Research Article

Received: 26 December 2017 Revised: 9 April 2018 Accepted article published: 24 April 2018 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.9101

# Incidence of bacterial diseases associated with irrigation methods on onions (*Allium cepa*)

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## Abstract

**BACKGROUND: In the last decade, diseases of bacterial origin in onions have increased and this has led to significant losses in production. These diseases are currently observed in both the Old and New Worlds. The present study aimed to evaluate whether the irrigation method influences the incidence of diseases of bacterial origin.**

**RESULTS: In cases where the inoculum was natural, the initial incidence of soft bacterial rot did not manifest in any treatment in the first year, whereas, at the end of the conservation period, all treatments had increased incidences of infection. Sprinkler irrigation (8%) was statistically differentiated from the other treatments, for which the final incidence was similar (4.5%). For all irrigation treatments, the final incidence of bacterial soft rot decreased or remained stable towards the end of the cycle, with the exception of sprinkler irrigation in 2015, which increased.**

**CONCLUSION: From the results of the present study, it can be inferred that the irrigation method does have an influence on the incidence of diseases of bacterial origin in the post-harvest stage for onions.**


**Keywords:** *Allimun cepa*; drip irrigation; plasticulture; onions; bacterial diseases

## INTRODUCTION

Onion is an important vegetable crop whose distinctive flavor is appreciated throughout the world. The distinctive taste of onion (*Allium cepa L.*) has made it a common ingredient in world cuisine. The plant has been cultivated for more than 4000 years[1] and is currently produced in 139 countries.[2]

Bacterial blight of onion is a severe disease that has emerged over the past decade in several onion-producing areas. This disease is currently observed in both the Old and New Worlds.[3]

The main vegetable cultivated in the Lower Valley of the Rio Negro (VIRN, Argentina) is onion. Some 2000 ha[4] of Valcatorce INTA cultivars are grown annually along with, to a lesser extent, Golden Grain cultivars. Both varieties are long-day onions that have coppery cataphiles, a spicy taste and good tolerance to long periods of conservation.[5,6,7,8] These onions are usually marketed in a deferred form up to 3 or 4 months after harvest. The bulbs are mainly stored in the open field, in structures called piles or trojas (Fig. 1). It is in this post-harvest stage where a series of diseases that deteriorate the quality of onions are triggered. Although the pathogenic microorganisms most frequently identified are of fungal origin, such as *Aspergillus niger, Penicillium* spp., *Fusarium* spp. and *Botrytis* spp.,[9] in recent years, there has been a change in the prevalence of diseases. The incidence of diseases of bacterial origin, such as *Pectobacterium carotovorum* (syn: *Erwiniacarotovora*) and *Burkholderia cepacia* (syn: *Pseudomonas cepacia*), has increased markedly. These pathogens have reached a concerning level, resulting in losses in yield and profitability. It is very common to detect these diseases during storage and even during transport of the bulbs.[10]

Sweet onions (*Allium cepa* L.) are typically grown on bare soil and irrigated with high-pressure systems such as sprinklers or center-pivots. In the VIrN, rain-fed fields have been incorporated into the production of onion and these are non-systematized. The crops are irrigated by spraying and, in some small-area family horticulture ventures, gravitational irrigation has been replaced by drip irrigation. These technological changes have generated cultivation conditions that differ from traditional ones and the impact of these systems has to be evaluated. Several studies have shown that the irrigation method influences the incidence of diseases in onions.[11,12] At the regional level, however, information is not available. The present study aimed to determine whether the irrigation method has an influence on the incidence of diseases of bacterial origin in onions cultivated in the Lower Valley of the Rio Negro.

