EVALUATION OF A *QUILLAJA SAPONARIA* SAPONIN EXTRACT FOR CONTROL OF POWDERY MILDEW OF WHEAT AND SQUASH

EVALUACIÓN DE UN EXTRACTO DE SAPONINAS DE *QUILLAJA SAPONARIA* PARA EL CONTROL DE OÍDIOS DE TRIGO Y ZAPALLO.

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ABSTRACT

Key words: *Ampelomyces quisqualis*, *Bacillus subtilis*, saponins, *Quillaja saponaria*, powdery mildews.

Powdery mildew are important diseases in Chile, especially on wheat and cucurbit crops, and their management mainly depends on the use of chemical fungicides. However, new control alternatives have been developed, including extracts of quillay (*Quillaja saponaria*). In this study three dosages of saponins extracted from quillay (QL 1000®, Natural Response S.A., Quilpué, Chile) were evaluated for their effect on wheat and squash powdery mildew. For comparison, AQ-10® (*Ampelomyces quisqualis*), Serenade® (*Bacillus subtilis*), sulfur, and a standard chemical fungicidal mixture of azoxystrobin plus propiconazol/ fenpropidin were used. To evaluate the control efficacy of the saponins against *Blumeria graminis* f.sp. *tritici*, an experiment on wheat was conducted in humid chambers in a greenhouse. QL 1000® at a dose of 100-ppm of saponins exhibited a control level statistically similar to AQ-10®: (44% and 31%, respectively). Serenade® showed a control level comparable to that obtained with a standard chemical fungicide mixture (75 vs. 80%). The experiment to evaluate the control efficacy of saponins against *Golovinomyces cichoracearum* and *Podosphaera fusca* in squash was carried out under field conditions. In this case 200 ppm of QL 1000® saponins, Serenade® and sulfur exhibited control levels of 51%,

RESUMEN

Palabras claves: *Ampelomyces quisqualis*, *Bacillus subtilis*, saponinas, *Quillaja saponaria*, oídios.

Los oídios son enfermedades importantes en Chile, especialmente en trigo y cucurbitáceas, por lo cual su manejo sanitario depende del uso de fungicidas químicos. Sin embargo, nuevas alternativas de control han sido desarrolladas, incluyendo el uso de extractos de quillay (*Quillaja saponaria* Molina). En este estudio fueron evaluadas tres dosis de saponinas provenientes de un extracto de quillay (QL 1000®, Natural Response S.A., Quilpué, Chile) contra el oídio del trigo y del zapallo. En los experimentos fueron evaluados de forma comparativa AQ-10® (*Ampelomyces quisqualis*), Serenade® (*Bacillus subtilis*), azufre y una mezcla estándar de los fungicidas químicos azoxystrobin más propiconazol / fenpropidin. El experimento con trigo fue realizado en cámara húmeda dentro de un invernadero para evaluar el control sobre *Blumeria graminis* f.sp. *tritici*. La dosis de 100 ppm de saponinas exhibieron niveles de control promedio similares a AQ-10®: 43% y 31%, respectivamente. Serenade® presentó un control comparable a los obtenidos con fungicidas químicos: 75 vs. 80%. La evaluación del efecto de control sobre *Golovinomyces cichoracearum* y *Podosphaera fusca* en zapallo fue realizada en el campo. En este caso, 200 ppm de saponinas, Serenade® y azufre exhibieron niveles de control de 51%, 50% y 70%, respectivamente. En los

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50% and 70%, respectively. In these tests, for both wheat and squash, spray applications of QL 1000® saponins provided powdery mildew control levels comparable to those obtained using AQ-10® and Serenade.

experimentos en trigo y zapallo, la aplicación de saponinas de QL 1000® mostraron niveles de control comparables a los obtenidos con AQ-10® y Serenade®.

INTRODUCTION

Powdery mildew occurs commonly in cereals, horticultural, fruit trees and ornamental plants worldwide (Elad *et al*., 1996; Paulitz and Bélanger, 2001). Among the causal agents of these diseases, the wheat powdery mildew fungus, *Blumeria graminis* f.sp. *tritici* (DC.) E.O. Speer, is described as a secondary disease of wheat in Chile (Latorre, 1992; Apablaza, 2000), but its severity has increased in southern Chile (Hewstone, 2001, personal communication).

