ensures continuous host availability and eliminates the need for lengthy overwintering strategies for the obligate biotrophic pathogen. The unrestricted ability of the pathogen to produce inoculum and cause disease is presenting farmers with year-round challenges in disease management. Disease prediction models based on the aerodynamics of pathogen multiplication are urgently needed to mitigate costly chemical spraying during critical disease periods and to develop regional integrated pest management programs (Ojiambo *et al*., 2011).

However, some effective factors in the development of the disease by the dispersion of airborne sporangia have not been evaluated for use in the CDM forecasting system (Ojiambo *et al*., 2011).

CDM disease severity is directly related to production and release of sporangia by the pathogen. A disease outbreak can be evaluated with consideration of these two parameters (Neufeld *et al*., 2013). Combining the inoculum data with weather data assists disease prediction models to assess the risk of infection more accurately (West *et al*., 2008). This risk assessment allows growers to foresee possible disease outbreaks and adjust fungicide applications accordingly (Gent *et al*., 2013; Gent *et al*., 2009; Granke and Hausbeck 2011; Granke *et al*., 2014). Knowledge of temporal changes in *P. cubensis* populations during a single growing season can promote effective management strategies.

The aim of this study was to evaluate and relate climatic variables and concentrations of airborne sporangia of *P. cubensis* to CDM disease severity.

2. MATERIAL AND METHODS

2.1. Experimental plots and Meteorological measurements

The plots were maintained according to the standard production practices for cucumber (*Cucumis sativus*) production in Iran.

The population of *P. cubensis* sporangia were observed in cucumber fields in two regions of Marand during the growing seasons of April–October 2014 and 2015. In each region, the plot was planted with the same cultivar (Superstar, Semo, Czech). The cultivation area of each plot was 2 hectares. The fields were naturally infected and no fungicides were applied during the study.

The weather parameters were evaluated daily using a local meteorological station 1.5 m above the soil level less than 10 m outside the experimental plot. The temperature, relative humidity, rainfall, precipitation, wind speed and radiation were recorded. The wind speed was measured approximately 2 m above the ground. Data was recorded every 3 min to 15 min by the weather station.

2.2. Monitoring sites and aerobiological studies

The monitoring was done at two regions of Arbatan, a village 10 km west of Marand, located in northwest of Iran (38°10'29''N, 46°56'14''E or 38.174722°, 46.937222°). The spore trapping was carried out 24 hours a day from April 8 to October 9 in 2014 and 2015.

On each assessment day, the aerial concentration of *P. cubensis* sporangia, C (sporangia m^{-3}), was monitored above the cucumber canopy, using whirling arm trap (Burkard trap, Hirst-type U.K). The spore trap was installed 0.5 m above the canopy of the plants. The sporangia were trapped on sticky-coated tape (adhesive tape) mounted on the leading edge of the rotating arms. The tape strips were made sticky with a petroleum silicone grease coating. After use, tapes were mounted on microscope slides (sampler). A rain-shield was mounted above the coated-tape (Lacey and West, 2006). The tape strips were exposed to the air at 8 am until 6 pm daily for 117 days in 2014 and 132 days in 2015. After exposure, strips were transferred to the laboratory for identification and enumeration of sporangia. The sporangia were identified and quantified using a microscope equipped with a 100X lens (Nikon Optiphot II, Japan). Concentrations of the sporangia were expressed as the number of spores per cubic meter of air. The first sporangia were trapped on April 29, 2014 and April 21, 2015. Sampling was conducted every three days until October 20, 2014 and October 19, 2015.

2.3. Evaluation of disease severity

To evaluate CDM disease severity, 10 plants were selected randomly in each 20×10 m^2 plot. Foliar disease severity was determined using the 0 to 9 index developed by Thomas *et al.* (1987) with some modification (Table 1). The following design was used to evaluate the disease severity for each plant or plot:

$$DS = [\Sigma (ni \times vi)/ N \times V] \times 100$$

Where DS: disease severity, ni: number of leaves with the same score, vi: disease score from 0-9 for each leaf, N: total number of evaluated leaves, and V: highest disease score (9).

The disease severity was recorded 3 days after the last sampling date to assess the number of sporangia, and was carried out from May 2 to October 23 (39 times) and from April 24 to October 22 (42 times) for 2014 and 2015 respectively.

