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Project leader:	Hayley Jones, RHS
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Location of project:	RHS Garden Wisley, Surrey
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Date project commenced:	[Date Month Year]
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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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GROWER SUMMARY

Headline

The agapanthus gall midge has a long and persistent active season and can infest many tissues of *Agapanthus* flower heads. Currently only detection and destruction of infested material can be recommended to growers. Further research into biological and chemical controls is needed.

Background

The agapanthus gall midge (*Enigmadiplosis agapanthi*) is a recently described pest affecting *Agapanthus*. It was discovered in the UK in 2014 at which time it was new to science. The larvae of this gall midge develop inside the individual flower buds or inside the closed flower head sheaths of *Agapanthus*. The midge can cause the bud to be deformed and discoloured and usually fail to open. The severity of this can range from a few buds failing, to collapse of the entire flower head. This poses a threat to the containerised plant and the cut flower industry. The pest was new to science and as such, very little was known about its biology and life cycle, and it was unknown which control measures could be effective against it.

This project aimed to determine the pest's life cycle and biology, in order to help target control and to ascertain the midge's current distribution both in the UK and abroad. The project also aimed to test the effectiveness of some currently available pesticide and biological control products under laboratory conditions.

Summary

Objective 1. Determine and describe the life cycle of agapanthus gall midge.

The midge was studied through observations in RHS Garden Wisley and by rearing in rearing tubes and rearing cages during 2015 and 2016.

The gall midge has a very long active period, between mid-June and early October. Active larvae in *Agapanthus* flower heads in the garden were confirmed between 30th June and 10th October 2015 and between 24th June and 6th October 2016. Larvae have also been found on 13th June 2017, in agapanthus plants flowering earlier than usual (possibly due to a warm spring). Fortnightly measures of infestation severity were taken from an 'award of garden merit' trial of agapanthus cultivars in 2015. This showed a long and consistent period of activity, indicating multiple overlapping generations.

Rearing showed that the larvae feed and develop inside the flowers and when fully grown emerge and drop into the soil or growing media. They bury themselves to pupate and take between ten days to two weeks from larvae dropping to adult emergence during the summer. It is likely that they bury themselves deeper to overwinter and pupate in the spring, but this has not yet been confirmed. Larvae left in rearing tubes over winter started to emerge in April, but these tubes were kept in sheltered conditions. In RHS garden Wisley active larvae were found as soon as any *Agapanthus* plants had well developed buds, which indicates that the midge starts to emerge before its host plant flowers.

Observations of rearing cages failed to observe mating and oviposition (egg-laying) behaviour

Agapanthus flower heads can be infested at different stages of growth and the associated symptoms severity is therefore quite variable. If only a few buds are infested on a flower head the infestation may go undetected. If infestation is very severe, or occurs before the flower head sheath opens then it may completely fail to flower. Flower dissection in 2016 also showed that larvae can survive inside senesced flowers, further prolonging their active period and making infestation hard to detect. A single flower head can host hundreds or even thousands of larvae; the average number of larvae found across 51 flower heads dissected was 719, with a maximum number of 3465.

Objective 2. Confirm the distribution and host range.

Distribution

The midge is most likely native to South Africa, as this is the native range of *Agapanthus* and there are reports of symptoms on wild and commercially grown plants there. There is currently no confirmation that this is the same species but samples of midge from SA have been obtained, so DNA sequencing will allow us to confirm whether they are the same species.

The only reports of the midge outside of the UK are on the islands of Guernsey and Jersey, where there are established populations. The International Plant Sentinel Network has circulated surveys to botanic gardens worldwide but currently only negative reports have been returned.

In order to confirm the UK distribution the RHS made a call to the public, through social media, gardening press, the RHS website and *The Garden* magazine. The distribution was ascertained by collating observations from the public and growers, supported by sample or photographic evidence.

These records, combined with those from a survey of commercial premises carried out by APHA in 2015 can be seen in Figure 1.

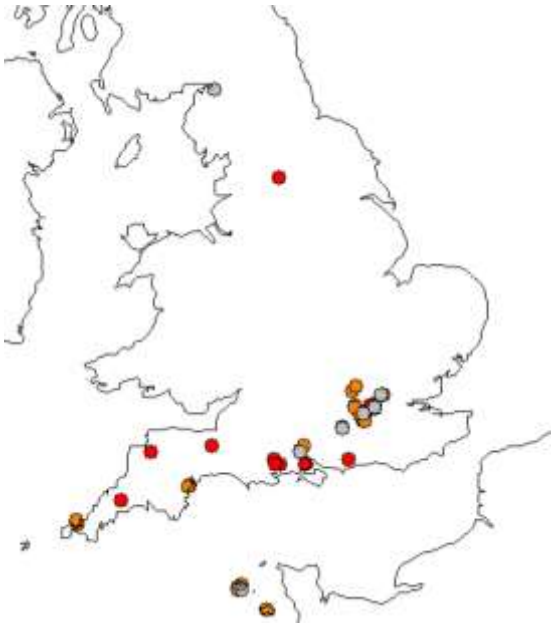

Figure 1. Agapanthus gall midge distribution 2014-2016; 2015 records in orange, 2016 records in red and unconfirmed in grey.

The map shows that the midge has a widespread distribution, mostly restricted to the South of England but with isolated cases in the north and established populations on Guernsey and Jersey. The West Yorkshire record was confirmed by a sample with larvae present, the owner of the *Agapanthus* had purchased a plant in Guernsey in spring 2014.

