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# A new highly effective hybrid fungicide containing difenoconazole and tea tree oil for managing scab of apple, pecan and almond trees and as a tool in resistance management

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**Abstract**

Management of scab diseases of apple, pecan and almond is mostly based on fungicide applications according to host phenological stage and wetness events. Resistance to widely used locally systemic fungicides including the methyl benzimidazoles, demethylation inhibitors (DMIs) and quinone outside inhibitors (QoIs) has been reported. We evaluated the activity of a new premixed hybrid fungicide containing the DMI difenoconazole and essential tea tree oil (TTO) against mycelial growth of *Venturia inaequalis* in vitro and against scab of apple, pecan and almond in field trials. Mycelial growth of 24 isolates of *V. inaequalis* collected from commercial apple orchards varied in their sensitivity to difenoconazole ($EC_{50}$ values of 0.0002–1.29 mg/L). Difenoconazole–TTO was significantly more inhibitory to some of the isolates than difenoconazole alone, primarily to those that were less sensitive to difenoconazole. Protective foliar applications of difenoconazole–TTO in field trials were highly effective in controlling scab of apple and generally provided significantly higher disease control than difenoconazole alone. On apple and almond, difenoconazole–TTO treatments were similarly or more effective than applications with other synthetic fungicides belonging to the DMI, QoI and succinate dehydrogenase inhibitor (SDHI) groups, or their mixtures. Difenoconazole–TTO completely prevented scab on pecan trees. Results suggest that difenoconazole–TTO can be integrated into scab control programmes for these crops and be used as a strategic approach in fungicide resistance management in orchards.

KEYWORDS
almond scab, apple scab, difenoconazole, pecan scab, tea tree oil, *Venturia* species

## 1 | INTRODUCTION

Scab is an important disease of apple, pecan and almond trees worldwide. Severe crop losses in susceptible cultivars can occur when appropriate control measures are not taken, especially with wet or high-humidity environmental conditions and favourable temperatures in the spring and summer seasons. Apple scab, caused by the fungus *Venturia inaequalis* (*Spilocea pomi*), is one of the most economically important diseases of cultivated apple (*Malus domestica*) worldwide (Biggs & Stensvand, 2014; MacHardy, 1996). The fungus infects leaves, shoots, buds, flowers and fruit and can reduce fruit quality and size. It can also cause premature fruit drop and defoliation (Biggs & Stensvand, 2014). In the northern region of Israel, estimates of crop loss for susceptible cultivars like Red Delicious and Pink Lady were as high as 60% in some orchards (Gur et al., 2021). In some locations, disease control was unsatisfactory even after 14 fungicide applications starting at the beginning of the season (at green tip stage) as preventive treatment and including various fungicides belonging to demethylation inhibitor (DMI) or quinone outside inhibitors (QoIs; strobilurin) groups. This could have been the result of multiple rain events in the spring, improper application timings and/or ineffective fungicides (Gur et al., 2021).

Pecan scab caused by *Venturia effusa* (synonym *Fusicladium effusum*) is the most economically important fungal disease of pecan (*Carya illinoinensis*) in the south-eastern United States (Gottwald & Bertrand, 1982, 1988), one of the major pecan-producing regions in the world. The pathogen causes lesions and tissue death on twigs, petioles, leaves, nuts and shucks, starting in early spring, and multiple infection cycles occur until late summer (Gottwald & Bertrand, 1988). Almond scab caused by *Venturia carpophila* (anamorph *Fusicladium carpophilum*) affects twigs, leaves and fruit (González-Domínguez et al., 2017). Symptoms develop in late spring and summer, and leaf infections may result in premature defoliation of trees. Almond scab is common in orchards with high humidity and poor air circulation (González-Domínguez et al., 2017; Palacio-Bielsa et al., 2017).

Pecan and almond scab can be severe in the United States but are not common in Israel. This may be related to different climatic conditions and orchard management practices. Indeed, in the eastern United States, where most of the pecans are grown, rain is prevalent from spring to late autumn with concomitant warm temperatures; in contrast, Mediterranean climate countries like Israel have cold and rainy winters, mild springs with few rain events, and dry summers. In California, a high incidence of almond scab develops in high-density plantings with poor air circulation and in sprinkler-irrigated orchards with poor soil drainage; this creates high humidity and frequent dews that are ideal for scab development (Palacio-Bielsa et al., 2017).

Fungicides are the primary means for control of scab diseases on susceptible cultivars. There are several classes of fungicides that can be used including dithiocarbamates, methyl benzimidazoles, DMIs, QoIs, guanidines, dithiocarbamates, succinate dehydrogenase inhibitors (SDHIs) and organometals, as well as phosphites (Bock et al., 2012; Villani et al., 2016), which are thought to elicit a systemically acquired resistance response. DMIs were introduced in the early 1980s, and they remain the main class of fungicides for the control of scab (Villani et al., 2016). Due to intensive fungicide spray programmes for most of the growing season, fungicide resistance has developed in *Venturia* spp. that infect apple (Biggs & Stensvand, 2014), pecan (Seyran et al., 2010) and almond (Adaskaveg, 2002; Palacio-Bielsa et al., 2017). Resistance has developed to DMIs in Canada, the United States and Chile (Braun, 1994; Braun & McRae, 1992; Henriquez et al., 2011; Seyran et al., 2010) as well as Israel (Shabi et al., 1997) and to some other fungicides with different modes of action, such as dodine (guanidines), methyl benzimidazole carbamates and QoIs (Ishii & Hollomon, 2015; Reynolds et al., 1997; Seyran et al., 2010; Standish et al., 2021; Stevenson et al., 2004).

