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Quantitative studies on downy mildew (*Peronospora destructor* Berk. Casp.) affecting onion seed production in southern Uruguay

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Abstract Onion downy mildew (Dm) symptoms and damage on seed production fields in southern Uruguay were quantified during two seasons as the progress of incidence, severity, and as the effect of the level of seed-stalks infections on seed yield and quality. In addition, the effects of two plantation dates and two plant densities on Dm were studied in a factorial experiment. Maximum incidence along the season ranged from 15 to 65% in four commercial fields in 2005 and two fields in 2006. Maximum severity ranged from 4.5 to 9.3% of leaf area affected in 2005, and 0.35 to 1.17% in 2006. Whereas Dm incidence varied among studied fields, disease progress as Dm severity varied mainly between studied years. Crop rotation, crop vigour and plant density were identified as major factors affecting Dm variation in the field. Intensive fungicide schedules did not control Dm when other conditions favoured the disease. A high level of Dm severity defined as large necklace spots on seed-stalks significantly reduced seed yield in comparison with healthy seed-stalks in 2005, as well as seed yield and germination in 2006. Weight of 1,000 seeds was not significantly reduced by Dm infections on seed-stalks. Six genera of fungi were recovered from seeds harvested on highly infected seed-stalks (*Stemphylium, Fusarium*, *Alternaria*, *Penicillium*, *Aspergillus* and *Botrytis*), but not *Peronospora*. Late planting date and low plant density had significantly lower Dm incidence and severity. This finding questioned the early planting dates and high densities previously recommended in order to achieve high yielding crops. The combination of several practices to reduce initial inoculum, susceptibility status of the host, and environmental conditions promoting the disease is discussed as forming a basis for effective disease control.

Keywords *Allium cepa* · Epidemiology · Integrated disease management · Seed-borne pathogens

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Introduction

Onion (*Allium cepa* L.) is one of the main vegetable crops in farming systems in southern Uruguay, with an annual acreage in the order of 2,000 ha. Production of onion seeds in small households has been a traditional practice that led to the development and maintenance of germplasm adapted to local conditions (Galván et al. 1997, 2005). Although seed production is a widespread activity, on-farm produced seed lots have dissimilar physiological and sanitary qualities (Cardani et al. 1990). Recently, onion breeding activities in Uruguay released cultivars (Vilaró et al. 2005) which currently hold more than 50% of the cultivated onion area (MGAP 2006). The uptake of these cultivars depended on their availability in the required quantity and quality. Consequently, there was the development of a seed certification programme involving seed growers (González et al. 2007).

Downy mildew (Dm) caused by *Peronospora destructor* Berk. (Casp.) is a major leaf disease for the production of onion bulbs, as well as for the production of onion seeds in the second year of this biennial species (Schwartz and Mohan 2008). Yield reductions of onion bulbs due to Dm outbreaks may range from 30% up to 70% when the environment is conducive for the disease (Van Doorn 1959; Maude 1990). Yields are strongly reduced by *P. destructor* during seed production, where 30–97% losses have been reported in Brazil (Verona et al. 1996) and 60–70% in India (Sharma et al. 2002). The spraying with (not always effective) chemicals adds to the economic losses. Forecast systems like 'Downcast' (Sutton 1986) reduce the number of chemical interventions during the season (Lorbeer et al. 2002), although not significantly in Uruguay because environmental conditions for Dm sporulation and infection frequently occur (Maeso 2005). A high level of host resistance does not occur within *A. cepa* (Kofoet and Zinkernagel 1989). Introgression of Dm resistance from *A. roylei* was recently achieved, and the development of onion resistant cultivars is continuing (Scholten et al. 2007). However, use of this resistance in onion cultivars adapted to diverse regions is a long term process. Knowledge on cultural practices influencing Dm leading to an integrated disease management is still important.

Typical symptoms of *P. destructor* are pale green to yellowish areas on leaves and seed-stalks, ovate to cylindrical in shape, which become covered by masses of gray fruiting bodies under appropriate environmental conditions (Schwartz and Mohan 2008). *P. destructor* may have high sporulation rates capable of destroying crop foliage quickly. As infected leaf areas turn necrotic and leaf tips collapse, plant growth and crop yields are negatively affected (Maude 1990). Loss of foliage results in thick onion necks and reduction in the keeping quality of onion bulbs (Schwartz and Mohan 2008). Stored bulbs can remain infected, and act as a primary source of inoculum for seed production (Virányi 1974). Dm is especially harmful for seed production because the supply of water and nutrients to the umbel can be interrupted when seed-stalks are infected, which may weaken and break down (Schwartz and Mohan 2008). Moreover, seeds may be infected by *P. destructor*, acting as primary inoculum for the next season (Rondomanski 1971).