It is likely that the midge has been present in the UK for some years before 2014, evidenced by photographs of symptoms in a private collection in 2012 and reports of possible symptoms from 2011.

Host range

Observations in 2015 of 149 cultivars of *Agapanthus* in the RHS 'award of garden merit' trial indicated that there may be some differences in midge infestation between different cultivars of *Agapanthus.* The pie chart in Figure 2 shows the proportions of cultivars with no, mild or severe symptoms.

In another experiment by Matthew Everatt (Defra), developed in collaboration with the RHS, six widely grown cultivars of *Agapanthus* were tested for susceptibility to the midge. This experiment found that Northern Star had much higher levels of infestation than the others tested, measured by number of larvae found inside a flower head. This was measured on

plants that were uninfested at the start of the season and had regular infestation pressure applied for twenty days prior to measurement.

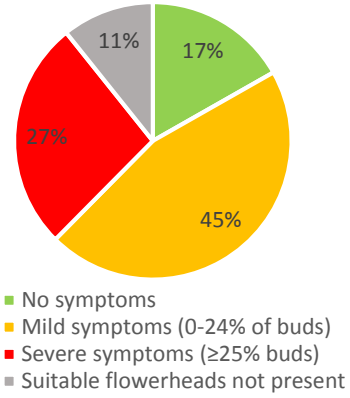

Figure 2. Proportions of cultivars showing different levels of agapanthus gall midge symptoms. 149 cultivars were observed.

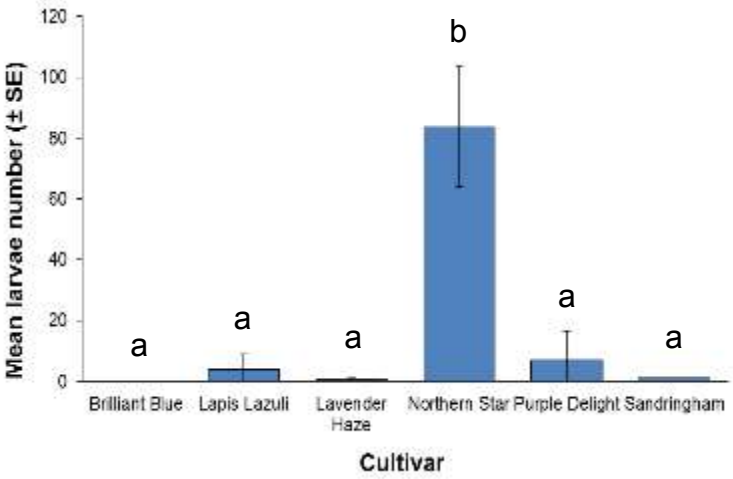

Figure 3. Mean number of agapanthus gall midge larvae associated with flower heads of six *Agapanthus* cultivars, following 20 days exposure to the agapanthus gall midge. Means with the same letter are not significantly different from each other.

Objective 3. Quantify the effectiveness of potential chemical and biological controls.

The following pesticides and biological controls were tested for their efficacy in controlling the midge:

1. *Steinernema kraussei* (Nemasys® L)
2. *S. feltiae* (e.g. Nemasys®) – both treatments 1 and 2 are entomopathogenic nematode species which can be used on any crop.
3. *Metarhizium brunneum (anisopliae)* (Met52 granular and Met52 OD) – A fungal entomopathogen. The OD product is approved for use as a spray on ornamentals in the UK but not yet commercially available or approved for use as a drench but an EAMU could potentially be sought if shown to be effective.
4. Spinosad (Conserve/Tracer) – an insecticide that has approval for use as a foliar spray on protected ornamentals (Conserve) and an EAMU for use on outdoor ornamentals (Tracer).
5. Thiacloprid (Calypso) – systemic insecticide that has an EAMU for use as a drench on protected ornamentals for control of vine weevil and sciarid fly.
6. Deltamethrin (Decis Protech) – a contact-acting pyrethroid insecticide that has approval for use as a foliar spray on both protected and outdoor ornamentals.
7. Cypermethrin (Cythrin Max EC) – a contact-acting pyrethroid insecticide that has approval for use as a foliar spray on both protected and outdoor ornamentals.
8. Water as a control.

The first experiment tested foliar sprays against flower-dwelling larvae. Cut stems with infested agapanthus flowers were gathered from RHS garden Wisley and inserted into plastic bottles of water through a parafilm seal. The eight treatments were then applied using an Oxford Precision sprayer. Each bottle was then placed in a large saucer of soapy water, to catch any larvae dropping from the flower heads (fully fed larvae drop to the ground to pupate) After 14 days the number of larvae in the saucers was counted, and the flowers dissected and numbers of dead and alive larvae within were counted.

None of the treatments tested had a significant effect on the percentage of live larvae in the petals or the number of larvae that dropped into the saucers. Gall-inhabiting organisms are often difficult to treat with foliar sprays as contact-acting products may not penetrate far enough into the plant tissue to reach the target pests.

The second experiment tested the effectiveness of treatments to growing media against the larvae that drop to the ground to pupate. Small pots of growing media were treated with drenches of the same treatments used in the first experiment on the flowers. Ten mature midge larvae were then added to the surface of the growing media in the pots, to mimic the larvae dropping to the ground. The underside of each of the snap-on lids to the pots were covered with a yellow sticky trap. The number of adult midges emerging was monitored on both the sticky traps and the surface of the growing media. The only treatment that significantly reduced the mean number of emerged adults (zero midges emerged) compared with those in the water controls (mean of 0.9 midges emerged per pot) was the drench of Calypso. However as only a mean of 10% of the larvae added to the water control pots successfully emerged as adults, indicating a mean of 90% natural mortality during the late larval or pupal stages further experimentation is necessary to improve survival of the midges in control pots in order to give a more robust result testing drench treatments for control in the growing media. It is possible that the growing media was too wet for successful adult midge emergence.