Australian tea tree oil (TTO) derived from the *Melaleuca alternifolia* plant contains many components, mostly terpenes (*p*-cymene, terpinen-4-ol, terpinolene, 1,8-cineole, α-pinene, α-terpinene, γ-terpinene), sesquiterpenes and their respective alcohols (monoterpene alcohol-terpineol) (Carson et al., 2006). It contains up to 15% 1,8-cineole and at least 30% terpinen-4-ol, which are the main active constituents (Carson et al., 2006). TTO has been shown to be an effective antiseptic, bactericide and fungicide (Carson et al., 2006; Hammer et al., 2004; Reuveni et al., 2009; Shao et al., 2013; Yu et al., 2015). The fungicidal activity of TTO is based on its ability to inhibit respiration and disrupt membrane permeability (Carson et al., 2002; Cox et al., 2001).

To reduce the risk of resistance to fungicide and the chemical load of the environment, a new concept of hybrid fungicide is being introduced with the development of a premixture containing the DMI difenoconazole (200 g/L) and TTO (400 g/L). This study was undertaken to evaluate the activity of this hybrid fungicide against mycelial growth of *V. inaequalis* in vitro and its efficacy in orchards compared to other registered synthetic fungicides or fungicide mixtures for controlling apple scab, as well as pecan and almond scab that are caused by other *Venturia* spp. Its use as a tool in resistance management is also discussed.

## 2 | MATERIALS AND METHODS

### 2.1 | Fungicides

Fungicides and fungicide mixtures used in laboratory and field studies are listed in Table 1. Rates used in field studies were based on the fungicide label or were otherwise recommended by the registrant. Rates of fungicides applied in field trials are given in grams of active ingredient/ha.

### 2.2 | Effect of TTO on the in vitro toxicity of difenoconazole to a scab pathogen

*V. inaequalis* was chosen as a representative species for this study. Twenty-four isolates collected in the spring of 2011 from apple fruit and leaves in 11 commercial orchards in four main growing regions in central Chile (located at Maule, 35°43′41″ S, 71°33′93″ W; Ñuble 36°33′00″ S, 71°33′00″ W; Bio-Bio, 37°40′00″ S, 72°01′00″ W; and Araucania, 39°20′59″ S, 71°34′45″ W) were assessed for their sensitivity to difenoconazole and difenoconazole–TTO. To obtain monosporic cultures, a single fruit or leaf lesion was placed into 50 µl of sterile distilled water (SDW), vortexed for 5 s, and the suspension was plated onto water agar. Plates were incubated for 24 h at 20°C in the dark. Using a dissecting

**TABLE 1** Fungicides used in this study

| Active ingredient | Active ingredient (g/L) | Product name | Producer | Fungicide group | Formulation | FRAC code[a] |
|---|---|---|---|---|---|---|
| Difenoconazole | 250 | Score | Syngenta Crop Protection | DMI | SC | 3 |
| Difenoconazole + azoxystrobin | 125 + 200 | Quadris Top | Syngenta Crop Protection | DMI + QoI | SC | 3 + 11 |
| Difenoconazole + tea tree oil | 200 + 400 | Regev | STK Bio-Ag Technologies | DMI + multisite | EC | 3 + BM 01 |
| Dodine | 396 | Elast | UPL | Guanidine | FL | U 12 |
| Fluxapyroxad + pyraclostrobin | 212.6 + 212.6 | Merivon | BASF | SDHI + QoI | SC | 7 + 11 |
| Propiconazole | 418 | Orbit | Syngenta Crop Protection | DMI | EC | 3 |
| Polypeptide | 200 | Fracture | FMC Corporation | Multiple modes of action: plant extract | EC | BM 01 |
| Polydimethylsiloxane | 999 | Kinetic | Helena Agri-Enterprises | Nonionic surfactant | WS | – |
| Mefentrifluconazole | 400 | Cevya | BASF | DMI | SC | 3 |
| Mefentrifluconazole + fluxapyroxad | 174 + 174 | Mibelya | BASF | DMI + SDHI | SC | 3 + 7 |
| Tea tree oil | 238 (114) | Timorex Gold (Act) | STK Bio-Ag Technologies (Summit Agro) | Multiple modes of action: plant extract | EC | BM 01 |
| Triphenyltin hydroxide | 800 | Super-Tin | UPL | Organotin compound | | 30 |
| Tebuconazole | 250 | Toledo | Rotam | DMI | WP | 3 |
| Polyoxin-D | 113 | Ph-D | Arysta LifeScience | Polyoxins | WDG | 19 |

Abbreviations: DMI, demethylation inhibitor; QoI, quinone outside inhibitor; SDHI, succinate dehydrogenase inhibitor.

[a] Fungicide Resistance Action Committee.

**TABLE 2** Field trials conducted in Israel to assess the efficacy of difenoconazole and difenoconazole–tea tree oil against apple scab caused by *Venturia inaequalis*

| Year | Location, region | Cultivar | Orchard age (years) | Rootstock | No. of sprays | Beginning of sprays[a] | End of sprays |
|---|---|---|---|---|---|---|---|
| 2015 | Hulla Valley, Galilee, Israel | Odem | 3 | MM 106 | 4 | 21 April (first scab lesions observed) | 22 May |
| 2015 | Masa'ade Golan, Israel | Starking | 8 | MM 106 | 5 | 1 April (BBCH-09) | 6 May |
| 2016 | Hulla Valley Galilee, Israel | Odem | 4 | MM 106 | 2 | 11 April (BBCH-11) | 8 May |
| 2020 | Bara'm Galilee, Israel | Sandowner | 3 | MM 106 | 3 | 16 March (BBCH-09) | 25 April |