Cucurbit powdery mildew is another important disease in Chile (Bruna, 1989; Apablaza, 2000), causing necrosis in the affected tissues and early defoliation when the attack is severe (Latorre, 1992; Apablaza, 2000). There are six powdery mildew species, belonging to three genera affecting cucurbit crops (Stadnik and Rivera, 2001). The two most damaging and frequently found powdery mildew in Chile and worldwide (Bruna, 1989; Elad *et al*., 1996; Stadnik and Rivera, 2001), are *Golovinomyces cichoracearum* (formerly *Erysiphe cichoracearum*) DC. V.P. Heluta, and *Podosphaera fusca* (Fr.) Braun & Shishkoff (formerly *Sphaerotheca fuliginea* (Schlecht ex Fr.) Poll.).

Successful management of these powdery mildew depends mainly on fungicides that suppress the development of the disease (Daayf *et al*., 1995; McGrath *et al*., 1996; Fraaije *et al*., 2002), although some resistant cucumber and melon cultivars are already available (Czembor and Czembor, 2002; Paris and Cohen, 2002). However, the repeated application of these fungicides may cause phytotoxicity and may results in the selection of populations that are fungicide resistant (Elad *et al*., 1996; Pasini *et al*., 1997; McGrath, 2001), as has already been reported for *P. fusca* (McGrath *et al*., 1996; McGrath, 2001; Brown, 2002) and *B. graminis* (Fraaije *et al*., 2002; Felsenstein *et al*., 2010). For this reason, there is increasing interest in the application of mineral salts, oils, plant extracts, and the use of biological control agents either in combination with or to replace synthetic fungicides (Elad *et al*., 1996; Pasini *et al*., 1997; Bettiol and Stadnik, 2001; Nuñez *et al*., 2009; Savvas *et al*., 2009).

Several plant extracts have been tested in order to determine their effectiveness against powdery mildew (Daayf *et al*., 1995; Elad *et al*., 1996; Pasini *et al*., 1997; Konstantinidou-Doltsinis and Schmitt, 1998; Newman *et al*., 1999; Coventry and Allan, 2001; Nuñez *et al*., 2009). Research in Chile has been conducted with different quillay (*Quillaja saponaria* Mol.) extracts (Apablaza *et al*., 2002; Apablaza *et al*., 2004). These products contain bidesmosidic (two sugar chains in the molecule) high molecular weight glycosylated triterpenoid compounds called saponins, which exhibit a fungicidal activity through a membranolytic action on the sterols present in cell membranes (Segal and Schlösser, 1975; Schönbeck and Schlösser, 1976). These quillay extracts have reached up to 52% control in an evaluation on cucurbit powdery mildew in cucumber plants under greenhouse conditions (Apablaza *et al*., 2002).

The objective of the present study was to evaluate three dosages of quillay saponin extracts for the control of wheat and squash powdery mildew in comparison with other biological control agents and chemical fungicides that are commonly used to control powdery mildew.

MATERIAL AND METHODS

Treatments

The treatments used in the experiments were: i) untreated control treatment (UTC); ii) three dosages of saponin (50, 100 and 200 ppm) obtained as an aqueous quillay extract concentrate (QL 1000®, Natural Response S.A., Quilpué, Chile), with a saponin concentration of 8% w/w, deter-

mined by RP-HPLC (Rouhi, 1995; San Martín and Briones, 2000); iii) AQ-10® (*Ampelomyces quisqualis*, Ecogen Inc., Langhorne, Pennsylvania, USA.), isolate M-10, in dosage of 11.56 g · ha^{-1}; iv) Serenade® (*Bacillus subtilis* QST 713 strain, AgraQuest Inc., Davis, California USA) in 8 Kg · ha^{-1}; and, v) standard control treatment. In the experiments with wheat the standard control was a mix of standard synthetic fungicides composed of azoxystrobin (Priori® Syngenta Agribusiness, Las Condes, Chile) and propiconazol / fenpropidin (Zenit® 425 EC, Syngenta Agribusiness, Las Condes, Chile) in dosage of 250 ml · ha^{-1} and 750 ml, respectively. In the case of squash the standard control fungicide treatment was 7.5 kg · ha^{-1} of sulfur (Magnetic Sulfur 95 W®, Anasac, Providencia, Chile).

