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MANAGING POWDERY MILDEW *(Oidium* sp.*)* OF BLACKBERRY *(Rubus glaucus)*. A PARTICIPATORY RESEARCH APPROACH

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ABSTRACT

Blackberries are a key source of income for many families, including small and medium-sized producers in Ecuador. There has been a growing need to contribute to research on controlling a disease, powdery mildew, that has been causing significant production losses in recent years. The country has approximately 5,000 hectares of blackberry crops, involving around 15,000 producers in the Ecuadorian highlands. This research was conducted to evaluate the chemical control of powdery mildew (*Oidium sp.)* in blackberry (*Rubus glaucus*) using participatory research. A completely randomized block design was used with 3 treatments, 3 repetitions at each parcel of seven participant farmers. Three fungicide molecules were tested: Accord (difenoconazole), Sulflox (micronized sulfur), and ZT (hydrogen peroxide–27 %). The applications of fungicides were carried out two times and incidence and severity data were taken at 5 and 15 days. For yield, blackberries were counted and weighted. Initial and final surveys to farmers were carried out, accompanied by training. Analysis of variance and Tukey's test ($\alpha = 0.05$) were done for incidence, severity, and yield. Participatory research was detailed by means of previous knowledge and the assimilation of chemical treatment for powdery mildew. The best product for control of powdery mildew in all localities was Accord (difenoconazole). San José location had the best control of incidence (15.7 %). Farmers successfully complied with re-entry period in their orchards, in addition to adding Accord to their chemical control of powdery mildew.
Additional keywords: Disease control, incidence, severity

RESUMEN

Manejo de mildiú *(Oidium* sp.*)* de la mora *(Rubus glaucus):* Abordando el problema con investigación participativa

La mora es un cultivo generador de divisas para muchas familias de agricultores a pequeña y mediana escala en Ecuador. Existe la necesidad de contribuir con la investigación del control de una de las enfermedades que en los últimos años ha estado causando pérdidas significativas en la producción, el mildiú. El país tiene un aproximado de 5000 hectáreas de mora con cerca de 15000 productores entre los cuales se encuentran agricultores de la serranía ecuatoriana. Esta investigación se llevó a cabo para evaluar el control químico del oídio (*Oidium* sp.) de la zarzamora (*Rubus glaucus*) utilizando la investigación participativa. Se utilizó un diseño de bloques completamente al azar con 3 tratamientos, 3 repeticiones en cada parcela de siete agricultores participantes. Se probaron tres moléculas fungicidas: Accord (difenoconazol), Sulflox (azufre micronizado) y ZT (peróxido de hidrógeno–27 %). Las aplicaciones de fungicidas se realizaron dos veces y se tomaron datos de incidencia y severidad a los 5 y 15 días. Para determinar el rendimiento, se contaron y pesaron las moras. Se realizaron encuestas iniciales y finales a los agricultores, acompañadas del respectivo entrenamiento. Se realizaron análisis de varianza y pruebas de Tukey ($\alpha = 0{,}05$) para incidencia, severidad y rendimiento. La investigación participativa se detalló mediante los conocimientos previos y la asimilación del tratamiento químico para el oídio. El mejor producto para el control del patógeno en todas las localidades fue Accord (difenoconazol), el cual, en la localidad de San José tuvo el mejor control de la incidencia (15,67 %). Los agricultores cumplieron con éxito el periodo de reentrada en sus huertos, además de añadir Accord a su control químico del oídio.
Palabras clave adicionales: Control de enfermedades, incidencia, severidad

INTRODUCTION

Blackberry (*Rubus glaucus* Benth.) is a species native to the Andean region cultivated by small and medium-sized producers in Ecuador (Viteri et al., 2016). Blackberry is best established in a temperate to cold climate with an average annual temperature

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of 14 to 19 ^{0}C, with 14 ^{0}C being the best. Its development is optimal at an altitude of 1800 to 2500 meters above sea level, for its good development it needs a sandy-clay to clay-loam soil with good drainage and good humidity with a pH of 6.5 to 7.5 (Calero, 2010).