## MATERIALS AND METHODS

### Experimental

The experiments were carried out during the 2013/2014 and 2014/2015 growing seasons on plot A 93 of the IDEVI colony (Lower Valley Development Institute), which is located in the east

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**Figure 1.** Piles of conservation of onion bulbs to field.

of the province of Río Negro, at 40° 48′ south latitude and 63° 05′ west longitude. The experimental design was based on the installation of the irrigation equipment. Three treatments were proposed: (i) irrigation by sprinkling, (ii) drip irrigation and (iii) furrow irrigation, with four replications in each case. The experimental units were composed of six 20-m long ridges, planted in double rows spaced 0.80 m apart (96 $m^2$) with a planting density of 4.5 kg $ha^{-1}$.

The onions were stored by stacking them with the bulbs inwards and the leaves out, in heaps that were 1 m in height and 1.5 m in width. The length of each stack was variable. These clusters were covered with polyethylene to protect them from precipitation. During this stage of conservation, the external temperature of the pile ranged between 19.7 °C (maximum temperature) and 1.4 °C (minimum temperature) for the 2015 campaign, and between 17.5 °C (maximum temperature) and 2.7 °C (minimum temperature) for the 2016 campaign. The relative humidity in both campaigns was between 40% and 60%.

### Materials and management

The soil in this area is a Mollisol[13] that is formed from alluvial sediments and has a clayey texture, good organic matter content (slightly more than 3%) and practically neutral pH values. Seeds of the cultivar Valcatorce INTA were planted directly in September 2013 and 2014.

In the drip irrigation treatment, hoses were spaced at 0.05 m apart in the sowing line, with a dripping spout every 0.20 m. In the sprinkler system, the sprinklers were located at a distance of 6 m from each other, with an irrigation diameter of 8 m. For these two methods of irrigation, three weekly irrigations were established on fixed days depending on the occurrence and amount of precipitation. In the whole season of 2013/2014, 29 irrigations were carried out and, in the 2014/2015 cycle, 32 irrigations were recorded. Water for the sprinkler and drip irrigation systems was supplied from the tertiary canal of the plot by a pump with a filter. In the experimental units irrigated by furrow, irrigation was performed every week with a total of 13 irrigations in the first cycle and 16 irrigations in the second cycle. To simulate, under the same growth conditions, two levels of incidence of diseases of bacterial origin, three furrows in each experimental unit were irrigated with an inoculum of *P. carotovorum* (Bacterial Soft Rot: BSR) and *B. cepacia* (Acid Skin: PA) (106 bacteria m$L^{-1}$) approximately 30 days before harvest. A backpack was used to achieve a homogeneous distribution on the seedbed. The other subplot was sprinkled with water. The volume used for each treatment was 20 L.

The trial was conducted under the conventional mode of the zone and the harvest was performed manually on 30 March 2014 and 20 March 2015. In both seasons, the bulbs were kept for 10 days on the furrow in the open for drying (curing). After completing this process, 120 bulbs from each experimental unit (treatments and replicates) were sampled. In total, 60 bulbs were used to record the values of the analyzed parameters at harvest and the other 60 were pocketed, perfectly identified, and incorporated into the pile to be analyzed at the end of the conservation (i.e. 4 months later in July).

### Measurement variables and statistical evaluation

The parameters measured at the two points discussed above were (i) health and (ii) incidence of diseases of bacterial origin. For this analysis, the bulbs were cut longitudinally and the evaluation was performed by eye as follows:

- Health: The percentage of healthy bulbs in the total sample (60 bulbs) was calculated using the formula: (number of healthy bulbs/total number of bulbs) × 100.
- Incidence of diseases of bacterial origin. The percentage of diseased bulbs with bacteriosis was calculated on the total of the sample (60 bulbs). The only disease observed was PBB.

The values recorded were submitted to analysis of variance (ANOVA) and Tukey's multiple comparison test ($P < 0.05$) and, where necessary, correlation factors were obtained. Because the analyzed parameters were calculated as percentages, the statistical analyses were performed with the actual data and with the transformed data in arcosene. The variations in the Fisher coefficients in the ANOVA tests were not significant and the relationships between the treatments when applying the multiple comparison tests were not altered. As a consequence, the reported data correspond to the values of the real parameters. Only in the case of the incidence of BSR were the data transformed into a square root of $Y + 0.5$ because, when null values were recorded in some experimental plots, the assumption of variance homogeneity was not satisfied. The INFOSTAT package[14] was used for statistical analysis.