Experiments

The experiment with wheat (*Triticum aestivum* L.) was carried out with the cv. Claudia, in a greenhouse in humid chamber conditions from October 2001 to January 2002, to evaluate the control of *B. graminis* f.sp. *tritici*. The plants were grown in pots containing 400 g of a previously sterilized substrate, at a ratio of 3:2:1 of sandy-loam soil, humus and fine sand, respectively. Eighteen seeds were sown per pot, and ten days after emergence they were thinned to 10 plants per pot. Experimental units consisted of a plot of four pots with four replicates. Each pot was fertilized with 0.62 g of N as granulated urea 46% N (Cargill, Santiago, Chile) at the three-leaf stage and again at heading. Aphids were controlled with two applications of pirimicarb (0.5 g · L^{-1}, Pirimicarb® Syngenta Agribusiness, Las Condes, Chile). Plants were watered at two-day intervals. The plants were placed in a humid chamber one week before the first application of the treatments. The chamber was ventilated daily to maintain the temperature under 28°C.

Another experiment was carried out with squash plants (*Cucurbita maxima* Duch.), biotype "de guarda" or "camote", grown in the field to evaluate the control of *G. cichoracearum* and *P. fusca*. The plants were grown from October 2001 to January 2002 in the Central Region of Chile (Curacaví, 33º27' Lat. S.; 70º38' Long. W.). The experimental unit was a row of 11 plants in a plot of 35 m^2 (5 x 7 m) with four replicates. Management was that used by local farmers, except for the fungicide applications.

Inoculation

Wheat plants were inoculated twice (November 16 and 28) with a spore suspension of 4.5 x 10^6 per ml of distillated water, measured with hemocytometer (Boeco, Hamburg, Germany). Spores were obtained from the field and sprayed on the plants prior to the first application with the treatments under study. In the squash experiment, the plants were naturally infected and were not inoculated.

Product application

In wheat, the fungicidal spray applications began when the infection was severe in the bottom leaves (25 to 75% of disease severity on the leaves), and slight to moderate in the basal half of the plant (<25% of disease severity on the leaves). Three applications were performed starting on December 03, 2001, at 12 and 14 day intervals. A total volume per spray of 390 L · ha^{-1} was used, until runoff.

In squash, four applications were made after the first signs of the disease appeared, starting December 21, 2001, with 8 to 10 day intervals. Treatments of 600 L· ha^{-1} per spray application were applied until runoff. Sprays were applied with a 5 L capacity manual sprayer (SOLO 456; Kleinmotoren GMBH, Sindelfingen, Germany), fitted with a pressure gauge and a hollow conical tip nozzle, at a pressure of 2 to 3 bar.

Only 3 or 4 applications were used per experiment as this is the number of applications commonly used by the farmers in the area.

In both experiments disease severity was measured with the Horsfall - Barratt scale (Horsfall and Barratt, 1945), where the highest rating represents the highest percentage of fungus-covered tissue. The percentages corresponding to each rating are: 1= 0; 2= 1 – 2.9; 3= 3 – 5.9; 4= 6 – 11.9; 5= 12 – 24.9; 6= 25 – 49.9; 7= 50 – 74.9; 8= 75 – 86.9; 9= 87 – 93.9; 10= 94 – 96.9; 11= 97 – 99.9; and 12= 100%. The evaluation of wheat powdery mildew was carried out by determining the disease severity on ten leaves selected arbitrarily among the plants of a single pot of the experimental unit per each replication (40 leaves per treatment). This pot was discarded

after reading and another pot was used for the following reading. Evaluations were carried out on December 18 and 28, 2001 and January 03, 2002. The evaluation of powdery mildew in squash was conducted on six treated leaves per replicate (24 leaves per treatment), selected randomly from the middle part of the plant. The readings were carried out on December 27, 2001 and on January 07, 17 and 25, 2002. The evaluation and application dates were the same after the first application, because sampling was performed prior to a new application.

