Monitoring races of *Bremia lactucae* in the State of São Paulo from 2003 to 2012

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Abstract

Lettuce is one of the most widely cultivated leafy vegetables in Brazil, which encourages the increase in infestation of production areas by the phytopathogen *Bremia lactucae* Regel, the causative agent of mildew, making it one of the principal crop diseases. In view of the quantity of races adapted to growing regions, the objective of this work was to do a survey of the races of *Bremia lactucae* found in the state of São Paulo between 2003 and 2012. On the basis of the identification system "sextet code" in studies of races in 2003 and 2004 in São Paulo, we identified the first race of *B. lactucae*, designated SPBl:01. In surveys in 2006 and 2007, three new races were found, namely SPBl:02, SPBl:03, and SPBl:04. In 2008 and 2009, we noted the emergence of two more new races, SPBl:05 and SPBl:06. In 2010, 2011 and 2012, the monitoring of races in São Paulo made it possible to identify five more new races: SPBl:07, SPBl:08, SPBl:09, SPBl:10, and SPBl:11. For lines of lettuce to acquire resistance to all races of *B. lactucae* found up to 2012, the resistance factors RF-17, RF-18, and RF-38 should be incorporated in these lines. According to monitoring, we can conclude that, from 2003 to 2012, there have been 11 races of *B. lactucae* that affect the productivity and quality of the lettuce crop in the state of São Paulo.

Keywords: mildew, resistance, differentiating cultivars, lettuce, genetic improvement

INTRODUCTION

Advances in management systems, cultivation treatments, irrigation, spacing, and harvest and post-harvest techniques, together with development of the crop, have boosted farming, making lettuce the most consumed leafy vegetable in the country. Lettuce is planted on approximately 35,000 hectares in Brazil, where the crop is characterized by intensive production and cultivation in small areas and by small family farms (Costa and Sala, 2005).

In the state of São Paulo, the Green Belt region is known as the largest production area, where 28.8155 tons of lettuce were produced between the months of January and July of 2013 and received at CEAGESP (Agrianual, 2014), the main central marketing authority. However, a large volume of lettuce is sold without going through CEAGESP, since the highest volume of lettuce is marketed by direct sales to major supermarket chains. Small areas of this crop are distributed around the principal cities of the state, showing a gradual increase over the years (Castoldi, 2011).

Along with intensification of production, difficulty in growing this vegetable crop has also increased, even under conditions of fall and winter, periods of mild and adequate temperatures for production. This has been mainly due to the infestation of farm areas by the phytopathogen *Bremia lactucae* Regel, the causative agent of downy mildew (Yuri et al., 2004). This is one of the most important and severe diseases of the crop in both conventional and greenhouse systems.

The genus *Bremia* includes oomycetes of the family *Peronosporaceae*; the species *B.*

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lactucae, a biotrophic parasite, inhabits live cells of the host and can thus be cultivated on live lettuce plants (Michelmore and Ingram, 1982). This phytopathogen is currently controlled by a combination of genetic resistance, farming practices, and chemical protection.

Among the methods used in the control of *B. lactucae*, the application of systemic fungicides is done regularly, but the use of these agents can lead to the selection of resistant and difficult to control pathogens. The development of resistant cultivars is an effective and economical control measure, reducing the utilization of chemical products with risks to health and environment, consequently reducing the cost of production (Castoldi, 2011).

Currently on the market, there are commercial lettuce cultivars with resistance to *B. lactucae*, but the emergence of new races, by means of introduction or mutation of novel genes, is capable of breaking the resistance of these commercial cultivars. Therefore, knowledge of races of *B. lactucae* that occur annually in different lettuce-growing regions is of great importance for the development of resistant lines. Accordingly, the objective of this work was to monitor the races of *B. lactucae* present in the state of São Paulo for the years 2003 to 2012.

MATERIALS AND METHODS

During the years 2003 to 2012, between the months of June and October, leaves with sporangia of *B. lactucae* were collected from the main lettuce-producing regions of the state of São Paulo: Ribeirão Preto, Jaboticabal, Pirangi, Catanduva, São Jose do Rio Preto, Atibaia, Salesópolis, Biritiba Mirim, Mogi das Cruzes, Itapira, Mogi Mirim, Cândido Mota, Presidente Prudente, Echaporã, Marília, Botucatu, and Bauru.

The samples were stored in plastic bags identified with the sampling region, name of producer, name of property, and name of cultivar, and placed in isothermal boxes for transport to the Laboratory of Genetics and Breeding of Vegetables, Department of Crop Production (UNESP-FCAV), and each sample was considered an isolate.

After completing all the collections, to obtain sufficient quantities of sporangia for utilization in the identification test, sporangia were propagated in the susceptible cultivar 'Solaris', which is a widely available commercial cultivar that is susceptible to all races of *B. lactucae*, according to Braz et al. (2007).

