Project title:	**New approaches to aphid control in strawberries combining botanicals and natural enemies.**
Project number:	CRP_RES_0148
Project leader:	Dr Tom Pope, Harper Adams University Glen Powell, NIAB EMR
Report:	Annual report, October, 2020
Previous report:	N/A
Key staff:	Ross George
Location of project:	Harper Adams University NIAB EMR
Industry Representative:	**Harriet Duncalfe**
Date project commenced:	**23/09/2019**

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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CONTENTS

GROWER SUMMARY

Headline

Initial laboratory bioassays indicate that botanical biopesticides may be effective against the potato aphid (*Macrosiphum euphorbiae*) on strawberry.

Background

The strawberry plant is a host for 30 aphid species, some of which are economically important pests. Whilst control of these aphid pests has historically relied upon conventional synthetic insecticides, this situation is changing. Reasons for this change include widespread insecticide resistance, approvals for conventional synthetic insecticides being withdrawn, concerns about the impact of synthetic pesticides on the environment and human health and difficulties in integrating many synthetic insecticides with biological control programmes. These reasons together with pressure from consumers and retailers to reduce the use of synthetic pesticides are leading growers to consider alternative control options.

Previous research has highlighted the efficacy of biopesticides against aphid pests. It has been suggested that biopesticides, including those based on plant extracts, may complement or even replace conventional synthetic pesticides because of their specificity and the reduced risk of resistance developing in target pests. The role of parasitoid wasps and other natural enemies in controlling aphid pest populations has also been well established, however, there is a lack of published research exploring the compatibility between botanical biopesticides and aphid natural enemies within an integrated pest management programme.

Summary

Mortality bioassays were performed on potato aphid (*Macrosiphum euphorbiae*) populations on strawberry leaves using a selection of biopesticides. These products were FLiPPER (Bayer Crop Science, UK) (active ingredients: fatty acids C7–C20) and two physically acting biopesticides coded AHDB9811 and AHDB9810. Additionally, a widely used conventional synthetic pesticide, Batavia (Bayer Crop Science, UK) (active ingredient: spirotetramat), was included for comparison. The products were applied to potato aphid-infested strawberry leaves at the highest application rate recommended by the manufacturers and their efficacy was compared with two controls: leaves that were treated with water and leaves that were untreated.

All of the products had the effect of increasing aphid mortality when compared to the negative controls. This suggests that it may be possible to use botanical biopesticides in place of conventional synthetic pesticides in order to control aphid populations in strawberry crops.

Financial Benefits

According to figures from DEFRA (2020), in 2019 the UK strawberry industry was worth £347.8 million domestically, with exports amounting to a further £4 million. Improved control of strawberry pests, including aphids, provided by a wider range of control products will lead to a reduction in crop damage.

Action Points

There are no grower action points at this stage.

SCIENCE SECTION

Introduction

Strawberry, *Fragaria x ananassa* Duchesne (Rosales: Rosaceae), is an important soft fruit crop, with exports in 2019 contributing £4 million to the UK economy and domestic sales amounting to £347.8 million (DEFRA, 2020). Strawberry is a host plant for 30 aphid species across 16 genera, of which some are economically important pests (Blackman & Eastop, 2000; 2017). Control of these aphid pests has relied on the use of conventional synthetic pesticides, however aphid resistance to these pesticides is becoming more widespread, especially in *Aphis gossypii* Glover populations (Marshall *et al.*, 2012; Gong *et al.*, 2014). Additionally, there are concerns surrounding conventional synthetic pesticides regarding environmental contamination and effects on human health, leading to pressure from consumers and in turn retailers for alternative control measures (Chandler *et al.*, 2011). The efficacy of biopesticides for the control of pests as part of an integrated pest management system used alongside natural enemies has been highlighted, however comparatively little research has been carried out into the compatibility of these two controls (Biondi *et al.*, 2013).

Materials and methods

The aphids selected for this project are three strawberry pest species: the strawberry aphid, *Chaetosiphon fragaefolii* (Cockerell), the potato aphid, *Macrosiphum euphorbiae* Thomas, and the melon-and-cotton aphid, *Aphis gossypii* Glover. In the experiment presented here, *M. euphorbiae* was selected due to the large population available in culture. Subsequent experiments will focus on the two other species.