Peronospora destructor occurs all over the world but is important in temperate regions (CMI 1990). Nevertheless, it requires very specific environmental conditions and therefore the timing and relevance of Dm outbreaks in a location may vary as a consequence of weather differences between years and crop management practices influencing micro-environment. Sporulation of Dm requires relative humidity above 95% or dew, temperatures from 4–7°C up to 22–25°C and darkness, though a previous period of intense light promotes sporulation (Hildebrand and Sutton 1982). As a consequence, sporulation occurs during the night when optimal conditions prevail for at least 6 h. Rainfall prevents sporulation. Release of sporangia takes place during the day when relative humidity decreases (Leach et al. 1982). New infections occur with a film of water present on the leaves for at least two to 6 h and temperature between 3 and 14°C. Latency period up to the development of new sporulation lasts from 9 to 16 days (Maude 1990; Schwartz and Mohan 2008).

The effects of onion downy mildew on yields and quality of onion seed in Uruguay have not been evaluated previously, although the presence of seed-stalk diseases and the lack of epidemiological information are major constraints in seed production. Plant diseases add complexity to crop management and uncertainty about the level of production. This research aims to quantify symptoms and damage caused by *P. destructor* in onion seed production in southern Uruguay. By surveying commercial fields across two seasons, Dm incidence and severity were evaluated, as well as the effect of seed-stalk infection levels on yield and quality of onion seeds. In addition, an experiment was set up to study the effects of two planting dates and two plant densities on the development of the disease. Factors influencing Dm differences between commercial farm fields were analyzed.

Materials and methods

Disease incidence and severity

The prevalence of downy mildew was assessed by monitoring commercial onion seed producing fields in the seasons 2005 and 2006 in Canelones, Uruguay (Table 1). Cultivar 'Pantanoso del Sauce CRS' was grown in all cases. Planting date was between August 1st and 12th, onset of anthesis took place in the second half of November, and harvesting in the first half of January. In the studied fields, weeds were controlled by herbicides and manual clearing, and did not influence the development of diseases, nor seed yields. Production as yield per plant, yield per hectare and seed germination are presented in Table 1. Farmers used similar combinations of fungicides and 7 to 10 days spraying schedule during October to December, except for DP in 2005 and JL in 2006, who sprayed fewer times after monitoring a low presence of Dm (Table 1).

Disease incidence was quantified as the percentage of plants with Dm symptoms, and disease severity was estimated as the percentage of leaf area infected per plant. Only Dm typical pale green to yellowish spots, or leaf areas covered by typical Dm sporulation were assessed for incidence and severity. Necrotic areas caused by old infections on leaf areas were disregarded, with only Dm active infections considered. Atypical symptoms of Dm were assessed on leaves and seed stalks by removing samples from the field and placing in a humid chamber before observation using a binocular microscope.

In 2005, Dm incidence was evaluated in four commercial fields that differed in crop rotation, plant density, use of fertilizers and fungicides (Table 1). The observed level of Dm disease in the previous season as perceived by farmers also varied and was recorded, although the piece of land within the farm exploited for onion seed production differed year to year. Each crop was divided into four quadrant sections, and five plants were randomly chosen within each section. Plants were assessed every 2 weeks from October 18th till December 27th (six times). Only symptoms developed on leaves were assessed for incidence and severity.

In 2006, Dm incidence and severity were evaluated in two fields selected for their contrasting prevalence of Dm disease in the farm previously(Table 1). One percent of the plants were randomly chosen within each quadrant (five and eight plants per quadrant per farm). The plants were assessed weekly from the 4th October to 22nd November 2006 (nine times). Dm severity on leaves and severity on seed-stalks were estimated separately.

Effects of seed-stalk infections on seed yield and quality

The effects of the level of Dm infections on seed-stalks were investigated by sampling inflorescences in commercial fields. A field with intermediate to high infestation was selected in the season 2005, and another in 2006 (Table 2), which permitted the differentiation of three infection levels on seed-stalks, defined as follows: (0) healthy stems; (1) 25–50% of infected tissue; and (2) 25–50% of infected tissue, with necklace spots extending over the circumference of the stem. Plants corresponding to each infection level were randomly selected and marked at the end of anthesis (early December). Six replications by infection level were set, each consisting of three stems (18 stems in total). In the season 2005, the sanitary status of seed-stalks was evaluated again at harvest (January 12th 2006), as well as the diameter of the umbels. Seed yield per plant and yield per hectare were calculated (with 4.5 stems per plant in 2005 and 4.0 in 2006). Seed quality was evaluated following ISTA protocols (1976) as percentage of seed germination at 12 days from the beginning of the test, seed vigour as percentage of germinated seedling at 6 days, and weight of 1,000 seeds. Genera of fungi that developed on the seed surface during germination tests were identified and quantified.