[a] BBCH-09, green leaf tips about 5 mm above bud scales. BBCH-11, first leaves unfolded.

microscope, a single germinated conidium was transferred to a potato dextrose agar (PDA; Difco) plate and incubated at 20°C. Subsequently PDA plates were prepared that were amended with 0, 0.01, 0.1 or 1 mg/L difenoconazole without or with the addition of 0, 0.02, 0.2 or 2 mg/L of TTO, respectively. Plates were inoculated with 18-day-old 5-mm diameter mycelial agar plugs and incubated in the dark at 20°C for 28 days. Colony diameters were measured, and percentage inhibition at each concentration was calculated based on growth on control plates. $EC_{50}$ values for each isolate were obtained by regressing percentage inhibition against $\log_{10}$-transformed concentrations (Henriquez et al., 2011). Each combination of isolate and fungicide concentration was evaluated three times.

### 2.3 | Field studies on apple scab

Five field trials with cvs Anna, Odem, Starking and Sandowner that are susceptible to apple scab were conducted in commercial orchards in the Golan and Upper Galilee regions in northern Israel during 2015 to 2017 and 2020 (Table 2). Spacing between rows and trees was 4 × 2 m and 4 × 1.5 m, respectively. Application of fungicides started when green leaf tips were about 5 mm above bud scales (BBCH-09) or at first observation of scab lesions, and subsequent applications were given before forecasted rain events, as detailed in Tables 2 and 3. Fungicides were sprayed to run-off with a Turbo 400 (100 L, 1400 kPa) gun sprayer (Degania Sprayers) at a volume of 1000 L/ha. Treatments in all trials were arranged in

**TABLE 3** Efficacy of difenoconazole and difenoconazole–tea tree oil for management of apple scab in field studies in Israel

| Treatment and rate (g/ha) | Incidence of disease (%) | | | | |
|---|---|---|---|---|---|
| | Hulla Valley, cv. Odem[a] | | Mas'ade, cv. Starking[b] | Bara'm, cv. Sandowner[c] | |
| | 2015 | 2016 | 2015 | 2020 | |
| | Leaves | Leaves | Leaves | Leaves | Fruit |
| Control | 68.5 a | 40.5 a | 37.4 a | 52.7 a | 21.0 a |
| Difenoconazole 50 | 6.0 b | 9.8 b | 7.3 b | 30.0 b | 11.6 b |
| Difenoconazole 50–tea tree oil 100 | 1.3 c | 3.3 c | 3.1 c | 12.5 c | 4.0 c |

*Note*: Values within columns followed by the same letter are not significantly ($p < 0.05$) different according to Fisher's LSD K-ratio *t* test.

[a] In Hulla Valley in 2015, four foliar sprays were done starting 21 April when the first lesion was observed and then on 1, 8, 22 May before rain events. Disease was rated on 4 June 2015. In the trial in 2016, applications were done on 11 April and 8 May before rain events. Disease was rated 16 days after the last application.

[b] At Mas'ade, five foliar sprays were done starting at green tip on 1 April 2015, and then on 8, 14, 24 April and 6 May before rain events. Disease was rated on 15 May 2015.

[c] At Bara'm, five foliar sprays were done starting at green tip on 16 March 2020, and then on 25 March, 4, 14, 25 April before rain events. Disease was rated on 7 May 2020.

a randomized complete block design (RCBD). Each replicate comprised three trees, and there were four replicates for each treatment. Disease was assessed 10–14 days after the last application on 10 shoots randomly selected from each two sides of each central tree of each replicate. Foliar incidence of apple scab was evaluated by examining the five terminal leaves of each shoot and determining the number of infected leaves (a total 100 leaves evaluated per replication, 400 leaves per treatment). Incidence of apple scab on fruit was assessed by counting the number of infected fruit out of 25 randomly selected fruit from each two sides of the central tree of each replicate (200 fruits per treatment).

## 2.4 | Field studies on pecan scab

Two trials were carried out in 2017 and 2018 on pecan trees in the United States. The trial in 2017 was performed with cv. Cunard in Quitman, GA, and evaluated the efficacy of difenoconazole–TTO at 120 + 240 g/ha compared to 720 g/ha triphenyltin hydroxide + 1425.6 g/ha of dodine. The trial in 2018 was performed in a cv. Cunnard orchard in Kahira, GA, that was planted in May 2014, and evaluated the efficacy of difenoconazole–TTO applied at 70 + 140, 90 + 180 or 100 + 200 g/ha compared to 720 g/ha triphenyltin hydroxide + 712.8 g/ha of dodine. In both trials, seven foliar applications were made for each fungicide. In Quitman, applications were started on 18 April and then were applied on 2, 16, 30 May, on 12, 27 June and on 11 July 2017; in Kahira, applications were started on 18 April and then were applied on 2, 15, 30 May, on 13, 26 June and on 10 July 2018. Treatments were applied with an orchard airblast sprayer calibrated to deliver 1000 L/ha, and there were four single-tree replicates in a RCBD. In both orchards, pecan trees were grafted on Elliott rootstock and spacing between rows and trees was 12 × 6.75 m. Scab severity on leaves and nuts was evaluated in June and July by estimating the percentage of area affected on each of 50 randomly collected leaves or nuts from each tree (Yadav et al., 2013).

