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Effects of agro-pedo-meteorological conditions on dynamics of

2 temperate rice blast epidemics and associated yield and

3 milling losses

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1 Abstract

2 Rice blast disease is a threat for European rice growers, who apply chemical treatments each year to 3 limit its impact on rice yield and milling quality. Good agronomic practices such as varietal choice 4 and reduced nitrogen fertilization can also be effective in limiting the impact of the disease, which 5 largely varies across sites and growing seasons. Here we present a three-year experiment (2013-6 2015), in which blast disease severity was dynamically sampled on four varieties grown with two 7 nitrogen doses (standard and double farmer fertilization) in three sites located in Northern Italy (i.e., 8 the largest European rice district). No chemical treatments were applied on these experimental 9 plots, which were compared to blast-treated controls. Field yield and yield after milling (t ha⁻¹) were 10 measured to assess the impact of rice blast. Disease progress curves of leaf and panicle blast were 11 analysed via F-test for site, nitrogen dose, rice variety, and year. The areas under disease progress 12 curves were correlated with yield losses via linear regression. Finally, a 4-way analysis of variance 13 was performed using field yield losses and head rice yield as dependent variables. Results showed 14 that blast epidemics were significantly affected by all the factors considered, with rice variety and 15 year as the most important sources of variability. Areas under disease progress curves were 16 significantly correlated with losses in field yield and even more in yield after milling, with panicle 17 blast proving to be the most impactful symptomatology. Year and variety ranked first and second 18 among the factors explaining yield losses, both in field and after milling. These results confirm the 19 effectiveness of varietal choice to reduce blast impact, indicating that fungicide applications should 20 be conditional to the conduciveness of weather conditions.

21

22 Keywords

23 Disease severity, disease progress curves, field yield, head rice yield

1 Highlights

- 2 A three-year experiment to assess rice blast disease impact on yield is presented.
- 3 Rice variety, nitrogen dose, year and site are tested factors of variability.
- 4 Leaf and panicle blast impacts on field and milling yield are distinctly considered.
- 5 Variety and year are the main sources of variability in blast disease progress curves.
- 6 Panicle blast is the symptomatology most correlated to field and milling yield losses.

1 1. Introduction

2 Rice blast disease (causal agent Magnaporthe oryzae B.C. Couch) is present in 85 rice-growing 3 countries (Kato, 2001), and represents a global threat to food security and farmers' income (IRRI, 4 2006). It is responsible of yield losses up to 50-100% (Ou, 1985; Liu et al., 2016), with annual losses representing food for 60 million people (Pennisi, 2010). Recent estimates report that the blast 5 6 fungus is responsible for up to 30% of losses in global rice production (Skamnioti and Gurr, 2009; 7 Nalley et al., 2017), and the annual cost of chemical control can reach over \$70 ha⁻¹ (Nalley et al., 8 2016), leading to the largest fungicides expenses among all fungal plant diseases (Illana et al., 9 2013).

10 The pathogen can colonize all the aerial plant organs at all growth stages (Wang et al., 2014), and 11 leads to distinct symptoms when it attacks the leaves and the panicles (Kobayashi et al., 2001). On 12 the leaves, it causes necrotic elliptical-shaped lesions, which vary in number and size according to 13 environmental conditions and cultivar resistance (Piotti et al., 2005). The effect of leaf blast (LB) on 14 yield losses is indirect, and it is mainly due to the reduction of photosynthetic rate and the increase 15 in leaf respiration, both affecting CO₂ assimilation of the single leaves (Bastiaans, 1991; Bastiaans 16 et al., 1994). Leaf blast impact on leaf tissue was estimated to extend roughly three times beyond 17 the area covered by the visible lesion (Bastiaans, 1993a).

18 Panicle blast (PB) is considered to be the most serious symptomatology of the disease (Goto, 1965; 19 Zhu et al., 2005). Its symptoms appear when the fungus develops on the neck node at early grain 20 filling stage, determining necrosis that leads to a premature death of the entire panicle (Gianessi and 21 Williams, 2011). It causes direct yield losses (Shim et al., 2005) due to a reduction in grain weight 22 and in the number of ripe spikelets and fully mature grains (Teng et al., 1990). The lesions in the 23 upper leaves are the main inoculum source for the asexual fungal spores causing PB (Ghatak et al., 24 2013; Kobayashi et al., 2016). In case of late outbreaks during rice maturity phase, the fungus can 25 also colonize panicle branches, spikes, and spikelets, reducing the remobilization of carbohydrates

to the rice grains, which is often subject to breakages (Agarwal et al., 1989) and therefore present
lower milling quality (Webster and Gunnel, 1992). PB impacts are known to be larger in temperate
environments than in the tropics (Ou, 1985; Bonman, 1991), even if the number of monocycles
during a growing season is roughly half in the former (Teng, 1994).

5 Italian rice agriculture is a typical example of a temperate environment where blast largely affects 6 the variability of the national rice production (Bregaglio et al., 2016). Italy is the top rice growing 7 country in European Union, contributing to 55% of the total production (Casati, 2013), with a 8 harvested area of 234,134 ha in 2016 (Ente Nazionale Risi, 2016a) and a total production of 9 1,386,100 t (FAOSTAT, 2016). Rice cultivation is performed in paddy fields under continuous 10 flooding during most part of the crop cycle (Hill et al., 1991), with two to four water drainages to 11 allow rooting during crop establishment, top-dressing fertilizations at tillering and/or panicle 12 initiation, herbicide spraying and harvesting (Fusi et al., 2014).

13 Italian rice growers can control blast epidemics combining agronomic practices and chemical 14 sprays. The former includes low doses of nitrogen (Piotti et al., 2005) and adopting partially 15 resistant varieties (Faivre-Rampant et al. 2011). However, resistant or partially resistant varieties 16 are not currently widespread, as rice in Italy is mainly produced for traditional "risotto" dishes, 17 leading farmers to grow blast susceptible varieties which are more suitable for the preparation of 18 these dishes and have high quality value (Titone et al., 2015). The impact of blast disease on rice 19 yields does vary among years, being strictly dependent upon the agro-pedo-meteorological 20 conditions during the growing season, with conducive weather represented by durable presence of 21 leaf wetness and optimal temperature in the range 19-24°C (Nunes Maciel, 2011, Kim et al., 2015). 22 Nonetheless, rice growers typically apply chemical control two times during the growing season, at 23 early-boot stage and right after heading to limit the occurrence of PB (Padovani et al., 2006), based 24 on the specific rice variety, nitrogen management, and the pedo-environmental conditions that can 25 largely modulate the impact of blast disease.

