

Confidential Report

PERFORMANCE PROFILE

**Laboratory and whole plant efficacy evaluations of PureCrop1
against aphid pests**



Laboratory trials were conducted in Davis and Sacramento, CA

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Evaluations were conducted to test the performance of PureCrop1 against four common aphid pests of landscape and interior grown ornamental plants. Laboratory bioassays were used to define the dose-mortality relationship between PureCrop1 and the melon aphid, *Aphis gossypii*, the rose aphid, *Macrosiphum rosae*, the green peach aphid, *Myzus persicae* and the bean aphid, *Aphis fabae*. Whole, potted ornamental plants infested with aphids were used to test the practical use of PureCrop1 for controlling aphids on plants and to evaluate any residual or repellent action attributable to PureCrop1.

The laboratory bioassays revealed that PureCrop1 at dilutions of 150 and 300:1 (6,666 and 3,333ppm) resulted in virtually 100 percent mortality of all aphid species tested. At the lowest concentration of 600:1 (1,666ppm), 90 percent or more of the pests were killed, but also indicated potency may begin to decrease at this concentration. It was also observed that aphids required approximately 36 hours to succumb to the PureCrop1 applications. The time to aphid death appeared to be independent of temperature but may be affected by PureCrop1 concentration.

Results of the potted plant trials demonstrated that as a practical pest control tool PureCrop1 effectively rescued aphid-infested plants using a single application of the product. Results of the residual activity and repellency tests though revealed no significant residual mortality to aphids when introduced to previously sprayed plants. These tests also indicated there was no repellency response to aphids within 24 hours of application.

From these evaluation trials it can be concluded that PureCrop1 was demonstrated to be an effective control agent of aphids at 600:1 (1,666ppm) or greater concentration. The tests also revealed that as a pest control tool, PureCrop1 consistently controlled aphid populations within 36 hours of application and was effective under a broad range of conditions consistent with many home and commercial applications. PureCrop1 proved easy to mix into solution and left virtually no visible residual on leaf surfaces.

Cover photo: The carcass of a rose aphid still attached to a rose leaf by its mouth parts 12 hours after a 300:1 PureCrop1 application.

Procedures

I. Laboratory Dose-Response Bioassays

PureCrop1 Treatments:

- 4) 0.166% PureCrop1 (1,660ppm or 600:1 dilution)
- 5) 0.333% PureCrop1 (3,330 ppm or 300:1 dilution)
- 6) 0.666% PureCrop1 (6,660 ppm or 150:1 dilution)
- 7) Water only control.

Laboratory trials were conducted in Davis and Sacramento, CA

Two separate laboratory dose-response experiments were conducted for the melon aphid, *Aphis gossypii* and one experiment each for the rose, *Macrosiphum rosae* green peach, *Myzus persicae* and the bean aphid, *Aphis fabae*. Four replicates of aphids were tested for each of the four treatments tested (16 replicates per experiment). Each replicate generally consisted of at least 20 to 50 aphids on a host leaf (see attached data). Chrysanthemum leaves were used for the melon aphid trials, rose leaves for the rose and green peach aphid trials and 'orange jasmine' leaves for the black bean aphid.

Materials were mixed to dilution in 1.0 liter bottles. Either 1.66, 3.33 or 6.66 ml of PureCrop1 were poured into bottles partially filled with water and filled to volume to obtain either a 600, 300, or 150:1 dilutions. Leaves with insects were dipped into paper cups containing the appropriate treatment solutions

for 5 seconds, allowed to air dry, and then placed in the bioassay arenas (figure 1a). Treatments were applied under indoor conditions generally between 68 and 72°F and 68 to 72% relative humidity. The bioassays were inspected and the numbers of live and dead individuals were recorded at 24 and 48 hours post-treatment application for the melon aphid trials and at 36 hours for the remaining trials. Prior to the second inspection period for the melon aphid trials, the arenas were opened and the insects and leaves ventilated for 4 hours prior to counting. The ventilation enhanced insect desiccation and provided a more accurate indication if insects were living or dead.

II. Whole plant evaluations

PureCrop1 Treatments:

5) 0.166% PureCrop1 (1,660ppm or 600:1 dilution)

6) 0.333% PureCrop1 (3,330 ppm or 300:1 dilution)

7) 0.666% PureCrop1 (6,660 ppm or 150:1 dilution)

8) Water only control.

