



# New fungicides for managing *Phytophthora* diseases of tree crops with foliar and soil applications

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## Abstract

Four new modes of action, oxathiapiprolin, fluopicolide, mandipropamid, and ethaboxam, have or are pending registrations on multiple tree crops in the USA including avocado, citrus, almond (and other tree nuts), and stone fruits to be used in sustainable anti-resistance rotation and mixture programs for managing *Phytophthora* diseases. All four fungicides are highly toxic in vitro against species of *Phytophthora* from different tree crops. Efficacy of foliar applications of oxathiapiprolin and mandipropamid for the management of citrus brown rot persisted for over 10 weeks. Still, residues could be removed to non-detectable levels by postharvest washing and brushing of fresh-market fruit. The four fungicides were highly effective in reducing root rot and crown/trunk cankers on tree crops and demonstrated acropetal systemic movement through the xylem after soil application and absorption by almond, citrus, and cherry roots. This was substantiated by reduced lesion size in stem inoculations of potted trees that were treated by soil applications or by in vitro bioassays and HPLC–MS–MS analyses of root, stem, and leaf extracts. Using an adapted Bromilow model that is based on the partition coefficient logP that indicates the lipo- or hydrophilicity and the acid dissociation constant pKa, mobility of ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin in plants was predicted, and this was supported by our studies. Mandipropamid and a mandipropamid–oxathiapiprolin pre-mixture are now registered as foliar applications for managing citrus brown rot, whereas oxathiapiprolin and fluopicolide are registered, and ethaboxam is pending registration as soil applications to control citrus root rot. Oxathiapiprolin has also been registered for soil use on almond and other nut crops, as well as avocado, and soil applications of mandipropamid are planned to be restricted to nursery use of multiple crops.

**Keywords** Soil-borne diseases · Oomycota plant pathogens · Disease management of tree crops

## Introduction

The major tree fruit and nut crops grown in California including almonds, avocados, cherries, citrus, pistachios, and walnuts are affected by numerous species of *Phytophthora* causing root and crown rots, trunk cankers, as well as brown rot of citrus fruits. These diseases can result in major economic losses from reduction in orchard health and yield. Historically, copper was used in foliar applications on citrus for managing brown rot. Since the introduction of the phenylamide metalaxyl (FRAC Code—FC 4), with subsequent marketing of the R-enantiomer mefenoxam in the early 1980s and the phosphonates (FC P07/33, e.g., fosetyl-AI,

phosphorous acid and its salt potassium phosphite) in the late 1980s for use in soil applications to prevent root and crown infections, no new active ingredients have been developed. Recently, ethaboxam, mandipropamid, fluopicolide, and oxathiapiprolin were made available for evaluation on tree fruit and nut crops. They all have a different mode of action, with FRAC Codes 22, 40, 43, and 49, respectively. This provides a unique opportunity to eventually integrate them into sustainable management programs to cope with pathogen resistance to phosphites that is increasingly being reported (Hao et al. 2021; Belisle et al. 2019) and to provide strategies to manage *P. mediterranea*, a newly emerging highly virulent pathogen especially on almonds that is inherently insensitive to phosphites. Resistance has also developed against mefenoxam (Timmer et al. 1998a; b), and additionally, this compound can be phytotoxic to young trees (see product label), thus limiting its use to lower, less effective rates. We conducted field and greenhouse studies

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to evaluate the effectiveness of ethaboxam, mandipropamid, fluopicolide, and oxathiapiprolin against *Phytophthora* diseases of citrus, avocado, and almond and determined their mobility in plants. This has resulted in approved and pending registrations on several of these crops.

## Materials and methods

### In vitro sensitivity of isolates of *Phytophthora* spp. from tree crops to four new fungicides

For determining sensitivities of mycelial growth, the spiral gradient dilution (SGD) method (Förster et al. 2004) was used. Stock concentrations of ethaboxam (Elumin; Valent USA), mandipropamid (Revus; Syngenta Crop Protection), fluopicolide (Presidio; Valent USA), and oxathiapiprolin (Orondis; Syngenta Crop Protection) were applied to 15-cm agar plates using a spiral plater. Fungal growth was measured after 2–3 days, and EC<sub>50</sub> values were calculated as described previously (Förster et al. 2004).

