



Article

Evaluation of Fungicides and Application Strategies for the Management of the Red Leaf Blotch Disease of Almond

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Abstract: Red leaf blotch (RLB) of almond, caused by *Polystigma amygdalinum*, is an important foliar disease of this nut tree in the Mediterranean basin and especially in Spain. In recent years, the control of this disease has become a key factor in the management of Spanish almond orchards. The management of RLB is not easy due to intrinsic factors of the disease (e.g., long infection and latency periods) and the low number of registered fungicides in this country. From 2015 to 2019, different field trials were conducted in the Lleida region, NE Spain, to evaluate the efficacy of several fungicide products and of application strategies to control this disease. Systemic fungicides, which included fluopyram, trifloxystrobin, and mixtures of fluopyram + trifloxystrobin and pyraclostrobin + boscalid, performed better than contact and penetrant products and showed up to 90% control against RLB. However, the efficacy of the tested fungicides varied depending on the year. In terms of application strategies, when fungicide applications were conducted following specific meteorological conditions (after 15 days from >15 mm rainfalls with ≈10–15 °C as the minimum average temperature), their efficacy was comparable to that of calendar-based treatments (every 14, 21, or 31 days from petal fall) but with fewer applications (depending on the year, 2–4 applications as compared with 5–9 for calendar treatments).

Keywords: almond; disease management; fungicide; *Polystigma amygdalinum*; *Prunus dulcis*; red leaf blotch disease



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1. Introduction

Almond (*Prunus dulcis* (Mill.) D.A. Webb) acreage and production has increased worldwide over the last few years, mainly due to the development of new cultivars with interesting agronomic characteristics, such as late-blooming and self-fertility, and the change towards new production models in irrigated areas that improve the agronomic and productive characteristics such as the bearing precocity and yield [1–3]. Spain, the country with the largest almond cropping area in the world, leads this acreage increase with over 140,000 ha planted within the last ten years for a total of 687,225 ha [4]. However, in the last decade, crop intensification and plantations in new areas are facing new challenges in the management of almond pests and diseases, such as the occurrence of new diseases and the reemergence of old ones. An example of a reemergent disease is the red leaf blotch (RLB) of almond [5]. The incidence of RLB has increased recently in Spain, mainly due to the expansion of new almond orchards to inland areas, where climatic conditions are more suitable for RLB development [6], and the use of more susceptible cultivars in new plantations [7]. Furthermore, the highly intensive production systems introduced in Spain during recent years, such as high-density plantations, have increased favorable conditions for the development of almond fungal diseases [8,9]. Therefore, disease management is a

key factor in achieving the maximum productive potential to ensure the economic viability of these new almond orchards [10].

RLB is caused by the ascomycete *Polystigma amygdalinum* P.F. Cannon and is one of the most important leaf diseases currently affecting almond trees in the Mediterranean basin and the regions of the Middle East, and particularly in Spain [7,11]. RLB only affects almond leaves, causing diffuse spots of different shapes and sizes, initially yellowish but later turning into reddish brown. In spring, under favorable temperature and humidity conditions, and especially after rain events, ascospores are released from the leaf litter and infect new almond leaves [7,12,13]. In severe RLB infections, early defoliation may occur [11,14], with a consequent decrease in the tree photosynthetic activity and a possible yield reduction, not only in the current season but also in the long term if the infection is persistent [6,15].

According to Almacellas [6], RLB management strategies are generally based on: (i) the use of tolerant cultivars, (ii) crop management practices intended to reduce primary inoculum and therefore the risk of infection, and (iii) the use of fungicides. Cultivar tolerance to RLB should be part of a long-term control strategy, as it has been observed that tolerant cultivars such as ‘Mardía’ or ‘Vairo’ can help in reducing RLB infections and thus facilitate the control of the disease [7]. However, ‘Guara’ and ‘Tuono’, two highly susceptible cultivars [7], are the most widely planted in Mediterranean countries [3,16], which makes it necessary to consider other management strategies besides cultivar selection. Some cultural practices are focused on eliminating the primary inoculum from the infected leaves that have fallen in the previous year [11], e.g., through the application of crystalline urea on the leaf litter [17]. However, in-season applications of fungicides are still needed to prevent infections and their use is a common control practice in Mediterranean almond orchards.

Regarding the control of RLB with fungicides, the evaluation of plant protection products has been mainly conducted in Middle East countries, mostly in Iran [12,18–22]. According to the results obtained by these studies, mancozeb, copper hydroxide and oxychloride, Bordeaux mixture, and triforine were the most effective fungicides.

One of the major concerns regarding RLB control in Spain is the low number of registered products currently available to use [23]. Moreover, it is difficult to implement an annual fungicide management program without applying the same registered products repeatedly, which can eventually lead to an emerging resistance in pathogen populations [24]. Therefore, it is important to explore new compounds and application strategies that are able to be used in the management of RLB with fungicides.

This study aimed to improve the current control strategies for RLB management in Spain based on the optimization of the usage of fungicides. To achieve this overall goal, some systemic and non-systemic fungicides were tested for their efficacy in a first assay. In a second assay, different application strategies with the best product were evaluated for RLB control.

