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RESEARCH

Control of Mal secco disease in lemon by drip irrigation with fungicide

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Abstract Mal secco disease of citrus is caused by the mitosporic ascomycete fungus *Plenodomus tracheiphilus* (formerly *Phoma tracheiphila* [Petri]). Mal secco is a highly destructive vascular disease of lemon and other citrus which is presently confined to the Mediterranean basin and has a marked economic impact on the citrus industry. The fungal pathogen infects the host tree by penetrating through wounds in the roots or canopy. Infection spreads quickly into the main branches and trunk and tree mortality usually ensues. The most typical symptoms are veinal chlorosis, leaf wilt, red discoloration of the xylem and dieback of twigs and branches. Current accepted control of the disease is mainly by sanitation of infected wood, and copper application during the winter to prevent germinating spores from infecting the plant. No effective chemical control for this disease has been reported. We present a drip-irrigation protocol to protect trees and control the disease in which we apply 250 g/ha of the triazole fungicide flutriafol five times a year. Progression of disease symptoms in the treated trees was inhibited by up to 81% compared to the untreated control, thereby significantly shortening the sanitation process and making it less costly than in untreated trees. Moreover, disease symptoms became less severe as the duration of treatment increased. We have treated orchards for 3 years, and present an effective commercial protocol for the growers which will help them control Mal secco disease.

Keywords Lemon · Fungal disease · Control · Irrigation

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Introduction

Mal secco disease caused by *Plenodomus tracheiphilus* (Petri) Gruyter, Aveskamp & Verkley (formerly *Phoma tracheiphila* [Petri] (Kant. & Gik.) is a devastating disease of citrus in the Mediterranean basin, around the Black Sea and in Asia Minor (EPPO & CABI, 1990; Palm, 1987; Solel, 1976; Timmer et al., 1988). Almost all citrus species are susceptible to *P. tracheiphilus* if inoculated artificially. In the field, the disease is highly destructive to lemons (*Citrus x limon)*), citron (*Citrus medica*) and lime (*Citrus aurantifolia*) (Graniti & Perrotta, 1988). The fungus and the disease have been studied and documented extensively in work focusing on epidemiology, the toxins involved in the pathogenicity of the fungus and possible methods of disease management (Chorin & Chorin, 1956; Fogliano et al., 1998; Graniti & Perrotta, 1988; Migheli et al., 2009; Nicasio et al., 2000;

Palm, 1987; Rotem et al., 1998; Solel, 1976; Solel et al., 1972; Timmer et al., 1988).

In Israel, the pathogen usually infects the host tree by penetrating through wounds in leaves and branches (Horin et al., 1966). Typical symptoms include chlorosis of leaf veins, leaf wilt, red discoloration of the xylem and dieback of twigs and branches. The pathogen proceeds slowly downward from the infected young shoots to the branches and main limbs; when the trunk and roots are infected, the tree dies. This movement is aided by phialoconidia, which are translocated in the xylem via the transpiration flow (Palm, 1987; Timmer et al., 1988). When infection occurs through the roots, sudden death of the tree, caused by rapid infection of the trunk, is typically observed (Graniti & Perrotta, 1988).

Control of the disease is mainly based on sanitation of diseased plant tissue, accomplished by cutting and burning dry and symptomatic branches and uprooting trees. This method is time-consuming and very expensive in most countries where the disease is found, and is only partially effective. Copper-based chemicals are applied during the winter in an effort to control the germinating spores of the fungus, which are disseminated by rain and wind. However, due to the very low persistence of these compounds on the canopy during rain events, this method of control is ineffective. No effective application of other chemicals has been shown, probably due to the location of the pathogen in the xylem, which protects it from the chemicals when it is inside the plant (Migheli et al., 2009). In the case of woody plants, systemic chemical products are not effective in the woody tissue but may have a limited effect when applied on leaves and wounds (Wu et al., 2020). El Boumlasy et al. (2022) demonstrated the effect of a new superabsorbent copper-containing polymer on the viability of the pathogen in infected lemon twigs under laboratory conditions, demonstrating its potential as a new control method for the disease in orchards. In addition, recent studies have explored the application and use of microorganisms for biocontrol and management of the disease (Aiello et al., 2022; Leonardi et al., 2023), as well as for induction of the plant defense system against the pathogen (Sicilia et al., 2023). However, to date, biological control of the disease has not been shown to be more effective than copper application (Aiello et al., 2022; Leonardi et al., 2023). The idea of drenching trees, including citrus, with chemicals is not new (Fares et al., 2009; Farih et al., 1981; Mondello et al., 2018), but it has not proven effective against Mal secco.