### Field efficacy studies

Field trials were conducted at experimental and commercial orchard sites with the fungicides copper hydroxide (FC M1), mefenoxam (FC 4), potassium phosphite (FC P 07, formerly 33), ethaboxam (FC 22), fluopicolide (FC 43), mandipropamid (FC 40), and oxathiapiprolin (FC 49). In addition to natural rainfall, trees were watered by micro-sprinkler or drip irrigation systems. For management of citrus brown rot, foliar air-blast sprays with fungicides were done to orange and lemon trees with ripening fruit in January before the onset of a rainy period. Treatments were applied at volumes of 935 or 1870 L/ha. Fruit were harvested after 5, 8, and 10 weeks, non-wound drop-inoculated with zoospores of *P. citrophthora*, and incubated for development of decay. For evaluation of citrus root rot, navel orange trees on ‘Carrizo citrange’ rootstock were inoculated at planting in spring 2016 with *P. citrophthora*, fungicides were applied to the soil around the base of the tree in spring and late summer/fall (timed with root flushes) of 2016 and 2017, *Phytophthora* soil populations were quantified, and the incidence of root rot determined by plating onto a semi-selective agar medium. Studies on *Phytophthora* root rot of ‘Hass’ avocado grafted on ‘Dusa’ rootstock were initiated in an 8-year-old orchard naturally infested with *P. cinnamomi*. The initial incidence of root rot was assessed for each tree. Fungicides were applied in the last 4 h of an irrigation cycle around the base of each tree in the spring of 2022, and the incidence of root rot was evaluated in the fall by root plating as described for citrus above. For evaluation of fungicide efficacy against *Phytophthora* crown rot and trunk cankers of almond, the

soil at the base of ‘Nonpareil’ trees on ‘Nemaguard’ or ‘Hansen’ rootstocks was inoculated with *P. cactorum* 1 and 2 years after planting that was done in 2017. Soil treatments were made biannually in the spring and fall of 2018–2020. Trees were visually evaluated for symptoms of gumming and canker formation on the crown and trunk in fall of 2021, and re-isolations were done from diseased tissue to confirm that disease symptoms were caused by *P. cactorum*.

### Greenhouse studies on the mobility of Oomycota fungicides in almond

The soil of potted ‘Nonpareil’ almond trees grafted on ‘Krymsk 86’ rootstock was treated with ethaboxam, fluopicolide, mandipropamid, oxathiapiprolin, or mefenoxam. Scions (16 cm above the graft union) and rootstocks (8 cm above the soil line) were wound-inoculated with *P. cactorum* after 2 weeks. Canker length was measured after another 3 weeks and was used as an indication of fungicide uptake and translocation. Results of the translocation studies with the Oomycota fungicides used were related to predictions using the Bromilow model (Bromilow et al. 1990) that was originally developed to predict the mobility of herbicides based on the acid dissociation constant (pKa) and lipophilicity (LogP) of the compound.

### Statistical analysis of data

Treatment responses to determine fungicide performance in reducing the incidence of fruit and root rots, as well as canker size on crowns and tree trunks, were evaluated statistically using GLIMMIX and LSMmeans separation (SAS version 9.4; SAS Inc., Cary, NC, USA).

## Results and discussion

### In vitro sensitivity of isolates of *Phytophthora* sp. from tree crops to four new fungicides

There was a wide range of EC<sub>50</sub> values for mycelial growth inhibition of ten species of *Phytophthora* with a total of 332 isolates evaluated for most fungicides. Values ranged from 0.001 to 0.190 µg/ml for ethaboxam, 0.020–0.461 µg/ml for fluopicolide, 0.001–0.016 µg/ml for mandipropamid, and 0.00006–0.0015 µg/ml for oxathiapiprolin (Table 1). Single isolates were less sensitive to ethaboxam and fluopicolide, but no resistance was detected. Overall, oxathiapiprolin and mandipropamid were the most toxic to the isolates evaluated.