2. Materials and Methods

2.1. Experimental Plots

Field trials were conducted in two commercial orchards located in the Lleida region, NE Spain: Alcarràs (UTM coordinates: WGS84 Datum, 31 T x = 283381, y = 4608774) and Vilagrassa (x = 341313, y = 4612125). The orchard in Alcarràs was a standard open-vase of ‘Guara’ cultivar grafted onto ‘INRA GF 677’ rootstock, planted in 2000 with a tree spacing distance of 5 m × 5 m. The orchard in Vilagrassa was a standard open-vase of ‘Tarraco’ cultivar grafted onto ‘INRA GF 677’, planted in 2007 with a tree spacing distance of 7 m × 6 m. ‘Guara’ and ‘Tarraco’ cultivars were chosen for the trials because they are the most susceptible cultivars to RLB that are commonly planted in Spain [7]. All trials were designed as a randomized complete block design with four replicates and four trees per experimental unit.

2.2. Fungicide Products Application

All the fungicide compounds used in this study were purchased as commercial products (Table 1). The products in all trials were applied as recommended by the manufacturers (Table 2). The rates for untested or non-registered fungicides on almonds in Spain were evaluated using similar doses to those used on other stone fruits or vegetables (Table 2) [23]. No other fungicides were applied in the orchards during the experimental period.

Table 1. Fungicide products evaluated for the control of the almond red leaf blotch between 2015 and 2019 in the Lleida region, Spain.

| Active Ingredient | Chemical Group ¹ | FRAC Group ² | Commercial Name | Manufacturer | Formulation ³ | Registered Concentration ⁴ |
|--------------------------------|-----------------------------|-------------------------|-----------------|------------------------------|---|---|
| Captan | Phthalimide | M4 | Capteran 50 | Adama Agriculture España SA | 500 g kg ⁻¹ WG | 2.5–3.0 g L ⁻¹ (peach) |
| Captan | Phthalimide | M4 | Blancado 85 | Comercial Química Massó SA | 850 g kg ⁻¹ WG | Not registered ⁵ |
| Copper oxide | Copper | M1 | Nordox 30/30 | Comercial Química Massó SA | 338 g kg ⁻¹ WG | Not registered ⁵ |
| Copper oxide | Copper | M1 | Nordox 45 | Comercial Química Massó SA | 450 g kg ⁻¹ WP | 1.5–2 g L ⁻¹ (vegetables) |
| Cyflufenamid | Amidoxine | U6 | Siz | Sipcam Iberia SA | 51.3 g L ⁻¹ EW | 0.5 mL L ⁻¹ (almond) |
| Cyproconazole | Triazole | 3 | Caddy 10 petite | Bayer CropScience SL | 100 g kg ⁻¹ WG | 0.1–0.2 g L ⁻¹ (peach) |
| Dodine | Guanidine | M7 | Syllit Flow | Arysta Lifescience Iberia SL | 544 g L ⁻¹ SC | 1.1–1.3 mL L ⁻¹ (almond) |
| Fenbuconazole | Triazole | 3 | Impala Star | Dow Agrosciences Iberica SA | 25 g L ⁻¹ EW | 3.0–8.4 mL L ⁻¹ (almond) |
| Fenbuconazole | Triazole | 3 | Impala | Dow Agrosciences Iberica SA | 50 g L ⁻¹ EW | 1.5–2.0 mL L ⁻¹ (almond) |
| Fenpyrazamine | Pyrazolium | 7 | Prolectus | Kenogard SA | 500 g kg ⁻¹ WG | 0.8–1.2 g L ⁻¹ (peach) |
| Fluopyram | Carboxamide | 7 | Luna Privilege | Bayer CropScience SL | 500 g L ⁻¹ SC | 0.3–0.5 mL L ⁻¹ (peach) |
| Folpet | Phthalimide | M4 | Folpan 80 | Adama Agriculture España SA | 800 g L ⁻¹ WG | 2.0 g L ⁻¹ (vegetables) |
| Myclobutanil | Triazole | 3 | Sythane 25 | Dow Agrosciences Iberica SA | 25 g L ⁻¹ EW | 2.0–6.0 mL L ⁻¹ (vegetables) |
| Penthiopyrad | Carboxamide | 7 | Fontelis | Dupont Iberica | 200 g L ⁻¹ SC | 1.5 mL L ⁻¹ (vegetables) |
| Tebuconazole | Triazole | 3 | Orius | Nufarm España SA | 200 g L ⁻¹ EW | 0.9–0.1 mL L ⁻¹ (apricot) |
| Thiram | Carbamate | M3 | Tiram Flow | Exclusivas Sarabia SA | 500 g L ⁻¹ SC | Not registered ⁵ |
| Trifloxystrobin | Strobilurin | 11 | Flint | Bayer CropScience SL | 500 g L ⁻¹ WG | 0.2 g L ⁻¹ (peach) |
| Fluopyram + trifloxystrobin | Carboxamide, strobilurin | 7 11 | Luna Sensation | Bayer CropScience SL | 250 g L ⁻¹ and 250 g L ⁻¹ SC | 0.6–0.8 mL L ⁻¹ (vegetables) |
| Isopyrazam + difenoconazole | Pyrazole, triazole | 7 3 | Embreli | Adama Agriculture España SA | 100 g L ⁻¹ and 40 g L ⁻¹ SC | 1 mL L ⁻¹ (peach) |
| Pyraclostrobin + boscalid | Strobilurin, carboxamide | 11 7 | Signum | Basf Española SL | 67 g kg ⁻¹ and 267 g kg ⁻¹ WG | 1.0 g L ⁻¹ (almond) |
| Tebuconazole + trifloxystrobin | Triazole, strobilurin | 3 11 | Flint Max | Bayer CropScience SL | 500 g L ⁻¹ and 250 g L ⁻¹ WG | 0.3 mL L ⁻¹ (peach) |

¹ Lewis et al. [25]. ² Fungicide Resistance Action Committee (FRAC). List of fungicides' common names [26]. ³ WP, wettable powder; WG, water dispersible granule; EW, water emulsion; SC, suspension concentrate. ⁴ Registered concentrations in Spain [23]. ⁵ Not registered use in Spain [23].