Blackberry orchards are distributed in the inter-Andean alley, specifically in the provinces of Carchi, Imbabura, Pichincha, Cotopaxi, Chimborazo, Bolívar and Tungurahua. The latter is the second province with the highest production of blackberries nationwide, contributing a national production of 33 % with a yield of 8 t·ha^{-1} (Barrera et al., 2017). Ecuador reports approximately 5000 ha of blackberries, with about 15000 small and medium-sized producers, obtaining an average of 5000 kg·ha^{-1} (Viteri et al., 2016). Five years ago, Sánchez et al. (2019) reported for the province of Tungurahua that blackberry crops covered 2,223 ha, which represented 42.36% of the total Ecuadorian planted area. The most frequent cultivars in the Ecuadorian highlands are the blackberry Colombian, with and without hawthorns, and Andimora (Barrera et al., 2017). A blackberry orchard in a small and well-managed area can produce 12 kg·year^{-1} per plant under a planting distance of 3x2 m, which represents an annual production of 18 t·ha^{-1} (Martínez et al., 2019).

Powdery mildew (*Oidium* sp.,) is a disease that drastically decreases blackberry production (Botero and Franco, 2007). The main symptom is deformation, curling of young leaves, irregular, diffuse and chlorotic spots that resemble a smooth mosaic on the leaf surfaces. Occasionally, a white powder can be observed on the underside of the leaves, which corresponds to the sporulating growth of the fungus that causes the disease (Tamayo, 2003). Yield losses could reach 42.5 % and symptoms occur at any stage of the crop cycle. Powdery mildew causes damage and loss to the foliage, and when the attack is intense, the leaves turn yellow and eventually dry out. On the other hand, indiscriminate use of fungicides to control diseases in blackberry has led to the detection of active ingredients in farms located in areas of the Cauca Valley, Colombia (Lagos et al., 2022).

The cultivation of blackberries is one of the economic sustenance’s of many families in both small and medium sized towns and villages, and it is necessary to contribute to research into the control of diseases that in recent years has been causing significant losses in production. Participatory research implies a complete and open inclusion of the participants immersed in the study, as collaborators in decision-making, committing themselves as equals to ensure their own well-being (Creswell, 2012). The methodological design involved in participatory action research includes initial or community contact, plan-making and implementation and evaluation of the outcomes of the intervention (Vaughn and Jacquez, 2020). It is vitally important to mention that the group of researchers must be involved with the community from the beginning, with the aim of fostering trust in the project and understanding that its execution is for the benefit of the community, to transform its reality. Participatory research was used in this research where a group of producers from the canton Tisaleo (Tungurahua), is involved in the production of new knowledge with a new management practice for powdery mildew of blackberry. Therefore, the objective of this study was to determine the best fungicide to control powdery mildew analyzing incidence and severity of the disease in seven localities (farmers) of blackberry producers.

MATERIALS AND METHODS

Experimental area. The study was carried out in seven orchards located in different sites of Tisaleo – Tungurahua- Ecuador (La Libertad, Santa Lucia, Agua Santa, San José, La Dolorosa, El Calvario and El Chilco) (Table 1), belonging to the humid zone of the province. The most common soils in the area of study have sandy loam texture, with pH ranging from 5.8 to 7.1.

Identification of management with growers and selection of participants. Growers mentioned that management of the disease consists of preventive practices such as sanitation pruning, frequently removing affected stems, before the sporulation of the pathogen spreads. Seven farmers were selected based on the recognition of the necessity to new alternatives to manage the disease in established orchards that were in production stage, and where the disease was present. Previously, two surveys were carried out for the participatory research: one at the beginning of the trial and the other at the end, with the aim of knowing the management of the mildew in the orchards. The final training was dictated in the terms that the farmer had to know how to make a good application of the product and take care of his health, to then socialize the results obtained in the research and publicize a good grace period and re-entry period.