## RESULTS AND DISCUSSION

### Initial percentage of healthy bulbs

The initial evaluation, before entering the pile, showed that the bulbs were harvested in the two seasons with different levels of sanitation. Statistical analysis of the initial health of onions established that the interaction of irrigation methods by years was significant ($P = 0.0076$). Therefore, healthy bulbs obtained by methods were compared for each year. In the 2014 harvest (first year), the highest percentage of healthy bulbs was in the furrowed treatment (80%) and these differed statistically from the other two treatments, which were similar to each other (spray: 55%, drip: 45%) ($P < 0.0001$) (Fig. 2). In 2015 (second year), the differences between irrigation treatments were not significant (furrow: 63.33%, spray: 60.83%, drip: 52.50%) ($P = 0.214$) but the same trend was observed as in 2014 (Fig. 2). The most noticeable difference between the two seasons corresponds to the furrow treatment because the register of healthy bulbs decreased by approximately 21% in the second cycle. For the other two treatments (spraying and dripping), the sanitation also varied significantly between the two seasons ($P < 0.05$). This interannual difference can be attributed to climatic conditions during both seasons, in the stage of bulbification, maturation and harvest, taking into account

  

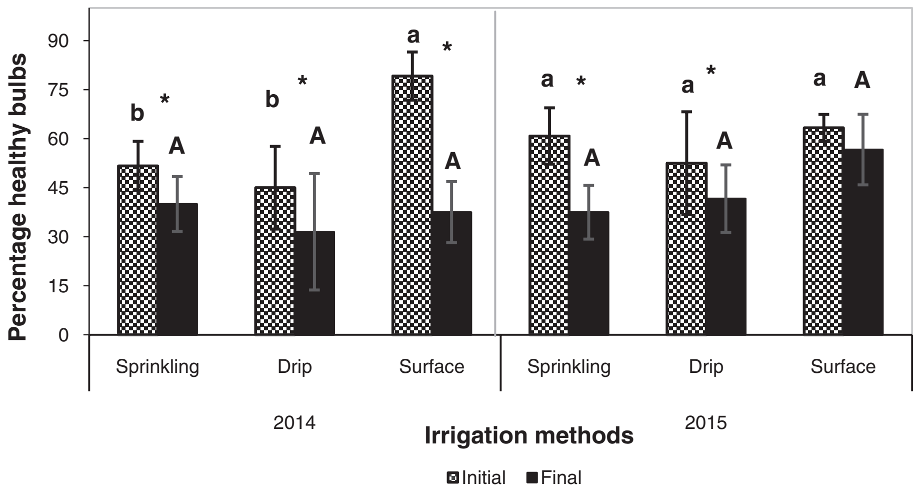


**Figure 2.** Percentage of healthy bulbs produced under different irrigation methods during 2013/2014 and 2014/2015. Bars with different letters, in each year, differ significantly from each other ($P < 0.05$). Lowercase letters compare initial states and uppercase letters compare final states. *Significant difference between initial and final health within the same irrigation method ($P < 0.05$).

**Table 1.** Distribution of pluviometric records according to the development status of onions during periods 2013/2014 and 2014/2015

| | | Development status | | |
|---|---|---|---|---|
| Years | Pluviometric records | Germination and foliar development | Bulbification and bulb growth | Ripening and havesting |
| 2013/2014 | Precipitation (mm) | 38.1 | 30.6 | 42.5 |
| | Frequency | 17 | 12 | 10 |
| 2014/2015 | Precipitation (mm) | 105.3 | 85.1 | 49.7 |
| | Frequency | 17 | 22 | 16 |

Data provided by the Meteorological Station INTA of the Inferior Valle of Río Negro.

that bacterial diseases, whose symptoms caused the decrease in health, are acquired during cultivation. The precipitations were greater, both in volume and in frequency, during the 2014/15 season, mainly from bulbification onwards (Table 1).