Tab. 1. The pattern used for the disease severity scaling of cucumber downy mildew.

Symptoms description	Score
No symptom	0
Visual spots without sporangium formation (incompatible)	3
Visual spots with a few sporangium (compatible)	5
Visual spots with scattered sporangium (5×10^3 spores per square cm of spot)	7
Spots covered the leaf surface (highly compatible) with a lot of sporangium (5×10^4 spores per square cm of spot)	9

2.4. Statistical analyses

All data obtained from the meteorological station and the number of sporangia registered during the seasons, as well as data on pathogenicity expressed as mean ± standard deviation, were analyzed using SPSS ver. 20 (SPSS Inc., Chicago IL). Paired sample t-tests for meteorological data, sporangium number and severity of disease were employed as the statistical tests, with the level of significance set at $p< 0.05$. The sum of the spores was calculated. A paired sample T-test (paired by the collection dates for the same site) was used to compare the mean sporangial concentrations collected in the growing seasons 2014-2015.

Pearson's correlation coefficients (r) analysis was carried out in order to assay the effect of meteorological factors, the maximum, minimum and mean temperatures (°C), the mean relative humidity (%) and rainfall (mm) on concentrations of sporangia and disease severity. The level of significance was set at ≤ 0.05 and ≤ 0.01. SPSS software was used to model and determine the mathematical relationship of the population of captured spores with climatic factors. The three-day sum of the captured spores was related to the weather data three days before (total rainfall, maximum and minimum temperature average, maximum and minimum relative humidity and total sunny hours in three days).

Regression statistical analysis was performed for the relationship between climatic factors and disease severity. A stepwise regression analysis was conducted on weather parameters and sporangia and disease severity data collected daily starting April 8 and ending October 9 in 2014 and 2015. Additionally, linear model analyses were conducted using in-season climate variables as predictors.

3. RESULTS

3.1. Disease severity and dynamism of sporangia

Symptoms of disease were first observed on April 29, 2014 and April 21, 2015 with a severity of 1.03 and 0.5%, respectively (Table 2). 10 leaves per plant were evaluated. The concentration of airborne sporangia differed significantly ($P < 0.05$) among months (May until October) and between years. Although based on the value of the Pearson's correlation coefficients (r), it has been shown that the dynamics of sporangia in the air in these monitoring years are similar in some respects (Table 3).

In the beginning of April 2014 and 2015, the daily concentration of *P. cubensis* sporangia increased slowly (Figure 1). In mid-May a rapid increase of daily sporangia density occurred. The highest spore density was observed on May 29, 2014 (3025 sporangia^{-3}) and May 27, 2015 (4528 sporangia^{-3}). After five weeks, the concentration of spores rapidly decreased (Table 2). On September 17, 2014 and September 1, 2015, the concentration of *P. cubensis* sporangia again increased and reached the highest value on August 1, 2014 and September 22, 2015 (Figure 1).

A three day average spore count peaks were recorded on May 26 and May 30 2014 and 2015 (Figure 1). The six days before these dates (time point to start infection), the average relative humidity of 59 and 54.5 %, maximum temperatures of 22.4 and 21.8 ˚C, minimum temperatures 11.2 and 11.2 ˚C, sun sum 8.6 and 6.6 h, wind speed of 17008 and 15005 m/s and rainfall 0.6 and 0.1 mm were recorded for 2014 and 2015 respectively (Table 2). The second period of high sporangia concentration occurred on Oct. 5 and Sep. 22 for 2014 and 2015 respectively, which could have been influenced by many features (Figure 1).

The diseases severity in the years 2014 and 2015 years were very similar, and a comparison between the two years showed almost similar climatic conditions in creating maximum spore (Table 2).

A series of Paired-samples t-tests conducted to compare all meteorological variables showed that was significantly associated with the number of sporangia during the years 2014- 2015. Similarly, there was a significant difference between the severity of the disease and the sporangia during the 2-year study period (Table 3).

In both years of study (2015-2014), the lowest and highest concentrations of sporangia at 0.5 m above the canopy were in the range of 198-310 sporangiam^{-3} with a disease severity of 0.5-1.03% and 3025- 4528 sporangiam^{-3} with a disease severity of 35%. (Complete data were not presented due to a large number of tables, but figure 1 shows the extent to which these numbers are somewhat consistent with the sporangia graph and the severity of the disease).