Table 2. Trials of fungicides against the almond red leaf blotch conducted in 2015–2017 in the Lleida region, Spain, using alternating products.

| Trial | Year | Location | Cultivar | Fungicide ¹ | Rate (%) | Alternate Product ² | Starting Time ³ | Timing between Sprays | Number of Sprays |
|-------|------|----------|----------|--|---|--------------------------------|----------------------------|-----------------------|------------------|
| 1 | 2015 | Alcarràs | ‘Guara’ | Captan 50% Cyproconazole 10% Dodine 54.4% Captan 50d% + dodine 54.4% | 0.250 0.015 0.100 0.250 0.100 | Thiram 50% at 0.250 | Petal fall | Every 14 days | 4 |
| 2 | 2016 | Alcarràs | ‘Guara’ | Cyproconazole 10% Fenbuconazole 5% Fluopyram 20% Fluopyram 20% + trifloxystrobin 20% Penthiopyrad 20% Pyraclostrobin 6.7% + boscalid 26.7% Tebuconazole 20% Thiram 50% Trifloxystrobin 50% | 0.015 0.150 0.040 0.040 0.150 0.100 0.100 0.250 0.015 | Captan 50% at 0.250 | Petal fall | Every 14 days | 4 |
| 3 | 2017 | Alcarràs | ‘Guara’ | Fenbuconazole 2.5% Fenpyrazamine 50% Fluopyram 25% + trifloxystrobin 25% Isopyrazam 10% + difenoconazole 4% Penthiopyrad 20% Pyraclostrobin 6.7% + boscalid 26.7% Tebuconazole 20% Thiram 50% Trifloxystrobin 50% | 0.300 0.120 0.040 0.150 0.150 0.100 0.100 0.250 0.015 | Folpet 80% at 0.200 | Petal fall | Every 21 days | 4 |

¹ Untreated control (UTC) included in all trials. ² Product used between each spray of tested fungicides. ³ Phenological stage [27].

Products were applied to runoff using a manual sprayer (Gaysa, Librilla, Spain) with a single nozzle. The volume of the fungicide solution was calibrated to approximately 1000 L/ha, which is a common commercial rate used in Spanish almond-growing regions. An untreated control (UTC) was included in each trial using tap water instead of fungicide solutions.

2.3. Experimental Trials

2.3.1. Fungicide Selection Trials

In the first stage, different fungicide products were evaluated in the period of 2015–2019 in different trials (Tables 2 and 3). All the fungicide applications were made in the spring-summer period (March–July), starting at petal fall [27], which usually occurs during the second half of March. The spray timings were set on a calendar basis (every 14 or 21 days), and the number of applications was established (4 to 5) before trials onset.

The fungicides were tested by being alternated with other fungicides in 2015, 2016, and 2017 (Table 2), and by being tested as single products in 2015, 2018, and 2019 (Table 3).

Table 3. Trials of fungicides against the almond red leaf blotch conducted in 2015–2019 in the Lleida region, Spain, using single products.

| Trial | Year | Location | Cultivar | Fungicide ¹ | Rate (%) | Starting Time ² | Timing Between Sprays | Number Of Sprays |
|-------|------|----------|----------|--|---|----------------------------|-----------------------|------------------|
| 1 | 2015 | Alcarràs | ‘Guara’ | Captan 85% Copper oxide 30% Copper oxide 45% Copper oxide 30% + captan 85% | 0.150 0.075 0.050 0.075 0.150 | Petal fall | Every 14 days | 5 |

Table 3. Cont.

| Trial | Year | Location | Cultivar | Fungicide ¹ | Rate (%) | Starting Time ² | Timing Between Sprays | Number Of Sprays |
|-------|------|------------|-----------|--|----------|----------------------------|-----------------------|------------------|
| 2 | 2018 | Alcarràs | 'Guara' | Cyflufenamid 5.13% | 0.500 | Petal fall | Every 21 days | 4 |
| | | | | Dodine 40% | 0.200 | | | |
| | | | | Fenbuconazole 2.5% | 0.300 | | | |
| | | | | Fenpyrazamine 50% | 0.120 | | | |
| | | | | Folpet 80% | 0.200 | | | |
| | | | | Fluopyram 20% + trifloxystrobin 20% | 0.040 | | | |
| | | | | Penthiopyrad 20% | 0.150 | | | |
| | | | | Pyraclostrobin 6.7% + boscalid 26.7% | 0.100 | | | |
| | | | | Trifloxystrobin 25% + tebuconazole 50% | 0.030 | | | |
| 3 | 2019 | Vilagrassa | 'Tarraco' | Dodine 54.4% | 0.125 | Petal fall | Every 21 days | 4 |
| | | | | Fenbuconazole 2.5% | 0.300 | | | |
| | | | | Myclobutanil 2.5% | 0.060 | | | |
| | | | | Penthiopyrad 20% | 0.150 | | | |
| | | | | Pyraclostrobin 6.7% + boscalid 26.7% | 0.100 | | | |

¹ Untreated control (UTC) included in all trials. ² Phenological stage [27].