In this study, we report the development of a drip-irrigation protocol for a chemical control product that can protect trees from, and control Mal secco disease in lemons.

Materials and methods

Lemon grove type and location

In 2016–2017, we evaluated chemicals' possible influence on disease development when applied through drip irrigation. The experiment was conducted in a 1-ha, 3-year-old lemon grove located in the village of Oza in the northeast Negev desert in Israel. The eastern half (0.5 ha) of the lemon grove was planted with lemon cv. VillaFranca grafted on Volkamer lemon (*Citrus volkameriana*) rootstock and the western half with cv. VillaFranca on Macrophylla (alemow, *Citrus macrophylla*) rootstock. Tree spacing was 6 m between rows and 4 m between trees in a row. The western part of the orchard was chosen for the experiment because a large number of trees expressed Mal secco symptoms, whereas the trees in the eastern half, grafted on Volkamer lemon rootstock, did not express symptoms.

Experimental design and chemicals used

The experimental plot consisted of 419 trees at planting (2014). By the second year (2016), about 30% of the trees had been uprooted due to Mal secco disease. During the 2016–2017 season, we tested the influence of four different fungicides: flutriafol (250 g/ha), azoxystrobin (800 g/ha), myclobutanil (480 g/ha) and carbendazim (5000 g/ha), by applying them five times at 1-month intervals from mid-September to mid-October 2016 (two times) and from the first week of March to the first week of May 2017 (three times). The experiment had three repeats (tree rows) for each treatment, each row with 17–23 trees. Together with the untreated rows, there were 15 rows in the experiment. The experiment was conducted in a block design. The fungicides were applied via the drip-irrigation system with a power pump (self-manufactured, with 3–6 bar pressure buildup). The fungicides were applied after the plot had been watered with 10 m^3/ha and the drip-irrigation system was washed post-application of the fungicides with 50 m^3/ha water. Disease symptoms were evaluated 10 times (every 2 weeks) from the beginning of March 2017 to the beginning of June 2017.

In 2017, we planted 128 new 1.5-year-old trees to replace the dead, uprooted ones. We could not purchase VillaFranca grafted on Macrophylla trees because most of the commercial nurseries in Israel had stopped producing this combination, so we planted Villafranca grafted on Volkamer lemon instead. The grove had 15 rows with 26 to 29 trees per row. At least 16 trees in each row were of the VillaFranca–Macrophylla combination (Table 1). Each row served as a repeat for a treatment, with three repeats (tree rows) per treatment. The experiment and treatment application were conducted for 2 years, in the 2017–2018 and 2018–2019 seasons.

Table 1 Structure of the experimental plot in the village of Oza (2017–2018)

Row number	Number of trees in row	Number of VillaFranca on Macrophylla*	Treatment
1	26	20 (77%)	Control
2	27	21 (78%)	Hosen 2 L/ha
3	27	22 (81%)	Hosen 1 L/ha
4	27	20 (74%)	Hosen 2 L/ha**
5	27	13 (48%)	Hosen 4 L/ha
6	27	20 (74%)	Control
7	28	22 (78%)	Hosen 2 L/ha
8	28	17 (60%)	Hosen 1 L/ha
9	28	16 (57%)	Hosen 2 L/ha**
10	28	18 (64%)	Hosen 4 L/ha
11	29	20 (65%)	Control
12	29	21 (72%)	Hosen 2 L/ha
13	29	20 (69%)	Hosen 1 L/ha
14	29	19 (65%)	Hosen 2 L/ha**
15	29	21 (72%)	Hosen 4 L/ha

Each row served as a repeat for a treatment, with three repeats (tree rows) per treatment.

*Percentage of trees grafted on Macrophylla rootstock.