It should be noted that the symptoms observed at harvest (initial health) were those produced by early infections (bulbification and maturation) because those produced towards the end of the cycle cannot be expressed before entering the pile.

### Final percentage of healthy bulbs

At the end of the conservation period, the percentage of healthy bulbs decreased for all treatments and in both seasons; however, the interaction between irrigation methods by years was not significant ($P = 0.078$). On analyzing each factor without considering the other, it was found that the health of the bulbs was statistically similar for all irrigation treatments ($P = 0.073$). In the case of furrow-treated onions, at the end of the treatment, they did not differ from the other two cases despite having started the storage period with the best health records (Fig. 2).

Sanitation, however, was different for the different years ($P < 0.039$). In 2014, at the end of the conservation period, 36% of the bulbs remained healthy and, in 2015, this parameter reached 45%. These differences could also be related to the climatic conditions in the final days prior to harvest and also to the conditions during the conservation process. If the climate remains dry in the days prior to harvest (i.e. without rain or spray, with high temperatures, preferably windy and with low relative humidities), then the external cataphiles of the bulbs dry well, their tissues cease to be functional and the collars close easily. Under such conditions, the chances of late infections are markedly reduced because bacteria can enter the plant through natural openings such as stomata or wounds but require free water on the tissue surface to enable entry[15] and such tissue needs to remain functional. If suitable levels of precipitation and appropriate temperatures occur at the end of the cycle or during curing days, late bacterial infections (and fungal diseases, although not considered in the present study) can occur. Such late infections cannot be expressed and are therefore not computed at the beginning of conservation. If the internal conditions of the pile are subsequently conducive to the development of these diseases, the percentage of healthy bulbs decreases markedly on opening piles after conservation.

Significant differences were detected on analyzing the climatic parameters for the 15 days prior to harvest and the subsequent 10 days (curing) for both seasons. In the first cycle, the harvest was carried out on 30 March 2014. During the previous 15 days, a total of nine rainfall events was recorded (total of 32 mm) and, during the 10 subsequent days (cured), there were six very abundant precipitations (total of 75 mm). The average temperature was between 16 and 17 °C. In the second cycle studied, harvesting was carried out on 20 March 2015. There were six pluviometric events during the previous 15 days (total of 10 mm) and only four subsequent events that barely reached 6 mm. The average temperature was 22 °C (Data provided by the Meteorological Station INTA of the Inferior Valle of Río Negro).

The climatic conditions prior to storage were markedly more conducive to late infections in 2014 than in 2015. In addition, the higher temperatures and the lower occurrence of rainfall in March 2015 probably contributed to improved crop delivery and the curing of the bulbs. The climatic records (external to the pile) during the conservation process were markedly different for the two seasons (Table 2).

  

**Table 2.** Climatic records (outside the pile) during the conservation period

| Climatic parameters | | First and second month of conservation 2014 | First and second month of conservation 2015 | Third and fourth month of conservation 2014 | Third and fourth month of conservation 2015 |
|---|---|---|---|---|---|
| Temperature (°C) | Maximum | 17.5 | 19.7 | 14.3 | 14.1 |
| | Average | 11.7 | 13.5 | 7.9 | 7.7 |
| | Minimum | 6.7 | 7.7 | 2.7 | 1.4 |
| Precipitation | (mm) | 110.6 | 81.8 | 27.1 | 6.3 |
| | Frequency | 22 | 17 | 17 | 12 |

Data provided by the Meteorological Station INTA of the Inferior Valle of Río Negro.

The total rainfall registered in the 4 months of conservation in 2014 was 137.7 mm, with a frequency of 39 records distributed throughout the conservation stage. The total rainfall record for 2015 was 88.1 mm, with a frequency of 29 records distributed in the first 2 months of the conservation process (Table 2).