Evaluations were conducted in Davis and Sacramento, CA

Potted plant trials were conducted to evaluate PureCrop1 as a practical pest control tool for rescuing heavily pest infested ornamental plants. For the melon aphid evaluation, potted chrysanthemum plants were used (Figure 1b). For the rose and green peach aphid, potted tea hybrid rose plants were used, and for the bean aphid 'Orange' jasmine plants were used. The potted chrysanthemum plants ('Regan') and potted roses used for whole plant trials were grown 'pest free' under greenhouse conditions. The Chrysanthemums were grown from cuttings in six-inch pots (3 plants per pot) until plants had grown 5 to 7 inches prior to pest infestation. Rose plants were pruned to four rose canes and allowed to grow until enough growth occurred to support pests. Aphid colonies were maintained separately on the same host plant types and transferred to the test plants approximately 7 to 10 days prior to the trials. Test plants were infested with aphids by removing leaves and aphids from the colony plants and placing them on the clean test plants. This forced healthy adults and nymphs to move to the test plants as the leaf dried.

For the melon aphid trial, 4 pots of chrysanthemums (3 plants each) were used for each experiment. Each plant in a pot represented a replicate and 3 leaves were sub-sampled from each plant to estimate aphid mortality. For the aphid trials on roses, 3 potted rose plants were used for each experiment. Each branch constituted a replicate and at least three leaves or stems were of the rose branch were subsampled to estimate pest mortality.

The PureCrop1 dilutions were mixed to in 1.0 liter bottles as described in the bioassay section above. Leaves with insects were sprayed with the appropriate treatments using a hand-held atomizer (figure 1b). Treatments were applied to leaf-surfaces until spray run-off (approximately 1.0ml/leaf side). Applications were made during the afternoon under clear conditions away from direct sunlight and generally between the temperatures of 65 and 72°F and 51 to 61% relative humidity (see attached data).

III. Residual efficacy / repellency

Two of the potted rose plants used in the whole plant evaluations above and one previously uninfested rose were used to evaluate the residual or repellent activity of PureCrop1 treated leaf surfaces. Twenty-five adult rose aphids were placed on preferred feeding sites on the rose plants (near terminal bud). Treated and untreated rose canes with aphids were observed for 10 days. At the end of which time the density of aphids on the treated plants were recorded and the aphid re-infestation rate estimated. Otherwise, aphids were counted as described above.

Results

Laboratory Dose-Response Bioassays

Result of the first bioassays for the melon aphid revealed that PureCrop1 at dilutions of 150 and 300:1 (6,666 and 3,333ppm) resulted in virtually 100 percent mortality of adult and nymph aphids. At the lowest concentration of 600:1 (1,666ppm), greater than 90 percent of the aphids were killed, but also indicated potency was beginning to decrease at this concentration. Comparison of the 24 and 48 hour aphid census indicated that aphids required approximately 36 hours to succumb to the PureCrop1 applications (Figure 2). The time to aphid death appeared to be independent of temperature but may be affected by PureCrop1 concentration.

Additional experiments for the rose, green peach and bean aphid showed similar responses as the melon aphid (Figures 3). As seen previously, at the lowest concentration tested (600:1), mortality averaged more than 90 percent, but this concentration also indicated that potency was beginning to decrease for the rose and green peach aphid. However, the bean aphid remained equally susceptible at all concentrations tested. The rose and green peach aphid also showed similar mortality time as the melon aphid (36 hours). In contrast, the bean aphid required only from 6 to 12 hours before mortality was complete.

II. Whole plant evaluations

Results of the potted plant trials demonstrated that PureCrop1 can be used as a practical pest control tool by effectively rescuing aphid infested potted chrysanthemum, rose and orange jasmine plants with a single application of the product (Figures 4, 5 & 6). The whole-plant trials showed similar performance as the laboratory trials resulting in virtually complete elimination of aphids at all treatments within approximately 24 hours. The time to aphid mortality was similar as the indoor trials except the dryer outdoor conditions desiccated the aphid carcasses more rapidly and therefore mortality was more apparent. Before and after the PureCrop1 applications, photographs of the rose and green peach aphid (Figure 7a & b) and the black bean aphid (Figure 8a & b) illustrate the impact of a single rescue treatment on aphid populations 36 hours after application.