Fungicide treatments. The following three fungicides were used to control the powdery mildew: Accord (difenoconazole, 1.25 $mL{\cdot}L^{-1}$), Sulflox (fungicide based on micronized sulfur, 5 $mL{\cdot}L^{-1}$, and ZT (hydrogen peroxide–27%, 1.25 $mL{\cdot}L^{-1}$), plus a control treatment without fungicide. For the selection of plants, the edge effect was avoided by selecting 3 plants for each fungicide treatment, with a total of 9 plants per plot. On the plant, a branch was chosen and marked, as well as a leaflet of the same branch to measure incidence and severity. Before applying the treatments, all the fruits present in 3 plants per batch were counted, as well as 3 fruits were collected from each plant and weighed. This same procedure was repeated at the end of the trial. Two applications were made in the trial, the first on day 0 and after 15 days the second application. Data collection was carried out at 5 and 30 days after fungicide application. A modification of the scale of Villarreal (2022), consisting of 6 classes, where class 0 indicates healthy plants without symptoms and Class 5 more than 50 % of the leaf area affected (Figure 1), was used to measure the incidence and severity of mildew. For that purpose, images were acquired with a phone camera with different percentages of disease.

Monitoring and disease evaluation. A database for digital registration was created to streamline the data registration process as the initial step in monitoring productivity. Farmers underwent training on utilization and data registration procedures. They had an active role in taking data and observations during the study at the seven sites. During this training, farmers practiced reporting data under the supervision of researchers to ensure the acquisition of high-quality data. Crop monitoring during harvests was conducted, with the frequency varying based on the harvest time of each site. Regular communication between farmers and researchers was maintained to ascertain the readiness of fruits for harvest. At the time of fruit harvest, the weight of the fruits was measured. Harvests were typically scheduled on Fridays and involved gathering products for sale during Saturdays at the local markets. Before applying the treatments, a count of all the fruits present on three plants, from their green state to their mature state, was carried out for each plot. Three mature fruits were collected from each plant to calculate the average weight. Average production per plant was obtained using the average weight per fruit by the number of fruits per plant. Production per treatment was calculated using production per plant by number of plants per treatment. This procedure was repeated at the end of the trial.

Experimental design and data analysis. A completely randomized design with four treatments, and three repetitions in the seven locations was used. The experimental unit was one blackberry plant.

Table 1. Geographical location of the orchards of this study in Tisaleo (Tungurahua), Ecuador

Site	Grower	Longitude	Latitude	Altitude (masl)	Planting distance (m)	Experimental area (m^2)
Libertad	Gloria Tipan	78°39'06"	1°21'25"	3117	1.3 × 2.0	1300
Santa Lucia	Jaime Merchan	78°39'41"	1°21'56"	3236	1.2 × 2.0	1440
Agua Santa	Lida Cuno	78°40'11"	1°21'59"	3218	1.0 × 2.2	990
San Jose	Edgar Tipan	78°40'34"	1°21'35"	3294	1.5 × 2.0	4500
La Dolorosa	Miguel Toapanta	78°40'40"	1°21'27"	3322	1.0 × 2.3	920
El Calvario	Martha Banda	78°40'33"	1°20'58"	3334	1.0 × 2.0	800
El Chilco	Felipa Panimbosa	78°41'03"	1°20'54"	3371	1.3 × 2.5	1625

CLASS	IMAGE	BLADE (%DAMAGE)
0		Healthy with no visible symptoms
1		<5% of affected area
2		5-10% of affected area
3		11-25% of affected area
4		26-50% of affected area
5		The affected area is >50%

Figure 1. Adjusted severity scale for powdery mildew of blackberry

To evaluate the effectiveness of treatments, productivity and the overall count of produced fruits were measured. Phytopathological variables consisted of incidence and severity taken at 5-day intervals. To identify significant differences analysis of variance and Tukey test were applied, using transformation to handle the percentages. All statistical analyses were conducted using the program InfoStat (Di Rienzo, 2005).

RESULTS AND DISCUSSION

The most prevalent disease in the studied localities was powdery mildew and a summary of its incidence and severity is presented in Table 2. For incidence, the coefficients of variation (C.V.) at 5 and 30 days after fungicide application were 7.7 % and 9.9 %, which indicate that the data on incidence are reliable and homogeneous. The interaction between fungicides and localities was not significant on day 5 (p = 0.860) and highly significant on day 30 (p = 0.008), meaning that at day 30 the effect of the fungicide varies according the specific location. In a similar way, for severity, the interaction between fungicides and localities was not significant on day 5 (p = 0.854), but became significant at day 30 (p = 0.014). For fungicides, as main factors, both incidence and severity were affected with a probability highly significant (p = 0.000), except for the incidence on day 5. The mean values of the products were statistically different across all locations.