Effect of planting date and plant density

An experiment aiming to evaluate the effects of two planting dates and two plant densities on Dm incidence and severity was set at the Centro Regional Sur (Progreso, Canelones). Onion bulbs cv. 'Pantanoso del Sauce CRS' were planted on July 27th and August 29th 2005 at two plant densities (4.45 and 8.90 plants$\cdot$m^{-1}), following a factorial split-plot design with four replications. Planting dates were the large experimental plots (6.0×3.2 m), and plant densities were assessed as small plots (3.0×3.2 m) within planting dates. Four

Table 1 Description and productive results of onion seed producing fields in southern Uruguay surveyed for downy mildew in 2005 and 2006

Characteristics	Season 2005				Season 2006	
	JL	CM	JM	DP	JL	NZ
Description						
Location	Cuchilla de Sierra	Cañada Grande	Cañada Grande	San Jacinto	Cuchilla de Sierra	Paso Garúa
Field size (m^2)	500	600	1032	3394	620	1700
Plant density (m^{-2})	4.00	2.80	1.55	2.80	4.00	3.20
Plant distribution	Simple rows 0.25 m between plants	Double rows 0.45 m between plants	Simple rows 0.40 m between plants	Double rows 0.45 m between plants	Simple rows 0.25 m between plants	Simple rows 0.25 m between plants
Manures and Fertilizers	Chicken manure and Superphosphate	Ammonium phosphate	Ammonium phosphate	No fertilizers	Chicken manure and Superphosphate	NPK 15-15-15 and Urea
Fungicides sprayed	Copper oxichloride, mancozeb, Propamocarb metil, metalaxil	Copper oxichloride, mancozeb, Propamocarb metil, metalaxil	Copper oxichloride, mancozeb, Propamocarb metil, metalaxil	Mancozeb +Metalaxil	Copper oxichloride, mancozeb, Propamocarb metil, metalaxil	Copper oxichloride, mancozeb, Metalaxil
Nr. of spraying times and period	6 Oct–Dec	6 Oct–Dec	6 Oct–Dec	1 Nov	2 Oct–Dec	6 Oct–Dec
Previous crop	Onion	Sweet potato	Maize	Tomato	Sweet pepper	Potato
Downy mildew in the previous season	Low	High	No detected	Low	High	Low
Productive results						
Yield per plant (g)	26	18	18	19	18.1	18.6
Yield (kg ha^{-1})	1040	504	279	532	744	579
Germination (%)[a]	96	93	86	89	93	92

[a] Test performed after seed cleaning

Table 2 Description of onion seed producing-fields in which the effects of the level of downy mildew infection on seed-stalks were investigated

Characteristics	Season 2005–06 AR	Season 2006–07 MR
Location	Canelón Chico, Canelones	La Paloma, Canelones
Field size (m^2)	495	1650
Plantation date	July 25–30	August 4
Plant distribution	Double rows 0.3 m between plants	Double rows 0.6 m between plants
Plant density (m^{-2})	3.85	2.18
Fertilization	Chicken manure 10 Mg ha^{-1}	Ammonium phosphate 200 kg ha^{-1}
Fungicides sprayed	Copper oxichloride, mancozeb +metalaxil	Copper oxichloride, mancozeb +metalaxil
Nr. of spraying times and period	3 Oct–Nov	4 Oct–Nov
Previous crops	Potato	Fallow
Downy mildew in the previous season	No detected	High
Productive results		
Yield per plant (g)	8.33	5.83
Yield (kg ha^{-1})	320	127
Germination (%)[a]	87	66

[a] Test performed after seed cleaning

plants per plot were assessed weekly for Dm incidence and severity (16 plants per treatment-combination). The effects of these treatment-combinations on seed yield, seed germination, seed vigour, weight of 1,000 seeds, and the presence of fungal infections on seeds were also assessed.

Data analysis

For the survey of commercial fields, standard deviations for Dm incidence means *(p)* were calculated using the four quadrants as replicates (n) and normal approximation for a binomial distribution, as: $SD = \sqrt{(p) \times (1-p)/n}$ (Box et al. 1978). Pearson correlation and Spearman rank correlation between Dm variables and onion seed yield in commercial fields were performed. For the experiment designed to assess two plantation dates and two plant densities, Dm incidence and severity were analyzed for each evaluation time as variables with respectively binomial and multinomial distributions, using residual maximum likelihood analysis in Genstat Discovery Edition 7.2.0.220 (VSN International Ltd., Lawes Agricultural Trust, UK). Seed yield and seed quality traits were studied by analysis of variance, and significant differences were established with Fischer's-protected test (LSD). Before analysis, seed germination, seed vigour and the presence of rotten seeds expressed as percentages were transformed by natural logarithm.

Results

Disease incidence and severity

In 2005, Dm incidence in the four studied fields increased until November and decreased in December (Fig. 1). Maximum incidences of 50 and 65% in two farm fields (JL, JM) contrasted with 15 and 20% reached in the other two fields (CM, DP). Dm severity increased from October 18th until November 29th. Evaluations of severity during December were disregarded because necrosis caused by natural leaf senescence and by *Stemphylium vesicarium* and *Alternaria porri* infections become predominant. Maximum Dm severity ranged from 4.45 up to 9.25% of leaf area affected in the four fields. The farm field JM with maximum Dm incidence also had maximum severity. The farm field CM had disease distribution in patches, and consequently standard deviation values were larger than other fields and as large as severity mean values (data not shown).