1	The main objective of this study is to quantify the effects of agro-pedo-meteorological conditions
2	on temperate rice blast epidemics, and on the associated yield losses. We divided our analyses in
3	three parts. Our first objective is to characterize the dynamics of LB and PB in three sites located in
4	Northern Italy, using field data collected in a three-year experiment testing alternative nitrogen
5	applications and rice cultivars. The second objective is to quantify the contribution of LB and PB
6	blast severity in explaining field (FYL, %) and milling (MYL, %) yield losses. The final objective
7	is the assessment of the impact of the different agro-pedo-meteorological factors on the variability
8	of FYL and MYL.

10 **2. Materials and methods**

11 **2.1 Description of the field experiments**

12 2.1.1 Study area and experimental sites

The experimental trials were carried out in the 2013, 2014 and 2015 cropping seasons in three Italian sites located in the provinces of Pavia (Confienza), Vercelli (Collobiano) and Novara (Garbagna Novarese) (Figure 1). The total rice area covered by these provinces accounts for 81% of the total Italian rice area (82% of national production) and 47% of the EU rice cropped area (39% of EU production) (Ente Nazionale Risi, 2016a).



2 Figure 1. Location of the three experimental sites and synthetic statistics of the rice area and 3 production in the Italian provinces where trials were carried out (Ente Nazionale Risi, 2016a). 4 5 The pedo-meteorological conditions of the experimental sites are presented in Table 1. Weather 6 data were retrieved by the weather stations of the Regional Agency for Environmental Protection 7 (ARPA, Assessorato Agricoltura - Settore Fitosanitario). The average temperature during the rice 8 growing season (May-September) was similar in the three years and ranged between 20.16-22.55 9 °C, however precipitation amounts varied, with 206.4 mm in Garbagna Novarese in 2013 as the 10 driest cropping season and 424.2 mm in Confienza as the wettest one in 2013. Confienza and 11 Garbagna Novarese have a silt loam soil, the former with a lower percentage of soil organic matter (1.8% compared to 2.16%) and a higher cation exchange capacity (14.7 cmol kg⁻¹ clay, compared to 12 13 10.4 cmol kg⁻¹ clay). The soil texture in Collobiano is loam, with a high percentage of soil organic 14 matter (2.76 %) and a medium cation exchange capacity (11.2 cmol kg⁻¹ clay). 15

Table 1. Latitude (Lat.), longitude (Long.), pedological characteristics (texture, soil organic matter,
 cation exchange capacity), and main meteorological conditions in the rice cropping seasons 2013,

¹

1 2014 and 2015 (from May to September) in the three experimental sites. T_{ave} = average air

2	temperatur	re (°C), R _{cum}	a = cumulat	ed rainf	all (mn	n), RH _{av}	e = averag	ge air re	lative l	numidi	ty (%), 0	Э.М.
3	= soil orga	nic matter (%); C.E.C	= cation	excha	nge capa	acity (cmo	ol kg ⁻¹ c	lay).			
	Site	Lat. N	Long. E	Year	Tave	R _{cum}	RHave	Sand	Clav	Silt	O.M.	C.E.C

Site	Lat. N	Long. E	Year	Tave	R _{cum}	RH _{ave}	Sand	Clay	Silt	O.M.	C.E.C
			2013	21.48	424.2	71.65					
Confienza	45° 33'	8° 56'	2014	21.04	372.6	73.72	27.6	14	58.4	1.80	14.7
			2015	22.55	307.8	68.62					
Corboano			2013	20.96	206.4	72.00					
Noverage	45° 38'	8° 66'	2014	21.4	514.2	71.2	36.9	6.3	56.8	2.16	10.4
Inovalese			2015	22.86	325.6	73.28					
			2013	21.2	461.2	77.80			45.6	2.76	
Collobiano	45° 40'	° 40' 8° 35'	2014	20.16	329.4	81.61	42.6	11.8			11.2
			2015	21.58	249.2	72.80					

4

5

2.1.2 Field experiment design and management

In each site, four Italian rice varieties (i.e., Gladio, Balilla, Deneb and Vialone nano) were grown
with two nitrogen levels (8 combinations). Nitrogen levels corresponded to the fertilizer dose
applied by the farmer (N1), and to a double dose (N2). Each combination was tested in a plot of 8 m
× 6 m, all plots were located in a unique field in each site.

10 No fungicide treatments were applied in the three cropping seasons. In the same fields control plots 11 were grown, for each variety, with N1 with the application of chemical control against blast disease 12 (1 or 2 applications of trycyclazole at 75% a.i. w/w, wettable powder, of 0.5 l ha⁻¹ commercial formulation Beam[®]) to determine attainable yield (van Ittersum and Rabbinge, 1997). Experimental 13 14 trials were carried out under flooded conditions, with rice seeds soaked in water for 48 hours and 15 then broadcast sown. Weeds were controlled with pre-sowing (Oxadiazon, Ronstar FL, 0.75 l ha⁻¹) and post-emergence (Penoxulam, Viper, 21 ha⁻¹) treatments. Sowing operations in 2013-2015 were 16 17 performed in the first week of May, and two top-dressing fertilizations were performed during the 18 cropping seasons around tillering and at panicle initiation, with nitrogen doses similar to those 19 typically applied in the farms hosting the experimental trials (Table 2). The set-up of the field trials 20 was performed by the Center for Seed Testing and Certification of the Council for Agricultural 21 Research and Economics (CREA-SCS).

- 22
- 23

Management operation	Year	Collobiano	Garbagna Novarese	Confienza
Pre-sowing fertilization	2013	26	0	78
(kg N ha ⁻¹ applied)	2014	25	0	51
	2015	25	0	51
Sowing date	2013	May, 7 th	May, 7 ^h	May, 6 th
	2014	May, 7 th	May, 5 th	May, 6 th
	2015	May, 4 th	May, 5 th	May, 7 th
1 st top-dressing	2013	47	85	78
fertilization	2014	50	80	69
(kg N ha ⁻¹ applied)	2015	50	80	69
2 nd top-dressing	2013	48	55	14
fertilization	2014	43	60	73
(kg N ha ⁻¹ applied)	2015	43	60	76

Table 2. Pre-sowing fertilization rates, sowing dates and top-dressed nitrogen applications for the
 three studied years in the three experimental fields for the N1 treatment.