The Tukey test ($p \leq 0.05$) revealed that the lowest incidence was present in San Jose Location where the best control of mildew was achieved with the product Accord (initial incidence 32.7 % at day 5, versus 15.7 % at day 30 after application) (Table 3), representing a control of 17.0 %. The highest incidence was found in La Dolorosa where the Sulflox fungicide (starting at 74.0 % at days 5, versus 63.3 % at day 30 after application) obtained a control of 10.7 %.

Considering severity, the lowest percentage was found in Santa Lucia and the best control of mildew was achieved with the product Accord (6.7 % at day 5 versus 3.0 % at day 30). The highest severity was found in La Dolorosa location where Accord reached a control of 15 % (28.3 % versus 13.3 %) (Table 3).

The most effective product for controlling powdery mildew was Accord which exhibited a significant control of incidence with a reduction of 37.29 %, as an average between locations (Table 3). This demonstrated a substantial improvement in powdery mildew control compared to Sulflox and ZT, which recorded values of 48.00 and 44.90 %, respectively. Both Sulflox and ZT were statistically similar but differed significantly from Accord. The severity with Accord started at 21.19 % and ended at 10.90 %; this last figure was statistically different from Sulflox (14.90%) and ZT (12.90 %).

Since orchards under study are planted in a humid zone, the high relative humidity probably prevented the rapid dispersion of the fungus; but, on the other hand, the scarce precipitation occurred during the study may have led to higher presence of the disease. Likewise, the high farm elevation, between 3,100 and 3,400 m above sea level (Table 1), suggests somewhat similar climatic conditions for the pathogen development. In this sense, Zhai et al. (2021) indicated that the infection level of powdery mildew in cold environments increased as temperatures rise.

Table 2. ANOVA summary for incidence and severity of powdery mildew (*Oidium* sp.) in blackberry (*Rubus glaucus*) using a participatory research approach

		Incidence		**Severity**		**Incidence**		**Severity**	
Source	**d.f.**	**Mean square**				***p*-value**			
		5 d*	30 d	5 d	30 d	5 d	30 d	5 d	30 d
Fungicide	3	21.24	4141.24	254.98	2787.44	0.325	0.000	0.000	0.000
Localities	6	20004.6	1581.73	391.56	271.95	0.000	0.000	0.000	0.000
Fung * Loc.	18	11.6	57.25	5.54	11.26	0.860	0.008	0.854	0.014
Error	56	17.98	24.4	8.68	5.15				
C.V. (%)		7.7	9.9	14.8	12.5				

d*: days after fungicide application

Incidence and severity by location. The severity progress curves by location (Figure 2) showed that at day 0, the percentages of incidence and severity were equal or very close for each treatment (i.e. 25 % severity in Agua Santa, or 66-68 % incidence in El Chilco), which validate the comparison between treatments at each location; this evidences an appropriate selection of the experimental unit.

The percentages of incidence and severity showed a constant trend (even increasing or decreasing) along the 30 days after fungicide application. The control treatment always increased in the seven locations, while the disease decreased when fungicides were applied. For instance, at 30 days, when considering the location La Libertad, the incidence for the control group increased 11 % (from 58 to 69%), and the incidence for Accord decreased 20 % (from 61 to 41 %). Likewise, regarding severity, the control increased 10 % (from 60 to 70 %), and with Accord decreased 12 % (from 27 to 15 %). In both cases, the fungicides ZT and Sulflox occupied intermediate positions (Figure 2).

In some locations, the percentages of decrease for incidence and severity were similar for the fungicides Accord and ZT. For example, in Santa Lucía, the decrease was 15 and 13 % for incidence and severity, respectively, and in La Dolorosa the decrease was 5 and 15 %; those values were equal for Accord and ZT. A few years ago, Mora et al. (2020) found that incidence of *Oidium* sp. in blackberry reached 12.50 % in a pathological diagnosis conducted in the municipality Gutiérrez, Cundinamarca, Colombia. On the other hand, González et al. (2023) evaluated the presence of the main fungal diseases affecting commercial varieties of blackberry (*Rubus* spp.) and observed 48.15 % incidence of powdery mildew in the crop leaves in the municipalities of Pamplona and Pamplonita, Norte de Santander, Colombia

At day 30, incidence on the control increased 28 % in San José and 10 % in La Libertad, Santa Lucía y Agua Santa. Intermediate percentages were found in the other locations. Severity on the control increased 20 % in Santa Lucia and about 15 % in the rest.