The experimental population of *Macrosiphum euphorbiae* was taken from the laboratory culture maintained in the Jean Jackson Entomology Building laboratory at Harper Adams University. The aphids were maintained on strawberry, *Fragaria* × *ananassa* Duchesne, plants of the Elsanta variety. A total of 600 adult aphids were used in the present experiment. Ten aphids were used per leaf, with ten leaves used for each of the six treatments. An a priori power analysis was performed using G*Power (Faul *et al.*, 2009) for a 6x5 repeated measures ANOVA. For power at 95%, alpha at 0.05, f = 0.10 (small effect size) and correlation between repeated measures of 0.25, the total number of aphids required was reported to be 468.

The strawberry plants were obtained as cold-stored plants from R. W. Walpole and were potted up in John Innes No. 2 compost (KG Loach, UK) in the Jean Jackson glasshouse at the Crop and Environment Research Centre (CERC) at Harper Adams University. After

approximately four weeks, the plants were moved to insect cages (BugDorm, Taiwan) in a growth room set to 20ºC, 60% RH, 16:8 L:D cycle and potato aphids were introduced and reared. Additional strawberry plants were grown in the Jean Jackson glasshouse at the same time to be used in the experiment, and these plants remained in the glasshouse until the experiment began.

The pesticide products selected for use in the experiment were three biopesticides (FLiPPER, AHDB9811 and AHDB9810) and a conventional synthetic pesticide (Batavia) as a comparison. These products were all prepared to the highest concentration of the range recommended by their respective manufacturers. For Batavia, AHDB9811 and AHDB9810 this was 0.1% v/v (concentrated product in water), and the concentration of FLiPPER was 1.6% v/v.

Protocol

Sixty Petri dishes were prepared by using a heated cork borer to pierce a 27 mm hole in each lid, which was then covered with mesh to allow ventilation whilst preventing aphid escape. An 85 mm diameter disc of qualitative filter paper (Grade 601, Fisher Scientific, UK) was placed on the bottom of each ventilated Petri dish and these dishes were then labelled with a date and a number to identify their treatment condition. A single leaf taken from the stock of strawberry plants was removed by cutting through the petiole with a scalpel and was introduced to each of the Petri dishes (60 leaves in 60 Petri dishes). Ten adult aphids were introduced to each leaf (600 adult aphids in total). The Petri dishes were then taken to the chemical preparation room at CERC where they were sprayed with the products listed above. Ten leaves (100 aphids in ten Petri dishes) were sprayed to run-off twice with each product, once on the adaxial surface of the leaf and once on the abaxial surface, using a hand atomiser. The leaves were removed from the Petri dishes one at a time, sprayed, and then immediately returned to their respective Petri dishes whilst still wet. Once the Petri dishes were re-covered they were taken back to the Jean Jackson Entomology Building laboratory. Each leaf petiole was inserted into a 1.5 mL Eppendorf tube. The top of the Eppendorf tube had first been replaced with a piece of parafilm through which a small hole was pierced to allow the petiole to be inserted. This provided the leaves with a source of water to keep them fresh for the duration of the experiment.

Mortality of the adult aphids was recorded daily for five days (Figure 1). Observations were made using a stereoscopic microscope. If signs of life, such as walking, movement of limbs or antennae, or reproduction, were observed, the aphid was counted as alive (Figure

2, below). Aphids were counted as dead if they showed no signs of life as described above, and did not respond to physical contact with forceps. The number of offspring produced in each Petri dish (Figure 2) was also recorded daily for the same duration.

Figure 1. An example of mortality of a potato aphid observed during the experiment.

Offspring development was recorded by observing each leaf under a stereoscopic microscope and looking for second instar nymphs as well as shed cuticles. Moulting can be seen in Figure 3. Second instar nymphs, when found, were removed from the Petri dish. This reduced the numbers of aphids on each leaf in order to maintain leaf health for as long as possible.

Figure 2. An adult potato aphid giving birth to live offspring.

Figure 3. A potato aphid nymph moulting.