Experimental design and statistical analysis

The experiment with wheat corresponded to a split plot completely randomized design, while in the squash experiment, a split plot completely randomized block design was used. The split-plot principle was applied to both experiments, because an analysis of covariance was performed on the severity reading in each disease assessment using PROC MIXED with the REPEATED statement for repeated measures (SAS Institute Inc., Cary, NC, USA), where several covariance matrices were evaluated and the independent structure of covariance between each date of assessment was chosen because it had the lowest Akaike information criterion (AIC) score with the fewest number of covariance parameters. This implied that severity readings determined at each disease assessment were independent from one time of assessment to the next, and this permitted the use of a univariate analysis split plot in time. The treatment used in each spraying (treatment factor) was considered as the main plot and the assessment time after each spraying as the subplot (assessment time factor). A subplot in this case differed from the usual subplot in that it consisted of data taken from the entire main plot rather than from a designated portion of the main plot, as is the case with the usual split-plot. In the wheat experiment the product factor and assessment time factor were assessed on the leaves of a pot randomly chosen from the experimental unit in each evaluation, because we considered that the foliage might concentrate the active ingredient of the treatments on the leaves after each spraying. In squash, the same criterion was followed, considering the assessed leaves retrieved from each treatment plot as the treatment factor and the differences between each time of assessment as the subplot. Then, the assessment of the disease was not independent from the same experimental unit, but the experimental error could be assumed independent. The analysis of variance (ANOVA) for a split plot design in time was carried out using the statistical program SAS Version 6.0 (SAS Institute Inc., Cary, NC, USA). The analysis evaluated the performance among treatments, time of sprayings, and determined interaction between both factors. Treatment means were compared using the Tukey-Kramer test ($p<0.05$).

RESULTS

Results obtained in the experiment on wheat determined that treatments interacted with the time of assessment ($p<0.05$) and the treatments showed differences among each assessment performed after each treatment spraying. This situation influenced the variation observed among the treatments, and thus the comparison among means of the treatments was only considered for each individual assessment (Table 1). Overall means comparison for the treatments showed statistical differences ($p<0.05$) (results not showed), but they are not adequate to do statistical inferences within the analysis of result for the split plot design considering the interaction among treatment and time of assessment observed. This interaction could be observed on the untreated control (UTC), because during the experiment there was a gradual decrease of *B. graminis* f.sp. *tritici* infection on this treatment. Nevertheless, throughout the first two evaluations there was significant control of the disease with all treatments compared to UTC. In the second evaluation, saponin dosages of 100 and 200-ppm showed disease control levels of 53 and 54% respectively, and were statistically similar ($p< 0.05$) to the level obtained with AQ-10® (40%). In this assessment, the disease control levels obtained with Serenade® and azoxystrobin plus propiconazol / fenpropidin were significantly higher than with quillay extract and AQ-10®, reaching control levels of about 80%. In the third evaluation, there were no significant differences ($p<0.05$) among the saponin dosages of the extract, AQ-10® and

Table 1. Average severity and percentage of control of *Blumeria graminis* f.sp. *tritici* on wheat under humid chamber conditions (three evaluation dates, 2001-2002).
Cuadro 1. Promedio de severidad y porcentaje de control de *Blumeria graminis* f.sp. *tritici* sobre trigo bajo condiciones de cámara húmeda (tres fechas de evaluación, 2001-2002).

Treatments	Powdery mildew severity[3]				Relative control percentage (Φ)[2]			
	Dec 18	Dec 28	Jan 03	A.V.G.	Dec 18	Dec 28	Jan 03	A.V.G.
Untreated control treatment	7.5a[1]	6.8 a	4.5 a	6.3	-	-	-	-
Quillaja saponins (50 ppm)[4]	5.5 b	4.8 b	3.5 a	4.6	27	29	23	27
Quillaja saponins (100 ppm)	4.1 bc	3.2 b	3.2 ab	3.5	46	53	30	44
Quillaja saponins (200 ppm)	5.5 b	3.1 b	3.5 a	4.0	27	54	22	34
Serenade®[5]	1.8 d	1.3 c	1.5 bc	1.5	76	80	67	75
AQ - 10® [6]	4.8 bc	4.1 b	3.7 a	4.2	37	40	18	31
Standard control treatment[7]	1.3 d	1.2 c	1.2 c	1.2	82	83	74	80

