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Losses Caused by Downy Mildew (*Peronospora trifoliorum*) in Alfalfa (*Medicago sativa* L.) Growing on the Northern Altiplano, Bolivia

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Abstract

The cultivation of alfalfa (*Medicago sativa* L.) on the Northern Altiplano of La Paz, Bolivia, has increased due to the expansion of dairy farming. However, several leaf diseases affect the yield of this crop. The present work examines the effects in the field caused by *Peronospora trifoliorum* on 11 varieties of alfalfa in the above region during the 2011-2012 growing season. The results showed disease incidence, determined before flowering, to differ across these varieties. In the following growing season, the protective effects (in terms of disease severity and yield [dry mass ha^{-1}]) of the fungicides Ridomil (metalaxyl) and Curathane (cymoxanil) were examined in the alfalfa variety Bolivia 2000. Both fungicides significantly reduced disease severity (Ridomil by a mean 12.5%, and Curathane by a mean 7%). With Ridomil the yield obtained was 29.94% greater than under the control conditions, and with Curathane it was 28.37% greater. A strong correlation (R^2=0.858) was detected between disease severity and yield.

Keywords: Leaf spots; Susceptibility; Dry matter; Polycyclic diseases

Introduction

The cultivation of alfalfa (*Medicago sativa* L.) on the Altiplano (3900 m) of La Paz, Bolivia, dates from the late 1940s. In 1949, several Peruvian varieties were cultivated in the River Keka (Achacachi) microbasi, with varieties from Argentina, Peru, France and the USA arriving for research purposes some years later. The introduction of alfalfa varieties has been continually studied in Bolivia with the aim of selecting those best suited to the cold conditions of the Altiplano. Since the 1980s, alfalfa production in this region has increased to serve its growing herds of dairy cattle. Of Bolivia's total surface area under alfalfa (37,678 ha), 27% is now in the Department of La Paz [1-3].

Resistance to cold temperatures has been the most important factor in the selection of varieties for cultivation on the Altiplano [2,4]. Factors such as resistance to disease were not considered because it was believed these same cold temperatures would prevent diseases appearing. Indeed, it was not until 1982 that the first record of alfalfa leaf disease was noted on the Bolivian Altiplano. It is now thought, however, that the size of the area under alfalfa, the restricted number of varieties grown, and climate might be favoring the appearance of pests and disease [5-9].

Downy mildew caused by *Peronospora trifoliorum* is an important disease of alfalfa worldwide, reducing yields and crop quality. Its appearance is favored by damp, cold conditions [10,11]. In 2002, alfalfa plants in experimental plots at the Belén Research Station (Estación Experimental Belén, on the Northern Altiplano in the Province of Omasuyos, Dept. de La Paz), showed leaf symptoms of downy mildew caused by this pathogen for the first time [12]. The aim of the present work was: i) to examine the field response of 11 varieties of alfalfa to this downy mildew, and, ii) examine the protective effect of two fungicides on the Bolivia 2000 variety in terms of disease severity and yield.

Materials and Methods

Site location and environmental conditions

The present work was performed at the Belén Research Station (16°03´25´´S, 68°41´45´´W; altitude 3824 m). The mean annual temperature at this location is 9.21°C; annual rainfall is some 450-550 mm, falling largely in November-March.

2011-2012 growing season

Disease incidence and severity in 11 varieties of alfalfa: The incidence and severity of disease caused by *P. trifoliorum* was examined in 11 varieties of alfalfa (provided by the Forage Research Centre)-Africana, Altiplano, Bolivia 2000, Moapa, Pampa flor, Ranger, Reepan, Riviera, Tamborada, UMSS and Valador. Plants were grown in an experimental plot following a random block design with five replicates (experimental units 5 × 3 m; space between units 0.5 m). Disease incidence (expressed as a percentage) was determined during the vegetative growth stage before flowering as (number of plants with symptoms/total number of plants) × 100 (Campbell and Madden, 1990); data were collected from 1 m^2 sampling areas (repeated in triplicate) per variety and replicate. Disease severity was recorded as a percentage of the crop leaf area affected and linearized using the equation ln(y/1−y) [4,13-15].

2012-2013 growing season

Protective effects of ridomil and curathane: A plot established in the summer of 2009 in which the variety Bolivia 2000 had been sown freely (not in rows) was used to determine the effects of fungicide

treatment following a random block design with three treatments and three replicates (subplot dimensions 8×10 m). The treatments were: 1) Control (H_2O); 2) spraying with Ridomil MZ78 (metalaxyl +mancozeb) (dose 2 kg/ha); and 3) spraying with Curathane (cymoxanil+mancozeb) (dose 2.5 kg/ha). Treatments were applied using manual "Jacto" sprayers once every 20 days between flowering (January-March) and the start of the rainy season (December). Irrigation was provided between September and December and pests controlled with insecticides when the treatment solutions were applied.