The Pearson's correlation coefficient showed no significant correlation between the spore concentration in the sampler and the climatic factors. In contrast, there was a significant relationship between the climatic factors (except total sunshine, wind speed and rain) and the disease

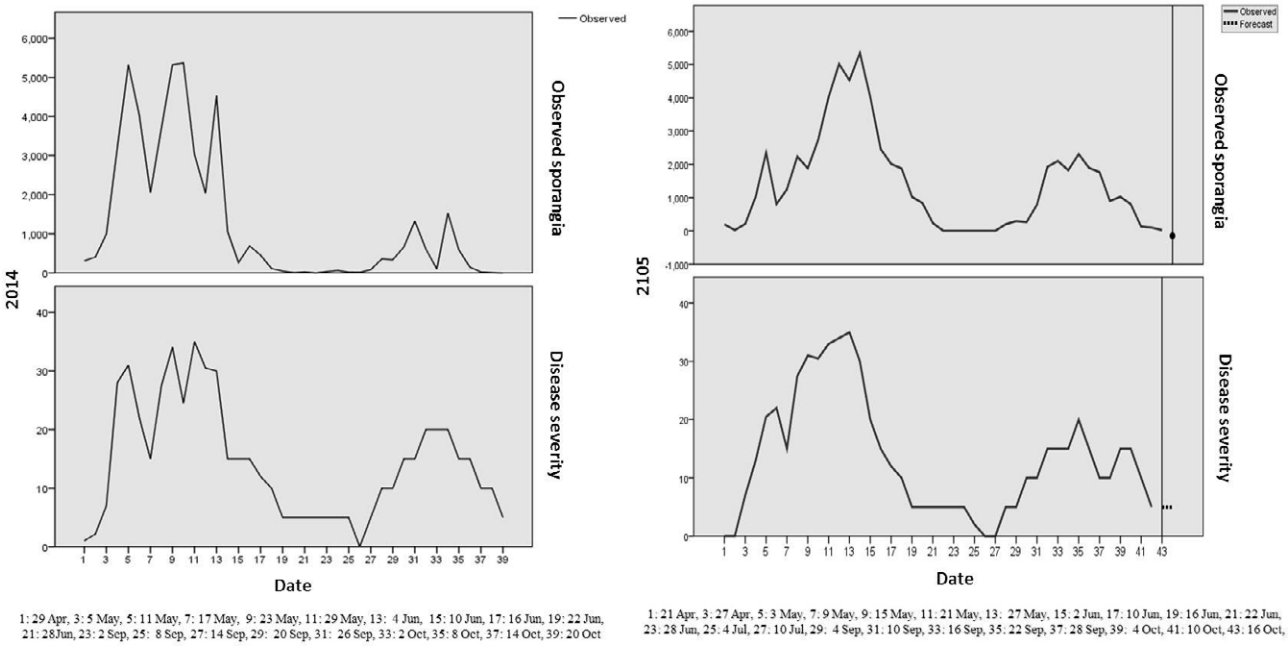

Fig. 1. Diurnal pattern of aerial concentration of *Pseudoperonospora cubensis* sporangia (m^3) above a cucumber canopy during downy mildew epidemics at Marand, North West, Iran. Curves are fitted by locally weighted regression to illustrate daily trends. Data are shown for disease assessment periods that represent the range of disease severity levels observed during 2014-2015.

Tab. 2. Standing crop of *Pseudoperonospora cubensis* sporangia escape and meteorological variables for the assessment periods in during the 2-year (2014-2015) study period.

	Date	Severity %[a]	No. of spores	RH[b]. max. (%)	RH. min. (%)	RH. av.(%)	T[c]. min. (°C)	T. max. (°C)	Sun(h)	Wind speed (m/s)	Rainfall (mm)
The first symptom	29 Apr. 2014	1.03	310	61	20	41	11	22.2	8.50	21009	0
	21 Apr. 2015	0.5	198	48	26	37	12.2	17.2	6.7	21027	0
Minimum disease severity	12 Sep. 2014	0.4	48	33	5	19	22.8	33.6	13.3	15009	0
	10 Jul. 2015	0.1	.00	35	12.00	23.5	25.8	37.6	12.7	16006	0
Maximum disease severity	20 May 2014	35	3025	71	47	59	11.2	22.4	8.6	17008	0.6
	24 May 2015	35	4528	74	35	54.5	11.2	21.8	6.6	15005	0.1

[a] Disease severity was assessed visually as the percentage of leaf area infected on each date when sporangia were collected.
[b] RH min, max and ave; maximum, minimum and average daily relative humidity
[c] T. max. and min.; maximum and minimum daily temperature

severity. In both years, the same result has been achieved. (Table 4).