**Table 1** Toxicity of four fungicides expressed as effective concentration values to inhibit 50% (EC<sub>50</sub>) of mycelial growth against species of *Phytophthora* causing root, crown, and trunk cankers of almond, citrus, and avocado in California

| Host    | <i>Phytophthora</i> spp.    | No. isolates | EC <sub>50</sub> values for mycelial growth (µg/ml) <sup>a</sup> |              |               |                 |
|---------|-----------------------------|--------------|--|--------------|---------------|-----------------|
|         |                             |              | Ethaboxam  | Fluopicolide | Mandipropamid | Oxathiapiprolin |
| Almond  | <i>P. cactorum</i>          | 9            | 0.013–0.079  | 0.111–0.275  | 0.004–0.009   | 0.0005–0.0007   |
|         | <i>P. citricola</i> complex | 11           | 0.046–0.158  | 0.046–0.069  | 0.005–0.007   | 0.0003–0.0005   |
|         | <i>P. niederhauserii</i>    | 31           | 0.031–0.105  | 0.041–0.070  | 0.003–0.009   | 0.0001–0.0004   |
|         | <i>P. syringae</i>          | 19           | 0.017–0.190  | 0.021–0.318  | 0.001–0.006   | 0.0002–0.0004   |
|         | <i>P. mediterranea</i>      | 23           | 0.006–0.036  | 0.034–0.078  | 0.002–0.007   | 0.0001–0.0004   |
|         | <i>P. megasperma</i>        | 4            | 0.040–0.079  | 0.082–0.240  | 0.002–0.005   | 0.0003–0.0005   |
| Citrus  | <i>P. citrophthora</i>      | 62           | 0.005–0.130  | 0.031–0.087  | 0.002–0.005   | 0.0002–0.0015   |
|         | <i>P. syringae</i>          | 71           | 0.015–0.038  | 0.020–0.461  | 0.002–0.016   | 0.00006–0.0003  |
|         | <i>P. nicotianae</i>        | 31           | 0.001–0.050  | 0.039–0.095  | 0.002–0.008   | 0.0003–0.0010   |
| Avocado | <i>P. cinnamomi</i>         | 71           | 0.017–0.069  | 0.046–0.330  | 0.003–0.011   | 0.0002–0.0007   |

<sup>a</sup>EC<sub>50</sub> values for mycelial growth (µg/ml) were determined using the spiral gradient dilution method (Förster et al. 2004); values for citrus and avocado isolates are from Gray et al. (2018) and Belisle et al. (2019), respectively

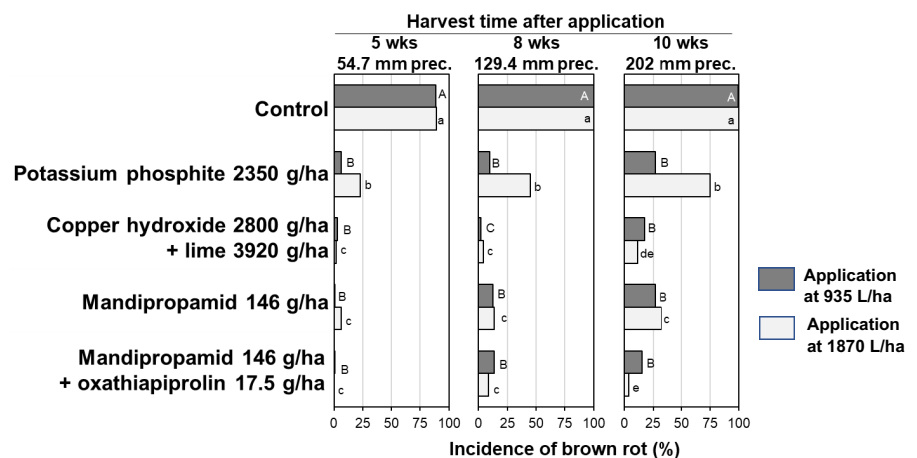
### Field studies on management of citrus brown rot

Annual field studies at different trial sites and under various environmental conditions were conducted on the management of citrus brown rot that may be caused by *P. citrophthora*, *P. syringae*, or much less often, by *P. nicotianae* or *P. hibernalis*. The disease commonly occurs during wet periods of the winter season in California. It has become problematic for growers and shippers because *P. syringae* and *P. hibernalis* are quarantine organisms in China which is a major export destination for California citrus fruits. Although potassium phosphite is registered as a postharvest treatment of citrus in the USA, postharvest uses of this treatment are not allowed in most export markets. Therefore, an effective field management program has to be in place that reduces brown rot in harvested fruit to non-detectable levels.