After obtaining sufficient quantities of sporangia, seeds of the differentiating cultivars of lettuce were sowed separately in plastic boxes (Gerbox, 11×11×3.5 cm), lined with moistened Germitex paper, divided into four equal parts. A total of 40 seeds were sown in each part for each differentiating cultivar.

The differentiating cultivars were divided into four groups, with the susceptible being 'Green Tower,' where values of 1, 2, 4, 8, 16, and 32 were given to each cultivar, within each group, according to the method of Van Ettekoven and Van der Arend (1999), as follows: Group I: 'Lednicky' [1]; 'UCDm-2' [2]; 'Dandie' [4]; 'R4T57D' [8]; 'Valmaine' [16]; 'Sabine' [32]; Group II: 'LSE 57/15' [1]; 'UCDm-10' [2]; 'Capitan' [4]; 'Hilde II' [8]; 'Pennlake' [16]; 'UCDm-14' [32]; Group III: 'PIVT 1309' [1]; 'CG Dm-16' [2]; 'LS-102' [4]; 'Colorado' [8]; 'Ninja' [16]; 'Discovery' [32]; and Group IV: 'Argeles' [1].

After planting the differentiating cultivars, they were maintained for 15 days in a BOD (biochemical oxygen demand)-type incubation chamber at 13°C with a 12-h photoperiod. Afterwards, isolates were collected according to Ilott et al. (1987): sporangia were obtained by washing the infested tissues of the host and suspended in distilled water. The seedlings were inoculated with a suspension of 5×10^4 sporangia mL^{-1}, which was sprayed on the plants to the point of dripping.

After inoculation, the boxes were returned to the BOD-type incubation chamber at 13°C. The chamber was made dark for the first 6 h and, afterwards, the photoperiod was adjusted to 12 h. Monitoring occurred daily and, when the first sporulation appeared in the susceptible cultivar 'Green Tower' (Dm-0), which normally varied from 12 to 15 days, the differentiating cultivars were evaluated individually, determining the presence or absence of sporulation and necrosis, according to the method proposed by Van Ettekoven and Van der Arend (1999). This method is based on assignment of +, (+), - or (-), according to the

percentage of damage to vegetative tissues of lettuce, where + = more than 80% of seedlings showing sporulating lesions, (+) = more than 80% of seedlings showing necrotic points and many sporulating lesions, - = less than 5% of seedlings showing necrotic points, and (-) = seedlings showing necrotic points with few sporulating lesions.

Within the differentiating cultivars that appeared susceptible to a particular isolate, the values within each group (1 to 4) were summed. The sum of each group was separated by a bar, and this numeric sequence, called the sextet code, was compared with numeric sequences already identified. If the code was different from those already identified, the emergence of a new race is proposed.

RESULTS AND DISCUSSION

The results obtained in 2003 and 2004 for the isolates showed the predominance of Sextet code 63/63/51/00, identifying the first race of *B. lactucae*, designated SPBl:01, with the observation that the stable form occurred in all regions. The genes that confer resistance leading to this behavior are RF- (resistance factor) 17, RF-18, and RF-38 (Table 1). They are easily found in commercial cultivars, and their use is recommended in breeding programs. The gene RF-17 was found in genotype LS-102 (*Lactuca serriola*), a wild species (Braz et al., 2007).

In 2006 and 2007, three behaviors of *B. lactucae* were identified in the farming region of the state of São Paulo by the sextet codes 63/31/19/00, 63/63/19/00, and 63/63/03/00, characterizing three new races of *B. lactucae*, SPBl:02, SPBl:03, and SPBl:04, respectively (Table 1). The difference in behavior of *B. lactucae* consisted of resistance conferred by the gene Dm-14 (UCDM-14) of race SPBl:02, with dominant resistance (Souza et al., 2011).

Of the races identified in São Paulo in 2006, SPBl:02, SPBl:03, and SPBl:04, the only the first two also occurred in 2007. The behavior of the *B. lactucae* races SPBl:02, SPBl:03, and SPBl:04 differed from that of the race previously identified as SPBl:01 (Braz et al., 2007) in susceptibility conferred by the gene RF-37 in the sextet code.

The monitoring carried out in 2008 and 2009 identified four behaviors of *B. lactucae* in the main lettuce-growing regions of the state of São Paulo: 63/63/03/00, 63/63/33/00, 63/63/51/00, and 63/63/19/00 (Table 1). It should be pointed out that the sextet code 63/63/03/00 occurred with greater frequency. It was also found that there was a dispersion of this race through the lettuce-growing regions of São Paulo, where, in 2006, it was present only in the region of Bauru; in 2008 it was present in Ribeirão Preto, Bauru, Cândido Mota, Mogi Mirim, Itapira, Mogi das Cruzes, Biritiba Mirim, and Botucatu.