## 2.5 | Field studies on almond scab

The first trial was carried out in 2017 in Gridley, CA, on 11-year-old cv. Winters almond trees in a high-density orchard with a history of scab. The second trial was conducted in 2018 in Poplar, CA, on 20-year-old cv. Winters trees. In both orchards, trees were planted on peach rootstock with a spacing of 7 m between rows and 6 m between trees. Four applications of each fungicide were made in each trial. In Gridley, the efficacy of difenoconazole–TTO at 230 (77 + 153 g/ha) was compared to polypeptide at 290 g/ha and applications were made on 4 April 2017, at 5 weeks after petal fall, 20 April, 12 May and 12 June 2017. In Poplar, difenoconazole–TTO was applied at 70 + 140 g/ha and 120 + 240 g/ha, and was compared to a propiconazole/fluxapyroxad + pyraclostrobin programme, where propiconazole was applied at 125.4 g/ha in the first three applications and fluxapyroxad + pyraclostrobin at 96 + 96 g/ha in the three following applications; applications were on 16 February (pink bud stage), on 21 February (petal fall), 15 March and 21 April 2018. There were four single-tree replications, and treatments were arranged in a RCBD. Applications were made with mist blower backpack sprayers (model SR450, Stihl Inc.) calibrated to deliver 1000 L/ha.

Disease incidence was determined on 24 July 2017 for trial 1, and on 9 July and 10 August 2018 for trial 2 by randomly collecting 50 leaves and 50 nuts from the mid-canopy of each tree, and assessing the number of infected leaves or nuts.

Three additional trials were conducted in 12- to 15-year-old cvs Monterey and Carmel orchards on peach rootstock in Colusa Co., CA, in 2020 and 2021. These plantings had a spacing of 8 m between rows and 6 m between trees. Treatments were initiated at the onset of sporulating twig lesions, and application dates are indicated in Tables 4 and 5. Fungicides included TTO, difenoconazole–TTO, mefentrifluconazole, three premixtures (difenoconazole–azoxystrobin, fluxapyroxad–pyraclostrobin, or mefentrifluconazole–pyraclostrobin), as well as a rotation of polyoxin-D + tebuconazole with the premixture of difenoconazole and azoxystrobin. All were applied at a volume of 935 L/ha using mist blower backpack sprayers as described above, and a nonionic surfactant (DynAmic; Helena Agri-Enterprises) at 0.6 ml/L spray solution was added to all treatments except for TTO and difenoconazole–TTO. Disease was evaluated in mid-August or early September. For this, fruit were randomly collected from each of the four single-tree replications of each treatment that were arranged in a RCBD. Disease incidence was based on the number of fruit with lesions of the total number of fruit collected. Additionally, the average number of lesions per fruit was assessed.

### 2.6 | Statistical analysis

Data from repeated experiments were combined for analysis when the variance between experiments was homogeneous. All data were analysed with the JMP statistics package v. 12.1.0 (SAS). The $EC_{50}$ values (the concentrations of each fungicide causing 50% inhibition) for mycelial growth were calculated by means of probit analysis with the SPSS Statistics v. 20.0 software (IBM). For field trials, analysis of variance (ANOVA) was applied to arcsine-transformed data of the percentage of infected leaves or fruits in order to achieve normal distributions. Data in tables are reported in untransformed units. Fisher's LSD K-ratio *t* test was applied, to determine whether differences between treatments were significant at $\alpha = 0.05$.

## 3 | RESULTS

### 3.1 | In vitro sensitivity of *V. inaequalis* isolates to difenoconazole and difenoconazole–TTO

Testing the direct activity of TTO alone on mycelial growth revealed that TTO was ineffective and fungal growth was seen even at >100 mg/L (data not shown). Therefore, the sensitivity of the collected isolates was examined only against difenoconazole or difenoconazole–TTO. $EC_{50}$ values for difenoconazole of 24 isolates ranged between 0.0002 and 1.29 mg/L (mean 0.340 mg/L), whereas values for the same isolates using the difenoconazole–TTO mixture ranged from 0.000001 to 0.36 mg/L (mean 0.174 mg/L). The difenoconazole–TTO mixture, had a significantly increased toxicity to mycelial growth for some of the isolates tested compared to difenoconazole alone, particularly for those with the highest $EC_{50}$ values for difenoconazole (Figure 1). For example, for isolates 23 and 24 with the highest $EC_{50}$ values for difenoconazole of 0.87 and 1.29 mg/L, respectively, $EC_{50}$ values for difenoconazole–TTO were 0.2 and 0.36 mg/L, respectively. A pairwise *t* test comparison of the sensitivity of 24 isolates to difenoconazole alone or a difenoconazole–TTO mixture indicated a significant difference ($p = 0.023$) between the two populations.

**TABLE 4** Efficacy of fungicide treatments for management of scab of cv. Carmel almond in California

| Treatment and rate (g/ha) | 2020 Incidence (%)[a] | 2020 No. lesions/fruit | 2021 Incidence (%)[a] | 2021 No. lesions/fruit |
|---|---|---|---|---|
| Control | 65.1 a | 5.3 a | 84.5 a | 9.9 a |
| TTO 230 | Not done | Not done | 18.1 b | 1.9 b |
| Mefentrifluconazole 146 | 47.6 ab | 2.5 b | 37.8 ab | 3.0 ab |
| Difenoconazole 88–TTO 175 | 23.9 bc | 0.8 b | 20.5 b | 1.9 b |
| Fluxapyroxad 115–pyraclostrobin 115 | 44.3 ab | 1.4 b | 15.6 b | 1.3 b |
| Mefentrifluconazole 102–fluxapyroxad 102 | 9.7 c | 0.3 b | 22.4 b | 2.2 b |
| Rotation | 15.0 c | 0.5 b | 27.9 b | 2.8 b |

*Note:* Treatments were applied using an air-blast sprayer at a rate of 935 L/ha. All treatments except tea tree oil (TTO) and difenoconazole–TTO were applied in combination with a nonionic surfactant. In 2020, treatments were done on 5, 27 May and 18 June; in 2021 they were done on 5 May and 9 June. The rotation treatment included tank mixture of 50 g polyoxin-D/ha + 250 g tebuconazole/ha that was applied in the first treatment in both years and also in the third treatment in 2020 and 130 g difenoconazole/ha + 205 g azoxystrobin/ha that was used in the second application in both years.