[1] Average values of four replicates followed by the same letter in the column, do not exhibit significant differences according to Tukey and Kramer (P<0.05).
[2] Percentage of control (Φ) relative to the untreated control treatment, calculated from the data on average severity, according to the following relation $\Phi = 100 - ((T_x \times 100)/ T_{utc})$, where: T_x = Value in the Horsfall and Barratt scale (1945) for a given treatment and T_{utc} = Value in the Horsfall and Barratt scale (1945) obtained for the untreated control treatment, in each evaluation.
[3] Values in the table correspond to the averages obtained from the severity estimation made through the use of the Horsfall and Barratt scale (1945).
[4] QL 1000, quillaja products from Natural Response S.A., Quilpué-Chile
[5] *Bacillus subtilis* QST 713 strain (AgraQuest Inc., Davis, California U.S.A.), treatment dose 8 kg PC · ha^{-1}.
[6] *Ampelomyces quisqualis* isolate M-10 (Ecogen Inc., Langhorne, Pennsylvania, U.S.A.), treatment dose 11.56 g PC · ha^{-1}.
[7] Treatment with a mix of azoxystrobin plus propiconazol / fenpropidin (250 ml · ha^{-1} and 750 ml · ha^{-1}, of Priori® and Zenit®425 EC, respectively. Both products of Syngenta Agribusiness, Las Condes, Chile).

UTC. Only azoxystrobin plus propiconazol / fenpropidin and Serenade® exhibited differences ($p<0.05$) relative to UTC, reaching those two treatment levels of control with respect to UTC of 74 and 67%, respectively. Table 1 also shows that the assessments of saponin dosages of quillay extract and *A. quisqualis* were similar during the whole evaluation period. Although, 100-ppm saponin dose did not show significant difference with the other saponin dosages, this treatment was observed to have a markedly better control level than the other saponin dosages, and this performance was observed during the entire experimental period.

The squash experiment showed that treatment means maintained their performance levels throughout the evaluations, indicating that there was no significant interaction between time of assessment and the response in the control level to each treatment ($p=0.64$). Therefore, only the comparison among the overall averages of the treatments was performed. In this experiment, individual statistical analyses for each date of assessment did not have statistical implications on the analysis of the results for the split plot design and was not adequate to allow inferences from these data. Likewise, the individual analysis for each assessment time showed that treatment means showed significant differences ($p<0.05$) similar to the overall averages (results no shown). Table 2 shows the results for the control of *G. cichoracearum* and *P. fusca* in squash. Treatments with the 100-ppm saponin dose and AQ-10® showed no significant differences on the severity of the disease with respect to UTC. Sulfur, the standard control, Serenade® and the 50 and 200-ppm saponin treatments showed similar severity levels and were different ($p<0.05$) from the control. Sulfur, the standard chemical control, in Table 2 showed the highest percentage of control (70%), followed by the 200-ppm saponin dose and Serenade® with control levels of 51 and 50%, respectively. AQ-10® decreased its level of control from 51% to 29% during the evaluation period, and was the product with the lowest efficacy.

DISCUSSION

The three natural products evaluated (Serenade®, AQ-10 and *Q. saponaria* extract) ex-

hibited control effects on the pathogens under study: *B. graminis* f.sp. *tritici*, *P. fusca* and *G. cichoracearum*. However, the results of the three natural products showed variations and statistical differences in their performance and efficiency. This type of variation has been shown by biological control agents and natural products to protect crop plants from disease and explains why their commercial development has been slower or hampered in some cases (Trigiano *et al*., 2004). In this research, Serenade® gave the most effective control for wheat powdery mildew among the bionatural products tested, reaching an average control level of 75%. This level of control was not significantly different from that obtained with the standard chemical mixture that was used. Serenade® was not optimal in the control of squash powdery mildew (50% of control). Nevertheless, the severity assessment of powdery mildew at each evaluation was not different from that of plants treated with Magnetic sulfur 95W, the standard control. Serenade® treatment was about 20 percentage points below the percentage control level of sulfur (70%). It is possible that the low relative humidity existing under field conditions during the experiment affected the germination of spores of *B. subtilis* as has been reported by Romero and coworkers (2007). This result was different from that obtained by Keinath and DuBose (2004) on powdery mildew of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) in the southern United States and the results of Romero and coworkers (2007) on *P. fusca* in melon (*Cucumis melo* L.) in Spain. AQ-10® was the least effective in controlling powdery mildew on wheat and on squash (31 and 36% on average). Reduction of the severity of *P. fusca* in melon seedlings between 62 and 92% has been achieved by AQ-10® under different levels of relative humidity, and those reductions were achieved in a greenhouse with a relative humidity over 75% (Romero *et al*., 2007). Thus, relative humidity probably had a role in the efficacy of *A. quisqualis* on *G. cichoracearum* and *P. fusca* under field conditions, but this does not explain the results observed with *B. graminis*. On the other hand, the standard chemical controls, the mixture of systemic fungicides azoxystrobin plus propiconazol/fenpropidin, and the contact fungicide sulfur, as expected, showed the high-