3

4

2.1.3 Characteristics of the rice varieties

5 The main features of the four Italian rice varieties tested in this study are presented in Table 3. Gladio is the most resistant variety to blast disease among tested ones (3rd most cultivated variety in 6 7 Long B merceological class), with low and medium susceptibility to LB and PB, respectively. Balilla (6th most cultivated variety in *Round* merceological class) is highly susceptible to PB and 8 presents a medium resistance to LB. Deneb (Medium merceological class, not widespread) and 9 10 Vialone Nano (1st most cultivated variety among Medium group) are highly susceptible varieties to 11 both symptomatologies of blast disease. Gladio and Deneb present a short life cycle (135 days and 12 140 days from emergence to physiological maturity, respectively), whereas Balilla and Vialone Nano have a longer duration (155 and 160 days, respectively). The average height of the four 13 14 varieties ranges from 72 cm for Gladio to 110 cm for Vialone Nano, with Deneb and Balilla 15 presenting intermediate values (80-90 cm). 16

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- 1/
- 18
- 19

2	Table 3. Main characteristics of the varieties grown in the experimental field trials, with information
3	on the susceptibility to leaf and panicle blast, phenology, morphology and merceological class.

Feature		Unit	Variety					
			Vialone N.	Deneb	Balilla	Gladio	-	
Blast	leaf blast	-	very high	high	medium	low	1,2,3	
susceptibility	panicle blast	-	very high	very high	high	medium	4,5	
Crop cycle	vegetative	day	97	80	100	85	6,7	
length	reproductive	day	58	60	60	50		
	total length	day	155	140	160	135		
Morphology	plant height	cm	110	80-85	88	72	6	
	panicle length	cm	21	18	16	21		
	Weight of 1000 seeds	g	30	30.4	23.7	21.5		
Merceological	grain shape	-	medium	medium	round	long	6	
class	group	-	japonica	japonica	japonica	indica	6	

4 1: Faivre-Rampant et al. (2011); 2: Cavigiolo and Lupotto (2010); 3: Titone et al. (2015); 4: Paleari

et al. (2015); 5: Tamborini and Legnani (2006); 6: Ente Nazionale Risi (2016b); 7: Regional Agency
for Agriculture and Forestry Services (2008);

7 8

9

2.1.4 Field samplings of disease severity and yield determination

LB was weekly assessed in each growing season from July 1st to September 30th on the four top 10 11 leaves (Surin et al., 1991; Prabhu and Filippi, 1993) on 20 randomly selected plants. Two or three 12 operators carried out sampling of disease severity (DS, the percentage of diseased leaf area) using 13 the standard scoring system proposed by the International Rice Research Institute (IRRI, 1996; 14 Vasudevan et al., 2015). This ordinal scale presents ten classes assigned to leaves with a corresponding degree of blast infection. The 0 class corresponds to a healthy leaf, and classes from 15 16 1 to 4 to DS below 4%, with differences due to the observed symptomatology. Then DS increases in 17 class 5 (DS = 4-10 %), 6 (DS = 11-25%), 7 (DS = 26-50 %), 8 (DS=51-75 %) up to >75% in the 18 class 9. PB severity was visually assessed on 20 plants per plot starting from early ripening stage 19 using a disease index ranging from 0 (no diseased grains) to 10 (100% diseased grains) according to 20 Asaga (1981) and Ishihara (2014). Individual LB and PB severity values were then averaged 21 leading to a single value for each plot and sampling date.

Experimental plots were harvested at physiological maturity (22% grain humidity). A minced sample of 5g was then analyzed with an infrared thermogravimetric moisture meter (Sartorius MA 150) in order to determine grain water content. Dry field yield adjusted to the reference commercial humidity (14%) was then calculated. A sub sample of 100 g was milled by Ente Nazionale Risi, to calculate global milling yield. Sieves were then used to separate broken and whole kernels in order to determine head rice yield for both controls and diseased plots.

7 **2.2 Statistical analyses**

8 2.2.1. Characterizing the dynamics of leaf and panicle blast epidemics

9 All the four factors were considered in the statistical analyses on the disease severity (DS) dynamics 10 of leaf (LB) and panicle blast (PB): the nitrogen dose (two levels: N1 and N2), the rice cultivar 11 (four levels: Gladio, Deneb, Balilla and Vialone Nano), the experimental site (three levels: 12 Confienza, Collobiano and Garbagna) and the year (three levels: 2013, 2014, 2015). 13 Three nonlinear growth functions (logistic, Gompertz, and Weibull) were fitted via least-squares 14 regression using DS dynamic data (Mohapatra et al., 2008). The choice of the best model was 15 driven by the Akaike Information Criterion (AIC), computed as N·ln(SS/N)+2K, where N is the 16 number of points, SS is the sum of the square of the vertical distances from the curve and K is the 17 number of model parameters (three for logistic and Gompertz models, and four for Weibull model). 18 The logistic model was selected as it obtained the minimum AIC in most conditions (i.e., fitting 19 data divided by factor modalities). After model selection, we used F statistics to compare the 20 disease progress curves as indicated by Motulsky and Christopoulos (2003), and Ciliberti et al. 21 (2015). The null hypothesis H₀ states that a single curve can fit all the DS data better than multiple 22 curves fitted on subsets of data divided according to each factor level (alternative hypothesis H₁). 23 To test H₀, we optimized the three parameters of the logistic function on all DS data (global model) 24 via non-linear least squares fitting. We used the same technique to fit the logistic function on the 25 data belonging to each modality of a factor, thus obtaining distinct parameter values for each

modality (single-modality models). The ratio between the relative difference of the sum of squares and the degrees of freedom in the global and single-modality models were tested via F-test, and the corresponding p-value was calculated. The DS at flowering (only for LB, DS_{flo}, %) and the area under disease progress curve (AUDPC, % days) were calculated according to the midpoint rule method (Campbell and Madden, 1990) to compare disease progress curves. Curve fitting and statistical analyses were performed with GraphPad Prism, version 7.00 (GraphPad Software Inc.).

7

8

2.2.2. Correlation between blast disease severity and yield losses

9 The AUDPC values computed for leaf (AUDPC_{LB}) and panicle (AUDPC_{PB}) blast were used
10 separately as predictors to perform linear regressions using field (FYL, %) and milling (MYL, %)
11 yield losses as dependent variables.