Values within columns followed by the same letter are not significantly ($p > 0.05$) different based on an analysis of variance using Fisher's LSD K-ratio *t* test.

[a] For disease evaluation (3 September 2020 or 11 August 2021), 40–60 random fruit were collected from each tree and the number of lesions on each fruit was determined. Disease incidence was calculated based on the number of fruit with scab lesions of the total number of fruit evaluated.

## 3.2 | Field trials on apple scab

In the four field trials on apple scab conducted in Israel in 2015, 2016 and 2020, disease incidence on leaves of the untreated controls ranged from 37.4% to 68.5% and was 21.0% on fruit in the 2020 study at Bara'm (Table 3). Difenoconazole at 50 g/ha, significantly reduced the incidence of scab to between 6.0% and 30.0%, corresponding to a 91.2% to 43.1% reduction in incidence compared with the controls. In all trials, the mixture of 50 g difenoconazole/ha with 100 g TTO/ha significantly increased the efficacy compared with using difenoconazole by itself, with reductions in incidence in comparison to the control of between 98.1% and 76.3%. Both treatments were most effective in the 2015 Hulla Valley study and less effective in the 2020 Bara'm trial (Table 3).

TABLE 5 Efficacy of fungicide treatments for management of scab of cv. Monterey almond in California in 2020

| Treatment and rate (g/ha) | Incidence[a] (%) | No. lesions/ fruit |
|---|---|---|
| Control | 93.1 a | 14.0 a |
| TTO 200 | 69.6 b | 9.6 b |
| Difenoconazole 88–TTO 175 | 38.0 c | 3.2 c |
| Difenoconazole 130–azoxystrobin 205 | 23.0 c | 2.1 c |

*Note*: Treatments were applied using an air-blast sprayer at a rate of 935 L/ha on 13 May and 3, 24 June 2020. The premixture of difenoconazole and azoxystrobin was applied in combination with a nonionic surfactant. TTO, tea tree oil.

Values within columns followed by the same letter are not significantly ($p > 0.05$) different based on an analysis of variance using Fisher's LSD K-ratio *t* test.

[a] For disease evaluation on 3 September 2020, 25–30 random fruit were collected from each tree and the number of lesions on each fruit was determined. Disease incidence was calculated based on the number of fruit with scab lesions of the total number of fruit evaluated.

## 3.3 | Field trials on pecan scab

In two field studies on pecan scab conducted in Georgia, USA, in 2017 and 2018, scab pressure was characterized as severe. On untreated control trees in the 2017 study, 41.2% of leaf area and 83.7% of fruit area showed symptoms at the end of the experimental period in mid-July (Figure 2a,b). Both difenoconazole–TTO (applied using three rates) and the industry standard triphenyltin hydroxide-dodine at registered rates completely inhibited scab development on leaves (Figure 2a) and nuts (Figure 2b). In the 2018 trial, high disease levels with 55.8% of leaf area and 100% of fruit area affected on control trees were observed by 10 July, and disease severity on leaves increased slightly to 57.9% by 24 July (Figure 2c,d). Triphenyltin hydroxide-dodine significantly reduced disease severity and at the last rating on 24 July provided 80.1% and 93.4% efficacy on leaves (Figure 2c) and nuts (Figure 2d), respectively, compared with the control. Difenoconazole–TTO applied at three rates completely inhibited scab development on leaves and nuts.