Based on the correlation results, the prediction of capacity of the climate parameters and combination of several parameters were evaluated. The sporangia density was calculated every three days for obtaining time-series models and to forecast the *P. cubensis* airborne sporangia on the cucumber plot (Figure 1). Figure 1 illustrates the observation and prediction of the *P. cubensis* sporangia by the adjusted model for each cucumber crop cycle. The lines indicate the number of observed spores and the severity of the disease. Only in 2015, as shown in Figure 1, prediction time was determined based on the number of spores and the severity of the disease. In the figure, the sporangia dynamism are marked with the line and severity of the disease marked by dots.

The regression analysis of weather variables and disease severity data from April 8 to October 9 in 2014 and 2015 showed that linear regression was significant at $P < 0.01$. (Table 5).

Tab. 3. Paired-samples t-test to compare all meteorological variables and sporangia *Pseudoperonospora cubensis* and disease severity for the assessment periods in during the 2-year (2014-2015) study period.

Paired Samples Test										
		Paired Differences					t	df	Sig. (2-tailed)
		Year	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Lower	Upper			
Pair 1	Sporangium – RH[a] max.	2014	1191.22	1705.60	273.12	638.32	1744.11	4.36	38	.000
		2015	1347.85	1450.95	221.26	901.31	1794.38	6.09	42	.000
Pair 2	Sporangium – RH min.	2014	1218.99	1706.41	273.25	665.83	1772.15	4.46	38	.000
		2015	1377.04	1454.43	221.79	929.48	1824.65	6.21	42	.000
Pair 3	Sporangium – RH av.	2014	1205.11	1706.01	273.17	652.08	1758.13	4.41	38	.000
		2015	1362.44	1452.68	221.53	915.37	1809.51	6.15	42	.000
Pair 4	Sporangium – T[b] min.	2014	1237.92	1711.46	274.05	683.13	1792.72	4.58	38	.000
		2015	1387.89	1455.26	221.92	940.02	1835.75	6.25	42	.000
Pair 5	Sporangium – T max.	2014	1228.50	1710.96	273.97	673.86	1783.13	4.48	38	.000
		2015	1379.28	1454.78	221.85	931.56	1826.99	6.22	42	.000
Pair 6	Sporangium – SS[c]	2014	1243.21	1710.67	273.93	688.67	1797.751	4.54	38	.000
		2015	1394.87	1453.85	221.71	947.44	1842.305	6.29	42	.000
Pair 7	Sporangium - Wind	2014	-16317.72	9379.97	1501.99	-19358.36	-13277.09	-10.86	38	.000
		2015	-16020.27	11045.71	1684.45	-19419.63	-12620.89	-9.51	42	.000
Pair 8	Sporangium - Rain	2014	1251.39	1710.31	273.86	696.97	1805.80	4.56	38	.000
		2015	1402.75	1454.19	221.76	955.22	1850.28	6.33	42	.000
Pair 9	Sporangium – Disease severity	2014	1238.15	1701.99	272.53	686.43	1789.87	4.54	38	.000
		2015	-1424.01	1446.51	223.20	-1874.76	-973.22	-6.38	41	.000

[a] Relative humidity, [b] Temperature, [c] Sunshine sum.

The statistical significance of regression equations was checked by F-test and the analysis of variance (ANOVA) for models was summarized in Table 6. Based on the 2014 model, with an increase in 1 sporangia unit and 1 mm of rainfall, the severity of the disease will increase by 0.72 (0.005 + 0.715). However, in the 2015 model, the same amount of sporangia unit and rainfall will increase the severity of the disease by 0.393 (0.006 + 0.195 + 0.192). A regression model was showing correlation between sporangium and severity (Figure 2).

4. DISCUSSION

Determination of environmental factors that affect the pathogenic contamination and sporulation allows the time of fungicide application to control the disease (Yang *et al*., 2007).