2.3.2. Application Strategies Trials

Different application strategies were evaluated in 2017–2018 (Table 4) using pyraclostrobin + boscalid, one of the registered products for almonds in Spain that performed better in the above screening trials. All strategies started at petal fall. In all cases, the spray programs did not include any alternating fungicide, and the final seasonal number of fungicide applications was dependent on the tested strategy. UTC sprays with tap water were performed at each cadence interval. We compared the calendar-based applications (every 14, 21, or 31 days) with the spray programs based on the meteorological data. For the latter, sprays were conducted after >15 mm rainfall, or 15 days after >15 mm rainfall with ≈10–15 °C as the mean minimum temperature. These action thresholds were set according to the results obtained by Miarnau et al. [7] and Zúñiga et al. [28], who showed that temperature and rainfall data from specific periods were related to the seasonal RLB incidence and the primary inoculum dynamics, respectively.

Table 4. Trials of fungicide application strategies against the almond red leaf blotch conducted in 2017–2018 in Alcarràs, Lleida region, Spain.

| Trial | Year | Fungicide | Rate (%) | Strategy ¹ | Starting Time ² | Application Timing | Number of Sprays |
|-------|------|--------------------------------------|----------|-----------------------|----------------------------|--|------------------|
| 1 | 2017 | Pyraclostrobin 6.7% + boscalid 26.7% | 0.100 | Cadence 1 | Petal fall | Every 14 days | 9 |
| | | | | Cadence 2 | Petal fall | Every 21 days | 6 |
| | | | | Meteorological 1 | Petal fall | After >15 mm rainfalls | 5 |
| | | | | Meteorological 2 | Petal fall | 15 days after >15 mm rainfall with ≈10–15 °C as mean minimum temp. | 3 |
| | | | | | | | |
| 2 | 2018 | Pyraclostrobin 6.7% + boscalid 26.7% | 0.100 | Cadence 1 | Petal fall | Every 21 days | 7 |
| | | | | Cadence 2 | Petal fall | Every 31 days | 5 |
| | | | | Meteorological 1 | Petal fall | 15 days after >15 mm rainfall with ≈10–15 °C as mean minimum temp. (applications until June) | 2 |
| | | | | | | 15 days after >15 mm rainfall with ≈10–15 °C as mean minimum temp. | 4 |
| | | | | Meteorological 2 | Petal fall | | |

¹ Untreated control (UTC) included in all trials. ² Phenological stage [27].

2.4. Disease Assessment

In each trial, a sample of 100 leaves per replicate block (50 leaves per each two central trees in each experimental unit) of each treatment was evaluated once in the summer (July or August, depending on the year). Fully expanded leaves were randomly collected from new shoots at different heights and orientations located in the outer canopy of each tree. The disease incidence and severity were evaluated according to the procedures described by Miarnau et al. [7]. The RLB incidence was recorded as the percentage of leaves showing at least one identifiable RLB lesion regardless of its size, whereas RLB severity was estimated from the mean proportion of the affected leaf surface. The efficacy of the products was calculated from the severity data using a modification of Abbott's formula [29]: $\text{Efficacy} = 100 \times (1 - (S_T/S_{UTC}))$, where S_T stands for the mean severity index in a given treatment and S_{UTC} stands for the mean severity index in UTC.

2.5. Data Analysis

Experimental data were analyzed using JMP (Version 16.0.0, SAS Institute Inc., Cary, NC, USA). Each trial was analyzed separately. Linear mixed models, including fungicide treatment or application strategy as fixed factors and block as a random factor, were fitted to the dependent variables of disease incidence, severity, and fungicide efficacy. The percentage data were arcsine-transformed prior to analysis. The mean comparisons among treatments were evaluated by Tukey–Kramer's test at $\alpha = 0.05$.

3. Results

RLB incidence and severity in the UTC were the highest among treatments in nearly all trials, with values roughly in the range from 60 to 90% and from 20 to 40%, respectively. In trials conducted in 2018 in Alcarràs, the UTC showed the lowest values of RLB incidence (35% and 45% for the fungicide selection and application strategy trials, respectively) and severity (12% in both trials) from among all experiments and years.

3.1. Fungicide Selection

Several fungicides were evaluated in 2015–2017 in alternating sprays with contact compounds such as thiram (in 2015), captan (2016), and folpet (2017) (Table 2, Figure 1). The trial conducted in 2015 with cyproconazole, dodine, captan, and a mixture of dodine and captan confirmed that cyproconazole and dodine significantly reduced RLB incidence ($p < 0.001$), whereas only cyproconazole additionally reduced severity ($p < 0.001$) (Figure 1A). In terms of efficacy, cyproconazole performed the best in this trial (over 90%), and the rest of the products showed less than 40% efficacy (Figure 1B). Captan and the mixture made of dodine and captan showed the lowest efficacies (3.6 and 12.4%, respectively), with no significant differences found between them. Cyproconazole and dodine showed efficacy values that were significantly higher ($p < 0.001$) than the rest of the products. In 2016, significant reductions in RLB incidence and severity between all the products and the corresponding UTC were detected (all $p < 0.001$) (Figure 1C). All the fungicides showed efficacy indices greater than 50% (Figure 1D). The five best products this season, in the order of efficacy, were fenbuconazole, trifloxystrobin, pyraclostrobin + boscalid, fluopyram, and fluopyram + trifloxystrobin; all of them showed efficacies greater than 75%. In 2017, four products significantly reduced disease incidence and severity with respect to the UTC ($p < 0.001$), namely fluopyram + trifloxystrobin, fenpyrazamine, pyraclostrobin + boscalid, and trifloxystrobin (Figure 1E). Four additional fungicides (tebuconazole, penthiopyrad, isopyrazam + difenoconazole, and thiram) showed significant differences in their mean severity values with respect to the UTC ($p < 0.001$). These values resulted in overall efficacies in the range between 60 and 90%, except for fenbuconazole, with an overall efficacy of only 43% (Figure 1F).