Table 2. Average severity and control percentage for treatments for the control of powdery mildew *Golovinomyces cichoracearum* and *Podosphaera fusca* in squash leaves under field conditions (four evaluation dates, 2001-2002).

Cuadro 2. Promedio de severidad y porcentaje de control de tratamientos para el control de los oídios *Golovinomyces cichoracearum* y *Podosphaera fusca* en hojas de zapallo bajo condiciones de campo (cuatro fechas de evaluación, 2001-2002).

Treatments	Powdery mildew severity[2]					Relative control percentage (Φ)[3]				
	Dec 27	Jan 07	Jan 17	Jan 25	A.V.G.	Dec 27	Jan 07	Jan 17	Jan 25	A.V.G.
Untreated control treatment	5.0	7.7	7.4	7.0	6.8 a[1]	-	-	-	-	-
Quillaja saponins (50 ppm)[4]	3.2	3.6	5.0	4.7	4.1 b	36	53	33	33	39
Quillaja saponins (100 ppm)	3.8	4.1	4.8	4.7	4.3 ab	24	47	36	33	36
Quillaja saponins (200 ppm)	2.0	3.6	3.7	4.1	3.3 b	60	53	50	42	51
Serenade®[5]	2.6	2.9	3.9	4.3	3.4 b	47	63	47	39	50
AQ - 10®[6]	2.4	4.6	5.3	4.9	4.3 ab	51	40	29	29	36
Standard control treatment[7]	1.5	2.2	2.1	2.4	2.1 b	70	72	71	65	70

[1] Averages of four replicates followed by the same letter in the column, do not exhibit significant differences according to Tukey and Kramer ($P<0.05$).

[2] Values in the table correspond to the averages obtained from the severity estimation made through the use of the Horsfall and Barratt scale (1945).

[3] Control percentage (Φ) relative to the control treatment, calculated from the information on average severity, according to the following relation $\Phi = 100 - ((T_x \times 100)/ T_{utc})$, where: T_x = Value in the Horsfall and Barratt scale (1945) for a given treatment and T_{utc} = Value in the Horsfall and Barratt scale (1945) for the untreated control treatment, in each evaluation.

[4] QL 1000, quillaja products from Natural Response S.A., Quilpué-Chile

[5] *B. subtilis,* QST 713 strain (AgraQuest Inc., Davis, California U.S.A.), treatment dose 8 kg PC · ha^{-1}.

[6] *Ampelomyces quisqualis* isolate M-10 (Ecogen Inc., Langhorne, Pennsylvania, U.S.A.), treatment dose 11.56 g PC · ha^{-1}.

[7] Treatment with sulfur (Magnetic sulfur 95 W®, Anasac, Providencia, Chile), treatment dose 7.5 kg PC · ha^{-1}.