The external environmental conditions have a significant influence on the conditions within the pile.[16,17] This fact suggested that the highest percentage of healthy bulbs recorded at the end of the 2015 conservation period, compared to 2014, was a result of the external temperatures of the pile being lower, thus avoiding an increase in the internal temperature to critical values for the expression of BSR. The rainfall recorded in 2015 was less than that in the 2014 conservation period. Although the structure of the pile protects the internal bulbs from rainfall, water penetration and wetting of internal bulbs are inevitable. The external temperature of the pile and the occurrence of precipitation during the conservation stage modify the internal environment of the pile. In some cases, these factors give rise to optimum conditions for the development of pathogens and, in other cases, they inhibit or slow down the pathogen activity.[16–18]

According to Pearson's correlation coefficient, the initial sanity could only account for 42% of final healing. The occurrence of such a low correlation value could be a result of the different pathogens not yet being expressed during the initial evaluation of health. Within the piles, the components of the internal atmosphere vary gradually[19] and, as a consequence, bulbs that are apparently healthy at the beginning of conservation may be registered as diseased 3 or 4 months later.

In agreement with Echegaray *et al.*,[20] irrigation by sprinkling can be inferred to favor the development of BSR, as a result of its mechanical action on the tissues, together with the architecture of the onion plants.

### Incidence of diseases of bacterial origin

In the main onion production zone (Bonaerense valley of Río Colorado), the incidence of BSR was manifested in the years in which there was abundant rainfall at harvest time.[21,22] As already noted, these bacteria require free water to enter the tissue and produce the infection.[23] As a result, the frequency of rainfall in the final month of the crop cycle is a fundamental factor that influences the health status of the bulbs.

The main sources of inoculum for bacterial diseases that affect onions are contaminated soils and decaying organic matter.[23] The spray irrigation system generates favorable conditions for the pathogen to enter and produce the infection in the bulb because the impact of the drops on the tissues usually causes small imperceptible wounds in a similar way to torrential rain. The free water on the leaf surface of the plant (Fig. 3a) and the soil particles deposited on the foliage (Fig. 3b) favor the dispersion of the bacteria. The architecture of the onion plants, mainly the pseudostem formed by the optical position of the sheeting leaves, contributes to water retention between the ligulas and this prolongs the period of infection for several hours more than the period of rain.

Epidemiological studies of bacterial diseases in crops, carried out under natural conditions in the field, may take several years of observations because the expression of these pathogens is strongly influenced by a combination of climatic factors, agricultural practices, genetic peculiarities of crops, nd reservoirs of inocula, amongst other factors. In the 2 years during which the present study was carried out, the only bacterial disease that was manifested and detected by isolates in specific culture media[24] was BSR (*P. carotovorum*), although, in previous seasons, the symptoms of other bacterial infections were evident. It was envisaged that these

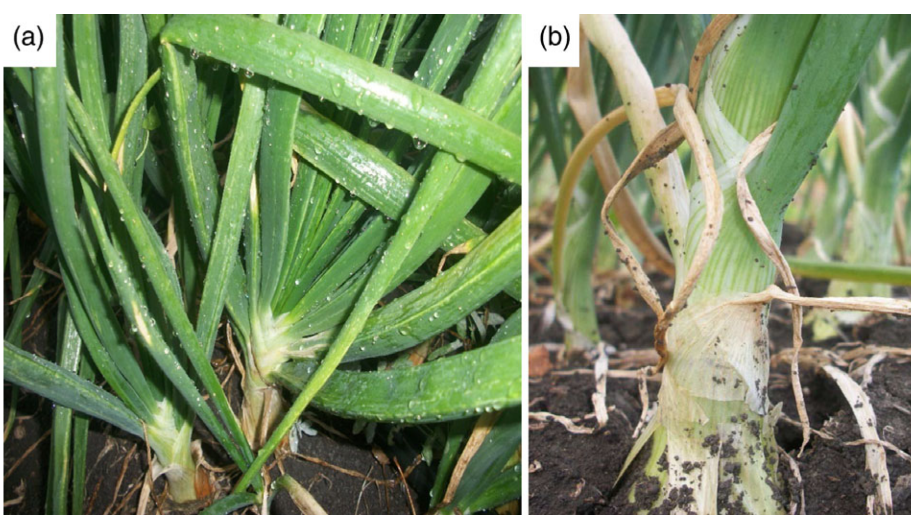


**Figure 3.** (a) Drops on the structure of the bulbs caused by sprinkler irrigation. (b) Deposit of soil particles in the pseudoculum of a bulb irrigated by sprinkling.