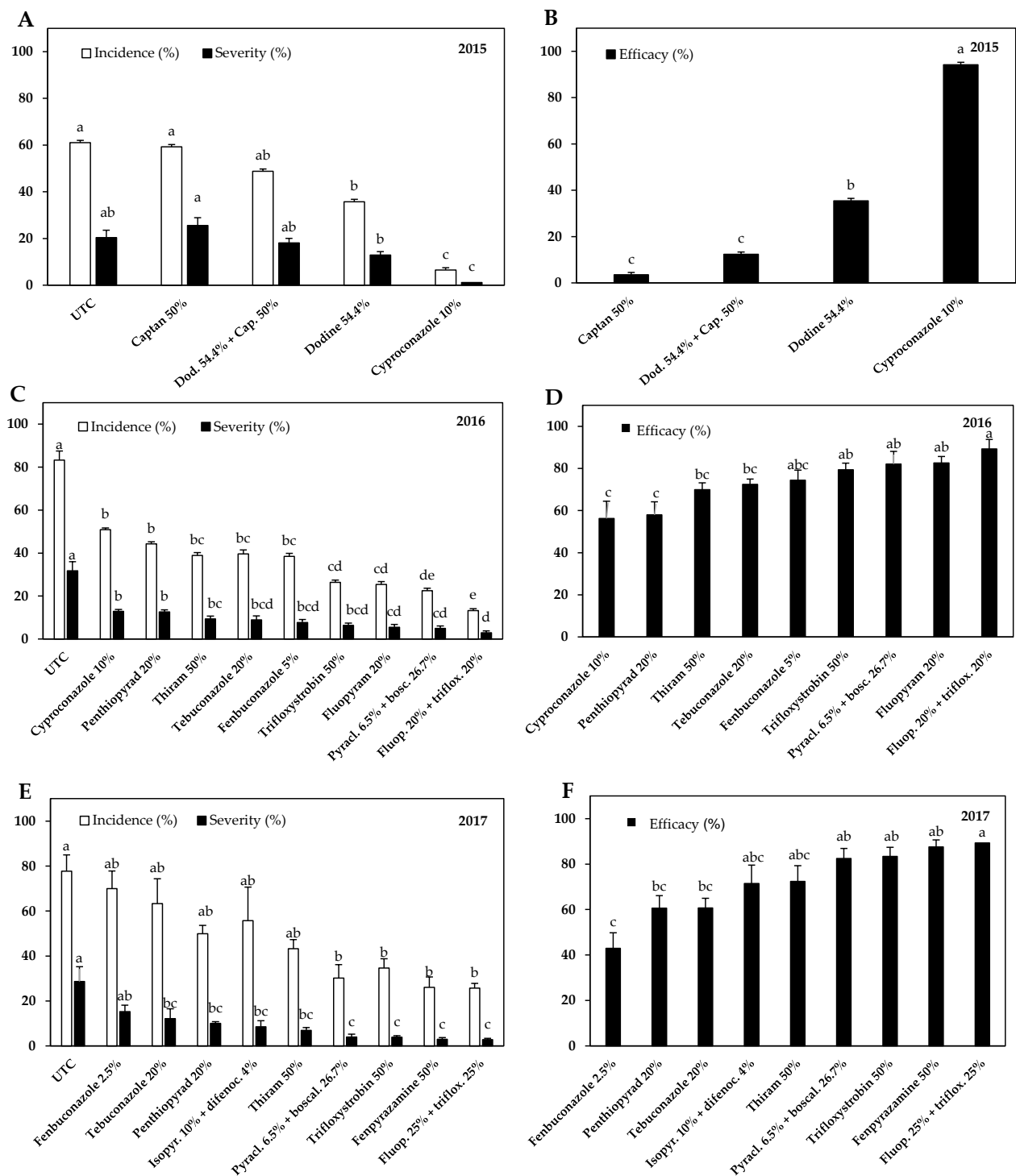


Figure 1. Disease incidence and severity (A,C,E) and control efficacy (B,D,F) of red leaf blotch in almond trees managed with different contact and systemic fungicides in combination with alternate products (2015–2017). The efficacy was calculated using Abbott’s formula [29]. Treatment means with different letters are significantly different according to Tukey–Kramer’s test ($p < 0.05$). The error bars indicate the standard error of the mean.

Regarding the fungicide trials without alternate products, a trial conducted in 2015 involving different copper-based products and an additional mixture of copper/captan indicated that copper products showed an overall disease reduction of less than 25% (Figure 2A,B).