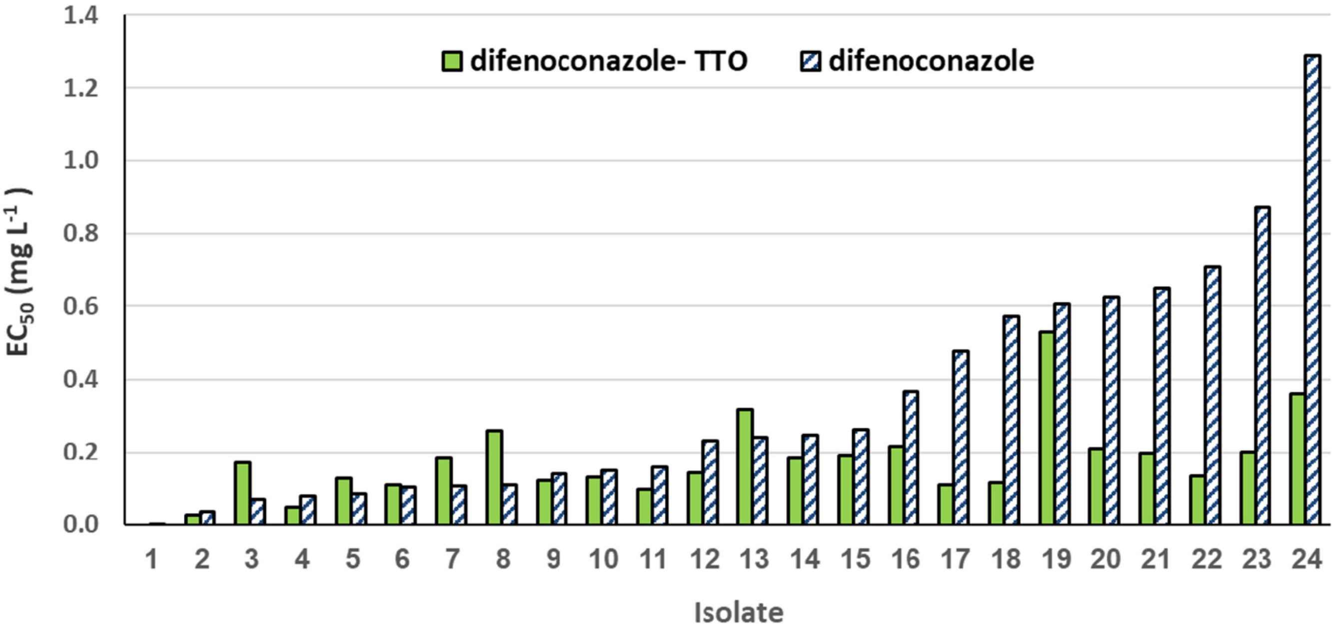

FIGURE 1 Sensitivity of 24 isolates of *Venturia inaequalis* from commercial orchards in central Chile to difenoconazole and difenoconazole–tea tree oil (TTO). Effective concentrations to inhibit mycelial growth by 50% ($EC_{50}$ values) were determined using an agar dilution method. Potato dextrose agar was amended with 0, 0.01, 0.1 or 1 mg/L difenoconazole without or with the addition of 0, 0.02, 0.2 or 2 mg/L of TTO, respectively. The concentrations shown are for difenoconazole. Isolates are arranged based on their sensitivity to difenoconazole with the most sensitive isolate on the left. A *t* test pairwise comparison of the sensitivity of 24 isolates to difenoconazole alone or a difenoconazole–TTO mixture indicated a significant difference ($p = 0.0231$) between the treatments.

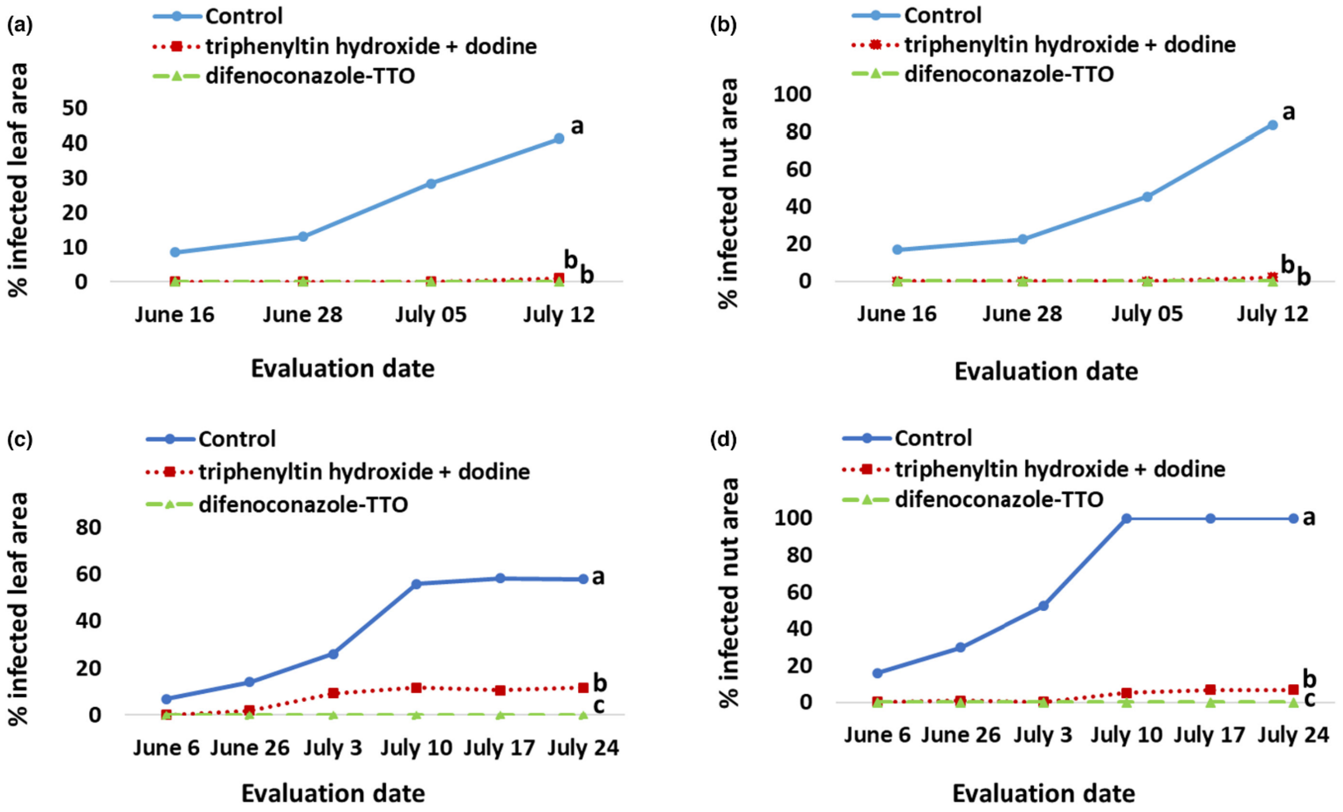

FIGURE 2 Efficacy of difenoconazole–tea tree oil (TTO) and triphenyltin hydroxide + dodine in controlling scab on leaves (a,c) and nuts of pecan trees (b,d) in Quitman, GA, USA, 2017 (a,b), and Kahira, GA, USA, 2018 (c,d). In Quitman, seven foliar sprays of difenoconazole–TTO at 120 + 240 g/ha or 240 + 480 g/ha triphenyltin hydroxide + 1425.6 g/ha dodine were applied from April to July 2017. In Kahira, seven foliar sprays of 70 + 140 g/ha difenoconazole–TTO or 720 g/ha triphenyltin hydroxide + 712.8 g/ha dodine were applied from April to July 2018. Different letters indicate significant difference ($p < 0.05$) according to least significant difference (LSD) test.