  

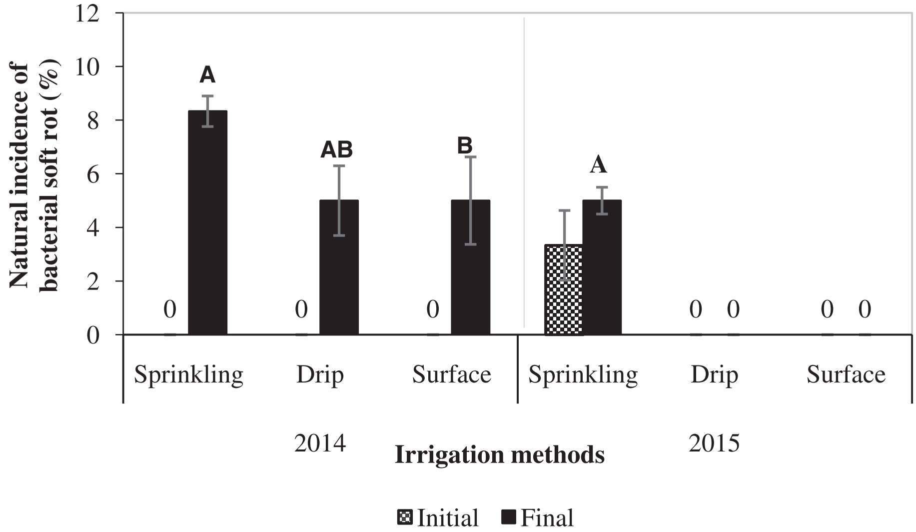


**Figure 4.** Incidence (initial and final) of *Pectobacterium carotovorum* (BSR) in onions cultivated in soil with natural inoculum, under different irrigation methods during 2013/2014 and 2014/2015. Bars with different letters differ significantly from each other ($P < 0.05$).

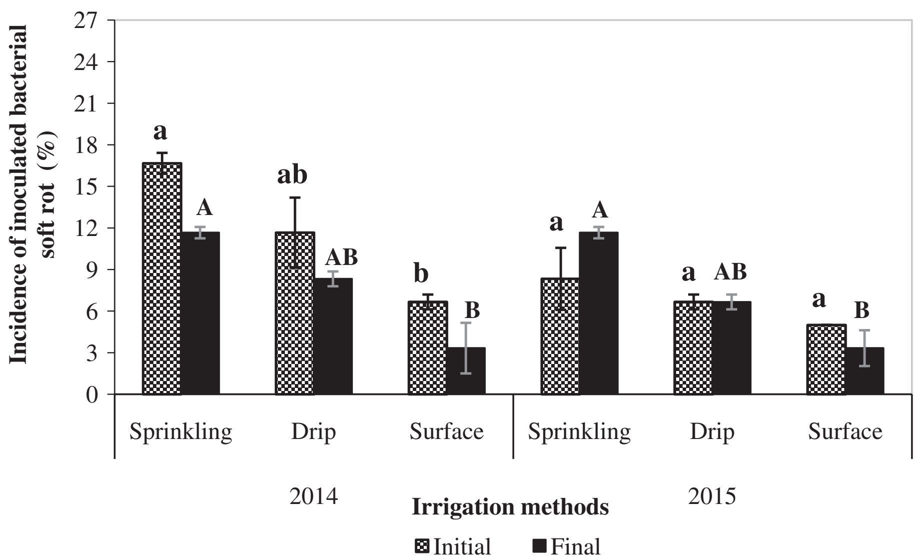


**Figure 5.** Incidence (initial and final) of *Pectobacterium carotovorum* (PBB) in onions grown on inoculated soil under different irrigation methods during 2013/2014 and 2014/2015. Bars with different letters, in each year, differ significantly from each other ($P < 0.05$). Lowercase letters compare initial states and uppercase letters compare final states. *Significant difference between initial and final health within the same irrigation method ($P < 0.05$).