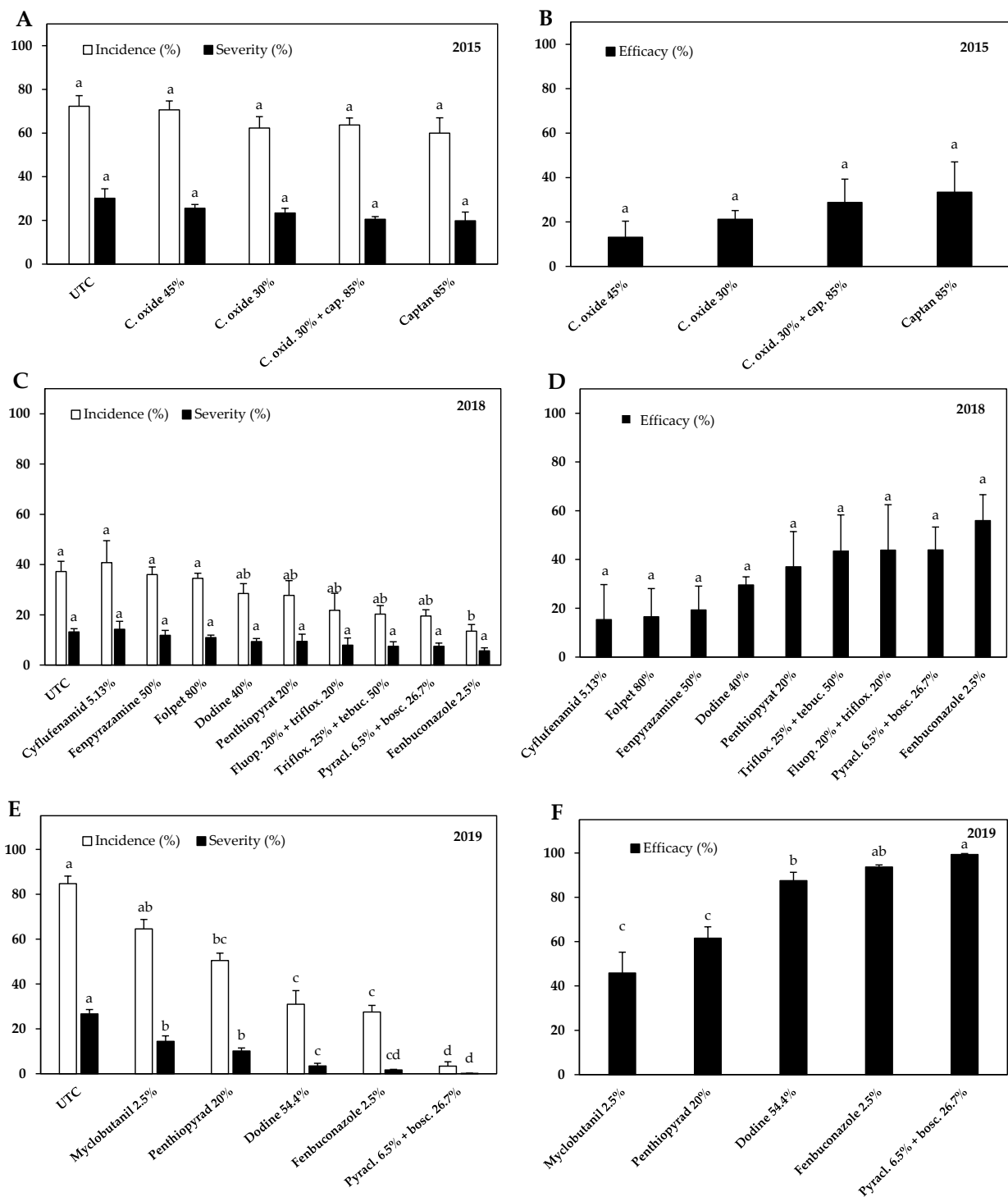


Figure 2. Disease incidence and severity (A,C,E) and control efficacy (B,C,F) of red leaf blotch in almond trees managed with different contact and systemic fungicides in non-alternating sprays (2015, 2018, and 2019). The efficacy was calculated using Abbott's formula [29]. Treatment means with different letters are significantly different according to Tukey–Kramer's test ($p < 0.05$). The error bars indicate the standard error of the mean.

The highest efficacies were obtained with captan and the copper/captan mixture (33.4% and 28.8%, respectively). However, no significant differences were detected between treatments and the UTC in terms of RLB incidence, severity, and treatment efficacy (Figure 2A,B). In 2018 and 2019, trials mostly included systemic fungicides. In 2018, the

incidence and severity of RLB were low in the UTC (less than 40% and 20%, respectively) and in the fungicide treatments (Figure 2C). Among the tested products, only fenbuconazole showed a significant reduction in incidence compared to the UTC ($p < 0.001$), which corresponded to a 56% efficacy. Pyraclostrobin + boscalid, fluopyram + trifloxystrobin, and trifloxystrobin + tebuconazole showed similar, higher efficacies (43–44%). However, no significant differences in terms of efficacy were detected among fungicides in 2018 (Figure 2D). In 2019, a trial was conducted to evaluate some of the best products tested in previous years, which included the most efficient, newly registered products in Spain for the management of almond diseases. All tested products significantly reduced both RLB incidence and severity ($p < 0.001$), except for myclobutanil, which only reduced RLB severity (Figure 2E). Pyraclostrobin + boscalid, fenbuconazole, and dodine, listed in a decreasing order of efficacy, showed efficacy indices greater than 85% (Figure 2F).

3.2. Application Strategies

All the evaluated strategies showed a moderate to high control of the disease, with higher efficacies in 2017 (over 70% on average) than in 2018 (40–50%). In 2017, significant reductions in RLB incidence and severity were found between all treatments and the UTC ($p < 0.001$) (Figure 3A).