III. Residual efficacy / repellency

Results of residual activity and repellency observations revealed no significant residual mortality or repellency to aphids at the rates tested. Of 48 aphid adults released, 89.6% (43 out of 48) settled on terminal shoots or buds and began to reproduce on the untreated rose plants. For the treated roses, 81.4% (35 out of 43) settled and reproduced within 10 days. In both treatments, adults migrated to preferred feeding sites (terminal shoots of major rose canes), no reluctance on the part of aphids was noted to settle down in previously treated areas.

Conclusions

Under the test conditions of the laboratory trials it can be concluded that PureCrop1 has proven itself to be an effective control agent at 600:1 dilutions or greater. Furthermore, the degree of aphid control was consistent across replicates, experiments and aphid species. These evaluations suggest PureCrop1 could operate as a reliable and effective aphicide. The results of the laboratory trials also suggested that the optimal concentration for PureCrop1 for aphid control could go lower (>300: 1) without sacrificing efficacy. This could further reduce the per acre cost of the product while minimizing any threat from phytotoxicity.

The whole plant trials clearly demonstrated that PureCrop1 could be used as an effective pest control tool for aphids on plants. These evaluations demonstrated that under more realistic and variable outdoor conditions, a single application of PureCrop1 was sufficient to eliminate virtually all aphids from plants in 24 to 36 hours. This control was achieved despite any residual mortality or repellency of the product.

Overall, the results of these evaluations provide encouraging evidence that PureCrop1 could be developed into an effective, reliable and easy-to-use commercial insecticide against aphid pests.



Figure 1a & b. Insect bioassay arenas used to evaluate the dose-mortality between PureCrop1 and aphid pests (above). Test plants used for the melon aphid whole-plant trials (below).

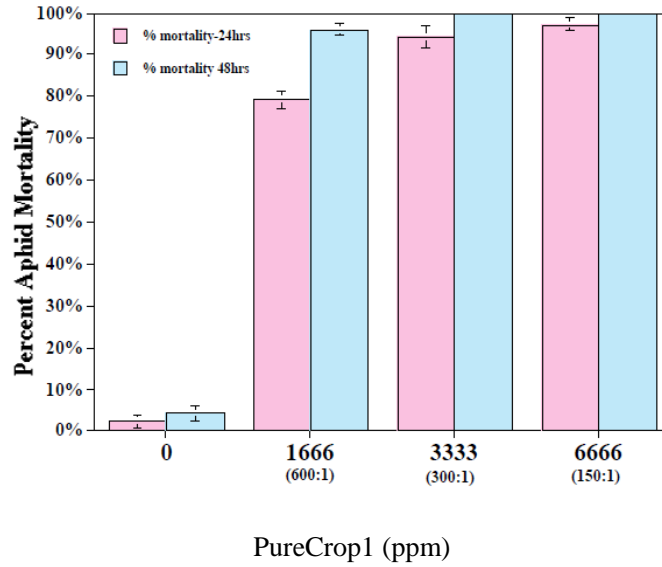


Figure 2. Melon aphid mortality at three concentrations of PureCrop1 at 24 and 48 hours after treatment application