**Rows that received Hosen for an additional year prior to the beginning of the experiment.

Concentration and timing of applications

In 2017–2018 and 2018–2019, an experiment was conducted to evaluate the effect of fungicide concentration, using the most effective of the four tested products. Five treatments were applied to the Oza orchard: (i) water control – untreated, (ii) Hosen (flutriafol 125 g/L, Cheminova, Denmark) 2 L/ha (250 g/ha, 2nd year of application), (iii) Hosen 2 L/ha (250 g/ha), (iv) Hosen 1 L/ha (125 g/ha), (v) Hosen 4 L/ha (500 g/ha). The treatments were applied five times in total per year: on 19 Oct and 28 Nov 2017 (1 month apart) and later on 13 Mar (right after the first spring irrigation of the orchard), 11 Apr and 15 May 2018 (1 month apart); and on 14 Oct and 16 Nov 2018 (1 month apart) and later on 5 May (right after the first spring irrigation of the orchard), and 28 May 2019 (note that there were only two applications in the spring of 2019 due to technical reasons). The product was applied by injection into the drip system using a pump. The fungicide was added after a short irrigation with water (about 30% of the amount given to the orchard at the relevant time of the year). At the end of the Hosen application, the trees were further irrigated with 50 m^3/ha of water. Each treatment was applied to three rows of trees, 26 to 29 trees/row. Each row was considered a repeat. The experiment was set up in a block design (Table 1). The same treatment was applied in 2017 to rows used as untreated controls and rows treated with 2 L/ha Hosen in 2016 to evaluate the fungicide's cumulative effect. Rows treated in 2016 with ineffective products were used for the other treatments. The same treatment design was repeated in seasons 2017–2018 and 2018–2019.

Mal secco symptom evaluation

The percentage of newly infected trees in the different treatment groups at each evaluation point was calculated by marking the new dry twigs and branches displaying at least two additional symptoms, such as leaf fall, petioles with no leaf attached to the branch or orange-pink discoloration of xylem. Evaluations were performed every 2 weeks from the beginning of April to the end of June. Accordingly, the control or ineffectively treated trees had new symptomatic branches and twigs appearing with time of evaluation, whereas on the trees with effective treatment, less new symptomatic branches and twigs appeared as time progressed.

All trees in the experiment were subjected to sanitation by pruning and removal of symptomatic twigs and branches before the treatment at the beginning of the 2017–2018 season. No further sanitation was applied to the trees after the treatment during the years of the experiment to enable evaluation of new disease symptoms (new dry twigs and branches).

Hosen application in "model orchards"

Following the results obtained from the Oza experiment, in the 2018–2019 season, we treated three more infected orchards: two lemon orchards in Patish village in the Negev desert (Eureka helen lemon grafted on Volkamer lemon) and Paame Tashaz village in the south of Israel (VillaFranca grafted on Volkamer), and one lime orchard in Kibbutz Gonen in the Golan Heights, northern Israel (Tahiti lime grafted on Volkamer), with different concentrations of Hosen. All experiments were conducted for 2 years (2018–2019 Seasons). Hosen was applied in the spring (March, April, and May) and autumn (October, November). Whereas the Oza orchard was young (3-year-old trees at the beginning of the experiment) with relatively small trees, these three orchards were older (6 to 9 years old at the beginning of the experiments) with bigger trees.

Data analysis

Trees displaying symptoms were counted at each evaluation time and marked on the plot map. The cumulative percentage of trees with new symptoms was calculated for each treatment, based on all relevant repeats. Student–Newman–Keuls (SNK) test was used for statistical analysis with significance at $p < 0.05$ (JMP V. 14 software).

Results

Experimental design and chemical used

The most effective fungicide of the four tested in 2016–2017 was Hosen (flutriafol 125 g/L) applied at 2 L/ha (250 g/ha active ingredient), with a significantly lower percentage of trees displaying disease symptoms at the end of the experiment (first week of June 2017) (Table 2). The percentage of trees treated with the other chemicals that displayed new disease symptoms did not differ significantly from the untreated control (Table 2).