In 2008 and 2009, two more new races were identified, namely SPBl:05 and SPBl:06, with the codes 63/63/33/00 and 63/63/02/00, possessing the resistance genes Dm-15, RF-17, RF-18, RF-36, RF-37, and RF-38 (Table 1) (Castoldi, 2011).

In 2010, six codes of *B. lactucae* were identified in the main lettuce-producing municipalities of the state of São Paulo: 63/63/51/00, 63/31/19/00, 63/63/19/00, 63/63/03/00, 63/63/02/00, and 63/31/03/00 (Table 1). Only 63/31/03/00 was a new race. Accordingly, the new coding found was designated race SPBl:07 (Galatti et al., 2012). The new sextet code (63/31/03/00) does not hamper the resistance genes DM-14, RF-17, RF-18, RF-36, RF-37, and RF-38, differing from races SPBL:01, SPBl:03, SPBl:04, SPBl:05, and SPBl:06 by the resistance genes Dm-14, RF-36, and RF-37.

In 2011, six codes were found: 63/63/51/00/00, 63/31/19/00, 63/63/19/00, 63/63/03/00, 31/63/51/00, and 31/63/19/00. The last two were new races, designated SPBl:08 and SPBl:09, respectively (Table 1). The races SPBl:08 and SPBl:09 differed from all races previously identified, due to resistance conferred by the gene Dm-6 in the sextet codes.

Table 1. Races of *Bremia lactucae* found in the state of São Paulo between 2003 and 2012.

	Cultivar																				
	Green Tower	Lednicky	UCDm-2	Dandie	R4T57D	Valmaine	Sabine	LSE 57/15	UCDm-10	Capitan	Hilde II	Pennlake	UCDm-14	Num DM 15	CG Dm-16	Num DM 17	Colorado	Ninja	Discovery	Argeles	
Dm/RF		Dm	Dm	Dm	Dm	Dm	Dm	Dm	Dm	Dm	Dm	Dm	Dm	Dm	Dm	RF	RF	RF	RF	RF	
Resistance	-	1	2	3	4	5	6	7	10	11	12	13	14	15	16	17	18	36	37	38	
Sextet no.	-	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
Value	-	1	2	4	8	16	32	1	2	4	8	16	32	1	2	4	8	16	32	1	
Group					1						2					3				4	
Isolate	**Resistance response**																				**Sextet code**
SPBI:01	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	63/63/51/00
SPBI:02	(+)	+	+	+	+	(+)	+	+	(+)	(+)	+	+	-	+	+	-	-	+	-	-	63/31/19/00
SPBI:03	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	63/63/19/00
SPBI:04	(+)	+	+	(+)	(+)	+	+	(+)	(+)	(+)	(+)	+	+	+	+	-	-	-	(-)	(-)	63/63/03/00
SPBI:05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	63/63/33/00
SPBI:06	(+)	+	(+)	+	(+)	+	(+)	+	+	+	+	+	+	-	+	-	(-)	-	-	-	63/63/02/00
SPBI:07	+	+	+	+	+	(+)	+	+	+	(+)	+	+	-	+	(+)	-	-	-	(-)	(-)	63/31/03/00
SPBI:08	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+	-	31/63/51/00
SPBI:09	+	+	+	+	+	+	-	+	+	+	+	+	+	(+)	+	-	-	+	-	-	31/63/19/00
SPBI:10	+	+	+	+	+	+	+	(+)	+	+	+	+	(-)	(-)	+	-	(-)	(-)	(-)	(-)	63/31/02/00
SPBI:11	(+)	(+)	(+)	(+)	(+)	(+)	+	(+)	+	(+)	+	+	(+)	(-)	(+)	(-)	(-)	(+)	(-)	(-)	63/63/18/00

RF, Resistance factor.

In 2012, two new codes of *B. lactucae* were determined in growing regions of the state of São Paulo, with the sextet codes 63/31/02/00 and 63/63/18/00, giving rise to two new races of the phytopathogen, SPBl:10 and SPBl:11 (Table 1). The new races SPBl:10 and SPBl:11 did not differ in relation to the resistance genes of the plant when compared with the previously identified races, but did differ between each other in relation to the genes Dm-6, Dm-14, Dm-15, Dm-16, RF-36, and RF-37.

CONCLUSIONS

On the basis of the results obtained, the monitoring of *B. lactucae* in the state of São Paulo led to the detection of 11 races of *B. lactucae* between 2003 and 2012.

For lines of lettuce to exhibit resistance to all races of *B. lactucae* found up to 2012, the resistance factors RF-17, RF-18, and RF-38 should be incorporated into them.

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