Finally a set of 160 agapanthus plants were established at a field site at Wisley, and exposed to pest pressure. These plants will enable us to carry out a field tests of controls in subsequent years, with the potential to test up to eight different treatments.

Future work

None of the foliar sprays tested had a significant impact on the midge, and this is likely to be because the products cannot penetrate into the flower buds successfully. Future experiments could repeat the foliar sprays with the addition of wetters to aid penetration of the products and could potentially include additional novel insecticides with translaminar or systemic action.

Future work could include an experiment to determine the optimum growing media moisture level needed for successful midge emergence and then repeat the drench treatments to further test control of the ground-dwelling stages of the pest.

Financial Benefits

Recommendations available based on the results of this project are limited; as control methods are not yet available, prevention of outbreaks by reliable sourcing and inspection of incoming plant and regular and detailed monitoring of nursery plants is necessary. Prompt destruction of infested plants should limit potential crop loss, which is estimated at up to 70% in an infested nursery. The value of this 70% crop loss would be approximately £840,000 (based on estimates of £3 production cost per pot in a representative sized nursery).

Action Points

- Source agapanthus plants from uninfested nurseries
- As plants may not yet be showing symptoms when brought onto the nursery, monitor closely for symptoms as soon as they start to flower
- Remove and destroy infested flower heads
- Destroy badly infested plants
- The results of this project indicated that a drench of Calypso (used according to EAMU 2014/2153 for control of vine weevil and sciarid larvae in protected ornamentals) may give some control of the larvae or pupae in the growing media after the larvae have dropped to the ground to pupate. However this result needs validating in a repeated experiment before this can be recommended to growers for control of agapanthus gall midge.
- Avoid highly susceptible cultivars such as Northern Star

SCIENCE SECTION

Introduction

The agapanthus gall midge (*Enigmadiplosis agapanthi*) is a recently described fly (Diptera: Cecidomyiidae), with *Agapanthus* as it's only known host. The larvae of this gall midge develop inside the individual flower buds or inside the closed flower head sheaths of *Agapanthus*, causing deformation and discolouration, and usually causing the flowers to fail to open.

Symptoms of the agapanthus gall midge were first noticed in a UK private garden in Surrey and reported to the Royal Horticultural Society (RHS) in 2014. Liaison with Defra and cecidomyiid expert, Keith Harris, confirmed that the midge was new to science. At this time the midge was already well established in RHS Garden Wisley, Surrey.

A call to the public by the RHS and a survey of commercial premises by PHSI demonstrated that the midge's distribution was sparse but widespread in the UK and mostly restricted to the South of England. Defra implemented statutory action on the pest, so that infested plants were not allowed to be moved. Subsequent reports indicated that it had been present in the UK for at least two years and was also present in at least one location in the north of England. A Rapid Pest Risk Analysis was carried out and concluded that statutory action was inappropriate (Everatt, 2015). The midge is likely to have originated in South Africa, where *Agapanthus* is endemic. There are observations of a midge inducing identical symptoms in South Africa, but it had never been described or studied (Duncan, 2002). Specimens from the UK were used to describe the species (Harris *et al.* 2016).

This emergent pest poses a significant risk to the approximately 100 growers of *Agapanthus* plants in the UK, who sell more than 1.25 million stems to the cut flower trade and 400,000 containerised plants per year. Infestation of flower heads with agapanthus gall midge makes the plants and stems unsaleable as it ruins the appearance. The potential cost to an infested nursery, based on estimates of £3 production cost per pot and potential crop loss of 70% in an infested nursery, is approximately £840,000 (Fairweather & Carr, *pers comm)*

Being new to science, very little was known about the biology and life cycle of the agapanthus gall midge, and it was unknown which control measures could be effective against it. Currently the only control option for growers is to remove infested flower heads, and to experiment with potential control methods. If control is ineffective the plants cannot be sold until the following year. The RHS carried out initial observations in 2015 then collaborated with ADAS in 2016 in this research project.

The long-term aim of this research is to generate knowledge that will enable growers to

implement a control strategy for the agapanthus gall midge, ideally to prevent infestation as well as to control existing infestations. Information on the distribution, host range and varietal differences in the effects of the midge are also needed, to highlight which geographic areas and commercial cultivars are most at risk, enabling growers to be informed and monitor accordingly. Finally research is needed to determine which commercially available chemical and biological controls are effective against the midge. Understanding the biology and life cycle of the pest should enable targeted and timely use of control methods, so that control is efficient and cost-effective.

The objectives of this project (HNS PO 199) were:

- *Determine and describe the life cycle of agapanthus gall midge*.
- *Confirm the distribution and host range.*
- *Quantify the effectiveness of potential chemical and biological controls.*

Materials and methods

Objective 1. Determine and describe the life cycle of agapanthus gall midge.

The midge was studied in RHS Garden Wisley, Surrey, a 24 hectare garden that is open to the public. Wisley has many patches of established *Agapanthus* plants throughout the ornamental gardens, as well as a trial of *Agapanthus* cultivars. Widespread agapanthus gall midge infestation has been recorded here since 2014. The midge was studied both *in situ* in the garden and by collecting material for laboratory studies.