Concentration and timing of applications and Mal secco symptom evaluation

In the 2017–2018 and 2018–2019 seasons, we treated the trees with Hosen at different concentrations to find the lowest and most effective concentration in terms of decreasing Mal secco symptoms in the trees. Mal secco symptoms appear in Israel at the beginning of spring when the trees start to bloom, around the beginning of April to mid-June when the temperatures rise and disease development decreases. We evaluated the appearance of new Mal secco symptoms seven times, approximately every 2 weeks from mid-April to the end of June (Table 3). It should be noted that symptom evaluation was initiated before the last application of Hosen in mid to end of May, to be able to identify the development of symptoms or their delayed appearance due to the treatment effect over time.

We noted that at the beginning of spring 2018, there were fewer disease symptoms on the trees as compared to the end of spring (end of June 2018), when more trees expressed symptoms. The trees treated with Hosen 2 L/h for two successive years (year II) did not display symptoms, whereas on the trees treated with Hosen 2 L/ha (year I), we could see symptoms. Hosen 1 L/ha (year I) was the least effective treatment, whereas Hosen 4 L/ha (year I) was as effective as Hosen 2 L/ha (year II) (Table 3).

Table 2 Evaluation of new Mal secco symptoms in the Oza orchard in the 2016–2017 season

Treatment	Percentage of trees with new symptoms– cumulative result
Untreated control	24.19 a
Flutriafol 250 g/ha	3.05 b
Azoxystrobin 800 g/ha	31.14 a
Myclobutanil 480 g/ha	21.42 a
Carbendazim 5000 g/ha	17.84 a

Results are presented as percentage of infected trees out of all living trees in the respective treatment group. Different letters indicate significant difference according to SNK, $P < 0.05$.

Table 3 Evaluation of new Mal secco symptoms in the Oza orchard in 2018 (2017–2018 season)

Treatment	Percentage of trees with new symptoms – cumulative result						
	17 Apr	**3 May**	**15 May**	**28 May**	**8 Jun**	**20 Jun**	**1 Jul**
Control	1.65a	8.35 a	10.00 a	11.65 a	15.00 a	16.65 a	20.00 a
Hosen 2 L/ha (year II)	0.00 a	0.00 a	0.00 a	0.00 b	0.00 b	0.00 b	0.00 b
Hosen 1 L/ha (year I)	0.00 a	3.41 a	3.41 a	3.41 ab	3.41 ab	5.11 ab	6.76 ab
Hosen 2 L/ha (year I)	0.00 a	0.00 a	0.00 a	1.77 ab	3.54 ab	5.31 ab	5.36 ab
Hosen 4 L/ha (year I)	0.00 a	0.00 a	0.00 a	0.00 b	0.00 b	0.00 b	0.00 b

Results are presented as percentage of infected trees out of all living trees in the respective treatment. Different letters in a column indicate significant difference according to SNK, $P < 0.05$.

We applied the same treatments to the same trees during the second year of the experiment (2018–2019). The same results were found for the different treatments at the end of the second-year evaluation (Table 4). The Hosen 2 L/ha (year II) treatment resulted in a higher percentage of trees with symptoms than the Hosen 2 L/ha (year III) treatment, but this percentage was still significantly lower than the control. Similar to the first year, Hosen 1 L/ha (year II) reduced the percentage of symptomatic trees, but not as well as Hosen 2 L/h (year II or III). The most effective treatment was Hosen 4 L/ha (year II), with the lowest percentage of trees showing disease symptoms (Table 4).

Hosen application in model orchards

The aim of the model orchards was to examine the application of Hosen on trees in other locations that are of different size, age and type, and grown under different conditions. The lemon variety in Patish was Eureka Helen grafted on Volkamer. In Paame Tashaz village, VillaFranca was grafted on Volkamer and in Gonen in the Golan Heights (northern Israel), Tahiti lime was grafted on Volkamer, compared to VillaFranca grafted on Macrophylla in Oza. Although the varieties and orchard locations differed, we could still see a reduction in symptoms on the treated trees compared to the untreated controls; and the higher the concentration of Hosen applied, the larger the reduction in the percentage of symptomatic trees compared to the untreated control at the end of the experiment (Table 5). However, the reduction in disease symptoms in the Oza orchard was much higher than that in the three model orchards.