Disease severity and yield: Disease severity was recorded before the start of the chemical treatments and every 20 to 23 days thereafter. The yield (expressed as t. ha^{-1} dry mass) was determined at a first cutting on 24th December 2012, and again at a second cutting on 12th April 2013, according to the method of Campbell and Madden [15]. Dry mass values were determined by drying 200 g samples in an electric oven at 210°C oven until no further weight loss was recorded.

Statistical analysis: Data were analyzed by ANOVA, and F0.05 and F0.01 values determined.

Results

2011-2012 growing season

Incidence and severity: The incidence of disease differed across the 11 varieties tested. The lowest incidence values were recorded for Altiplano, Pampa flor, Ranger, Tamborada and Riviera, while the highest were recorded for Valador, Reepan, Moapa, UMSS, Africana and Bolivia 2000. Disease severity ranged between 20 and 35%. Following the linearization of the severity results, the varieties Altiplano (r=0.0379/day), Reepan (r=0.0383/day) and Moapa (r=0.0362/day) showed the lowest apparent infection rates, while Pampa flor (r=0.0664/day), Ranger (r=0.0658/day), Bolivia 2000 (r=0.0577/day), Valador (r=0.0554/day), UMSS (r=0.0489/day), Riviera (r=0.0475/day). Tamborada (r=0.0479/day) and Africana (r=0.0449/day) showed the highest (Table 1). The experimental error (rR^2) varied between 0.76 and 0.97 (Figures 1 and 2).

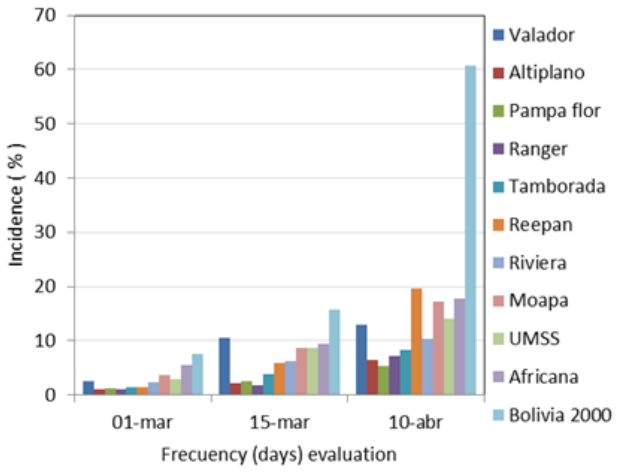

Figure 1: Disease incidence for the 11 test varieties in the 2011-2012 growing season.

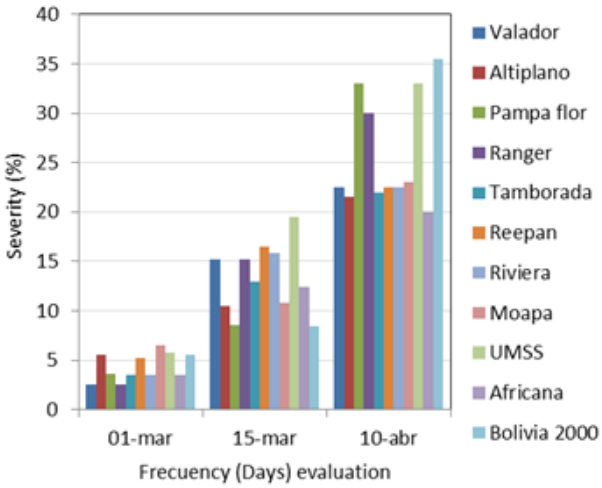

Figure 2: Disease severity in the 11 test varieties in the 2011-2012 growing season.

Variety	y=BX ± A	rR^2 (experimental error)
Valador	0.0554x-2068.8	0.7648
Altiplano	0.0379x-1418.9	0.9864
Pampa flor	0.0644x-2405.7	0.9999
Ranger	0.0658x-2456.9	0.8569
Tamborada	0.0479x-1791.7	0.8603
Reepan	0.0383x-1430.6	0.7926
Riviera	0.0475x-1776.2	0.7714
Moapa	0.0362x-1354.6	0.9988
UMSS	0.0489x-1826.1	0.8806
Africana	0.0449x-1679.7	0.8452
Bolivia 2000	0.0577x-2156.7	0.9766

Table 1: Linear equation and regression coefficient for disease severity in each variety in the 2011-2012 season.

2012-2013 growing season

Effect of fungicide treatment on disease severity in Bolivia 2000: Both fungicides significantly reduced disease severity in Bolivia 2000 (Ridomil by a mean 12.5%, and Curathane by a mean 7%). The linearization of the results clearly shows the fungicides to reduce the apparent rate of infection ($r_{control}$=0.04/day, $r_{curathane}$=0.032/day and $r_{ridomil}$=0.0145/day). Linear regression analysis revealed disease severity to be strongly related (R^2=0.858) to a lack of treatment (control conditions) (Figure 3).