In 2006, maximum Dm incidence for two studied fields was 30 and 45%, and severity was respectively 0.35 and 1.17%. Whereas disease progress in the former field was erratic, in the latter field disease kept increasing until November 29th, and in both cases decreased in December (Fig. 1). Dm symptoms on seed-stalks were observed less frequently than symptoms on leaves (data not shown).

The range of variation between fields for Dm incidence was similar between years, but Dm severity was higher in 2005 than that observed in 2006. Maximum Dm incidence, maximum Dm severity, and cumulative Dm severity in the surveyed fields were not (negatively) associated with seed yields per plant, yield per hectare, nor with seed germination (correlation tests not shown).

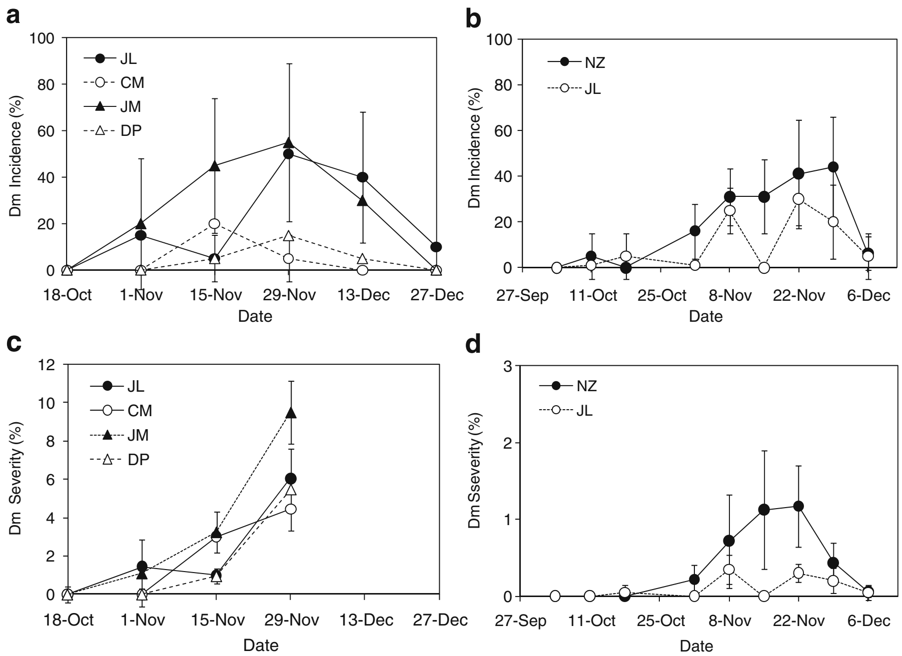

Fig. 1 Downy mildew evaluated in commercial onion seed producing fields along the season. Only typical symptoms caused by new infections and leaf areas covered by sporulation were accounted for. (**a**) Incidence and (**c**) severity in four fields surveyed in 2005. For simplicity, standard deviations were drawn only for the lowest and highest incidence means within each evaluation time, and JM and DP severity means. (**b**) Incidence and (**d**) severity in two fields surveyed in 2006

Effects of downy mildew on seed yields and quality

In 2005, onion seed yield obtained with severity levels 2 (25–50% of the seed-stalk infected with necklace spots) and 1 (25–50% of the seed-stalk infected without necklace spots) were lower than yields obtained from healthy stems ($p<0.05$) (Fig. 2). At harvest, seed-stalk area affected by Dm was estimated as 51, 44 and 0% for respectively infection levels 2, 1 and 0. Among stalks marked as infection level 1 at the end of anthesis, 56% of the stalks presented necklace spots at harvest due to secondary infections caused by *Stemphylium vesicarium* or *Alternaria porri* on lesions initially caused by *P. destructor*. Average diameters of umbels were 6.9, 7.2 and 7.3 cm for infection levels 2, 1 and 0 respectively, which were not significantly different.

In 2006, seed yields in the studied field were approximately one quarter of the yields obtained in 2005. Under these conditions, seed yield of seed-stalks with severity level 2 was significantly lower than yields obtained with infection levels 1 and 0 (Fig. 2).

Seed germination, vigour and weight of 1,000 seeds were not significantly reduced by the level of Dm infection on the seed-stalk in the season 2005 (Table 3). The effects of Dm infection levels on seed quality were more severe in 2006. For instance, germination means ranged from 76 to 71% in 2005 and from 55 to 42% in 2006 for the different infection levels. Germination tests produced sub-normal growth of seedling radicles and/or epicotyls, as well as the presence of other pathogens (rotten seeds). In the season 2006, seed germination for seed-stalks with infection level 2 was lower than levels 1 and 0, whereas seed vigour was lower for infection level 1 in comparison with levels 0 and 2 ($p<0.05$). Weight of 1,000 seeds was not significantly affected by Dm infection levels on seed-stalks (Table 3).