12 FYL (%) were computed according to Equation 1.

13
$$FYL = \frac{Y_{control} - Y_{blast}}{Y_{control}} \cdot 100$$
 [1]

where $Y_{control}$ (t ha⁻¹) is the field yield in blast-treated plots and Y_{blast} (t ha⁻¹) is the field yield in the experimental plots with no chemical control. MYL (%) were computed as in Equation 1, after the multiplication of $Y_{control}$ and Y_{blast} by head rice yield, i.e., the weight of entire kernels with respect to total kernels after milling.

18 The significance of the relationships was tested and a model selection procedure based on Akaike

19 Criterion (AIC) was performed versus the null model (constant model, i.e., with no predictor). Data

20 were fitted individually for each modality of the four factors (site, rice variety, nitrogen and year)

- 21 by linear regression, with intercept forced to zero, in the form $y = m \cdot x$, where Y = FYL or MYL
- 22 (%), $x = AUDPC_{LB}$ or $AUDPC_{PB}$, and m = slope. All regressions were performed with R software
- version 3.2.3, using *lm* function from the base package *stats* (R Core Team, 2015).
- 24
- 25

1 2.2.3. Analyzing the field and head rice yield losses

2 FYL and the reduction in head rice yield (HRL, % computed according to Equation 1 using HRY in 3 control and blast-treated plots) were analyzed with a 4-way ANOVA using the nitrogen dose, the rice 4 variety, the experimental site and the year as factors. The main effects, the second- and third-level 5 interactions were tested as fixed effects. The contribution of each factor was assessed by the Mean 6 Squared Error (MS), calculated as the sum of squares divided by the associated number of degrees of 7 freedom (df). MS was used to compare the contributions of the different factors to the total variability 8 in FYL (the highest the MS of a factor, the highest its contribution). The significance of each factor 9 was then evaluated using F-test. The analysis was performed with R software version 3.2.3 using the 10 anova function from the stats base package (R Core Team, 2015).

11

12 **3. Results**

13 **3.1. Dynamics of blast disease epidemics**

14 **3.1.1.** Disease progress curves of leaf blast

Figure 2 presents the dynamics of LB severity as divided by the modalities of the four factors considered. The logistic models were fitted using all the data in each sampling, but we show here the average values \pm one standard error.



Figure 2. Dynamics of leaf blast severity according to the modalities of the four factors (a) nitrogen
dose, (b) variety, (c) site, and (d) year. Points refer to the mean disease severity in each sampling
date, error bars are ± one standard error, lines correspond to the fitted logistic models. Besides the
modality name, the mean standard deviation of the replicates (SD_{REP}, %) is reported.



1	0.003%, Table 4) and at maturity (average final $DS = 3.21\%$), corresponding to low AUDPC _{LB}
2	(71.9). In 2014, the disease progress presented a steep increase since the second half of July, with
3	DS_{flo} = 5.04% and a final AUDPC _{LB} of 653.9 (Table 4). An intermediate disease progress was
4	observed in 2015, associated to a slow increase in July ($DS_{flo}=0.002\%$), with favorable conditions
5	for secondary epidemic cycles in August, with average maximum DS reaching 11.49% and
6	AUDPC _{LB} = 213.0 (Table 4). Doubling the nitrogen dose (N2) caused a steeper increase of the LB
7	disease progress curve ($DS_{flo} = 3.28\%$, Table 4), and it was associated with a higher dispersion of
8	the data (standard deviation of the replicates, $SD_{REP} = 18.59$, Figure 2a) with respect to the farmer
9	standard fertilization N1 ($DS_{flo} = 0.08\%$, $SD_{REP} = 9.28$). The associated AUDPC _{LB} were 144.4 for
10	N1 and 396.3 for N2. Fitting single-modality models for the three sites did not lead to significant
11	differences with respect to the global model ($F = 1.91$, Table 4), although Collobiano was
12	characterized by a different pattern than the other two sites with a delayed symptoms onset (DS $_{\rm flo}$ $<$
13	0.001) and a steeper increase in August (Figure 2c), reaching an average maximum value of
14	14.29%. The AUDPCLB associated to Collobiano (169.2) was slightly smaller than in Garbagna
15	(233.4), with Confienza presenting the highest AUDPC _{LB} (653.9).
16	

2	Table 4 Analysis of	f the dynamics of	leaf blast enidemics.	Estatistic na	rameters of the logistic
<i>L</i>	Table 4. Analysis of	the dynamics of	lear blast epidennes.	r statistic, pa	rameters of the logistic

3	model ($y0 = initial$	disease severity, $r = rate$ of	increase, asy = asymptote of	disease severity), value
---	------------------------	---------------------------------	------------------------------	--------------------------

of disease severity at flowering (DS_{flo}) and average area under disease progress curve (AUDPC)

computed for each modality of the four factors considered in the study. * p < 0.05, ** p < 0.01, ns p > 00.05

Factor	Employ	F value Modality	y0		r		asy		סס		
Factor	r value		best-fit	SE	best-fit	SE	best-fit	SE	DS_{flo}	AUDI CLB	
Niture and	0.42**	N1	1.77E-07	1.70E-06	0.35	0.22	9.04	1.26	0.08	144.4	
Nitrogen	9.42	N2	1.47E-03	4.19E-03	0.16	0.08	19.54	3.84	3.28	396.3	
		Balilla	2.68E-04	1.15E-03	0.20	0.11	3.68	0.68	0.39	66.9	
Vomoto	44.66**	Deneb	4.20E-05	2.48E-04	0.23	0.14	9.53	1.82	0.48	200.3	
variety		Gladio	1.86E-02	1.50E-02	0.09	0.03	0.80	0.22	0.19	16.6	
		Vialone	4.71E-04	1.30E-03	0.18	0.07	43.71	6.28	5.1	797.5	
	1.91 ^{ns}	Collobiano	1.19E-16	6.01E-15	0.82	1.17	14.29	1.94	< 0.001	169.2	
Site		Garbagna	3.50E-03	1.05E-02	0.14	0.09	16.74	4.17	2.06	233.4	
		Confienza	2.35E-03	9.84E-03	0.15	0.12	11.42	3.99	3.89	374.7	
	17.3**	2013	1.02E-14	6.25E-13	0.82	1.63	3.21	0.66	0.003	71.9	
Year		2014	2.93E-03	7.30E-03	0.14	0.07	28.65	6.99	5.04	653.9	
		2015	1.53E-13	4.70E-12	0.67	0.71	11.49	1.23	0.002	213.0	

3.1.2. Disease progress curves of panicle blast

Figure 3 presents the dynamics of PB severity starting from July 25th, corresponding to the average rice flowering date in our experiment. As for LB, we plot here the average values in each sampling date \pm one standard error.