### 3.4 | Field trials on almond scab

Several trials were conducted in California on the efficacy of difenoconazole–TTO against almond scab. In the 2017 trial, the incidence of scab on leaves and fruit of control trees was 24% and 23.3%, respectively, at evaluation on July 24 (Figure 3a). Difenoconazole–TTO and the standard polypeptide significantly reduced the incidence to 7.3% and 16% on leaves, and 3.3% and 3.7% on nuts, respectively. In the 2018 trial, 20% of nuts were found to be infected on control trees (Figure 3b). Both rates of difenoconazole–TTO (70 + 140 g/ha and 120 + 240 g/ha) were highly effective and reduced scab incidence on nuts to 1.8% and 0.5%, respectively, resulting in 90% and 97.6% efficacy, and was similar to the standard treatment of propiconazole/fluxapyroxad + pyraclostrobin.

In three additional studies on almond scab, TTO by itself and difenoconazole–TTO were compared to standard fungicide treatments. TTO significantly reduced the incidence of scab on fruit from 84.5% in the control to 18.1% in a study conducted in 2021 (Table 4). Difenoconazole–TTO was similarly effective as TTO, with an incidence of 20.5%, and disease severity was also significantly lower than in the control. In another study, TTO was less effective, and scab incidence was reduced from 93.1% in the control to 69.6% (Table 5). Difenoconazole–TTO was significantly more efficacious than TTO alone in this trial, resulting in an incidence of 38.0%, which was statistically similar to a standard treatment with difenoconazole-azoxystrobin (Table 5). In the third trial conducted in 2020, difenoconazole–TTO was compared to standard fungicide treatments and effectively reduced the disease from 65.1% in the control to 23.9% (Table 4). Among other fungicide treatments, the DMI mefentrifluconazole did not significantly reduce scab incidence from the control in two studies where it was used but significantly decreased disease severity in one trial in 2020 (Table 4). The premixture of pyraclostrobin with fluxapyroxad resulted in the numerically lowest incidence of scab in the 2021 trial (Table 4) but was not very effective in the 2020 study where only disease severity, but not incidence, was reduced significantly from the control. The premixture of mefentrifluconazole and fluxapyroxad and a rotation of polyoxin-D + tebuconazole with the premixture of difenoconazole and azoxystrobin showed consistent high efficacy in two studies where they were used (Table 4). Overall in these studies, difenoconazole–TTO was statistically as effective as the best treatments evaluated.

## 4 | DISCUSSION

Fungicides with different single-site modes of action (i.e., belonging to different FRAC codes) are often combined in mixtures to expand the spectrum of activity (provided the active substances have

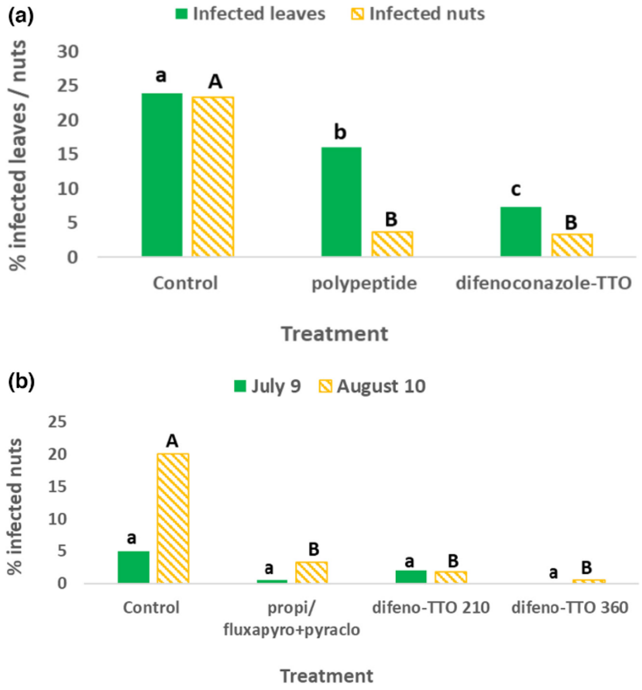

FIGURE 3 (a) Efficacy of difenoconazole–tea tree oil (TTO) at 230 (77 + 153 g/ha) and polypeptide at 290 g/ha in controlling almond scab in the field, California, 2017. Disease incidence of scab on leaves and nuts was assessed by randomly collecting 50 leaves and 50 nuts from the mid-canopy of each tree. (b) Efficacy of difenoconazole–TTO in comparison to other fungicide regimes against almond scab in the field, California, 2018. Treatment with difenoconazole–TTO at two different concentrations, termed 210 (70 + 140 g/ha) and 370 (120 + 240 g/ha), was compared to a propiconazole/fluxapyroxad + pyraclostrobin programme, where propiconazole was applied at 125.4 g/ha in the first three applications and fluxapyroxad + pyraclostrobin at 96 + 96 g/ha in the three following applications. Treatments were applied four times from February to April. Disease incidence was assessed as described in (a). Different letters indicate significant difference ($p < 0.05$) according to least significant difference (LSD) test.

different toxicities to target pathogens), to prolong persistence, and to improve disease control by exploiting additive or synergistic interactions between the components (van den Bosch et al., 2014; Brent & Hollomon, 2007; Elderfield et al., 2018; Gisi, 1996). Mixtures are also frequently advocated as a resistance management approach that slows the rate of development of fungicide resistance in the pathogen population and thus extends the effective life of the fungicides involved. An additional strategy can be the use of protectant multisite mode of action fungicides such as dithianon, mancozeb, dodine or captan.