Field observations

Throughout summer 2015 (prior to project HNS PO 199) observations were made on an RHS 'award of garden merit' (AGM) trial of agapanthus cultivars. This consisted of three plants from each of 149 cultivars of agapanthus planted in four rows. Fortnightly observations were made, recording the following for each plant of each cultivar:

- Flowering stage (no buds, flower head sheath present, buds present, flowering, senescing)
- Number of flower heads present
- Number of flower heads infested
- Severity of infestation (visual assessment of approximate proportion of buds infested across infested heads)

This data was then used to calculate the proportion of flower heads infested throughout the season. Observations of which plant tissues could be infested were made on plants gathered from the garden in 2015 and plants dissected as part of tests of foliar sprays against flower dwelling larvae in 2016.

Rearing

Rearing tubes such as those shown in Figure 5 were used. These tubes consisted of an opaque lower tube containing the growing media (coir mixed with vermiculite) where larvae can pupate and a transparent upper tube that the adult midges fly up into upon emergence. For rearing experiments larvae were gathered by taking cut heads of *Agapanthus* with severe symptoms from RHS garden Wisley. The larvae were extracted by placing the flower heads into polythene bags with a small amount of water sprinkled inside and leaving overnight at ambient temperature. Larvae which left the flowers were pipetted or transferred with forceps into the rearing tubes and placed on a slightly dampened piece of tissue to maintain moisture

levels in the tubes. The tubes were kept in an unheated room which was lit by an external window.

Figure 5. Rearing tubes used to observe pupation and adult emergence of agapanthus gall midge.

Figure 6. Rearing cages used to observe mating and oviposition behaviour of agapanthus gall midge

On three occasions in 2016 attempted observations of mating and oviposition behaviour were carried out in large rearing cages. These consisted of a 600 mm x 600 mm x 1000 mm cast acrylic box with an open bottom in a tray lined with capillary matting, which allowed the plants to be watered without opening the door. Each cage had a door attached by magnetic strips and two vents covered with insect-proof mesh. Three cages were set up with two uninfested *Agapanthus* plants in each cage, with each plant having at least one flower head.

On each observation occasion approximately 20 adult midges were transferred from the rearing tubes into each cage, using a pooter (aspirator). These cages were then observed for two hours, and any activity noted.

Objective 2. Confirm the distribution and host range.

Distribution

To gather records to map the distribution of the midge the RHS made a call to the public to submit their sightings, with samples or photographs. The call was disseminated via RHS social media posts in January, July and August, as well as further social media, blog posts and pod casts by RHS staff such as Hayley Jones and Andrew Salisbury. These communications asked gardeners to submit records by email or post, preferably accompanied by samples in sealed containers to RHS Garden Wisley, or photographs.

Host range

This experiment was carried out by Matthew Everatt, Defra, in collaboration with the RHS, not as part of HNS PO 199. *Agapanthus* plants from six cultivars were obtained as plugs (16

each of Lavender Haze and Purple Delight) or in 9 cm pots (16 each of Brilliant Blue, Lapis Lazuli, Northern Star and Sandringham) on 21st July 2016. The plants were re-potted into 2 litre pots of multipurpose compost and arranged in four blocks on weed-suppressant ground-cover matting at the RHS Field Research Facility. Each block consisted of four plants from each cultivar grouped together as one replicate, with the position of the cultivar within the block randomised using a random sequence generator. Infestation pressure was applied by placing pots of growing media at regular intervals throughout the blocks, and adding infested flower heads to these pots weekly for three weeks.

On 11th August 2016 the visual assessment of the level of infestation of the flower heads in each replicate was carried out. Flower heads from each replicate group were placed into sealable polythene bags with 20 ml water and transported back to Fera, York for assessment. The flower heads were left at room temperature for at least three days prior to counting. Each cultivar grouping was assessed individually by placing buds into a tray, dissecting them by hand, and counting any larvae that were found.

Objective 3. Quantify the effectiveness of potential chemical and biological controls.

The effectiveness of the following treatments were tested, as foliar sprays and/or growing media drenches:

1. *Steinernema kraussei* (Nemasys® L)
2. *Steinernema feltiae* (e.g. Nemasys®) – both treatments 1 and 2 are entomopathogenic nematode species which can be used on any crop.
3. *Metarhizium brunneum (anisopliae)* (Met52 granular and Met52 OD) – A fungal entomopathogen. Met52 granular is approved for incorporation into growing media or soil used for growing ornamentals. The OD product is approved for use as a spray on ornamentals in the UK but not yet commercially available or approved for use as a drench but an EAMU could potentially be sought if shown to be effective.
4. Spinosad (Conserve/Tracer) – an insecticide that has approval for use as a foliar spray on protected ornamentals (Conserve) and an EAMU for use on outdoor ornamentals (Tracer).
5. Thiacloprid (Calypso) – systemic insecticide that has an EAMU for use as a drench on protected ornamentals for control of vine weevil and sciarid fly.
6. Deltamethrin (Decis Protech) – a contact-acting pyrethroid insecticide that has approval for use as a foliar spray on both protected and outdoor ornamentals.
7. Cypermethrin (Cythrin Max EC) – a contact-acting pyrethroid insecticide that has approval for use as a foliar spray on both protected and outdoor ornamentals.
8. Water as a control.