Figure 3. Dynamics of panicle blast severity according to the modalities of the four tested factors.
Points refer to the mean disease severity in each sampling date, error bars are ± one standard error,
lines correspond to the fitted logistic models. Besides the modality name, the mean standard
deviation of the replicates is reported (SD_{REP}, %).



1 dynamic of PB increase associated to the two nitrogen fertilizations (Figure 3a) was similar to LB,

2 with N2 leading to higher final values (final DS=68.16%) and AUDPC_{PB} (1290.5) than N1 (final

3 DS=55.42%, AUDPC_{PB} = 911.8). The Site factor was not significant (Figure 3c, Table 5).

4 Table 5. Analysis of the dynamics of panicle blast epidemics: F statistic, parameters of the logistic

5 model (y0 = initial disease severity, r = rate of increase, asy = asymptote of disease severity), value

6 of area under disease progress curve (AUDPC) computed for each modality of the four factors

7 considered in the study. * p < 0.05, ** p < 0.01, ns p > 0.05

Fastar	Evolue	Modelity	y0	y0			asy			
Factor	r value	wodanty	Best-fit	SE	Best-fit	SE	Best-fit	SE	- AUDPCPB	
NI' da se	10 10**	N1	0.016	0.011	0.21	0.04	55.42	3.73	911.8	
Nitrogen	12.12	N2	0.025	0.013	0.22	0.04	68.16	3.53	1290.5	
		Balilla	0.011	0.008	0.22	0.04	67.62	4.17	1083.4	
Variatas	((= 0**	Deneb	0.020	0.012	0.20	0.04	76.27	4.77	1348.5	
variety	00.38	Gladio	0.009	0.015	0.19	0.09	20.35	4.49	228.6	
		Vialone	0.020	0.012	0.27	0.05	83.04	3.43	1744.1	
		Collobiano	0.025	0.020	0.22	0.06	67.62	3.64	985.6	
Site	2.23 ^{ns}	Garbagna	0.012	0.011	0.20	0.05	76.27	3.91	1052.4	
		Confienza	0.019	0.013	0.19	0.04	56.35	6.36	1226.9	
		2013	0.004	0.006	0.24	0.07	53.30	5.48	732.5	
Year	15.49**	2014	0.025	0.014	0.21	0.04	72.63	4.48	1304.7	
		2015	0.021	0.016	0.27	0.06	59.47	2.97	1334.1	

8

9 **3.2 Impact of blast disease severity on yield losses**

10 The adjusted R² computed with linear regressions using AUDPC_{LB} and AUDPC_{PB} as explanatory

11 variables and FYL and MYL as dependent variables are shown in Table 6. The regression equations

12 are presented in Appendix A.

1 Table 6. Adjusted R² of the linear regressions computed using field and milling yield losses as

2 dependent variables, and area under disease progress curve of leaf (AUDPC_{LB}) and panicle

3 (AUDPC_{PB}) blast as predictors. Bold font identifies the highest adjusted R^2 between the two

4	symptomatologies of the disease.	[°] p <0.05, ^{**}	p <0.01, *** ·	p < 0.001, ^{ns} $p > 0.0$	5

5

		Field yield l	losses (FYL)	Milling yiel	d losses (MYL)
Factor	Modality	AUDPC _{LB}	AUDPC _{PB}	AUDPC _{LB}	AUDPC _{PB}
Nitrogen	N1	0.485^{***}	0.678***	0.466^{***}	0.788***
	N2	0.395***	0.843***	0.345***	0.880***
Variety	Balilla	0.254*	0.523***	0.327**	0.735***
-	Deneb	0.577^{***}	0.847***	0.546^{***}	0.952***
	Gladio	0.672***	0.730***	0.756***	0.738***
	Vialone	0.634***	0.884***	0.607^{***}	0.872***
Site	Collobiano	0.780***	0.868***	0.831***	0.927***
	Garbagna	0.290^{**}	0.904***	0.226^{**}	0.825***
	Confienza	0.418^{**}	0.744***	0.355***	0.851***
Year	2013	0.261**	0.569***	0.131*	0.743***
	2014	0.402^{**}	0.939***	0.381**	0.922***
	2015	0.461**	0.827***	0.470^{***}	0.861***
All	data	0.384***	0.785***	0.332***	0.847***

6

7 Using all the experimental data, regressions were significant at p < 0.001, with adjusted R² ranging 8 from 0.332 (AUDPCLB vs. MYL) to 0.847 (AUDPCPB vs. MYL) (Table 6). PB was most correlated 9 with yield losses than LB in all considered conditions, but one (Gladio, milling yield losses). Deneb 10 was the cultivar showing the highest correlation between both symptomatologies of the disease and yield losses, with AUDPC_{PB} highly correlated both with FYL (adjusted $R^2 = 0.847$) and MYL 11 12 (adjusted $R^2 = 0.952$) (Table 6). Vialone Nano ranked second, with AUDPC_{LB} and AUDPC_{PB} 13 significantly correlated with FYL (adjusted $R^2 = 0.634$ and 0.884, respectively) and MYL (adjusted 14 $R^2 = 0.607$ and 0.872, respectively). Similar correlations were found for the moderately resistant variety Gladio, with adjusted R² always significant at p<0.001 and ranging from 0.672 (AUDPC_{LB} 15 16 vs. FYL) to 0.738 (AUDPC_{PB} vs. MYL). Balilla showed less significant correlations between AUDPC_{LB} and yield losses, whereas AUDPC_{PB} was correlated to FYL (adjusted $R^2 = 0.577$) and 17 18 MYL (adjusted $R^2 = 0.735$) at p < 0.001. 19 2014 was the year in which there were highest correlations between AUPDC_{PB} and FYL (adjusted $R^2 = 0.939$) and MYL (adjusted $R^2 = 0.922$), followed by 2015 and 2013. In 2013 – the year in 20 21 which blast disease severity was the lowest in our experiment -AUDPCLB was less correlated with 22 yield losses, with adjusted R^2 ranging from 0.131 (MYL, p<0.05) to 0.261 (FYL, p<0.01) (Table 6).