Six genera of fungi were identified from infected seeds on blotters prepared to evaluate seed germination (Table 4). Fungal infections increased from 8% for seeds produced on healthy stems to 23.67% for seeds collected on seed-stalks with the higher Dm infection level. *Stemphylium* and particularly its perfect state *Pleospora* (perithecia observed on seed surface and radicle) was the most frequently observed genus. *Fusarium*, *Alternaria*, *Penicillium*, *Aspergillus* and *Botrytis* were found at lower frequencies. No *Peronospora destructor* infection was detected following this methodology.

Effects of planting date and plant density

Overall, early planting date (July 27th) originated higher Dm incidence and severity than late planting

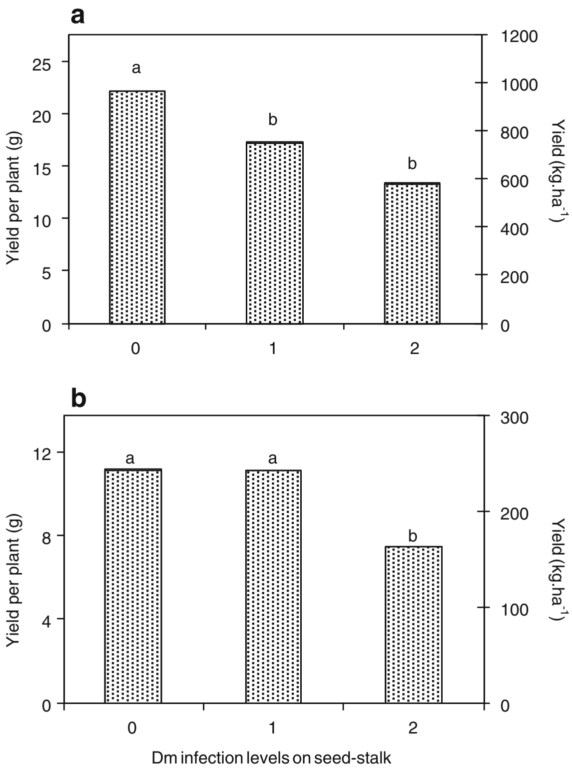

Fig. 2 Onion seeds yields per plant and per hectare for three levels of downy mildew infections on onion seed-stalks, defined as (0) stems without symptoms, (1) 25–50% of infected tissue, and (2) 25–50% of infected tissue with necklace spots extended over the circumference of the stem, in the seasons (**a**) 2005 and (**b**) 2006. Bars with the same letter on top do not differ (Fischer LSD test, $p<0.05$)

date (August 29th), but the effects of plant density were less relevant (Fig. 3). Dm incidence (active symptoms) for early planting date treatments were detected from September 20th, and increased until the end of October, before decreasing during the second half of November. A counterintuitive result was that Dm incidence increased earlier for the less dense cultivation and reached maximum incidence 1 week earlier. Nevertheless, severity was higher for the dense cultivation from October 25th until November 8th, with significant interaction between planting date and plant density within this three-week period (Fig. 3). At the end of the season (December), estimations of severity were disregarded because of high infestation of *Alternaria porri* and *Stempylium vesicarium,* and natural senescence of the leaves.

The combination of later planting date and lower plant density led to no Dm symptoms. In addition, development of *Alternaria porri* and *Stempylium vesicarium* infections at the end of the season were lesser in this treatment combination in comparison with the other three treatment combinations.

For early planting date, a seed yield of 20.4 g plant^{-1} for the less dense cultivation was higher than the yield obtained with the dense cultivation (14.8 g plant^{-1}) (Table 5). These yields were significantly higher than yields obtained for later planting dates (9.72 and 9.10 g plant^{-1} respectively for low and high density, without significant difference between them). Early planting and denser cultivation produced higher seed yield per hectare, without interaction between

Table 3 Effects of downy mildew infection levels on seed-stalks on germination, vigour and weight of 1000 onion seeds in the seasons 2005 and 2006

Infection levels[a]	Season 2005			Season 2006		
	Germination[b] (%)	Vigour[c] (%)	Weight of 1000 seeds (g)	Germination (%)	Vigour (%)	Weight of 1000 seeds (g)
0	76.4ns	26.6ns	4.33ns	55.2a	25.6a	3.25ns
1	72.0	23.0	4.21	51.8a	19.4b	3.13
2	70.7	21.2	4.02	41.6b	25.3a	2.73
C.V.%	4.4[d]	11.2[d]	7.4	4.8[d]	13.3[d]	14.4

[a] (0) stems without symptoms; (1) 25–50% of infected tissue; and (2) 25–50% of infected tissue, with necklace spots extended over the circumference of the stem

[b] Normal germinated seedlings 12 days after the beginning of the test, following ISTA rules (1976). Test performed before seed cleaning

[c] Normal germinated seedlings 6 days after the beginning of the test

[d] Data were transformed by natural logarithm before analysis of variance

Table 4 Fungi infecting onion seeds for the different downy mildew infection levels on seed-stalks in the seasons 2005 and 2006