Preharvest treatments with mandipropamid or a pre-mixture of mandipropamid and oxathiapiprolin were compared

to the grower standards copper hydroxide-lime and potassium phosphite. Results from the high-rainfall winter season of 2023 with total precipitation of 202 mm over the 10-week experimental period are presented in Fig. 1. At the three sampling times, harvested control fruit inoculated with *P. citrophthora* developed 89–100% brown rot decay. All treatments except potassium phosphite were mostly highly effective at the three harvest timings after application although there was a trend for slightly reduced efficacy of copper and mandipropamid at the 10-week evaluation time. This high and persistent efficacy of foliar applications of oxathiapiprolin and mandipropamid for the management of citrus brown rot is likely due to binding to the surface of the fruit flavedo. Residues, however, could be removed to non-detectable levels by postharvest washing and brushing of fresh-market citrus fruit (Adaskaveg unpublished). Potassium phosphite only remained very effective when applied as a more concentrated spray of 935 L/ha as compared with a diluted spray

**Fig. 1** Efficacy of preharvest treatments with fungicides for management of *Phytophthora* brown rot of navel oranges in a high-rainfall spring season. A single application of each fungicide was done on Jan. 12, 2023. Fruit were harvested after 5, 8, and 10 weeks, inoculated with *P. citrophthora*, and incubated for 10 days at 20°C

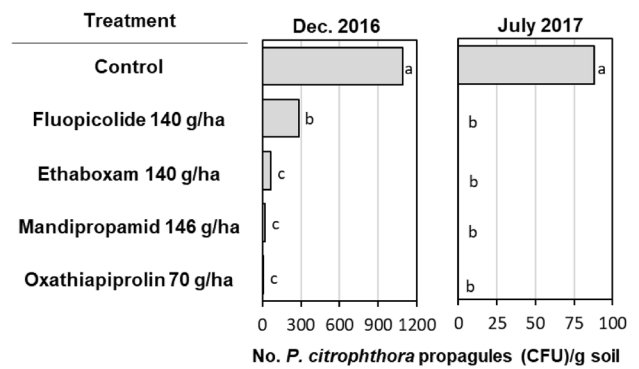


at 1870 L/ha (or 3740 L/ha—data not shown) that was less effective in reducing the incidence of brown rot from that of the control after 8 weeks and was only slightly effective in the 10-week harvest (Fig. 1). For the other treatments, differences between the two spray volumes were much less pronounced. These results are consistent with previous studies conducted in winter seasons with less rainfall (Hao et al. 2023), and additionally, similar or better results were obtained in fruit inoculations with *P. syringae*. *P. citrophthora* was used in most of the studies because successful inoculations with this species are easier to obtain than for *P. syringae* that requires specific incubation conditions at lower temperatures.

Our studies identified new highly effective treatment alternatives to copper and potassium phosphite. Copper treatments have become under regulatory scrutiny in California due to concerns about soil and watershed contamination after many decades of usage. As for phosphite, resistance has been found in *Phytophthora* species affecting citrus including those causing brown rot in California (Hao et al. 2021). Registration of mandipropamid and the mandipropamid–oxathiapiprolin pre-mixture allows development of rotation programs using the older two products and new modes of action to provide a sustainable, highly effective management strategy.

### Field studies on management of *Phytophthora* root rot of citrus

*Phytophthora* root rot can be another destructive disease of citrus and occurs worldwide (Erwin and Ribeiro 1996). In California, it is mostly caused by *P. citrophthora* and *P. nicotianae*. Management of *Phytophthora* root rot is especially critical in new plantings where trees may not become established. This requires an integration of rootstock selection, irrigation management, and fungicide applications. Members of the phenylamides and phosphonates were the sole fungicides available for many years, and resistance in *Phytophthora* species causing citrus root rot has developed in nurseries and in the field (Adaskaveg et al. 2017; Hao et al. 2021; Timmer et al. 1998a, b). In the field trial on evaluation of new Oomycota fungicides as soil treatments that is presented in Fig. 2, soil populations of *P. citrophthora* were very high in non-treated control soil in the first sampling 6 months after inoculation in December and decreased to lower levels in the second sampling. This decline in propagules of *P. citrophthora* can be expected during the warm summer season when the second sampling was done. Ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin significantly reduced soil populations to low levels, and 12 months after treatment, the pathogen was no longer detected. Rates used were within the registrants' recommended ranges, and lower rates often resulted in reduced efficacy (Hao et al. 2019).