9 3.3 Quantifying the sources of variability in field yield and head rice yield losses

The boxplots reported in Figure 4 show the data of FYL and HRL in each modality of the four
factors considered. The results of ANOVA performed with the linear models fitted using FYL and
HRL as dependent variables are presented in Table 7.





Figure 4. Boxplot presenting the dispersion of the percentage of field yield losses (left side) and the reduction in head rice yield (right side) according to each modality of the four factors considered (a) nitrogen, (b) variety, (c) site, and (d) year. GLA = Gladio, DEN = Deneb, BAL = Balilla, VIA =

17 Vialone Nano, COL = Collobiano, GAR = Garbagna, CON = Confienza. Upper whisker represents

1 75th percentile + 1.5 IQR, lower whisker represents 25^{th} percentile – 1.5 IQR, where IQR is the box 2 length (75th percentile – 25^{th} percentile).

3 For FYL, the main effects of three factors out of four were highly significant (p<0.001), with year > 4 variety > site, and nitrogen factor was not significant (Table 7), proving that they all contribute to 5 explain the variability of blast impact in the tested conditions. 2015 (average FYL = 32.15%, SD = 6 27.41%) resulted as the year with the highest impact of blast disease (Figure 4d), with respect to 7 2014 (average FYL = 28.8%, SD = 40.07%) and 2013 (average FYL = 7.82%, SD = 14.01%). 8 Among varieties, Vialone Nano (average FYL = 38.21%, SD = 34.36%) showed the highest FYL 9 values (Figure 4), followed by Balilla (average FYL = 27.8%, SD = 33.88%), Deneb (average FYL 10 = 25.48%, SD = 36.64%) and Gladio (average FYL = 6.73%, SD = 12.78%) (Figure 4b). For the 11 site factor, Garbagna (average FYL = 35.13%, SD = 35.81%) was the modality where FYL were 12 larger, followed by Confienza (average FYL = 22.86%, SD = 36.98%) and Collobiano (average 13 FYL = 12.99%, SD = 19.59%) (Figure 4c). 14 Doubling nitrogen fertilization did not lead to significant differences in FYL (Table 7), despite this 15 factor was significant in explaining LB and PB progress. The two level interactions of Site \times 16 Variety and Site \times Year were also significant at p<0.001 (Table 7), suggesting that (i) yield losses

17 were not constant in the four varieties across pedo-meteorological conditions, and (ii) the year-to-

18 year variability of meteorological conditions has a large impact, even in close sites (Figure 1). The

19 second level interaction Nitrogen \times Site was significant at p<0.05 (Table 7), thus indicating a site

20 effect of nitrogen fertilization on FYL. The other second level interactions (Nitrogen × Year and

21 Variety \times Year) were not significant at p=0.05, as well as the third level interactions (Site \times Year \times

22 Nitrogen and Site \times Year \times Variety) (Table 7).

23

24

1 Table 7. ANOVA table of linear models for field yield losses (FYL) and reduction in head rice yield

		Field yield losses (FYL)			Reduction of head rice yield (HRL)		
Source of variation	Df	MS	F	p value	MS	F	p value
Nitrogen	1	346.2	1.83	0.201	17.3	0.25	0.629
Site	2	2529.4	13.36	< 0.001	124.9	1.79	0.221
Variety	3	3267.3	17.25	< 0.001	2818.6	40.45	< 0.001
Year	2	3338.6	17.63	< 0.001	3944.9	56.62	< 0.001
Nitrogen × Site	2	1138.7	6.01	0.016	431.3	6.19	0.020
Nitrogen × Variety	3	141.7	0.75	0.544	135.7	1.95	0.192
Nitrogen × Year	2	463.4	2.45	0.128	111.2	1.595	0.256
Site \times Variety	6	1704.9	9.00	< 0.001	438.6	6.30	0.011
Site × Year	3	1912.0	10.09	< 0.001	565.1	8.11	0.006
Variety \times Year	6	518.1	2.74	0.065	463.3	6.65	0.006
Site \times Year \times Nitrogen	3	260.6	1.37	0.299	44.8	0.64	0.607
Site \times Year \times Variety	9	262.4	1.39	0.296	88.9	1.27	0.340
Residual	21	2272.8			69.7		

2 (HRL). df = degree of freedom, MS = mean sum of squares, F = F-statistic.

3

4	The average HRL in the whole dataset	(23.97%)) were slightly higher	than FYL (average HRL =
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5 22.55%) (Figure 4). Year and variety were the only significant main effects (Table 7).

6 2014 (average HRL = 36.5%, SD = 25.24%) and 2015 (average HRL = 33.57%, SD = 23.26%)

7 were the cropping seasons in which HRL were largest, with lower impact in 2013 (average HRL =

8 8.44%, SD = 15.54%) (Figure 4d). For Variety factor, Deneb determined the highest reduction in

9 head rice yield (average HRL = 45.66%, SD = 36.13%), followed by Balilla (average HRL =

10 29.37%, SD = 21.79%), Vialone Nano (average HRL = 28.47%, SD = 25.48%) and Gladio,

11 confirming its low susceptibility to blast (average HRL = 8.97%, SD = 7.23%) (Figure 4b).

12 Collobiano, which was the less impacted site both in FYL and HRL (average HRL = 21.20%, SD =

13 25.35%), followed by Garbagna (average HRL = 24.71%, SD = 21.97%) and Confienza (average

14 HRL = 27.08%, SD = 28.44%) (Figure 4c). Nitrogen factor, which was not significant according to

15 ANOVA (Table 7), presented very similar values for the two modalities, with N2 (average HRL =

16 25.33%, SD = 29.17%) > N1 (average HRL = 20.16\%, SD = 32.74\%) (Figure 4a).

17 Site \times Variety and Site \times Year interactions were significant at p < 0.05, confirming that the impact

18 of blast disease on HRL is prone to a huge variability due to pedo-meteorological conditions,

consistently with the ANOVA results for FYL. The second level interactions Nitrogen × Site and
 Variety × Year were significant at p<0.05.