Test against ground-dwelling larvae

In order to test controls for the ground-dwelling larval stage a laboratory test using pots of growing media was used (Figure 7). Eight different treatments including a water-treated control were tested with 10 replicates per treatment. Each replicate consisted of a 365 ml plastic pot (9.5 cm diameter at top of pot) with four ventilation holes near the top and one hole at the base (holes smaller than midge body size). These pots were filled with 280 ml moistened multi-purpose growing media (except in the case of Met52 granular – see below). They were housed in a controlled environment polytunnel at the RHS Field Research Facility at 21°C under a 16 hour day length.

The eight treatments were applied at the following rates, using the label or EAMU recommended rates for control of other pests:

No.	Treatment (justification for choice)	Rate
1	Nemasys L (gave some control of blackberry leaf midge in lab bioassays in SF 158 and used on HNS for vine weevil control)	*S. kraussei* – 1,000,000 nematodes/m^2 in 4 L/m^2 water-curative drench (rate for vine weevil control). Calculated this for 9.5 cm diameter pot. In 1 m^2 can fit 140.85 pots. Therefore 7100 nematodes in 28 of water is needed per pot .
2	Nemasys (included as a comparison with Nemasys L, used on protected HNS for sciarid fly control)	*S. feltiae* - 1,000,000 nematodes/m^2 in 4 L/m^2 water-curative drench (rate for sciarid fly control). Rate per pot as for Nemasys L above.
3.1	MET52 Granular mulch (has EAMU for control of midges with pupal stage in the ground on soft fruit crops)	500 g product / m3 of growing media applied as a mulch (EAMU 2011/1997) – substrate incorporation.
3.2	MET52 OD (approved but not yet commercially available and not yet recommended as a drench but may be possible to get EAMU if effective)	4.68 ml per L (converted from 62.5 UK fluid oz per 379 litres, converted from US rate of 60 US fluid oz per 100 gal). 28 ml of this rate added per pot.
4	Tracer (potential control of blackberry leaf midge shown in lab bioassay in SF 158, no recommendation for use as a drench on HNS but is used as a drench for cabbage root fly control on brassica modules so EAMU may be possible if shown to be effective)	200ml/ha (1000 L water/ha) - drench, using EAMU 2908/2008 (foliar spray rate). 28 ml of this rate added per pot.

5	Calypso (has EAMU for use as drench on protected ornamentals for control of vine weevil and sciarid fly and has shown potential against blackberry leaf midge as a foliar spray)	83 ml in 100 L per m^3 compost (per 1000L compost) (EAMU 2014/2153 drench for vine weevil and sciarid fly control). Equivalent to 0.83 ml in 1 L. 28 ml of this rate added per pot.
6	Decis Protech (no recommendation for use as a drench but included as has a wide spectrum of activity against a range of pests)	120 ml per 100 L water (label recommendation as foliar spray for other pests on outdoor amenity ornamentals). 28 ml of this rate added per pot.
7	Cythrin Max EC (as for Decis Protech)	10 ml per 100 L water (label recommendation as foliar spray on roses for aphid control). 28 ml of this rate added per pot.
8	Water-treated control	28 ml per pot

The MET52 mulch was applied by combining 1.75 g of Met52 granular with 3.5 litres of moistened multipurpose growing media in a lidded bucket. This was mixed thoroughly by rolling and turning the container for 10 minutes. 280 ml of this mix was then added to each of ten pots.

Before application the nematodes were tested for viability. 1 ml of the nematode suspension was extracted with a pipette and added to a 1 ml Hauxley haemocytometer counting chamber. A binocular microscope was used to verify the presence of approximately 250 nematodes per ml. Three replicates of this were carried out for each of the two nematode treatments.

The drenches were applied evenly across the surface in the pots using a syringe to deliver 28 ml per pot (using 10% of the volume of growing media per pot is standard grower practice

Figure 7. Pots of growing media used for tests of biological and chemical control of the ground-dwelling stage of the agapanthus gall midge. Ten larvae were added to treated compost and lids covered with yellow sticky added to catch emerging adults.

when applying drenches). Treated pots were then arranged in blocks across two benches in the CE polytunnels.

The treated pots were left overnight lightly covered by their lids. Over the next two days (26th and 27th August) ten mature midge larvae were added to the surface of the growing media in each pot, to mimic larvae dropping to the ground in order to pupate. Infested flower heads were dissected and mature larvae counted out into groups of ten in drops of water which were then transferred to the pots. Once larvae had been added the pots were sealed with snap-on lids which had yellow sticky trap on the underside.

As treatments 2 and 3.2 were not available when the experiment was set up, identical pots were used for these treatments and an additional set of water treated controls on 1st September and the midge larvae were added to each pot on 2nd September.

Five spare water–treated controls were also established to monitor emergence of the adult midges to help time the assessments. These were checked twice weekly for four weeks and then all pots were assessed from the first experimental set on 28th September and those from the second experimental set were assessed on 5th October. The numbers of adult midges were counted on both the sticky traps and the surface of the growing media, and the numbers of dead and alive larvae on the surface counted.

Statistical analysis was carried out in Genstat (VSN International, 2009) to determine if there were significant differences between the treatments, using ANOVA and Duncan's multiple range test.

Test against flower-dwelling larvae

In order to test foliar sprays of chemical and biological controls cut stems of *Agapanthus* with infested flowers were collected from RHS garden Wisley, in multiples of ten from each *Agapanthus* cultivar or patch (with stems from one patch forming a block for the treatments). The cut stems were inserted into plastic bottles of water through a parafilm seal (Figure 8).