Discussion

Controlling Mal secco disease in citrus is a challenging task. Extensive research has been conducted to find control solutions, but to date, the most effective treatment method remains sanitation, i.e., the pruning of symptomatic branches and twigs, and removal of infected trees displaying disease symptoms (Migheli et al., 2009; Nigro et al., 2011). The conventional

Table 4 Evaluation of new Mal secco symptoms in the Oza orchard in 2019 (2018–2019 season)

Treatment	Percentage of symptomatic trees–cumulative result				
	9 Apr	**10 May**	**24 May**	**6 Jun**	**30 Jun**
Control	20.00 a	46.67 a	68.33 a	68.33 a	71.67 a
Hosen 2 L/ha (year III)	0.00 b	0.00 c	4.92 bc	6.51 bc	6.51 bc
Hosen 1 L/ha (year II)	1.52 b	16.96 b	21.93 b	27.36 b	30.84 b
Hosen 2 L/ha (year II)	6.95 ab	8.89 bc	12.22 bc	15.56 bc	19.18 bc
Hosen 4 L/ha (year II)	2.56 b	2.56 bc	2.56 bc	2.56 bc	2.56 bc

Results presented as percentage of infected trees out of all living trees in the respective treatment. Different letters in a column indicate significant difference according to SNK, $P < 0.05$.

Table 5 Evaluation of new Mal secco symptoms in the Patish, Paame Tashaz and Gonen orchards compared to Oza orchard in 2019 (2018–2019 season)

Treatment	Percentage of symptomatic trees compared to untreated control at the end of the experiment*			
	Patish	Paame Tashaz	Gonen	Oza
Control	100	100	100	100
Hosen 1 L/ha (year II)	64.5	80.7	77.5	43.0
Hosen 2 L/ha (year II)	53	39.7	41.3	29.5
Hosen 4 L/ha (year II)	26.8	18.1	36.7	3.5

Results are given as percentage of trees displaying symptoms compared to the number of symptomatic trees in the respective control.

*Final evaluation of new symptomatic trees was conducted on 28 Jul 2019 for Gonen, 11 Jul 2019 for Paame Tashaz, 4 Aug 2019 for Patish and 30 Jun 2019 for Oza.

approach involves pruning symptomatic branches in spring and early summer when the symptoms are more easily recognized, below the area where symptoms are visible, primarily as dryness and discoloration of the xylem. A few years ago, we revealed that to prevent the fungus from remaining below the cut area, the branch should be cut approximately 0.5 to 1 m below the disease symptom (Ezra et al., unpublished results; Migheli et al., 2009). Branches, leaves, and all plant residues should be removed from the orchard and burned; otherwise, the fungal fruiting bodies (pycnidia) on the infected tissues can serve as a source of infection (Migheli et al., 2009; Nigro et al., 2011).

Other solutions explored over the years include developing and selecting resistant lemon varieties. Several resistant varieties have been reported in the literature, created by collecting field-resistant varieties (Thanassoulopoulos, 1991), classical breeding, mutation by irradiation or chemical induction (Gulsen et al., 2007; Uzun, 2008), or by more sophisticated methods, such as somaclonal variation (Nadel & Spiegel-Roy, 1987), somatic hybridization (Tusa et al., 2000) and genetic transformation (Gentile et al., 2007; Russo et al., 2009). However, in most cases, the new resistant or tolerant lines have inferior fruit quality and yield (Migheli et al., 2009). Another examined method is biological control of the disease, primarily using antagonistic microorganisms and induction of the plant's defense system (Sicilia et al., 2023). Several studies have shown that endophytic bacteria can reduce the intensity of infection by the pathogen (Aiello et al., 2022; Coco et al., 2004; Leonardi et al., 2023). Antagonistic soil microorganisms have also been found effective against the pathogen (Cirvilleri et al., 2005; Migheli et al., 2009). Inoculation of trees with viroids, such as Citrus exocortis viroid, resulted in a decrease in disease symptoms, likely due to the activation of the plant's systemic defense mechanism (Solel et al., 1995). But aside from sanitation, none of the mentioned approaches provide a repeatably effective method of controlling the disease.