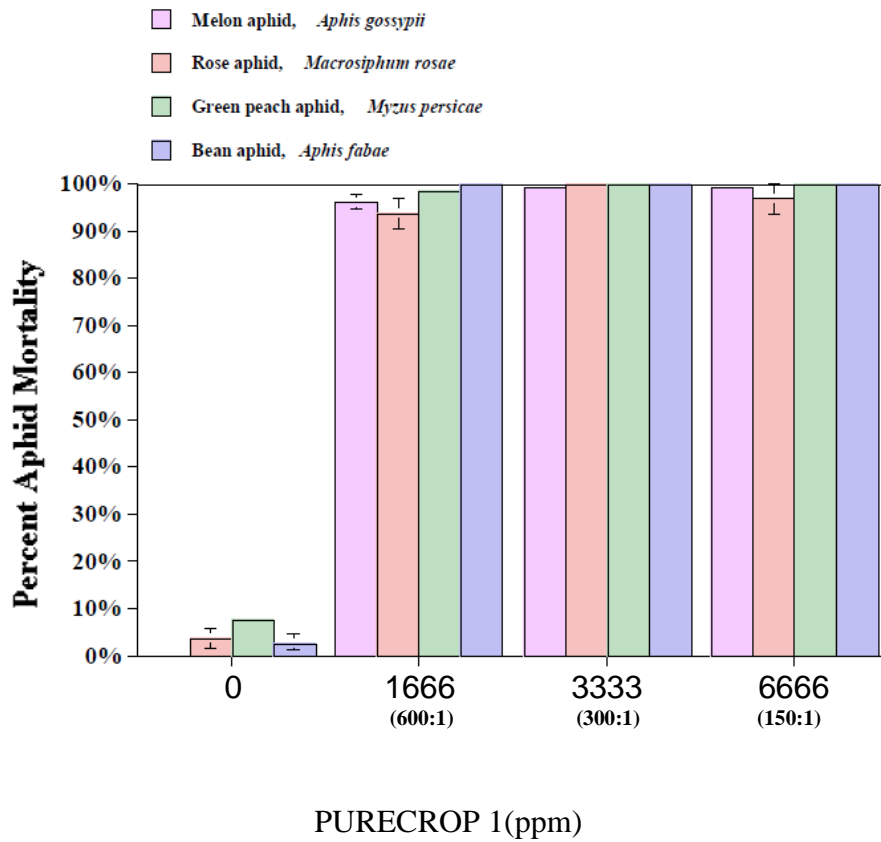
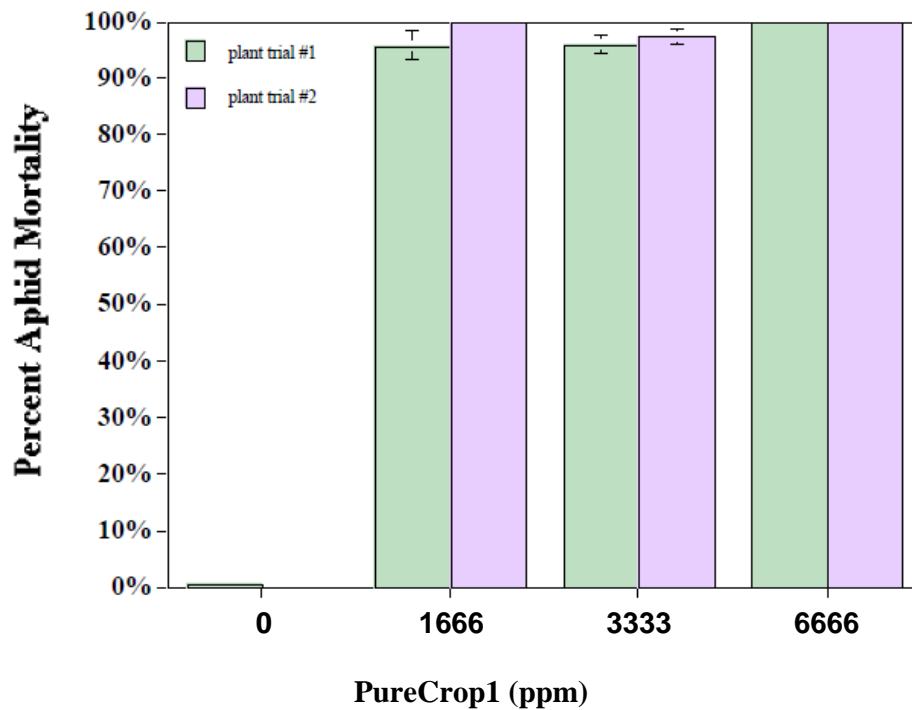


Figure 3. Comparative dose-mortality relationship among four aphid species 36 hours after PureCrop1 treatment



¹**Figure 4.** Result of two whole-plant trials demonstrating PureCrop1 control of the melon aphid on potted chrysanthemum plants.