3 **4. Discussion**

4 4.1. Agro-pedo-meteorological factors affecting leaf and panicle blast disease epidemics in 5 Northern Italy

6 We provide a ranking of the factors explaining LB and PB variability, i.e., rice variety, cropping 7 season (year), site (being significant only for PB) and nitrogen fertilization dose (being significant 8 only in interaction with site). In our experiments, we included Italian rice varieties with a 9 heterogeneous resistance to blast disease. The adoption of resistant or moderately blast resistant 10 varieties, such as Gladio, could constitute an effective means to reduce blast impact for rice growers 11 (Ashkani et al., 2015), but is constrained in many cases by economic reasons, given that rice 12 varieties with a high quality value as Vialone Nano are often highly susceptible to the disease. The 13 effect of varietal choice in modulating blast disease epidemics and impacts is confirmed by many studies testing susceptible and partially resistant varieties in almost all rice growing areas (e.g., 14 15 Chuwa et al., 2015 in Tanzania; Fujii and Hayano-Saito, 2007 in Japan; Zhu et al., 2000 in China). 16 The variability of weather conditions is also considered as a main source of variability in LB 17 epidemics, even in Mediterranean regions (Koutroubas et al., 2009; Bregaglio et al., 2016). In our 18 study, LB impact in 2013 was very low, and environmental conditions were favorable for PB only 19 after flowering. In 2014, the impact of LB and PB was huge as the favorable weather conditions 20 (i.e., high number of rainy days leading to prolonged leaf wetness and warm air temperatures) 21 extended from July to September. In 2015, the disease onset was recorded very early in the season, 22 but then few precipitation events occurred since early maturity stage, where DS steadily increased 23 both for LB and PB. This 3-year experiment demonstrated the large impact of the year-to-year 24 weather variability in determining blast disease epidemics and impacts, which are favored by long 25 periods of leaf wetness with high relative humidity, and temperatures in the range 19-24 °C (Kim et

1 al., 2015). It is widely known that nitrogen rates and timing of application largely affect the impact 2 of LB on rice (Amin and Venkatarao, 1979; Kurschner et al., 1992), and therefore the rationale 3 application of fertilizer is recommended as a good practice to limit the occurrence of the disease 4 (Webster and Gunnell, 1992). The physiological reasons for this effect are not yet completely 5 clarified, and comprise a reduction of silicon uptake and of hemicellulose and lignin in rice plant 6 cells (Ou, 1985), and the increased leaf area index creating a more conducive environment for blast 7 development because of higher humidity in the canopy (Hai et al., 2007). In our study, doubling the 8 nitrogen dose to maximize the impact of fertilization yielded a 10% and 13% average increase in 9 final LB and PB severity. In contrast, Long et al. (2000) found a nitrogen effect only on LB, testing 10 three nitrogen treatments with different doses and timing of application. 11 Different pedological features characterize the three sites in our experiment (Table 1) with 12 Collobiano soil presenting the highest sand content and organic matter, followed by Garbagna and 13 Confienza. Blast disease development is known to be favored by sandy soil with a low cation 14 exchange capacity, because of the faster release of nitrogen than on clay and silty soils (Inoue, 15 1943; Datnoff, 1994). Other studies report that poorly drained paddy soils with high soil organic 16 matter content are also conducive for the disease (Kozaka, 1964). Our findings show that in the 17 explored conditions the site factor was not significant in explaining the variability of LB progress 18 curves. A better understanding of the relative impact of the factors influencing blast severity could 19 serve as the basis to develop simple quantitative indicators of risk to help rice growers in adopting 20 cost-effective management decisions, as well as to provide educational tools to inform on the 21 benefits of management practices lowering the impact of the disease (e.g., varietal choice, nitrogen 22 fertilizations), as already done for many fungal diseases of peanut (Kemerait et al., 2017). The 23 choice of the logistic model to describe PB epidemics is consistent with findings of Mohapatra et al. 24 (2008), who showed its appropriate fit in 91.2% of 307 blast progress curves referred to rice 25 varieties originated in India, South and North America and West Africa. Similarly, Marchetti (1983) 26 used the logistic model to successfully model blast progress curves on susceptible and resistant

1 varieties in California. Koizumi and Kato (1987) also found that the logistic function better fitted 2 experimental data than exponential, monomolecular and Gompertz models in a blast nursery grown 3 in temperate conditions using a Japanese cultivar. In contrast from what observed in our study, there 4 is other evidence that, in Asian countries, LB epidemics undergo a steep increase from disease onset 5 until maximum tillering, and then a decrease to maturity (e.g., Hwang et al., 1987 in Korea; Pasha et 6 al., 2013 in Iran). The main reason for this decline is the death of diseased leaves and the formation 7 of new leaf tissue (Bastiaans, 1993b). However, in Italy the suitable environmental conditions for 8 LB epidemics occur later in the growing season (Rodolfi et al., 2006; Titone et al., 2015), 9 approximately between mid-July (around panicle initiation) and mid-August (early maturity), when the vegetative organs of rice plants are already fully developed and without the production of new 10 11 leaves. This could explain why such a decline in LB severity was not observed here.

12

13 **4.2.** Panicle blast is the symptomatology most correlated with yield losses

14 Many studies investigated the correlation between LB and PB on yield losses (Kingsolver et al., 15 1984). Most of them focus on the relation between AUDPC of the two symptomatologies of the 16 disease and FYL, and provide empirical equations built on experimental data. In our experiment, 17 average AUDPC values mostly varied according to the rice variety, and were in the range 18-1744, 18 corresponding to the two extremes in terms of resistance (Gladio, AUDPC_{LB} and Vialone Nano, 19 AUDPC_{PB}). The highest AUDPC values are in agreement with findings of Mohapatra et al. (2008), 20 who reported 1153 for a cluster of fast blasting varieties in India, and with Puri et al. (2006) who 21 found a maximum value of 1538 for the susceptible Masuli cultivar in Nepal. 22 Our regression analysis indicates that PB is the symptomatology most correlated both with FYL and 23 MYL. The variability explained by the linear models built with AUDPC_{LB} was 38.4% and 33.2% 24 on FYL and MYL, whereas the corresponding percentages explained by AUDPCPB were 78.5% and

25 84.7%. Many studies showed that both LB (Surin et al., 1986) and PB (Chuwa et al., 2015)