The fungicide Accord produced the higher decrease of the disease incidence along the time in La Libertad, Agua Santa, San José, El Calvario and El Chilco. The fungicides ZT and Sulflox presented intermediate results. Likewise, Accord produced the higher decrease of severity in Agua Santa.

Table 3. Effect of fungicides on powdery mildew on blackberry in seven localities evaluated at two different days using participatory research**.**

Fungicide	**Locality**	**Incidence (%)**		**Severity (%)**		**Yield**
		Day 5	**Day 30**	**Day 5**	**Day 30**	(kg·plant^{-1})
Accord	Libertad	60.7 a-c	40.3 b-f	26.7 a	15.0 a-d	1.57 a
Sulflox	Libertad	60.0 a-c	53.3 a-d	26.7 a	18.3 ab	1.03 ab
ZT	Libertad	62.7 a-c	53.3 a-d	26.7 a	18.3 ab	1.20 ab
Accord	Santa Lucia	42.0 d-f	27.7 fg	6.7 a	3.0 f	1.07 ab
Sulflox	Santa Lucia	42.3 d-f	38.0 c-f	6.7 a	4.3 ef	0.93 ab
ZT	Santa Lucia	41.3 ef	28.3 e-g	8.3 a	3.7 ef	1.13 ab
Accord	Agua Santa	56.7 b-d	41.0 c-f	25.0 ab	13.3 a-d	0.47 c
Sulflox	Agua Santa	60.3 a-c	50.0 a-d	25.0 ab	16.7 a-c	0.40 c
ZT	Agua Santa	60.0 a-c	49.3 a-d	25.0 ab	20.0 a	0.50 c
Accord	San Jose	32.7 f	15.7 g	16.7 b-d	8.3 d-f	1.27 ab
Sulflox	San Jose	33.0 f	28.0 e-g	15.0 b-d	10.0 c-f	0.97 ab
ZT	San Jose	30.7 f	23.7 fg	16.7 b-d	10.0 c-f	1.07 ab
Accord	Dolorosa	70.7 ab	55.0 a-c	28.3 a	13.3 a-d	1.57 a
Sulflox	Dolorosa	74.0 a	63.3 a	26.7 a	20.0 a	1.17 ab
ZT	Dolorosa	68.0 ab	55.0 a-c	26.7 a	11.7 b-e	0.57 c
Accord	Calvario	52.7 c-e	35.7 d-f	21.7 a-c	10.0 c-f	0.57 c
Sulflox	Calvario	57.9 b-d	49.3 a-d	21.7 a-c	16.7 a-c	0.50 c
ZT	Calvario	56.3 b-e	48.0 a-d	21.7 a-c	11.7 b-e	0.50 c
Accord	El Chilco	68.3 ab	45.7 a-e	23.3 a-c	13.3 a-d	1.83 a
Sulflox	El Chilco	66.0 a-c	54.0 a-c	25.0 ab	18.3 ab	1.07 ab
ZT	El Chilco	68.7 ab	56.7 ab	26.0 ab	15.0 a-d	1.60 a

Mean Square			
Fungicide	8.71ns	638.49**	84**
Location	1741.31**	1366.48**	212.31**

Means at each column with the same letter are not significantly different according to Tukey test ($p \le 0.05$).
ns: not significant, *: $p \le 0.05$, **: $p \le 0.01$

Accord performed better than Sulflox to control incidence at the seven locations, and, regarding severity, the same occurred in Agua Santa, La Dolorosa, El Calvario and El Chilco.

Overall, Accord was the most effective in controlling disease incidence, and had or shared with ZT the best efficiency in controlling severity at several locations.