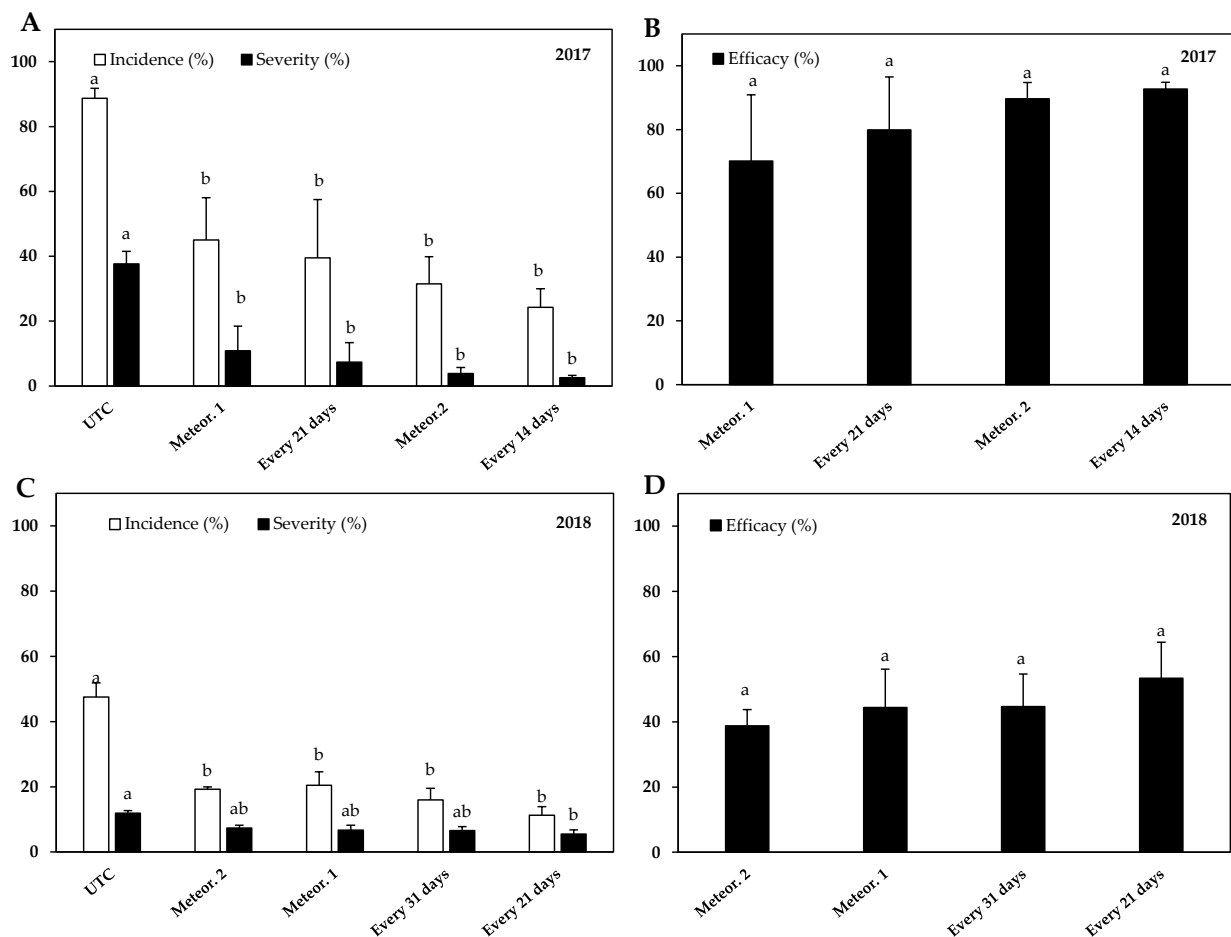


Figure 3. Disease incidence and severity (A,C) and control efficacy (B,D) of red leaf blotch in almond trees managed with different strategies of fungicide sprays (2017 and 2018). The efficacy was calculated using Abbott's formula [29]. Treatment means with different letters are significantly different according to Tukey–Kramer's test ($p < 0.05$). The error bars indicate the standard error of the mean.

In 2018, significant differences in RLB incidence were also found between all treatments and the UTC, but only for one of the treatments in the case of RLB severity (Figure 3C). In addition, no significant differences were found among treatment efficacies, although treatments with a fixed application cadence generally showed higher efficacies (80–93% in 2017 and 45–54% in 2018), as compared to the application strategies following epidemiological criteria (70–90% in 2017, and 39–44% in 2018) (Figure 3B,D). However, a smaller number of spray applications was used in the weather-based strategies (2 to 5 applications) as compared to the calendar-based ones (5 to 9) (Table 4).

4. Discussion

An effective management of almond RLB using fungicides demands that the most effective fungicides be used in improved application strategies, especially in those areas which are planted with highly susceptible cultivars to RLB. In Middle Eastern countries, previous research reported a good efficacy for the performance of triforine and a medium efficacy for some copper-based products, with one application at petal fall and two additional applications at 14-day intervals [12,18,19]. In Spain, captan, thiram, and mancozeb have been recommended for decades for use from petal fall to mid-summer [6,10,30], but all of these are no longer accepted [31]. In recent years, new systemic fungicide products have been marketed in Spain to control different fungal diseases in almonds other than RLB [23]. Therefore, we were interested in evaluating the suitability of these new products for the control of the RLB disease. In addition, we were especially interested in developing an optimized strategy for the timing of the application of these products.

P. amygdalinum is a hemibiotrophic pathogen [28] that cannot be isolated and grown on synthetic culture media [12]. Consequently, the evaluation of the efficacy of fungicides is better performed under natural field conditions that are suitable for pathogen development. This is a serious constraint for a number of fungicides and strategies to be tested under such conditions. However, our trials were conducted in an area where RLB occurs naturally [7,28,30]. In the current study, the UTC incidence levels during the experimental period (60–90%) were comparable to those reported by Miarnau et al. [7]. The results obtained in the fungicide selection trials confirmed the differential behavior of fungicides in terms of RLB control efficacy.