est average control levels with 80% on *B. graminis*, and 70% on *G. cichoracearum* and *P. fusca*, respectively. The statistical interaction between the treatments and the time of assessment showed that the pathogen infection was affected differentially for those factors, which was reflected in the disease severity on both crops. This detected interaction meant that the comparison of means between treatments was made with different sets of data generated from the experiments (means from individual assessment in wheat and overall means in squash). This result implies that other factors could have influenced the treatment's efficacy. The difference in the performance between both experiments could be explained by the type of infection that occurred during the experimental work. In the case of *B. graminis* on wheat, the disease severity did not increase over time; nevertheless, the level of powdery mildew infection on wheat was relatively high at the first treatment. This explains the poor efficacy of most biocontrol treatments as the disease had already reached its higher epidemic progress and the biocontrolers and quillay dosages did not show an eradicative effect. Additionally, the low values of disease severity observed over time on wheat, especially in the last assessment, were associated with the level of infection that the leaves showed between each single plot in the experimental unit. The reduction in the infection levels on the plant assessed in a single plot could be related to environmental effects observed through the experiment. The high temperatures registered during the development of the experiment, which when higher than 25°C can reduce conidia germination (Apablaza, 2000; Glawe, 2008) and the dissemination process (Martinelli, 2001), could explain the reduction of the infection during the experimental period. Moreover, the high relative humidity maintained inside the humid chamber, would have favored the appearance of chasmothecia (= cleistothecia), a state of resistance in which the pathogen is less vulnerable to control (Stadnik and Rivera, 2001; Glawe, 2008), and which was observed in the last assessment, may explain the lower levels of control obtained by the different treatments in the last evaluation, where quillay extract dosages and AQ-10® were similar to the UTC. On the other hand, our experience with squash crops in the central valley of Chile has shown that natural infection of *G. cichoracearum* and *P. fusca* occur relatively constantly until late in the season, when the severity of the disease increases, associated with the high temperature conditions presented during the summer that accelerate the vegetative growth and senescence of leaves, thus favoring the disease. During the period of experimentation, a constant pattern of severity of infection was also observed, but the experimental assessments were carried out during the period of constant severity infection and which could explain the similar level of infection observed between assessments on UTC.

This study determined that the 100 and 200-ppm saponins dosages showed higher control levels observed in both experiments (43% for wheat and 51% for cucurbit powdery mildews). These results confirmed studies carried out by Apablaza *et al*., (2002), who proposed that the best control dosages with quillay extracts for *G. cichoracearum* and *P. fusca* would be between 32 and 400 ppm of saponins. At these low concentrations, saponins are present mainly as monomers and not aggregated into micelles (Mitra and Dungan, 2001), which is a condition that probably decreases their antifungal activity. Also, it is important to indicate that in our experiments, for both wheat and squash, QL 1000® saponin treatments provided powdery mildew control levels comparable to those obtained using AQ-10® and Serenade, which are commercial biopesticides. Percentages of control of 22 to 60 observed when using quillay extracts might be considered too low for this product to be used alone in a control program for powdery mildew. However, with these levels of control, quillay extracts could be included in an integrated pest management program of the disease as has been reported for other biopesticides and powdery mildew pathosystems (Romero *et al*., 2007; Gilardi *et al*., 2008; Pertot *et al*., 2008). Quillay extracts could have a potential for use in a combined strategy with other plant protection products and this action will permit the incorporation of a new mode of action in these disease management programs. In this context, considering the membranolytic action of the quillay saponins on the sterols present in cell membranes of powdery mildew (Segal and Schlösser, 1975;

Apablaza *et al*., 2004), this could be an alternative to improve the performance of the standard chemical fungicides and to reduce the risk of resistance development.

Based on the above discussion, and considering the quillay extracts are approved for human consumption in the USA, the European Community and Japan (San Martín and Briones, 1999) and that saponins have been demonstrated to have a number of pharmaceutical effects, such as reducing plasma cholesterol in humans and antitumor and antimicrobial activity (Rouhi, 1995; Mitra and Dungan, 2001), it can be concluded that the effect of dosages of 100-ppm and 200-ppm from quillay saponins on *B. graminis* and on *G. cichoracearum* and *P. fusca* showed at least partial efficacy in controlling the disease on leaves. However, those results are promising, particularly considering the restrictions required for use of many synthetic fungicides and the necessity to develop new products with new modes of action (Lisansky and Coombs, 1994; Harman *et al*., 2010). Moreover, since saponins are naturally thermo-stable molecules and have surfactant characteristic (Mitra and Dungan, 1997), the performance of quillay saponins should not be affected by environmental conditions, as has occurred with biocontrol agents (Froyd, 1997). However, quillay degradation by the microbial community present in the phyllosphera could reduce the quillay saponin action. More studies need to be carried out to determine the possible interactions associated to the use of quillay extracts on leaves. On the other hand, Serenade® exhibited the highest control among the natural products evaluated, while AQ-10® did not show an adequate level of control in our studies. Considering that sulfur use has increased in some countries (Froyd, 1997) and use of biocontrol agents has shown promising results on powdery mildews (Bettiol and Stadnik, 2001; Romero et al., 2007; Pertot *et al*., 2008), the saponins from quillay could be considered in a integrated pest management program of powdery mildew on vegetable and cereal crops.

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