Fungicide management. At the time of the first survey, five farmers were aware of the pre-harvest interval and two already had a basic concept of the subject. After the training on the subject, people assimilated the concept and all farmers mastered the concept. Also, at the time of the first survey, six farmers were aware of the re-entry period and one farmer was aware of the concept of the topic. After the training covered the topic, all farmers assimilated and mastered the concept of the re-entry period. When carrying out the first survey, the farmers reentered the plots after 6 hours after making a phytosanitary application due to the lack of knowledge of the re-entry period; after the training to the group of producers, a 24-hour agreement was reached for the resumption of agricultural work after a phytosanitary application. When carrying out the first survey, farmers harvested blackberries 2, 4 and 6 days after making a phytosanitary application due to the lack of knowledge of the grace period; after the training, the group of producers reached an agreement of 7 days for the blackberry harvest after the product application. In summary, the terms grace period and re-entry period were unknown by more than 50 % of farmers, when conducting and reporting the results of the research; and after training, it was concluded that the period of re-entry into the orchards would be after 24 hours, and 7 days the waiting period.

With the participation of the farmers in the trial, the training carried out and the explanation of the results obtained, the group of blackberry producers decided that the best fungicide for the control of mildew in their plots was Accord.

When averaging the yield obtained with the use of each fungicide, it was obtained that ZT promoted plant yield to 0.93 $kg{\cdot}plant^{-1}$, while Sulflox induced a yield of 0.86 $kg{\cdot}plant^{-1}$; the production with these two fungicides were statistically the same. Accord was the product that obtained the best production after its application, with 1.19 $kg{\cdot}plant^{-1}$. The locality with the lowest final production was Agua Santa (0.46 $kg{\cdot}plant^{-1}$), followed by El Calvario (0.52 $kg{\cdot}plant^{-1}$). El Chilco was the locality with the highest production with 1.50 $kg{\cdot}plant^{-1}$. By performing the Tukey test, it was detected that when using Accord in the best location (El Chilco) yield reached 1.83 $kg{\cdot}plant^{-1}$, whereas Sulflox in Agua Santa induced the lowest yield (0.40 $kg{\cdot}plant^{-1}$).

In our study, the best control of powdery mildew was obtained with 15.7 % incidence using Accord at a dose of 1.5 $mL{\cdot}L^{-1}$ at day 30. Similarly, Ati (2022) conducted a study with the use of penconazole (Topas), a fungicide that belongs to the triazole group along with difenoconazole (Accord), and found a better result with the dose of 1 $mL{\cdot}L^{-1}$ (12.03 % incidence). The lowest value of severity (7.93 %) obtained by Tamayo (2003) using a dose of 1 $mL{\cdot}L^{-1}$ of penconazole is higher than the value obtained in the present study in Santa Lucia (3.0% severity) using difenoconazole 1.5 $mL{\cdot}L^{-1}$. The difference could be due to the use of the higher doses of the product in our study, or to the different climatic and topographic conditions of localities of each study.

On the other hand, the percentages of incidence and severity of the disease when using Sulflox in the present study showed the best values of 28.0 % for incidence in San José, and 4.33 % for severity in Santa Lucia. These values differed from those of Chacha (2023), who carried out a study using micronized sulfur (Azufrol) for the handling of powdery mildew and obtained a 13.74 % incidence and 9.37 % severity.

Accord was the best fungicide for the control of powdery mildew. In the incidence of the disease it reduced its value to an average of 37.29 %, and for the severity its value was 10.90 % (Table 3). These values represent a good control when compared to the other products such as Sulflox that ended with a 48.0% incidence and 14.90 % severity and ZT its completion was with a 44.90% incidence and 12.90 % severity. In the interaction of fungicide/locality, Accord continued to be the best product for powdery mildew control, the incidence was better controlled in San José with a 30-day value of 15.7 % and for the severity in Santa Lucia was where the control was obtained a low value of 3.0 %.