In the first stage, we were interested in the evaluation of contact (captan, copper-based fungicides, folpet, and thiram) and penetrant (dodine) products. Overall, these products presented a high variability in efficacy (4–88%), with 4–5 spray applications performed after petal fall every 14 or 21 days. Among these contact products, dithiocarbamates (thiram, with 70–72% efficacy in 2016 and 2017) performed better than phthalimides (captan and folpet, with 4–33% and 17% efficacy, respectively, depending on the year). Differences in the efficacy values for captan could likely be due to the different percentage of the active ingredient used in the formulated captan products (50% or 85%), as higher RLB control was observed in the latter case. Similarly, dodine performed better when applied alone (88% efficacy) at a higher concentration (54.4%) than when applied at a lower (40%) concentration (29.6% efficacy). Moreover, the efficacy of dodine at a higher concentration significantly decreased when it was applied alternately with thiram (35.5% efficacy) or mixed with captan (12.4% efficacy). It is suggested that the better efficacy performance of dodine, as compared to phthalimides, could be due to its different mode of action (i.e., as a membrane disruptor) and its additional penetrant action [25].

Copper-based products, commonly used as fungicides in autumn and winter applications [32], showed low levels of RLB control in seasonal applications (less than 30% efficacy). Similarly, results with low to moderate levels of RLB control were obtained by Banihashemi [12], Tork et al. [19], and Amanifar [18], thus confirming that copper-based compounds would not be the best products to choose for the management of RLB.

The systemic products evaluated in this study were tested in alternate combinations with captan (in 2016) and folpet (2017) or alone (2018 and 2019). The overall efficacy of systemic fungicides was higher than that observed with contact and penetrant products.

The systemic products used in this study are either included in FRAC groups 3 (sterol biosynthesis inhibitors), 7 (succinate dehydrogenase inhibitors), 9 (methionine biosynthesis inhibitors), or 11 (respiration inhibitors) [26]. The fungicides in FRAC group 7 (fluopyram, penthiopyrad, and boscalid) and FRAC group 11 (trifloxystrobin and piraclostrobin) were the most effective, with efficacies between 60 and 90% depending on the year and the type of trial. Furthermore, the efficacy was higher when the commercial product was a mixture of two active ingredients belonging to FRAC groups 7 and 11. To the best of our knowledge, this is the first time that these products have been tested in field conditions for the control of RLB. Fluopyram was reported to control the fungal diseases of vegetable and fruit crops caused by powdery mildews, *Botrytis*, *Monilinia*, and *Sclerotinia* [33]. The combination of Fluopyram + trifloxystrobin is registered in the USA for the control of the blossom blight, shot hole, rust, and hull rot of almonds [34]. Strobilurin products (pyraclostrobin and trifloxystrobin) have been also reported to be highly effective against different fungal diseases [35], and specifically against rust, brown rot, and the shot hole diseases of the almond [36].

Contact and penetrant products (captan, folpet, and dodine) may be good candidates for use in an alternated combination with systemic fungicides, as they could help in avoiding the emergence of resistant pathogen strains. The alternate use of captan and folpet in 2016 and 2017, respectively, in combination with several systemic fungicides, resulted in overall higher efficacies. Some of the efficacies obtained in 2016 and 2017 with the same fungicide (e.g., fluopyram + trifloxystrobin and pyraclostrobin + boscalid) but using two different alternate products, were similar. On the other hand, some systemic fungicides (e.g., fenbuconazole) performed differentially in combination with an alternate product, but further research is still needed to clarify the alternate use of fungicides in terms of RLB control efficacy. Finally, contact products may need multiple applications, as they can be washed off by rain, and new, growing shoots may need continued periodic protection.

In general, the efficacies of fungicides were lower in years with low levels of disease (e.g., in 2018) in both the fungicide screening and the application strategy trials, as compared to the rest of the experiments. This issue could be related to less precise measurements of both disease incidence and severity at low disease levels, which resulted in a higher variability and non-significant mean treatment comparisons and hence, in a disputed disease control efficacy [37].

As a general overview, we classified the fungicide products used in this study into three main categories depending on their overall efficacy during the trials. In the first category, higher efficacies (75–95%) were shown by fluopyram, trifloxystrobin and its mixture, pyraclostrobin + boscalid, trifloxystrobin + tebuconazole, and fenbuconazole. A second group with moderate efficacies (50–75%) included penthiopyrad, fenpyrazamine, thiram, isopyrazam + difenoconazole, tebuconazole, cyproconazole, and dodine. Finally, the third group showed the lowest efficacies (<50%) and included folpet, cyflufenamid, myclobutanil, captan, and copper products.

Regarding the evaluation of the different application strategies, all the treatments reduced the disease levels compared to the UTC, but no significant differences were found between strategies in terms of the disease incidence reduction. Treatments with a fixed application cadence showed higher efficacies as compared to the application strategies following the meteorological criteria, although the differences were not statistically significant. These results agree with different authors [12,18–22,38] who recommended one fungicide application at petal fall and two additional ones at 14 days as the most effective program in reducing the rate of disease. However, great differences in the number of total seasonal applications were observed among the strategies, which is relevant in view of the economic and environmental implications. In line with this observation, the most sustainable strategies followed the meteorological criteria, especially the strategy of combining hydrothermal variables (15 days after >15 mm rainfall, with ≈ 10 – 15 °C as minimum temperature). This strategy only accounted for 2–4 annual applications, which dramatically contrasted with the number of fungicide sprays conducted in the calendar-based strategies (5 to 9). These

meteorological strategies could prevent leaf infections, as hydrothermal parameters are likely related to ascospore outbreaks from ascocarps [12], and the progress of symptom expression [7].

This work is a first step in the developing of a sustainable fungicide program based on a prediction model for the management of RLB disease with fungicides. Further research is therefore needed to validate the best product combinations and their application at specific moments where disease infections may occur.

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