This study showed *P. cubensis* sporangia to be present in the atmosphere throughout the two years, with their concentrations becoming higher during the mid and last stages of the crop growth. The number of sporangia in the air decreases with warming and decreasing moisture in the summer.

The concentrations of the sporangia in the atmosphere were very low at the time of the crop emergence, as the growth and expansion of the plant's green areas prevent the rapid accumulation of inoculum. The sporangia often spread again on aging or dying leaves so that spore concentrations often peak in the mid-stage, when the leaves are drying out (Figure 1).

The current study found a significant positive correlation between climate factors and spore concentration. With the rise in the mean, maximum and minimum temperatures, the spore density in the air increases, this makes *P. cubensis* a temperature-dependent pathogen. In Marand (the site of the study), the most sporangia in the atmosphere were found when the minimum temperatures were over 11.2 °C and maximum temperatures fell between 21.8 and 22.4 °C. *P. cubensis* sporangia belong to parasitic species that germinate within the optimum temperatures of 10–20 °C (Cohen, 1977).

Maximum relative humidity during the study years (2014-2015) was 71-74%. Favorable conditions for the re-

Tab. 4. Pearson's correlation coefficients (r) between for the overall meteorological variables and sporangia *Pseudoperonospora cubensis* and disease severity in during the 2-year (2014-2015) study period.

2014 [1], 2015 [2]		Sporangium	RH max.	RH min.	RH av.	T min.	T max.	SSS	Wind	Rain	Severity
Sporangium[1]	Pearson Correlation	1	.263	.227	.258	-.255	-.134	-.179	-.205	-.048	.828**
	Sig. (2-tailed)		.105	.165	.113	.117	.416	.275	.210	.771	.000
	N	39	39	39	39	39	39	39	39	39	39
Severity[1]	Pearson Correlation	.828**	.403*	.348*	.396*	-.329*	-.181	-.291	-.124	.211	1
	Sig. (2-tailed)	.000	.011	.030	.013	.041	.271	.073	.451	.198	
	N	39	39	39	39	39	39	39	39	39	39
Sporangium[2]	Pearson Correlation	1	.179	-.053	.102	-.253	-.157	.011	-.130	-.038	.860**
	Sig. (2-tailed)		.252	.736	.514	.102	.313	.944	.405	.810	.000
	N	43	43	43	43	43	43	43	43	43	42
Severity[2]	Pearson Correlation	.860**	.454**	.236	.409**	-.447**	-.376*	-.161	05	.217	1
	Sig. (2-tailed)	.000	.003	.132	.007	.003	.016	.308	.977	.168	
	N	42	42	42	42	42	42	42	42	42	42

Tab. 5. Results of the analysis of variance (ANOVA) for the disease severity of cucumber downy mildew and weather variables.

Model		Sum of Squares	DF [a]	Mean Square	F	Sig.
2014	Regression	2761.558	2	1380.779	53.691	.000 [b]
	Residual	925.819	36	25.717		
	Total	3687.377	38			
2015	Regression	3510.438	3	1170.146	69.205	.000 [c]
	Residual	642.520	38	16.908		
	Total	4152.958	41			

[a] Degrees of freedom; [b] Predictors: (Constant), Sporangium, Rain; [c] Predictors: Sporangium, RH av., Rain.

Tab. 6. The regression models analysis of weather variables and disease severity of cucumber downy mildew during 2014 and 2015.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
2014	Constant	7.493	1.053		7.115	.000
	Sporangium	.005	.000	.840	10.051	.000
	Rain	.715	.238	.251	3.002	.005
2015	Sporangium	.006	.000	.833	12.879	.000
	RHav	.195	.059	.237	3.319	.002
	Rain	.192	.093	.147	2.075	.045

production and dissemination of the pathogen are warm temperatures (20–25°C) and high humidity (>85% RH) (Lebeda and Cohen, 2011), which are available during the growing season in many parts of East Azarbaijan, Iran. The highest concentrations of *P. cubensis* sporangia in this study were recorded under these conditions.

The temperature and moisture conditions for the dispersal of *P. cubensis* sporangia in cucumber were observed. They were similar to those reported Neufeld *et al.*, (2013) and Naegele *et al.*, (2016).