Identified genera	Season 2005 Infection levels on seed-stalk[a]		Season 2006 Infection levels on seed-stalk[a]		
	1	2	0	1	2
Stemphylium sp.[b, c]	21.9a	22.2a	4.33a	15.33b	17.00b
Fusarium sp.	2.9	5.4	1.67	1.67	4.00
Alternaria sp.	2.4	1.7	0.67	1.00	1.33
Penicillium sp.	3.4	5.4	0.67	0.33	1.00
Aspergillus sp.	–	–	0.67	0	0
Botrytis sp.	–	–	0	0	0.33
Others	5.5	4.4	–	–	–
Total[b]	22.8a	24.7a	8.00a	18.33b	23.67b

[a] (0) Stems without symptoms, (1) 25–50% of infected tissue, (2) 25–50% of infected tissue with necklace spots extended over the circumference of the stem. Infection level 0 was only evaluated in the season 2006

[b] Within each season and row, means followed by the same letter do not differ ($p<0.05$)

[c] *Stemphylium* and its perfect state *Pleospora* sp

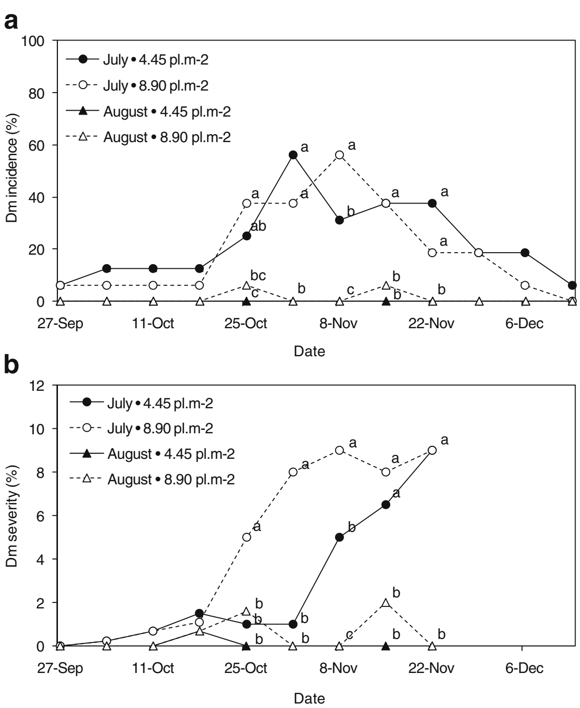

Fig. 3 (**a**) Mean incidence and (**b**) severity of downy mildew along the season in four treatment-combinations of planting date and plant density in onion seed production. Only typical symptoms caused by new infections and areas covered by sporulation were accounted for. Within each evaluation time, means labelled with the same letter do not differ (REML analysis, $p<0.05$)

these two factors. Conversely, weight of 1,000 seeds, seed germination and seed vigour were significantly larger for the late planting date in comparison with values obtained for the early planting date (Table 5). The presence of rotten seeds on blotter tests increased with the combined effect of early planting date and low plant density.

Discussion

Uruguay has 1,100 to 1,300 l m^{-2} as an average annual rainfall, that contributes to create a favourable environment for onion Dm caused by *Peronospora destructor*. Several seed companies from Europe and United States of America have established onion seed production abroad in search of dry climates. When this strategy is not possible, Dm may be particularly harmful in the production of onion seeds. This research carried out a case study on commercial fields in southern Uruguay, as knowledge on the pathogen, farming history and the effects of diverse factors within the agro-ecosystem are critical elements in designing effective disease management (Lorbeer et al. 2002).

Downy mildew was found as a significant disease for onion seed production, and symptoms developed mainly in the period October–November when plants had maximum vegetative development and seed-stalks evolved from onset until full anthesis. Nevertheless, Dm

Table 5 Seed yield per plant and seed quality traits as a function of planting date and plant density

Planting date	Plant density (m^{-2})	Seed yield (g $plant^{-1}$)[a]	Germination (%)[b]	Vigour (%)[c]	Rotten seeds (%)	Weight of 1000 seeds (g)
July 27	4.45	20.4a	77.25b	17.5b	6.35c	4.433b
	8.90	14.8b	80.25b	17.8b	4.83b	4.585b
August 29	4.45	9.7c	82.00a	24.0a	4.53b	4.882a
	8.90	9.1c	83.75a	25.8a	0.50a	4.800a
C.V.%		13.7	1.6	17.8[d]	14.2[d]	3.0

[a] Within each column, means followed by the same letter do not differ significantly

[b] Normal germinated seedlings 12 days after the beginning of the test, following ISTA rules (1976). Test performed before seed cleaning

[c] Normal germinated seedlings 6 days after the beginning of the test

[d] Data were transformed by natural logarithm before analysis of variance

incidence and/or severity differed between studied years and farm fields (Fig. 1). These differences could be caused by differences in environmental and micro-environmental circumstances. Either for infection, for the progress of the disease and sporulation, *P. destructor* requires the occurrence of specific conditions of relative humidity, temperature and darkness for a minimum period of time (Hilderbrand and Sutton 1982; Gilles et al. 2004). Differences between years in the fulfilment of these particular combinations of environmental factors probably gave rise to the observed variation, particularly for Dm severity. The number of days per year in which these conditions are achieved would cause variation in timing and severity of Dm outbreaks. A distinct result was observed for Dm incidence, as the range among fields was similar for the studied years. General conditions required for infection of *P. destructor* seemed to be more regularly achieved, but affected by factors at field level.