Results

Adult mortality

Mortality of potato aphids was recorded as a percentage of the experimental population. Data are presented in Figure 4. On day zero there were zero deaths in all of the treatments. By day one, three of the biopesticide products had already begun to take effect. Those treatments that work by physical mode of action demonstrate rapid lethality: AHDB9811 showed a mean mortality of 75%, FLiPPER showed a mean mortality of 81% and AHDB9810 showed the highest mean mortality of 90%. Batavia, by comparison, caused a mean mortality of just 34%. On the same day, some mortality was observed in the control dishes: 15% mean mortality in the dishes housing the leaves that were not sprayed and 27% mean mortality in the dishes housing the leaves that were sprayed with water. These are both lower than the pesticide or biopesticide-treated dishes, and could be a result of age. On day two of the experiment, the mean mortality observed in the Batavia-treated dishes was increasing whilst the other pesticide-treated dishes was beginning to plateau. By day four of the experiment all of the pesticide products, both systemic and physically acting, showed a high level of control: Batavia: 92%; AHDB9811: 92%; AHDB9810: 97%; FLiPPER: 95%. The controls (unsprayed leaves and water-sprayed leaves) showed mean mortality of 49% and 40% respectively.

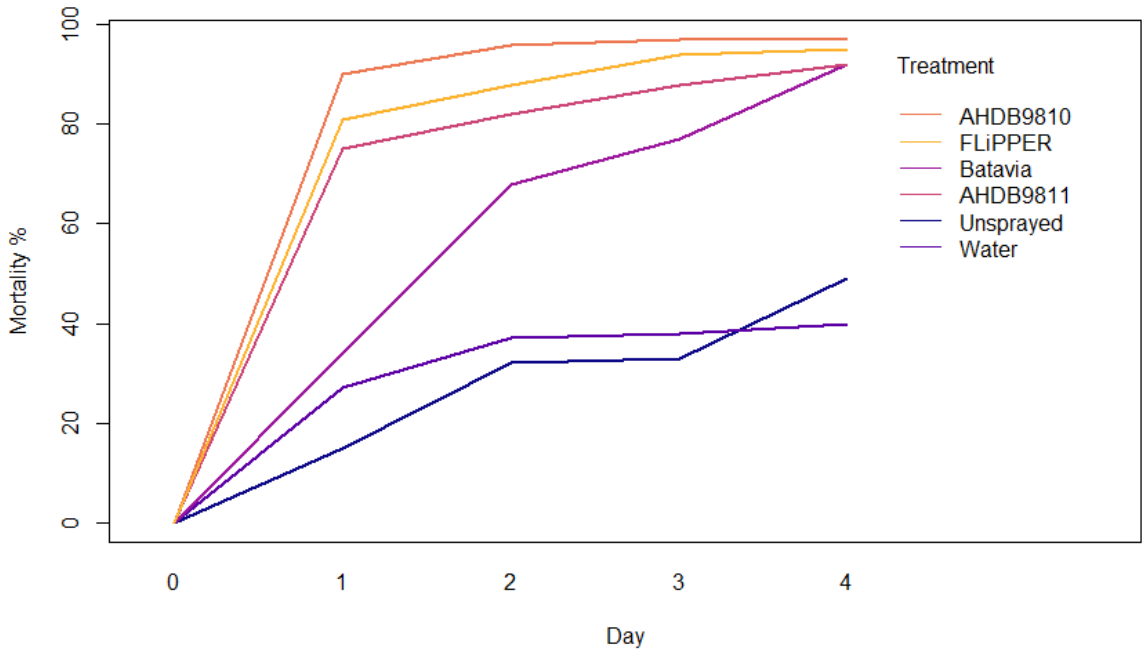

Figure 4. Potato aphid mean mortality percentage by day for each of the treatment conditions.

An ANOVA analysis found a significant difference between mean aphid mortality percentage on sprayed compared to unsprayed leaves ($F(5,290) = 82.542$, $p < 0.001$). Post-hoc t-tests comparing the effects of each individual treatment found significant differences between mean mortality percentages on unsprayed leaves and leaves treated with both pesticide and biopesticide products on day four (Batavia: $t = 8.286$, AHDB9811: $t = 12.138$, AHDB9810, $t = 14.647$, FLiPPER: $t = 13.363$; $p < 0.001$). No significant difference in mean mortality percentage was found between unsprayed leaves and those sprayed with water ($p > 0.05$).