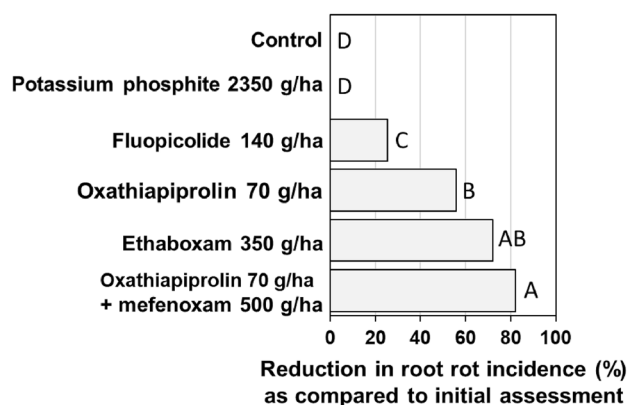
**Fig. 2** Management of *Phytophthora* root rot of citrus. Navel orange trees on ‘Carrizo citrange’ rootstock were inoculated at spring planting with *P. citrophthora*. Fungicides were applied to soil 1 and 10 months after planting, and *P. citrophthora* populations were determined by soil plating 2 months after the fungicide application

In other studies, a standard treatment of mefenoxam was included, and this fungicide performed significantly worse than the new compounds. This was attributed to the low rate used because higher rates of mefenoxam caused stunting and dieback of the newly planted trees (Hao et al. 2019). No tree injury was observed after treating trees with ethaboxam, fluopicolide, mandipropamid, or oxathiapiprolin, and these fungicides represent viable alternative rotation treatments in the management of *Phytophthora* root rot of citrus.

### Field studies on management of *Phytophthora* root rot of avocado

*Phytophthora* root rot caused by *P. cinnamomi* is the most destructive field disease of avocado and limits crop production worldwide. *Phytophthora*-tolerant rootstocks can be used to reduce the severity of disease. For chemical management of this disease, mefenoxam and phosphonate products were the only registered fungicides in the USA for many years. Similar as for *Phytophthora* spp. pathogens of citrus, resistance has been reported in the avocado root rot pathogen in California (Belisle et al. 2019). Potassium phosphite treatments are preferred by avocado growers because they are less expensive than mefenoxam. Its optimal application by trunk injection, however, can be labor-intensive and therefore costly, and injection sites provide entry points for insect pests.

Initial greenhouse studies on the evaluation of new fungicides established the high efficacy of soil treatments with ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin in reducing the incidence of root rot of potted, inoculated plants (Belisle et al. 2019), and this resulted in registration of oxathiapiprolin on avocado in the USA. Several studies at field sites naturally infested with *P. cinnamomi* were initiated starting in 2018 to provide further support for registration of



**Fig. 3** Efficacy of fungicides for managing *Phytophthora* root rot of ‘Hass’ avocado cultivar grown on ‘Dusa’ rootstock in a commercial field trial. Initial root rot assessment was done by sampling roots from each tree and plating onto a selective agar medium, a single soil application of each fungicide was made, and a root rot evaluation was done by plating root samples onto a selective medium 5 months after fungicide application

additional new fungicides. Results are presented for a trial with ‘Hass’ avocado grafted on ‘Dusa’ rootstock. Six months after the first treatment, the incidence of root rot was significantly reduced by fluopicolide, oxathiapiprolin, ethaboxam, or a mixture of oxathiapiprolin and mfenoxam as compared to initial pre-treatment assessments (Fig. 3). The mixture treatment was numerically the most effective, whereas fluopicolide was significantly the least effective. Treatment with potassium phosphite did not reduce the incidence of root rot in the presence of a high incidence (i.e., 90%) of phosphite-resistant isolates in the pathogen population at the trial site. This trial is ongoing, and root rot is being assessed after each of the biannual (spring and fall) treatments.