Downy mildew incidence and severity differed among farm fields within the same year (Fig. 1). Rudolph (1990) reported 15% Dm incidence on seed stalks as average among surveyed fields in Germany, with a range between 0 and 82%. This range is wider than the one found in the present research for Dm incidence scored on leaves. The fields JL and DP contrasted for their respectively high and low levels of disease in 2005, either in incidence or severity. This result was in line with the potential difference in initial inoculum (Table 1): JL had a previous onion crop, however, DP was a piece of land without an onion crop history. Plant debris in the soil may act as reservoir of *P. destructor* oospores, as well as onion bulbs used as planting material for seed production which could be infected in the previous season. Therefore, effective disease control of the crop in which bulbs are produced (Palti 1989), and long rotation with non-host crops would contribute to reduce Dm outbreaks during seed production. Elimination of volunteer onion plants is another proposed action to reduce Dm (Palti 1989).

Initial inoculum may explain the difference between JL and DP, but does not add to an understanding of the differences between the other two farm fields surveyed in 2005, nor between the fields surveyed in 2006 (Fig. 1). The observed relative levels of Dm incidence and severity certainly were not related with the background of disease in the farm, as subjectively reported by the farmers. Plant density, an agronomic practice that changes air circulation and relative humidity within the canopy, tended to be positively associated with maximum Dm incidence (r^2=0.59, n=6, n.s.). However, plant density does not explain a high Dm incidence for JM with 1.55 plants m^{-2}, and low incidence for CM with 2.80 plants m^{-2} (Table 1). Nor did the number of chemical interventions explain the differences between fields, as a more frequent spraying schedule was applied by those farmers having significant Dm outbreak. Fungicides did not eradicate Dm when other factors were conducive to the disease, but probably delayed disease development during the season in comparison with untreated fields. Many factors may contribute to explaining variation in Dm outbreaks among fields, such as micro-environmental conditions differing from farm to farm (topography, neighbouring forests, etc.), differences in initial inoculum, chemical interventions, and differences in agronomic practices (Rudolph 1990). For instance, Sharma et al. (2002) observed that higher density of weeds increased the presence of Dm. A single factor could not explain differences between fields in incidence or severity, but the combination of several factors determines Dm disease development.

For some of the studied cases (e.g., farm fields in 2006), an initial small maximum peak value for incidence was observed early in the season (October) before the main maximum disease outbreak that usually took place in November (Fig. 1). The occurrence of an early small incidence peak was also observed in the screening of onion germplasm to Dm (Colnago 2009), and may represent the polycyclic behaviour of the disease. In such a way, initial Dm infections probably occur during winter or early spring, and two main cycles of sporulation may be completed during spring in southern Uruguay. Using local onion germplasm and a local *P. destructor* isolate for inoculations in a growing chamber, latency period lasted 20 to 22 days (Colnago 2009). This time-span corresponds with epidemiological observations carried out in commercial fields in this research, and is longer than the period reported by Hildebrand and Sutton (1982). Monitoring crops for early detection of Dm symptoms and/or modelling early sporulation and infection phases would be additional relevant elements in disease management, in order to reduce maximum incidence and severity afterwards during the season (Lorbeer et al. 2002).

Seed yield is affected by agronomic practices and environmental conditions that determine plant growth, leaf development, differentiation of primary and secondary inflorescences, and the number of flowers per inflorescence finally developed. Among these conditions affecting seed yield, Dm reduces active leaf area and plant growth, as well as weakening seed-stalks causing yield losses. In contrast with this theoretical concept, yields obtained in the surveyed farm fields were not (negatively) related with Dm incidence or severity during the season. This lack of relationship would be the consequence of the complexity of factors involved in yield formation, and the measures applied by farmers to stop and overcome the potential negative effects of Dm disease.

Nevertheless, when a disease outbreak was out of control and numerous Dm infections developed on seed-stalks, seed yield and quality were negatively affected (Fig. 2). That was the case for the fields where the effects of the level of seed-stalk infections were studied (Table 2). Seed-stalks with necklace infections yielded 40% and 33% less seeds than healthy seed-stalks respectively in 2005 and 2006. These yield losses for severely infected seed-stalks are lower than those reported in Brazil (Verona et al. 1996) and India (Sharma et al. 2002) for seed producing fields. Furthermore, observed yield reductions cannot be strictly assigned to Dm infections, as apparently these Dm infections assist the occurrence of *Stemphylium* and *Alternaria* diseases late in the season. As a result, even in those farm fields where disease control was not effective at all, Dm in Uruguay seemed not to reach the aggressiveness and effects reported in other regions, probably because of differences in environmental conditions between locations.