The eight foliar sprays were applied at the following rates using ten replicate flowers per treatment:

No.	Treatment	Rate
1	Nemasys L	250,000 nematodes/m^2 (no recommendation as foliar spray so used same rate as Nemasys)
2	Nemasys	250,000 nematodes/m^2 (rate for thrips control as foliar spray)
3	MET52 OD	1.25 L/ha
4	Tracer	200 ml/ha (EAMU 2908/2008 foliar spray)
5	Calypso	375 ml/ha (EAMU 2149/2014 foliar spray)

6	Decis Protech	120 ml per 100 L water (label recommendation as foliar spray for other pests)
7	Cythrin Max EC	10 ml per 100 L water (label recommendation as foliar spray for other pests)
8	Water-treated control	600 L/ha All other treatments to be applied in the same water volume.

The nematodes were tested for viability using a Hauxley haemocytometer as for the experiment on the ground-dwelling larvae above. The eight treatments were then applied using an Oxford Precision sprayer fitted with a HC/1.74/3 nozzle, in 600 litres water per ha using 3 bar pressure.

Each bottle with its agapanthus flower and stem was then placed in a large saucer of soapy water, to catch any larvae dropping from the flower. They were housed in the same CE polytunnels as the pots used for the experiment on ground-dwelling larvae (21°C, 16 hour day length).

Figure 8: Cut stems of *Agapanthus* used for tests of biological and chemical control of the flower-dwelling stage of the agapanthus gall midge.

The majority of the treatments were applied on 25th August. Treatments 2, 3 and an additional set of water treated controls were treated on 1st September.

After 14 days (8th and 15th September respectively) the flower heads and saucers were sampled and stored for assessment. The contents of the saucers were strained through individual pieces of insect-proof mesh, which were then stored in the fridge until they could be assessed under a binocular microscope to count the numbers of larvae. The flower heads were placed into sealed polythene bags and stored in the fridge before being dissected and the numbers of alive and dead larvae counted. Dead larvae from the MET52 treatment were incubated at 23°C on damp filter paper for 7-10 days and any growth of Metarhizium recorded.

Statistical analysis was carried out in Genstat (VSN International, 2009) to determine if there were significant differences between the treatments, using ANOVA and Duncan's multiple range test.

Establish a field trial of infested Agapanthus for future tests of biological and chemical controls

160 *Agapanthus* 'Northern Star' were obtained as 9 cm container plants and re-potted into 2 L pots using multipurpose compost. These plants were arranged in five blocks of 32 plants on ground-cover matting at the RHS Field Research Facility. Each block consists of eight sets of four plants, with one set of four constituting an experimental unit for future experiments. The plants were exposed to agapanthus gall midge by placing pots of growing media at regular intervals throughout the blocks, and adding infested flower heads to these pots weekly throughout July and August. The pots were watered daily throughout the summer using overhead irrigation.

Results

Objective 1. Determine and describe the life cycle of agapanthus gall midge.

Field observations

The results of the preliminary work looking at midge presence throughout the *Agapanthus* growing season (carried out in 2015) are shown in Figure 9.

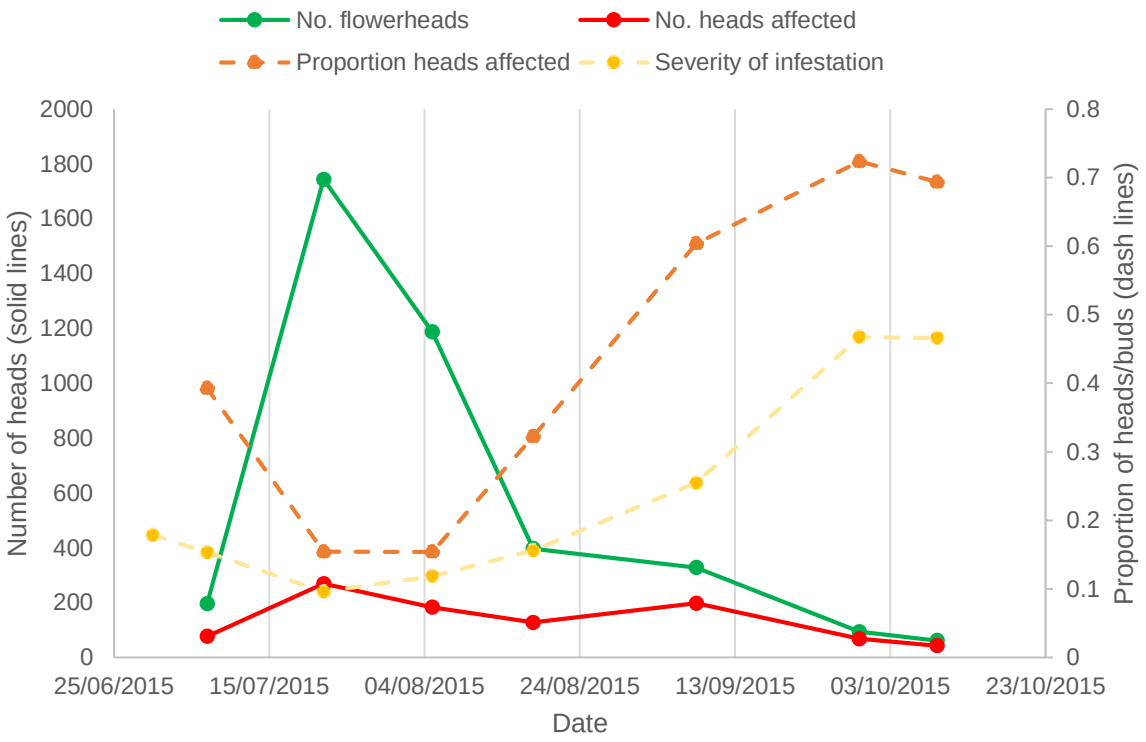

Figure 9. Line graph illustrating levels of agapanthus gall midge infestation on *Agapanthus plants* the RHS Award of Garden Merit *Agapanthus* trial at Wisley in 2015.