fluctuations in bacterial populations could cause the evaluations to fail and artificial inoculation was therefore carried out in the subplots of each irrigation treatment to guarantee the effect of these pathogens in a culture cycle with conditions conducive to disease.

### Initial incidence of BSR

Statistical analysis of the initial incidence of BSR, after curing and prior to admission to the pile, indicated that the triple interaction [irrigation method × years × inoculation ($P < 0.498$)] and double interactions [irrigation method × years ($P < 0.903$), methods of irrigation × inoculation ($P < 0.477$) and years × inoculation ($P < 0.091$)] were not significant. This enabled us to analyze the effect of factors separately. Given that the ‘inoculation’ factor was highly significant ($P < 0.0001$), the effects of the other factors were analyzed separately in both infection situations (natural inoculum and bioaugmented inoculum).

### Natural inoculation

It can be seen from Fig. 4 that in neither of the 2 years were symptoms detected at the start of the experiment, with the exception of the spray irrigation treatment in the year 2015. This allows us to infer that in crops grown on soils with a low inoculum load, bacterial infections produced in the crop are not usually manifested before harvest, either because they are late or because the environmental conditions do not allow them to reach a sufficiently high population rate to produce a massive colonization of the tissues.

### Artificial inoculation

As shown in Fig. 5, the initial incidence of BSR in the bulbs grown in the inoculated plots increased to values consistent with those reported in other studies.[25,26] The highest initial incidence of BSR in 2014 was recorded for sprinkler irrigation (16.67%) and

  

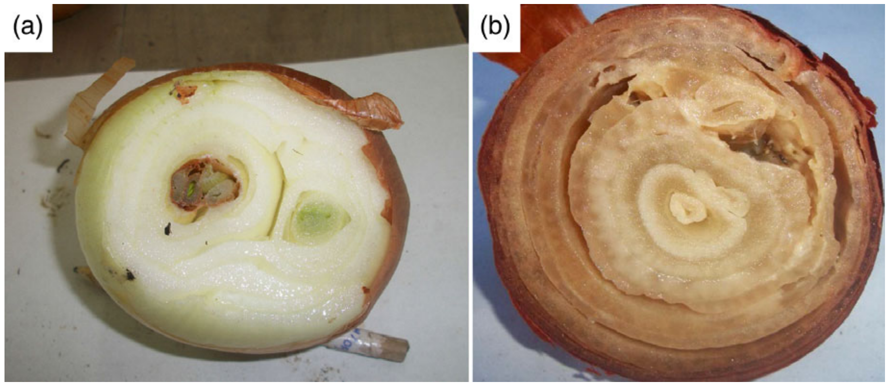


**Figure 6.** (a) Symptoms of bacterial soft rot (incipient injury). (b) Advanced symptom that represents generalized rot.

this differed statistically from that recorded for furrow irrigation (6.67%). In drip irrigation, the log had intermediate values ($P < 0.05$). In 2015, the initial incidence was lower and was not differentiated between irrigation methods, although the same trend was observed as in 2014 (spray: 8.33%, drip: 6.67% and furrow: 5%) (Fig. 5).

### Final incidence of BSR

At the end of the conservation period, the statistical analysis indicated that the triple interaction [irrigation method × years × inoculation ($P < 0.808$)] and double interactions [irrigation method × years ($P < 0.809$), irrigation methods × inoculation ($P < 0.409$) and years × inoculation ($P < 0.122$)] were not significant. When analyzing the factors separately, irrigation methods ($P < 0.001$), years ($P < 0.003$) and inoculation ($P < 0.0003$) showed significant differences. Once again, given that the 'inoculation' factor was highly significant, the effects of the other factors were analyzed separately for both infection (natural and bioaugmented inoculum) conditions.