1 severities can be used as predictor of FYL, even used in linear regression, as done by Torres (1986) 2 in the Philippines. However, PB is considered as the most destructive symptomatology, and our 3 study confirms these findings. Nevertheless, the strength of the correlations found in our dataset 4 largely varied across the modalities of the four factors, thus limiting the field of application of these 5 empirical relationships to the explored conditions. The development of process-based simulation 6 models to reproduce the impact of LB and PB severity as a function of different agro-pedo-7 meteorological conditions (e.g., Teng et al., 1989; Luo et al., 1997) could then represent a viable 8 mean to deepen our understanding on the complex interactions in the rice blast pathosystem. The 9 experimental data collected in this research match the requirements of the datasets needed for crop 10 and disease modelling (Donatelli et al., 2017), as they characterize the dynamics of the two 11 symptomatologies of the disease, as well as their impact on final yield. These datasets could be used 12 for multiple purposes, e.g., the simulation of the time course of the epidemics as affected by 13 environmental conditions to provide in-season forecasts, the ex-ante assessment of the impacts of 14 climate change on rice blast disease - after model calibration and validation with these datasets - or 15 the refinement of the formalisms used to represent the coupling of crop and disease models to 16 differentiate leaf and panicle blast impacts on yield. Depending on their level of mechanism, the 17 resulting models could be suitable for application in other agro-environmental contexts where blast 18 disease represents a threat for food security (e.g., Kihoro et al., 2013), rather than in Northern Italy, 19 where rice production is mainly devoted to international export and high quality internal 20 consumption.

21

4.3. Blast disease has a large impact both on field yield and on head rice yield

Three out of the four tested agro-pedo-meteorological factors (site, variety and year) proved to be significant in explaining yield losses due to blast disease at the field level. Nitrogen fertilization rates did not impact on FYL, despite their relevance in explaining the LB and PB dynamics.

1 Available estimates of FYL due blast disease largely depend on the Genotype × Environment × 2 Management components taken into account. Even considering only Asian countries, blast related 3 yield losses are largely discordant, as they are reported to vary e.g., from 5-10% (Pasha et al., 2013) 4 up to 50% in upland conditions in India (Widaswky and O'Toole, 1990), in the range 20-100% in 5 Japan (Khush and Jena, 2009) and 50-85% in the Philippines (Nuque et al., 1983). According to our 6 experimental design, average FYL present the highest variability between the tested rice varieties, being on average 6.73% for Gladio variety and 38.21% for Vialone Nano, with all the other 7 8 modalities of the other factors within this range. This is consistent with findings by Koutroubas et 9 al. (2009), who reported FYL up to 33% in a two-year experiment using Italian, Spanish and Greek 10 varieties in a Mediterranean environment. 11 We found a larger impact of blast disease on rice quality, synthesized by head rice yield, than on 12 field yield in all conditions tested. This qualitative attribute is considered as the main determinant of 13 rice market price at global level (Siebenmorgen et al., 2013) and it is known to be largely 14 influenced by the rapid moisture adsorption of kernels around harvest, which could easily break 15 after milling (Banaszek and Siebenmorgen, 1990). In Northern Italy, sale agreements are frequently 16 signed between growers and rice mills. In many of these agreements, selling price is determined 17 based on market price, referring to a minimum head rice milling yield of 60%. If head rice ratio is 18 lower than the stated reference level, selling price drops by 1% for each unitary decrement. Paddy 19 lots with a head rice milling yield lower than 54% can be rejected by the buyer or, if there is a high 20 demand in the market, lead to further negotiations that could greatly reduce selling price. 21 The impact of blast disease on head rice yield is well documented in literature. Candole et al. (2000) 22 reported a significant HRL due to blast disease in the range 7-12% on two rice varieties grown in 23 Arkansas. Koutroubas et al. (2009) in the same experiment cited above found HRL in the range 4-24 11% depending on rice cultivar and growing season. In our experiment, HRL values were larger, 25 and the rice variety was the most important factor explaining their variability.

5. Conclusions

1

2 An effective management of blast disease is required to limit the outbreaks of the epidemics and 3 subsequent economic losses suffered annually by rice growers in Northern Italy. This study 4 confirmed that the varietal choice is one of the most efficient practices to limit the disease 5 development and the impact on the crop. Further, it proves that the year-to-year weather variability 6 has a large impact on LB and PB development, thus highlighting the need of forecasting tools to 7 guide the application of chemicals in conducive years, and to limit their use in unfavorable growing 8 seasons for the disease. A major finding here is the evidence that the impact of blast disease is not 9 limited to the rice production at harvest, but it is even larger when rice is post-processed (after 10 milling), thus implying an additional economic damage to rice growers, who cannot sell broken rice 11 at the same price than normal one. This is even more important in Italy, where about one third of 12 the rice growing area is cultivated with traditional varieties that are highly susceptible to rice blast 13 but are requested by the market because particularly suited for the preparation of Italian typical 14 dishes.

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1 Appendix A. Regression equations using field and milling yield losses as dependent variables

2 and area under disease progress curve of leaf and panicle blast as predictors

3			y = field y	vield losses	y = milling yield losses	
	Factor	Modality	$x = AUDPC_{LB}$	$x = AUDPC_{PB}$	$x = AUDPC_{LB}$	$x = AUDPC_{PB}$
Δ	Nitrogen	N1	y=0.0982x	y=0.0285x	y=0.1222x	y=0.0389x
-		N2	y=0.0422x	y=0.0341x	y=0.0445x	y=0.0391x
_	Variety	Balilla	y=0.2334x	y=0.0293x	y=0.3265x	y=0.0416x
5		Deneb	y=0.1382x	y=0.0363x	y=0.1637x	y=0.0466x
		Gladio	y=0.5726x	y=0.0333x	y=0.8709x	y=0.0482x
6		Vialone	y=0.0396x	y=0.0306x	y=0.0417x	y=0.0327x
	Site	Collobiano	y=0.0899x	y=0.0199x	y=0.1475x	y=0.0330x
7		Garbagna	y=0.0508x	y=0.0418x	y=0.0496x	y=0.0434x
/		Confienza	y=0.0425x	y=0.0314x	y=0.0459x	y=0.0389x
_	Year	2013	y=0.0635x	y=0.0193x	y=0.0686x	y=0.0312x
8		2014	y=0.0413x	y=0.0469x	y=0.0430x	y=0.0495x
		2015	y=0.0939x	y=0.0272x	y=0.1195x	y=0.0350x
9	A	ll data	y=0.0476x	y=0.0321x	y=0.0520x	y=0.0390x
-						