The above demonstrates that the midge has a long and consistent period of activity with no distinct peaks and troughs, most likely multiple overlapping generations. The proportion of flower heads and buds infested was highest when available flower heads was lowest, in the earliest and latest part of the season.

Of the 149 cultivars assessed 16 never developed suitable flower heads, 25 showed no symptoms, 68 showed mild symptoms, and 40 showed severe symptoms (Figure 10). This was not a replicated trial so no firm conclusions about cultivar susceptibility can be drawn from it, but the results indicate that there may be differences in susceptibility. Therefore the collaboration with Matthew Everatt at Defra was developed to start to address this.

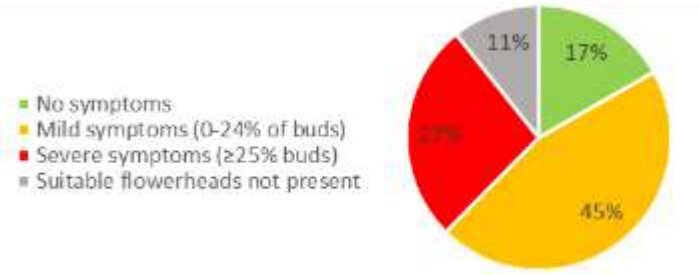

Figure 10. Proportions of cultivars showing different levels of agapanthus gall midge symptoms. 149 cultivars were observed.

During observations in 2015 and approximately fortnightly in 2016, a random selection of buds were opened to check for the presence of active larvae. Active larvae were confirmed between 30th June and 10th October 2015 and between 24th June and 6th October 2016. Larvae were also found on 13th June 2017, in three cultivars of *Agapanthus* plants, two of which were found in the RHS AGM plants and one in the 'Mixed Borders' area of the garden.

The *Agapanthus* flower heads can be infested at many different stages of growth (Figure 11). The developing flower stems can be infested; in this situation the larvae are usually found between the developing individual buds, making the entire flower head one gall. If infestation occurs early enough the flower head collapses rather than continuing to develop.

The most easily observed infestation is when individual buds of a flower head are infested and can be seen to be discoloured and deformed and failing to flower (Figure 12a).

Figure 11. Infestation in early stages of flower bud development leads to total **(a)** or partial **(b)** collapse of flower head with larvae feeding between developing individual buds **(c)**.

Figure 12. Flowering heads of *Agapanthus* with 'typical' agapanthus gall midge symptom. **(a)** Deformed and discoloured buds that fail to flower. **(b)** Generally healthy flower head with a small number of infested buds (bottom left of flower head).

Flower dissection in 2016 also showed that a limited number of larvae were living inside senesced flowers, either between the petals and the seed pod or inside the underdeveloped seed pod (Figure 13). In these cases no symptoms were visible from the outside of the flower. Fully developed seed pods were also dissected but no larvae were found inside.

Figure 13. Senesced flower bud of *Agapanthus* infested with agapanthus gall midge, forming a gall inside the seed pod.

The flower dissections also highlighted that extremely high numbers of larvae can survive in a single flower head. The average number of larvae found across 51 flower heads dissected was 719, with a maximum number of 3465. The number of larvae inside were not easy to predict based on appearance of the flower head, but this may be worth quantifying in future work.

Rearing

Larvae left in rearing tubes from autumn 2015 started to emerge in April 2016. Larvae added to rearing tubes from late June to early July provided adults for use in rearing cage observations.

Observations were carried out from 9.30am on 6th July, 3pm on 20th July and 8.30am on 15th August. During these observations no mating or oviposition activity was observed, and adult midges rested on the walls of the cages or became difficult to see on the floor of the cage.

Objective 2. Confirm the distribution and host range.

Distribution

As a result of the call to the public, the RHS received 36 responses, of which 24 were confirmed by sample or photograph, five were unconfirmed and seven were considered negative results. These records, combined with those from a survey of commercial premises carried out by APHA in 2015 can be seen in Figure 14.

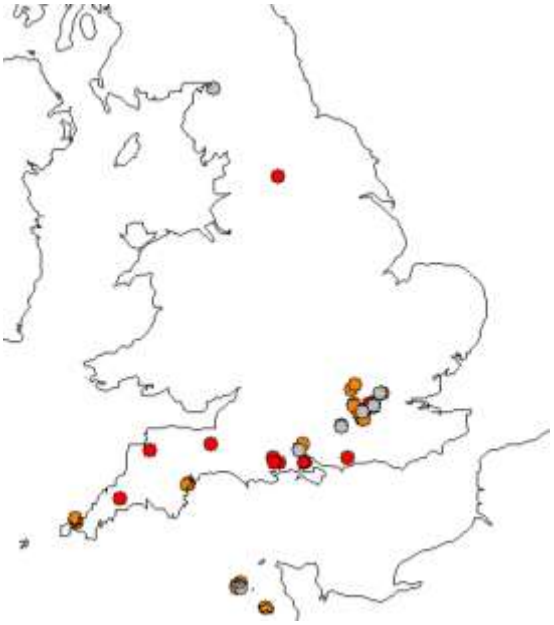

Figure 14. Agapanthus gall midge distribution 2014-2016; 2015 records in orange, 2016 records in red and unconfirmed in grey.