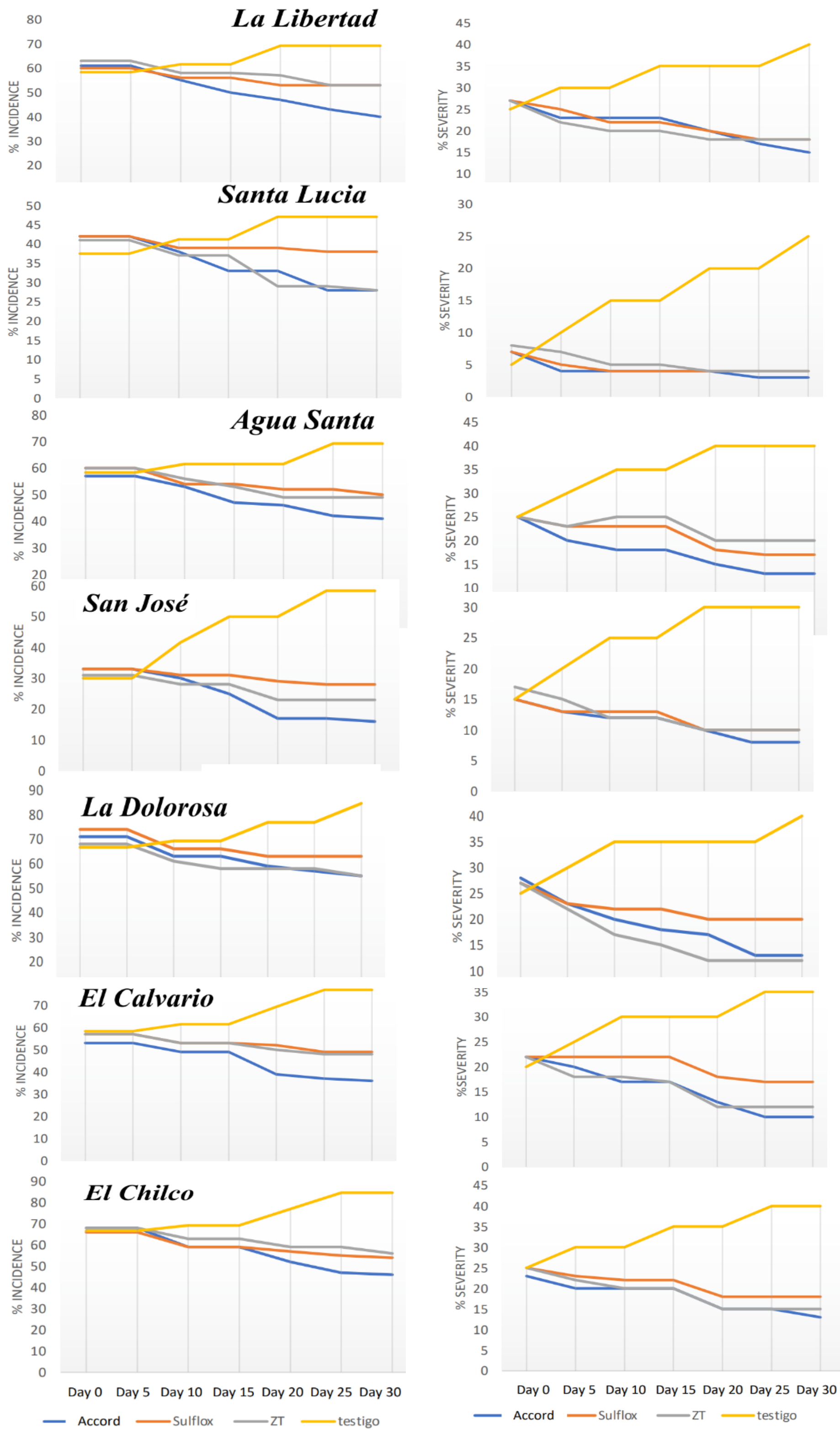

Figure 2. Effect of three fungicides on the severity progress curves of powdery mildew (*Oidium* sp.) of blackberry (*Rubus glaucus*) in seven localities using a participatory research approach.

CONCLUSIONS

Accord was the most effective fungicide for controlling powdery mildew, significantly reducing the incidence and severity of the disease compared to the control group, ZT, and Sulflox. In the interaction between treatment and location, Accord remained the best product for controlling powdery mildew.

Regarding blackberry production, Accord was the product that better promoted yield increase, achieving the highest production in Chilco location with 1.83 kg·plant^{-1}, followed by ZT with 1.60 kg·plant^{-1}. Sulflox, on the other hand, showed the lowest production, as seen in Agua Santa (0.40 kg·plant^{-1}), where this data should be considered for future research and product applications.

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