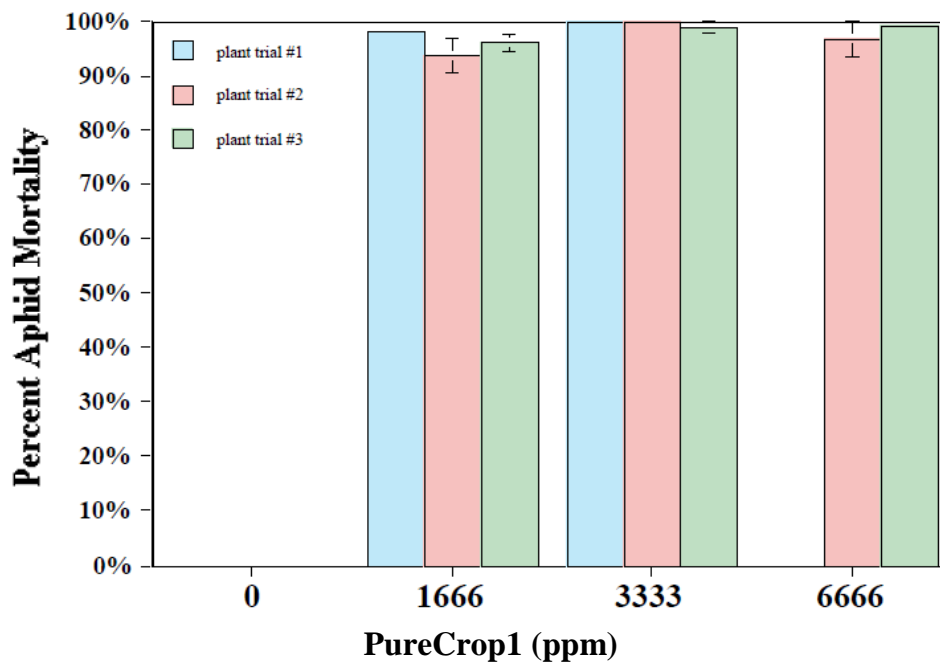


Figure 5. Result of three independent whole-plant trials demonstrating PureCrop1 control of the rose and green peach aphid on potted rose plants.

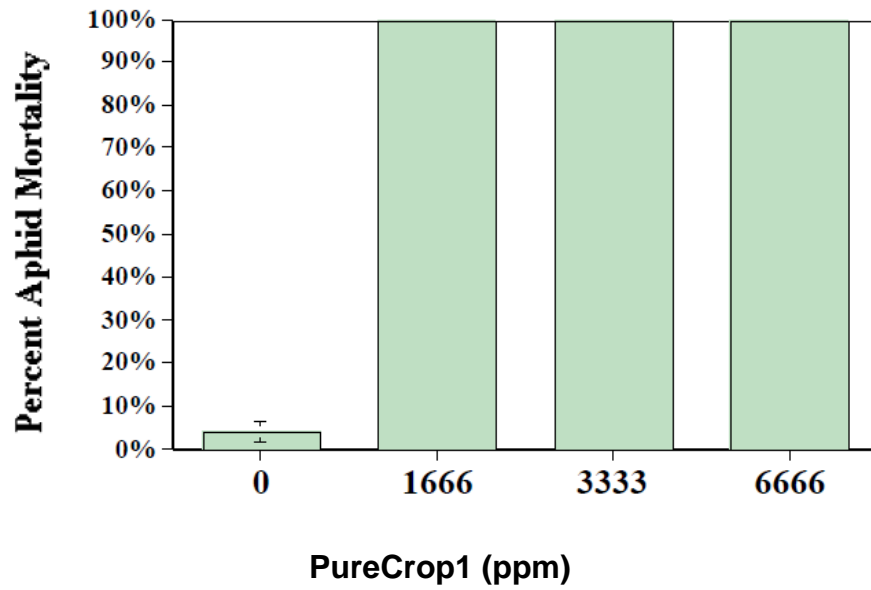


Figure 6. Result of a whole-plant trial demonstrating PureCrop1 control of the bean aphid on potted Jasmine plants.



Figure 7. Aphid infested rose leaves on rose plants used in the whole-plant trials (above). The same rose leaves 36 hours after a 300:1 application of PureCrop1 (below).



Figure 8. Jasmine plants infested with the bean aphid and used in the whole-plant trials (above). The same Jasmine plants 36 hours after a 300:1 application of PureCrop1 (below).