Reproduction

The presence of offspring was recorded as both new offspring produced each day and the total offspring produced since the start of the experiment. Figure 5 shows the mean number of new offspring produced on each day for each of the treatments. In control dishes larger numbers offspring were produced early in the experiment. On day one, both the population on the unsprayed leaves and the population on the water-sprayed leaves produced an average of 1.6 nymphs per leaf. In comparison, the pesticide and biopesticide-treated populations showed much lower numbers. The Batavia-treated adults produced a mean of just 0.1 nymphs per dish. The population treated with AHDB9810 was the next lowest, with a mean of 0.3 nymphs per dish. The FLiPPER-treated adults produced a mean of 0.5 nymphs per dish and the AHDB9811-treated populations produced a mean of 0.7 nymphs per dish. By the end of the experiment, all of the populations were producing much lower numbers of offspring. The AHDB9810-treated aphids stopped producing offspring entirely on day three. For context, the mean mortality for that treatment on that day was 97%. On the final day of the experiment, mortality was high for all of the pesticide and biopesticide-treated dishes which would explain the low numbers of offspring produced on that day. The populations in both control conditions produced an average of 0.4 nymphs (unsprayed) and 0.6 nymphs (water-sprayed), again likely owing to the increasing mortality in these dishes.

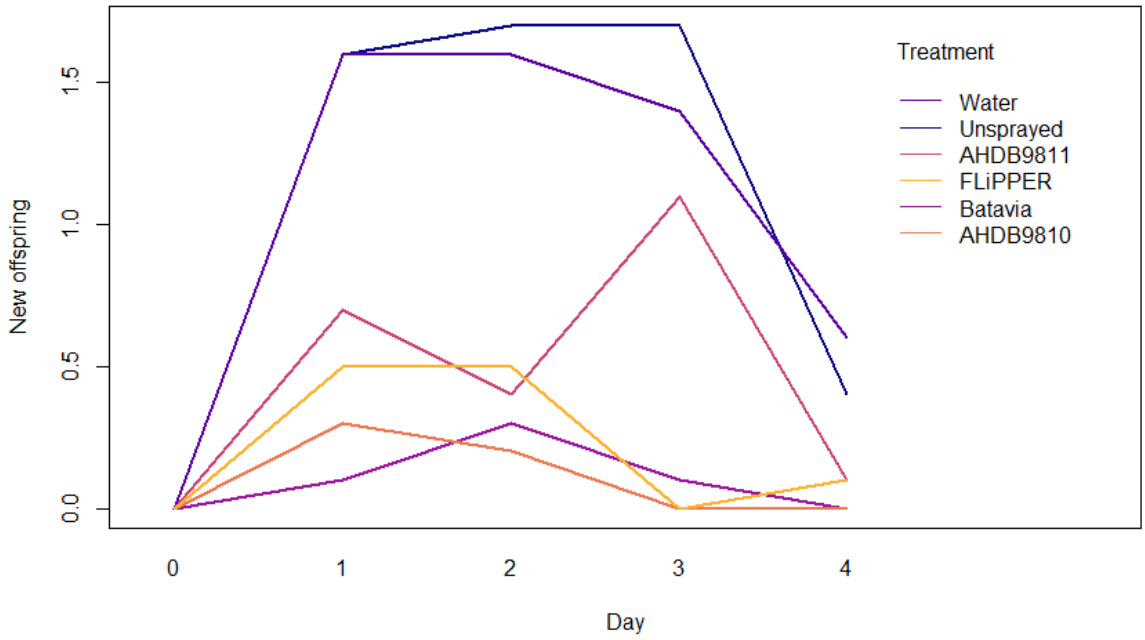

Figure 5. Mean new potato aphid offspring produced by day for each of the treatment conditions.