Improvement in Bolivia 2000 yield with fungicide treatments: The Bolivia 2000 yield (dry matter) obtained with the Ridomil treatment was 3.64 t. ha^{-1} while that obtained with the Curathane treatment was 3.56 t. ha^{-1}-29.94% and 28.37% greater respectively than the yield

obtained under the control conditions (2.55 t. ha^{-1}) ($F_{0.05}$) and high correlation between dry matter yield and disease severity (Figure 4).

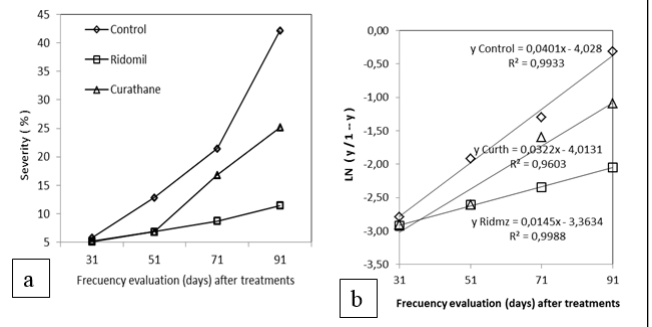

Figure 3: a: Effect of the fungicides on disease severity; b: Linearization of disease severity results.

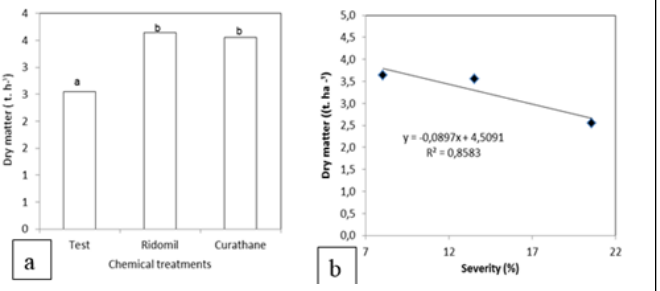

Figure 4: a: Dry mass yield of Bolivia 2000 under different treatment condition (F 0.05); b: Coefficient of regression between dry matter yield and disease severity in Bolivia 2000.

Discussion

The 11 varieties studied showed different resistance to *P. trifoliorum* in the field. In the present work the varieties Altiplano, Reepan and Moapa were the least affected by the Oomycete, and Valador, Pampa flor, Ranger and Bolivia the most affected. The differences in the reactions of the varieties with respect to the report of the Centro de Investigaciones Forrajeras might be explained by differences between the pathogen's characteristics on the Altiplano at 3824 m and the Valle de Cochabamba at 2465 m. According to McDonald and Linde, environmental conditions strongly affect the development of pathogen populations.

The apparent infection rates ("r") grouped the varieties Altiplano, Reepan and Moapa together, and Pampa flor, Ranger, Bolivia 2000, Valador, UMSS, Riviera, Tamborada and Africana in another group. The varieties with low apparent infection rates may have accumulated recessive genes of use to them in this respect. However, when temperature and humidity conditions favorable to the pathogen persist, it could become very destructive even in the varieties with low apparent infection rates. Downy mildews become destructive, polcyclic epiphytic fungi when environmental conditions allow [15].

Ridomil and Curathane both significantly reduced the apparent infection rate in Bolivia 2000, although the former appears to be the more effective. According to Bowden and Goldberg, Ridomil is an efficient systemic fungicide against downy mildew when applied to alfalfa seeds. To date there has been little work performed of the control of downy mildew on alfalfa in Bolivia. However, both leaf and stem disease are known to reduce the yield and quality of this crop. The Centro de Investigaciones en Forrajes has reported yields of 4-7 t. ha^{-1} for Bolivia 2000 when grown under Valle de Cochabamba conditions and two-cut management with no fungicide treatment. In the present work, following single cut management, a yield of 3.64 t. ha^{-1} was achieved with Ridomil treatment, 3.56 t. ha^{-1} with Curathane treatment, and 2.55 t. ha^{-1} under control conditions.

According to Campbell and Madden, yield losses are measured as the difference between the "reachable" and "actual" yields. In the present work, the reachable yields were those obtained under the Ridomil and Curathane treatments, and the actual yield that obtained under control treatment. Under these treatment conditions these yields were 29.94% and 28.37% higher. Indeed, a strong correlation (R^2=0.858) was detected between disease severity and yield. It should not be forgotten, however, that defoliation due to frost and winds, which are common on the Altiplano from April onwards, will also influence yield.

In conclusion, the 11 alfalfa varieties examined responded differently in terms of the resistance shown to *P. trifoliorum*. With Ridomil and Curathane treatment, the yields obtained with Bolivia 2000 we increased by nearly 30% [4,11,16,17].

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