Given the correlation between the climatic conditions and the spore levels, the observed pattern was similar during the two study periods. Aerobiological studies serve as an essential means of predicting the disease risk as they greatly contribute to the determination of the levels of inoculum (Neufeld *et al.* 2013).

Many aerobiological studies on the dispersion of plant pathogen have used daily measurements of the standing crop of spores on the study site as the measure of inoculum source strength (Aylor and Taylor, 1983; Aylor *et al.*, 2001, 2011; Andrade *et al.*, 2009). However, it is possible to base the disease management decisions on the assumed or actual identification of inoculum. In both regression models were presented, there was the correlation between percentage of disease severity and sporangia. This suggested the models could well predict disease severity of cucumber downy mildew. Linear logistic models are generally used in aerobiological studies to predict spore concentrations (Rodríguez-Rajo *et al.*, 2010).

The replication of this study by considering the relation of sporangia, rain and average humidity with the epidemic development could promote the development and/or improvement of the forecasters of cucumber downy mildew (CDM) disease in the East Azarbaijan.

This study was primarily aimed at generating data as a foundation for improving the CDM forecasting system that functions as a decision-making tool for controlling downy mildew. It more specifically focused on the data

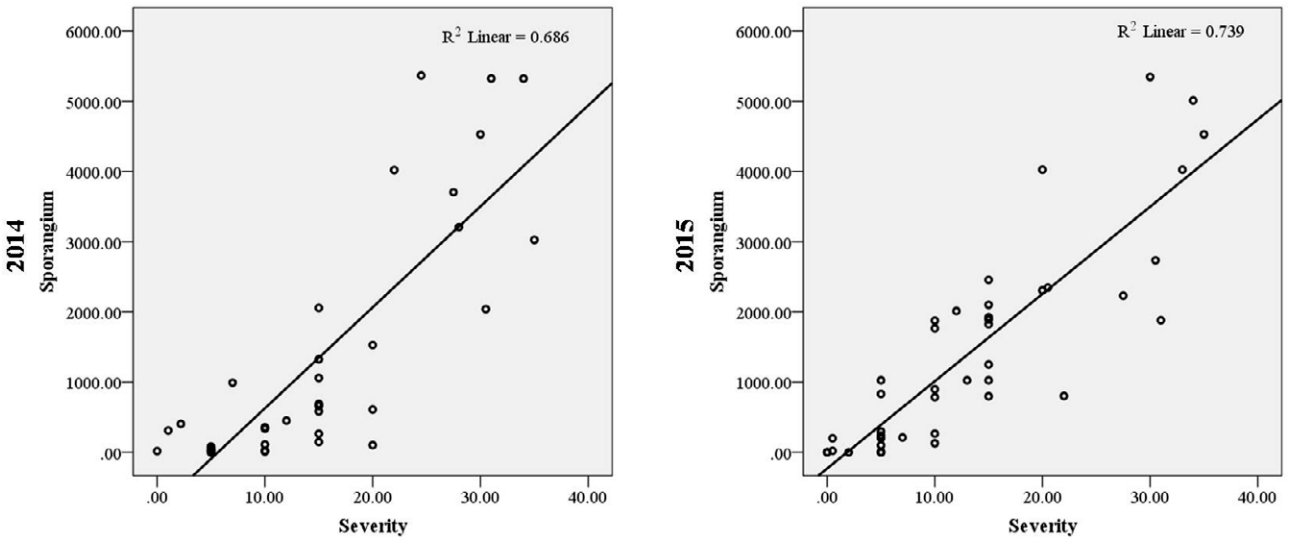

Fig. 2. Relationship between disease severity and sporangia due to downy mildew of cucumber during 2014 and 2015 in Iran- Marand.

that can be applied to adapt the aerial concentrations of sporangia and sporangia escape to the weather parameter along the projected pathways of sporangia transport and deposition for the risk prediction (Ojiambo *et al*., 2011). This forecasting system has reportedly led to a reduction of fungicide applications used by growers.

In Iran, farmers often use plastic houses, glass houses and net houses to grow cucurbits. They are confronted with the devastating attacks of downy mildew, especially on cucumber in winter, and are forced to frequent use fungicide applications (Fani *et al*., 2014, Pouzeshimiab and Fani, 2016). The relatively higher temperatures and humidity of these glass-covered houses in winter provides favorable conditions for the development of downy mildew, enabling the produced sporangia to stay inside and spread more infection.

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