An ANOVA analysis found a significant difference between mean number of offspring produced per day on sprayed compared to unsprayed leaves ($F(5,290) = 9.612$, $p < 0.001$). Post-hoc t-tests comparing the effects of each individual treatment found significant differences between mean number of offspring produced per day on unsprayed leaves and leaves treated with both pesticide and biopesticide products on day 4 (Batavia: $t = -4.738$, AHDB9810, $t = -4.738$, FLiPPER: $t = -4.158$; $p < 0.001$; AHDB9811: $t = -2.998$; $p = 0.003$,). No significant difference in mean number of offspring produced per day was found between unsprayed leaves and those sprayed with water ($p > 0.05$).

As shown in Figure 6, the population of adult aphids in both control conditions produced a larger number of offspring than the pesticide-treated populations. Similar to the results shown in Figure 5, the total numbers of nymphs increased steadily for the control populations until slowing on day three. The numbers of nymphs produced by pesticide-treated populations, however, began to slow down earlier in the experiment. The numbers of nymphs produced by aphid populations treated with either AHDB9810 or FLiPPER slowed after day one (0.3 and 0.5 mean total offspring per dish respectively). This remained the same for the AHDB9810-treated population for the duration of the experiment, and rose to 0.7 mean total offspring per dish for the FLiPPER-treated population by day four. For the AHDB9811-treated

population, the production of nymphs showed more of an increase up to day two (1.1 mean total offspring per dish) but then reached a plateau (1.3 mean total nymphs per dish by day four). The Batavia-treated population was slow to reproduce (0.1 mean total nymphs per dish by day one) with a slight increase in mean offspring number from day one to day two (0.1 mean total nymphs per dish increasing to 0.4 mean total nymphs per dish) where it remained at the same number for the rest of the experiment.

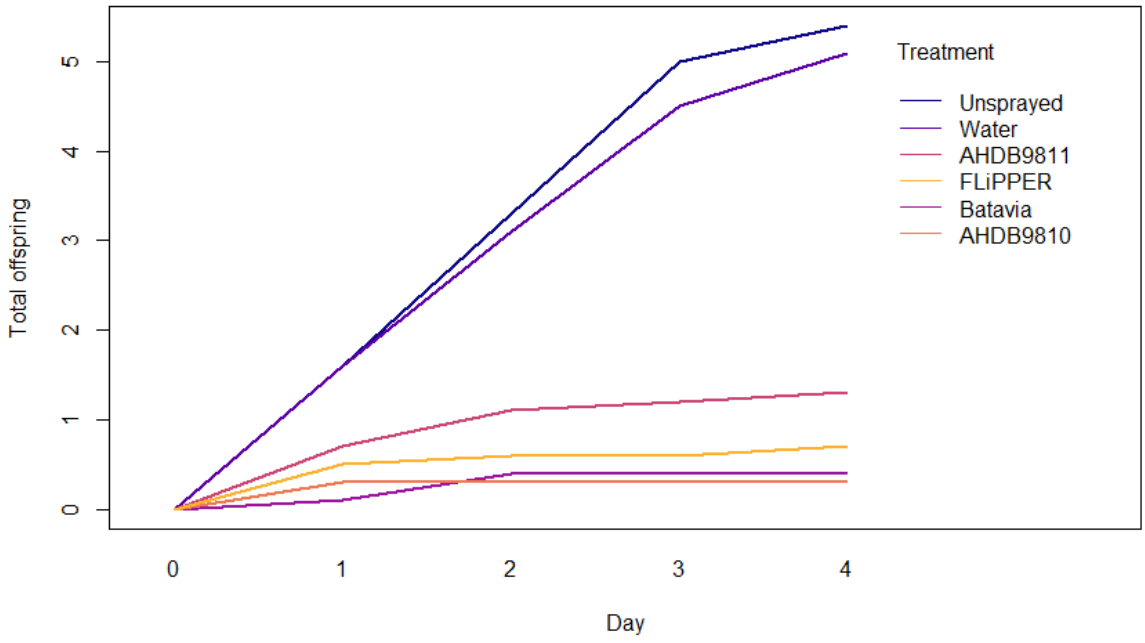

Figure 6. Mean total offspring of potato aphid nymphs produced across the duration of the experiment for each of the treatment conditions.