### Natural inoculum

It can be seen from Fig. 4 that, in 2014, although BSR symptoms were not observed at the beginning of the conservation, some bulbs with the different treatments did show symptoms 3 months later after having been subjected to the conservation conditions (internal and external). Bulbs treated by sprinkler irrigation had a percentage of incidence higher than 8% and this differed statistically from the other two treatments, in which the incidence was similar (4.5%) ($P < 0.05$). In 2015, the samples with different treatments remained symptom free except for spray-dried bulbs, which showed a low initial incidence (2.3%) that increased slightly towards the end (3.3%).

### Artificial inoculum

It can be seen from Fig. 5 that, for all irrigation treatments, the incidence of BSR decreased or remained stable towards the end of the cycle, with the exception of sprinkler irrigation in the year 2015, which increased.

The pathogen in question produces pectinolytic enzymes that can internally degrade tissue without external visible symptoms. These usually enter through the neck of the bulb when it is close to harvest. The affected cataphiles in the zone near the neck become watery, bright and take on a coloration that ranges from pale yellow to pale brown[27,28] and this usually goes unnoticed during harvest (Fig. 6a). As the conservation period progresses, if the disease is favored by the internal atmosphere of the heap,[16,29] the disease progresses and the symptoms are more pronounced (Fig. 6b). However, it is possible that an incipient lesion detected at the start of conservation does not progress and is stopped at the neck of the plant. At the end of storage, this is masked by closure and drying of the neck. This possibility could explain the decrease in the visual record of the disease (Fig. 6b). It is also feasible that, depending on the degree of progression of the initial lesion, the severity increases during the conservation, thus resulting in the total loss of the bulbs (Fig. 6b). This process may occur even if the initial incidence is not modified.

According to the Pearson correlation coefficient, the initial incidence of BSR can only account for 42% of the final incidence of BSR. However, there are other factors that have an influence and these include environmental conditions during the crop cycle, especially at the time of harvest, as well as conditions inside and outside the pile during conservation.

The differences between the results obtained in the inoculated and uninoculated plots indicate that the inoculation method was good and the data can be used to simulate situations of strong infection. This possibility demonstrated that the level of inoculum in the soil should be one aspect to consider when choosing the lot to be planted. It is important to have knowledge of the ancestral cultures and avoid the onion–onion sequence[30] when a high incidence of BSR has been recorded in the first cycle.

## CONCLUSIONS

The results obtained in the present study show that the irrigation method and climatic conditions during the growing season have a substantial impact on the incidence of post-harvest bacterial diseases; in particular, it is inferred that sprinkler irrigation, as a result of its mechanical action on the tissues, together with the architecture of the onion plants, favors the development of BSR. The level of incidence observed with other irrigation systems depends, amongst other factors, on the frequency of precipitation in the final stage of cultivation.

The external temperature of the cell and the occurrence of precipitation during the conservation stage modify the internal environment of the cell in such a way that, in some cases, they generate optimal conditions for the development of the pathogens and, in other cases, they inhibit or slow down the activity of said pathogens.

The differences between the results obtained for the inoculated and uninoculated plots indicate that the inoculation method was good and the resulting data can be used to simulate situations of strong infection. This allowed us to demonstrate that the level of inoculum in the soil should be one aspect to consider when choosing the lot to be planted. It is important to have knowledge of the ancestral cultures and avoid the onion–onion sequence when a high incidence of BSR has been recorded in the first cycle.

The incorporation of alternative irrigation methods, such as sprinkling and dripping, should be accompanied by management guidelines (date of sowing, fertilization, curing of bulbs, etc.), crop water requirements and sanitary management.

## ACKNOWLEDGEMENTS

This work was carried out under the auspices of the Secretaria de Ciencia y Tecnica of the Universidad Nacional del Comahue (Argentina).

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