Chemical control was proposed in as early as 1927 by Petri, who sprayed the soil with manganese sulfate and immediately afterward, the tree canopy with copper oxychloride, Bordeaux mixture and sodium arsenate (Petri, 1932). Attempts to apply substances such as calcium cyanamide and succinic acid to the soil were also made, but despite reports of disease symptom reduction, researchers also reported damage to tree growth and development (Nigro et al., 2011). Spraying copper-based and other synthetic compounds reduced the number of infections but failed to eradicate the fungus if the plants were already infected before treatment. The preventive treatment of spraying copper-based compounds in winter immediately after rain events is still commonly practiced in orchards today. However, this practice is still seen as not very effective, mainly because the rain during this period washes these substances off the leaves, requiring a large number of applications in winter to achieve effective infection reduction (Migheli et al., 2009; Solel et al., 1972). Other synthetic fungicides shown to reduce the disease in the 1970s include benomyl, which, according to researchers, is absorbed by the plant through the roots, leaves and cortex, travels through the plant's apoplast, and accumulates in the upper parts of the plant and leaf tips; and compounds sprayed on the tree canopy, including triphorine, thiocur, maneb, methyl-thiophanate, and methyl-benzimidazole. However, in field trials conducted by Somma et al. (1978), it was concluded that these substances do not reach effective amounts in the leaves and bark (Nigro et al., 2011). Attempts were also made to control Mal secco disease by injecting chemical control products directly into the xylem (Solel et al., 1972) or applying the compounds directly to the roots of the tree by pouring it onto the soil. As part of a large project in Israel (2014–2019) to find new approaches for the control of Mal secco in citrus, and mainly in lemon and lime, we tried applying chemicals through the irrigation system. The logic was that slow application through the water would influence the ability of the active ingredients to penetrate and migrate in the tree tissue. Chemigation, which is the use of irrigation systems for the application of chemicals with the water, has been used for decades as fertilizers, herbicides, insecticides and more (Johnson et al., 1986). Fungigation—the application of approved fungicides via the irrigation water system—is an alternative option to aerial and ground application and can be an effective method of applying agrochemicals without increasing field traffic, especially when crop height restricts ground applications or in order to reduce the amount of fungicide used or to try to refine its application to a limited area around the plant root (Yost et al., 2022). Fungigation is mostly described for the treatment of vegetables against soil borne and foliar diseases (Alaoui et al., 2021; de Aguiar et al., 2014; Johnson et al., 1986; Tolentino Júnior et al., 2011; Yan et al., 2023). The use of fungigation for the treatment of woody plants such as fruit trees to control trunk and foliar diseases is less common. In 2020, FMC Corporation announced that the fungicide Rhyme® (active ingredient flutriafol) is effective against trunk disease complex in grapes and tree nuts when applied by pressurized irrigation system (FMC, 2020). In another example, fungigation was used for the control of downy mildew and powdery mildew diseases in vine nurseries, and the economic impact of using fungigation vs. the conventional method of spraying was assessed (Dimitrova et al., 2022). In our experiments, we tested fungigation as a possible method to control Mal secco in citrus. First, we tested different fungicides in *in-vitro* petri plate experiments and found flutriafol, azoxystrobin, myclobutanil and carbendazim to be the most active against the pathogen *P. tracheiphilus*. We applied commercial products containing these active compounds via drip-irrigation-system delivery in the orchards and chose to continue our experiment with the one that was most effective at reducing the disease symptoms in the orchard: Hosen (flutriafol active ingredient). Over the next few years, we examined the effects of this compound's concentration and duration of application (1 year vs. 2 years, and even 3 years of application in 2019). As result of the observed influence of the treatments on the appearance of new symptomatic trees in the experimental orchard, we expanded the research by adding three more orchards in three different locations, to test the treatments under different conditions: orchard location, citrus type and tree age and size. Analyzing the results from all four orchards, we concluded that the size of the trees and the severity of infection at the beginning of the treatment were critical factors in the treatment's success. Low infection severity (less than 30% of the tree volume symptomatic) and relatively small tree size (height up to 2.5 m) at the beginning of the application resulted in better control of the disease symptoms. Trees with more than 30% infected volume were not affected by the treatment at any of the examined concentrations; they declined and died, even under the highest concentration of Hosen (data not shown). We recommend trimming the trees to no more than 2.5 m in height; this will allow better and easier sanitation of dry and infected branches. Trees with more than 30% infection should be uprooted and removed from the orchard because it is very difficult to cure them and they serve as inoculum for infection of other trees in the orchard. It is recommended to start applying Hosen 2 L/ha as soon as the first symptoms of the disease are diagnosed in the young orchard. Our experience is that in most cases when the pathogen is established in the orchard, it can take months to years for the symptoms to appear on other trees; however, when they do, symptoms often appear simultaneously on many trees in the orchard. We found that there is a cumulative effect of the treatment: the longer the treatment is applied (on the order of years), the less sanitation of dry symptomatic branches is needed and we see less trees dying from the disease. Sanitation of dry symptomatic branches should be performed based on inspection of the orchard once a month, and preferably every 2 weeks during the spring (March to end of June) and autumn (October to December) when conditions are favorable for symptom development. Pruning of symptomatic branches should be to at least 100 cm (1 m) below the observed disease symptom (dead wood, pink-orange coloration of the xylem). We found that continual application of Hosen 2 L/ha five times a year reduces symptoms significantly more than Hosen 1 L/ha (Tables 3–5). Although the Hosen 4 L/ha treatment significantly reduced disease symptoms, even compared to the Hosen 2 L/ha treatment, some phytoxicity was observed after the first year of application in the Oza orchard, expressed as dried out leaf edges. This phytotoxicity disappeared with the growth of new leaves, and we assume that it was due to application of the higher concentration at the first irrigation of the trees in the spring, when the upper layer of the soil is dry. We did not see phytotoxicity if the orchard was first irrigated with water and then Hosen 4 L/ha was applied (data not shown). However, in this latter case, we did notice a minor delay in blooming that disappeared completely at our second evaluation of disease symptoms in June.