An ANOVA analysis found a significant difference between mean total number of offspring on sprayed compared to unsprayed leaves ($F(5,290) = 21.176$, $p < 0.001$). Post-hoc t-tests comparing the effects of each individual treatment found significant differences between mean total number of offspring produced on unsprayed leaves and leaves treated with both pesticide and biopesticide products on day four (Batavia: $t = -6.946$, AHDB9811: $t = -5.458$, AHDB9810, $t = -6.996$, FLiPPER: $t = -6.400$; $p < 0.001$). No significant difference in mean total number of offspring produced was found between unsprayed leaves and those sprayed with water ($p > 0.05$).

Discussion

The results presented demonstrate the efficacy of the pesticide products, including the botanical biopesticides AHDB9811, AHDB9810 and FLiPPER. As growers move away from conventional synthetic insecticides, alternative control measures are necessary for crop protection (Chandler, 2011), and the evidence presented here demonstrates that botanical biopesticides could prove to be a viable option, if similar results were recorded under field conditions. Figure 1 shows the rapid speed of kill of these biopesticides, as compared to the controls and the Batavia. All of the pesticide products demonstrated high levels of control, with each leading to over 90% mean mortality four days after treatment application. Data presented in Figures 5 and 6, however, suggest that the physically acting compounds (AHDB9811, AHDB9810 and FLiPPER) are not as effective at limiting offspring production as the systemic Batavia. Populations of aphids treated with AHDB9811 or FLiPPER showed an increase in total number of offspring produced across the duration of the experiment. Batavia, however, showed a smaller increase in the number of offspring produced, primarily on day one and day two (Figure 5) before reducing again. Of the biopesticides, AHDB9810 also reduced the number of offspring produced by the affected aphids, with offspring production peaking on day one before decreasing again for the remainder of the experiment (Figure 5). AHDB9810 also gave the highest level of control on day one of the experiment, and so reduced offspring numbers compared to the other biopesticides are potentially a result of reduced adult numbers. Due to Batavia's mode of action as a lipid biosynthesis inhibitor (Nauen *et al.*, 2008), Batavia-treated populations showed comparatively low mortality, though mortality did increase across the duration of the experiment. The effect of biopesticides on aphid mortality, however, plateaued earlier, demonstrating reduced persistence (Copping & Menn, 2000) and possibly explaining the higher offspring numbers in those populations.

The main recommendations for improving the experiment involve standardisation of the experimental material. Firstly, standardising the age of the aphids. Though the first experiment utilised adult aphids, there was potentially a range of ages within the population used. This could have implications on the length of time that the adults survived during the experiment as well as offspring production. Older adults may not survive on the leaf for the full duration of the experiment due to age rather than treatment condition. Conversely, younger adults may not have started producing offspring by the time the experiment began and so this would impact the number of offspring produced during the experiment itself. In order to overcome this problem standardised cohorts of aphids could be produced. This could be done by taking adult aphids from the culture and placing these individuals on strawberry

plants in a separate enclosure. These adults would be left to produce offspring over the course of 48 hours before being removed from the plants. These offspring would then be allowed to develop into adults over the course of approximately one week to be used in the experiment. By doing this, it would be known that all of the aphids used in the experiment are of approximately the same age.

Conclusions

The products that were selected for this experiment have been shown to reduce the size of aphid populations that were exposed, as well as to reduce the number of offspring produced by any surviving aphids. In subsequent work with natural enemies, in particular parasitoid wasps, the compatibility between these control methods will be established in order to help inform IPM strategies in strawberry crops.

Knowledge and Technology Transfer

30.10.2019: Gave a presentation to introduce myself and my project to the students, supervisors and industry partners in the CTP at NIAB EMR.

14.11.209: Presented a poster introducing my project at the Berry Gardens Research and Agronomy Conference.

28.11.2019: Gave a presentation to introduce myself and my project to fellow postgraduate researchers at the Harper Adams University Research Colloquium.

29.01.2020: Gave a presentation detailing my work on the project to date to the students, supervisors and industry partners in the CTP at the AHDB Crops PhD Conference.

07.07.2020: A research note was made available online detailing my project.

04.08.2020: Gave a presentation detailing my work on the project to date to the students, supervisors and industry partners in the CTP at a virtual conference.

07.10.2020: Gave a presentation to introduce my project and report the results of my first experiment at a Harper Adams University entomology laboratory meeting via Microsoft Teams.

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