This study describes the activity of the first hybrid fungicide, that is, a combination of a natural product with broad-spectrum activity (TTO) classified as FRAC code BM 01 and a traditional site-specific chemical (difenoconazole). Our results show that difenoconazole–TTO can effectively control apple scab in a Mediterranean climate region such as Israel and can manage almond scab in California with similar efficacy as standard fungicides. Difenoconazole–TTO was also highly effective against pecan scab in the south-eastern United States where it completely inhibited disease development. Pecan and almond scab are not common in Israel, and therefore, the efficacy against these diseases was evaluated in other growing regions.

TTO, an essential oil, is regarded as a low-risk biopesticide with no known resistance in target pathogens (FRAC, 2021). TTO is registered as an active ingredient in the United States, Canada, China, and included in the List of Chemicals: Annex I of the European Chemical Agency. Several TTO-based products are registered as broad-spectrum fungicides for various crops and are in use in more than 30 countries. In addition, no residues of TTO were found in plants after 24 h and thus, no maximum residue level (MRL) is requested to be established for various crops (Reuveni et al., 2009; Reuveni, Barbier, et al., 2020).

TTO was shown to affect spore germination and significantly inhibited fungal growth, lesion development, formation of reproductive structures and sporulation of *Mycosphaerella fijiensis* in banana and *Bremia lactucae* in lettuce (Reuveni, Barbier, et al., 2020; Reuveni & Cohen, 2020). The antimicrobial activities of TTO against fungal pathogens are derived from its ability to inhibit respiration and disrupt membrane permeability of living organisms (Carson et al., 2002, 2006; Cox et al., 2001; Reuveni, Sanches, et al., 2020). TTO was found to exhibit strong curative activity against *M. fijiensis* in banana (Reuveni, Sanches, et al., 2020), enabling its use even when disease is already visible. In addition, TTO was found to be an activator of host defence mechanisms and systemically induced resistance in banana and tomato plants (Dalio et al., 2020). For example, application of TTO to field-grown banana mother plants showing symptoms of Fusarium wilt inhibited disease development in daughter plants. TTO enhanced pathogenesis-related (PR) proteins and expression of marker genes for systemic acquired resistance and induced systemic resistance (Dalio et al., 2020). The present study opens new questions such as the modes of action of TTO, its systemicity, and the optimum timing of applications in apple, pecan and almond trees.

As a suitable mixture partner for TTO, a chemical was needed that is stable over time, does not reduce the activity of TTO, and has a similar spectrum of activity against target pathogens as TTO. Additionally, like formulated TTO, it should not move systemically in the plant. If TTO was combined with a systemic fungicide such as the DMI triazole flutriafol, the two components could separate and be present in different locations inside the plant tissue, negating the benefit of a mixture treatment for resistance management. Thus, the non- or limited systemic fungicide difenoconazole was chosen as a mixture compound for TTO. However, although TTO itself is nonsystemic, if it induces systemic resistance in trees then its antifungal effect is systemic. In that case, other systemic fungicides may be considered suitable mixture partners for TTO. As a DMI compound, difenoconazole inhibits biosynthesis of ergosterol, a vital fungal cell membrane component. Specifically, DMIs interfere with C-14 demethylation of lanosterol or 24-methylenedihydrolanosterol, the immediate precursor of ergosterol in the biosynthetic pathway (Köller & Scheinpflug, 1987; Villani et al., 2016). Although the direct toxicity of TTO against mycelial growth of *V. inaequalis* was very low, additive or synergistic interactions between TTO and difenoconazole were observed in our in vitro studies. Growth of most isolates less sensitive to difenoconazole was effectively inhibited by the addition of TTO, indicating that populations of the apple scab pathogen with reduced sensitivity to DMIs may be effectively managed using this new hybrid fungicide. Furthermore, the additional effect of TTO in combination with difenoconazole was more pronounced when they were applied to plant tissue in the field than in vitro. This might be related to the indirect activity of TTO on the plant defence mechanism, as mentioned above. Difenoconazole–TTO could be alternated with fungicides that exhibit different modes of action and to which fungal pathogen populations have shown a loss of sensitivity, so that the population of individuals that are less sensitive to specific fungicides would be reduced, as demonstrated in our in vitro study.

The different modes of action of TTO and difenoconazole make difenoconazole–TTO an effective tool for managing resistance in integrated disease management programmes. Furthermore, the combination of a conventional fungicide with a plant-derived essential oil product results in a reduced synthetic chemical load in the environment, a characteristic that is highly favoured by today's regulatory agencies and consumers. Difenoconazole–TTO is currently registered in various countries including the United States and is used for controlling a broad range of diseases of arable crops including cereals, fruits, nuts and vegetables (Reuveni, 2019). With preventive and curative activities, an indirect effect on the host plant by inducing systemic resistance and reduced chemical residues in the commodity and the environment, difenoconazole–TTO has the potential to be an important component in resistance management and an effective control strategy for scab and various other plant diseases.

## AUTHOR CONTRIBUTIONS

M.R., L.G., G.C., J.F., J.L.H., E.T. and J.E.A planned the experiments. M.R., L.G., J.L.H., G.C. and J.E.A were responsible for data collection and analysis. M.R., L.G. and J.E.A. prepared the manuscript. All authors have read and agreed to the published version of the manuscript.

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## CONFLICT OF INTEREST

M. Reuveni is employed by STK Bio-Ag Technologies Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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