### Field studies on management of *Phytophthora* crown rot and trunk cankers of almond

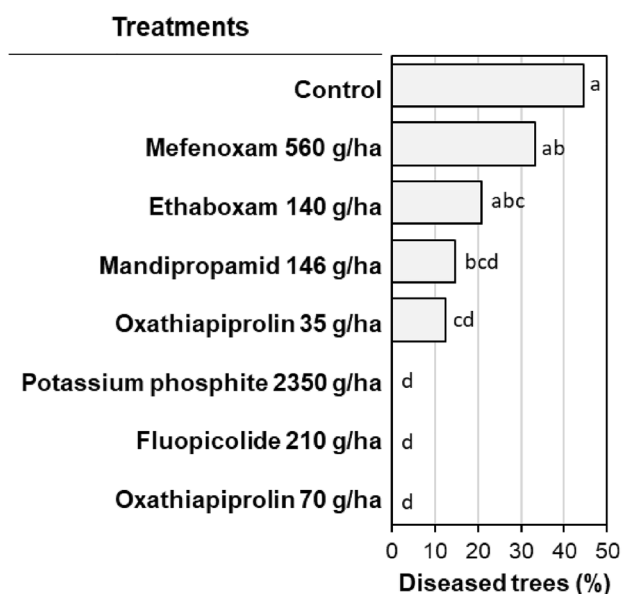
Crown and trunk cankers with subsequent decline and death of trees have become a widespread problem in the establishment of new almond plantings at many locations in California in recent years. This problem likely has been aggravated or even incited by new state regulations that limit the use of well water. As a result, irrigation is now often done with surface water from rivers, ponds, or lakes that may be contaminated with propagules of *Phytophthora* spp. as has been shown for other crops (Gevens et al. 2007; Hao et al. 2020; Stamler et al. 2016). The main species of *Phytophthora* currently involved in almond crown rot and trunk cankers are *P. niederhauserii* and *P. mediterranea* (previously identified as *P. cinnamomi* or *P. sp. ax*) that are both adapted to high summer temperatures, *P. syringae* that is most active in the cooler seasons, as well as *P. cactorum* and species in the *P.*

*citricola* complex (Browne et al. 2015; Browne and Viveros 1998; Nouri et al. 2018).

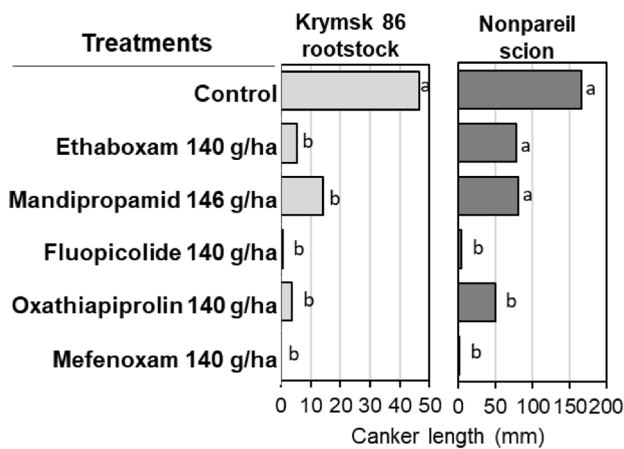
To help growers manage this disease problem on almond, field studies with new Oomycota fungicides were conducted using inoculated trees. Gummy cankers at the lower trunk were first observed on some trees 1 year after the first inoculation. Results for combined data for the two rootstocks in a final disease evaluation 2 years after the first inoculation and biannual soil treatments are presented in Fig. 4. Among non-treated control trees, 44.6% showed crown and trunk cankers, whereas all trees treated with fluopicolide, oxathiapiprolin (using 70 g/ha), or potassium phosphite remained healthy. Treatments that provided intermediate levels of control were mandipropamid and the 35 g/ha-rate of oxathiapiprolin, whereas ethaboxam and mfenoxam reduced disease incidence only numerically from that of the control.