The Oza orchard served us as the main experimental orchard: it was the first to be treated and the one in which the treatments were applied for the longest period. The other three orchards were older and less homogeneous in terms of disease severity, tree size and the ability to perform the recommended tree sanitation, even though the Hosen 2 L/ha treatment reduced symptoms to 40–53% of the number of trees exhibiting symptoms compared to the untreated control trees in each respective orchard. Thus, although the conditions in the orchards were not optimal for the proposed chemical control, we still observed a positive influence of the Hosen 2 L/ha on disease symptoms.

The model orchards were commercial lemon orchards treated by the owners with accepted methods and the addition of our Mal secco disease treatment. The model orchards were treated by Hosen 2 L/ha five times a year as described. At the end of each season, disease symptoms, number of dead trees and crop assessment (weight of fruit picked from 10 trees, from 5 different treated rows for a total of 50 trees, compared to same number of trees from untreated rows) were evaluated. We found, as expected, a reduction in the number of new symptomatic branches and dead trees with the treatment vs. no treatment. We found no significant differences in crop weight 2 years after the treatment had been applied to the orchard (results not shown). Along our experiments, the question of chemical residues in the fruit was raised. An analysis by GC/MS of flutriafol residue in fruit, sampled 26 days after last application, from treated and untreated trees in 2019, did not detect the chemical in the fruit. 0.02 ppm is the limit of quantification for the test using GC/MS, much lower than 0.2 ppm, the authorized limit in Israel (Itrolab Ltd, Nizan, Israel).

Hosen 2 L/ha was registered for use for the control of Mal secco in Israel in 2020 and is now recommended as part of the available treatments, and widely used by growers for the control of the disease in young and newly planted lemon orchards, as well as in mature orchards.

Conclusions

We treated lemon trees with flutriafol 250 g/ha five times a year, in October and November (1 month apart), March (right after the first spring irrigation of the orchard), April and May (1 month apart), via injection into the dripping system using a pump. In combination with strict sanitation of dry and symptomatic twigs and branches, and keeping the tree height under 2.5 m, this treatment can help control Mal secco disease symptoms and dramatically reduce drying out of branches, and tree death with consequent uprooting.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

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