Seed quality was also negatively affected by seed-stalk infections (Table 3). Seed germination tended to decrease, though only significantly in 2006, and the presence of diverse potentially pathogenic fungi on seed surface increased, particularly *Stemphylium* and its perfect state *Pleospora* (Table 4). Previous studies of commercial onion seed lots also detected *Alternaria, Aspergillus, Fusarium* and *Stemphylium* on seed surfaces (Cardani et al. 1990; Verona et al. 1996; Sharma et al. 2002), *Penicillium* (Cardani et al. 1990), and *Botrytis squamosa* (Ellerbrock and Lorbeer 1977), although *Aspergillus niger*, *Botrytis aclada* and *Fusarium oxysporum* f.sp. *cepae* were outlined as prevalent species (Özer and Köycü 2004). Seed cleaning aiming to remove the share of seeds with lower weight and seed treatments with fungicides can improve seed germination values when these are below acceptable standards (Cardani et al. 1990; Sharma et al. 2002; Özer and Köycü 2004). In addition, regarding the influence of seed-stalk infection on seed quality (Table 3), rejection of inflorescences from seed-stalks severely infected during hand-harvesting would be another action to improve seed quality.

Even when *P. destructor* was reported as a seed-borne pathogen (Rondomanski 1971), the importance of this phase in initiating the disease is not well documented. As Dm infections occur only on adult plants, when leaves have more than 20 cm in length (Maeso 2005), disease development occurs only late in the season. It is not straightforward to recognize Dm infections at seed or seedling stages. During the production of onion bulbs, the presence of dwarf plants with curved leaves and fully covered by Dm sporulation, also observed in Uruguay, allows speculation about systemic Dm infections from the beginning of the season (Virányi 1981; Maeso 2005). Recent and ongoing development of immunoassay-based detection of *P. destructor* (Kennedy and Wakeham 2008) and molecular tests as developed for other *Peronosporales* (Scott et al. 2004; Tsay et al. 2006) will contribute to elucidate the role of seed transmission in the dynamics of this disease.

Infections of *P. destructor* on seed-stalks helped ulterior infections by the *Stemphylium–Alternaria* complex. Indeed, healthy stems (infection level 0) remained as such at the end of the season, whereas half of the stems with infection level 1 moved to infection level 2 (necklace spots) caused by *Stemphylium* and/or *Alternaria* infections that developed on the lesions caused by *P. destructor*. Such interaction was also observed in the experiment in which planting dates and plant densities were studied. Early planting date treatments that were more affected by *P. destructor* developed later on *Stemphylium* and *Alternaria* lesions that increased general disease severity up to 100% of leaf surfaces. In contrast, the treatment combining late planting date and low plant density did not have Dm nor *Stemphylium–Alternaria* infections. The latter ones are weak opportunistic pathogens that, although they can also infect intact tissues, usually infect plant tissues through wounds caused by *Thrips tabaci* and necrotic spots caused by other diseases or abiotic factors (Maude 1990; Schwartz and Mohan 2008). Further research is needed to understand interactions between pathogens and their effects, a poorly studied topic of disease dynamics.

Planting in June–July was previously recommended in order to obtain maximum onion seed yields in Uruguay (Arboleya et al. 1987). Early planting date permits larger leaf development and adequate vernalization before emission of seed-stalks. However, a trade-off between seed yield and the development of Dm occurs, in such a way that early planting date is associated with larger development of Dm and other leaf diseases, as well as an increase in the presence of seed-borne pathogens (Table 5). This trade-off is the consequence of larger plants increasing relative humidity within the canopy, and holding larger proportions of older leaves that are more susceptible to Dm infections. Planting date and leaf age associated susceptibility is also observed in the production of onion bulbs (Maeso 2005).

Early August was already chosen in commercial seed production in Uruguay as a planting date that combines profitable yield and moderate conditions for development of Dm. Although results from a single experiment, planting at the end of August as studied in this research seemed to produce markedly lower yields regardless of the elevated sanitary status of the crops and the obtained seeds, and can still be an attractive alternative for the production of organic seeds. Plant density was found as another factor influencing seed yield and Dm development because ventilation within leaf canopy is modified, as well as the extent of the period in which conditions for infection and/or sporulation are fulfilled. Nevertheless, plant density effects were less evident than those observed for planting date.

Adaptation of Dm forecast systems applied in bulb production for the seed production phase would be a valuable element in disease management and spraying decisions. However, this cannot be the single element. An effective control of Dm in onion seed producing fields would be the result of the combination of several practices into an integrated disease management. These practices should be directed to either reduce initial inoculum, susceptibility status of the host, and environmental conditions promoting disease development.

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