### Greenhouse studies on the mobility of Oomycota fungicides in almond

Crown and trunk cankers are currently the most common *Phytophthora* diseases of almond. We observed that natural tree infections mainly originate near the tree crown and rarely on roots. Thus, in addition to field efficacy studies, we conducted greenhouse experiments to determine the mobility of new Oomycota fungicides in almond plants after soil treatments. Our results show that following



**Fig. 4** Management of *Phytophthora* crown rot and trunk cankers of ‘Nonpareil’ almonds grown on ‘Nemaguard’ and ‘Hansen’ rootstocks. Trees were planted in the fall and treated with each of the fungicides in the subsequent spring and fall. Trees were soil-inoculated with *P. cactorum* 1 month after each fungicide application. The third-leaf orchard was evaluated for crown rot in the fall



**Fig. 5** Greenhouse studies on the mobility of Oomycota fungicides in ‘Nonpareil’ almond grafted to ‘Krymsk 86’ rootstock. The scions and rootstocks were wound-inoculated with *P. cactorum* 12 weeks after fungicide soil application, and canker length was measured after an additional 3 weeks

wound-inoculations with *P. cactorum*, ethaboxam, mandipropamid, fluopicolide, and oxathiapiprolin significantly reduced canker formation on aboveground parts of the ‘Krymsk 86’ rootstock as compared to control plants, and this reduction was similar as for mefenoxam (Fig. 5). Cankers on the ‘Nonpareil’ scion were significantly reduced to small sizes only by fluopicolide, oxathiapiprolin, as well as mefenoxam although lesions were numerically smaller also for ethaboxam and mandipropamid as compared to the control. Mobility of these fungicides was also demonstrated in citrus and sweet cherry plants where residues in aboveground plant parts were detected by bioassays and analytical HPLC–MS–MS analyses (Belisle et al. 2023; Gray et al. 2020). Studies are now also being conducted in fields with different soil types.

These data indicate that the four new Oomycota fungicides have the potential to be translocated into aboveground portions of trees. Therefore, the high efficacy of soil applications in managing crown and trunk cankers can be explained by directly reducing pathogen populations in the soil and infection of crowns, but also by inhibiting spread of infections from crowns into the rootstocks, and for fluopicolide and oxathiapiprolin, into lower scion portions of the trunks. Our results on fungicide mobility align with the Bromilow model that was developed for predicting phloem translocation of herbicides in plants (Bromilow et al. 1990). Thus, using available information on lipophilicity (logP values) and dissociation constants (pKa values) for ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin that are 2.89/3.6, 3.26/0, 3.2/13.9, and 3.67/0.78, respectively, xylem mobility was predicted for ethaboxam, fluopicolide, and oxathiapiprolin and low xylem mobility for mandipropamid.

This resulted in acropetal movement of the fungicides in almond, as well as in citrus and cherry plants.

## Conclusions

The discovery of new Oomycota fungicides in the early 2000s opened the opportunity to potentially make them available for the numerous tree fruit and nut crops grown in California. At that time, few effective treatment options were available for the management of chronic *Phytophthora* diseases such as root rot of avocado and root rot and fruit brown rot of citrus, as well as of new epidemics such as crown and trunk cankers of *Prunus* spp. including almond and of other nut crops such as walnut and pistachio. Increasing restrictions on soil fumigation, regulatory changes in ground water allocations, and resistance to phosphites in local pathogen populations further stressed the need for new treatments. The high in vitro toxicity of ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin against numerous *Phytophthora* spp. validated their evaluation in field studies. Based on the high efficacy obtained in trials over several seasons, mandipropamid and a mandipropamid–oxathiapiprolin pre-mixture were registered for foliar applications on citrus for managing brown rot. Oxathiapiprolin and fluopicolide have been registered as soil applications to control *Phytophthora* root rot of citrus, and ethaboxam is pending registration. Oxathiapiprolin has also been registered on almond (and other nut crops) and avocado for soil application with pending registrations for fluopicolide and ethaboxam. Soil applications with mandipropamid are planned to be restricted to nursery use for multiple crops. Additional registrations have been approved for other nuts crops and were requested for stone fruits. These fungicides can be used in sustainable anti-resistance rotation and mixture programs and will set a new standard in the management of *Phytophthora* diseases of tree crops.

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## Declarations

**Ethical approval** This research and the information presented were done in an ethical manner. No animals or people were hurt while conducting this research, and the authors did not discriminate against any person or group based on the ethnicity, religion, sexuality, or political alignment.

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