The only confirmed record not in the south of England was in West Yorkshire, and was confirmed by a sample with larvae present. The owner of the *Agapanthus* had purchased a plant in Guernsey in spring 2014 which was the most likely source of the infestation. The owner would normally have moved the plants under protection over winter but decided to leave them outside in winter 2015 in an attempt to kill the midge. However, in 2016 further symptoms were seen, meaning the midge has successfully overwintered in the north of England.

Evidence that the midge has been present in the UK for some years before 2014 was given by a member of the RHS *Agapanthus* forum who provided a sample in 2016 along with photographs of symptoms in his private collection in 2012 and records in his log book of possible symptoms from 2011.

Host range

Matthew Everatt, Defra, tested six widely grown cultivars of *Agapanthus* for susceptibility to the midge. The visual assessments of symptoms did not clearly demonstrate differences in midge infestation, but the numbers of larvae within infested flower heads varied significantly.

Table 1. Adapted from Matthew Everatt Project for BASIS certificate in crop protection. Agapanthus gall midge damage assessment. Terminology for flowering stage: S = Flower head sheath developed, EF = Early flowering flower buds emerging from sheath), MF = mid flowering, buds emerged, F = Flowering, and G = Going over (flowers beginning to die back).

Cultivar	**Predominant flowering stage at the start of the experiment**	**Predominant flowering stage at the end of the experiment**	**Number of flower heads affected**	**Severity of damage of affected flower heads**
Brilliant Blue	MF	F/G	3-4/18	Low/medium
Lapis Lazuli	MF/F	F/G	1/16	Low
Lavender Haze	EF/MF	F	1/16	Low
Northern Star	S/EF	MF/F	4/16	Low
Purple Delight	EF	MF/F	2/16	Low/medium
Sandringham	MF/F	F/G	0/20	NA

The mean number of larvae found inside flowers of Northern Star (83.75) was significantly greater than for the other five cultivars, which had a mean of no more than seven larvae (Figure 15). The least infested Northern Star replicate had more than twice as many larvae inside as the highest number found in a replicate of any of the other five cultivars.

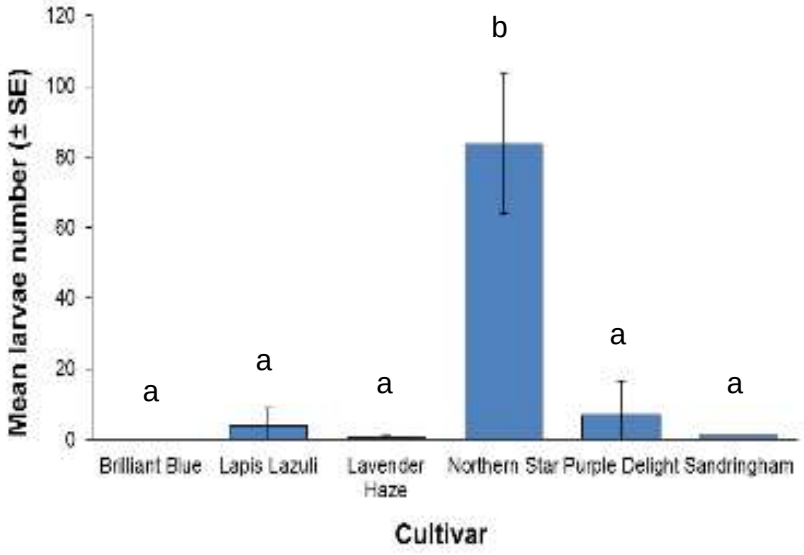

Figure 15. Mean number of agapanthus gall midge larvae associated with flower heads of six *Agapanthus* cultivars, following 20 days exposure to the agapanthus gall midge. Means with the same letter are not significantly different from each other.

Objective 3. Quantify the effectiveness of potential chemical and biological controls.

Test against ground-dwelling larvae

Eight biological and chemical controls were tested for their effectiveness against the ground-dwelling larval stage of the midge in a laboratory pot experiment. Overall there was very high mortality during the experiment, with a mean of only 1.3 adult midges emerging from the ten larvae added, across both sets of water controls (Table 2). This high mortality may have been due to high moisture levels in the pots, as there was a lot of visible condensation.

ANOVA was carried out in Genstat to test for significant differences between the treatments. Separate analyses were carried out for treatments tested on the two different application dates. Only 'mean adults emerged' showed a significant effect of treatment from the treatments tested on the first date ($F^{6,54} = 2.64$, $P = 0.025$). A Duncan's multiple range test showed that the only treatment with significantly fewer adult midges emerged than the water control was Calypso (Figure 16).

Table 2. Mean values ± standard error of adults emerged after treating growing media with before the addition of ten mature agapanthus gall midge larvae.

	Treatment	Mean adults emerged ± standard error		
1	Nemasys L	0.90	±	0.38
2	Nemasys (date 2)	2.80	±	0.70
3.1	Met52 Granular	0.20	±	0.13
3.2	Met52 OD (date 2)	1.70	±	0.56
4	Tracer	1.00	±	0.54
5	Calypso	0.00	±	0.00
6	Decis Protech	0.10	±	0.10
7	Cythrin Max EC	0.20	±	0.20
8	Water (date 1)	0.90	±	0.35
8	Water (date 2)	1.70	±	0.30

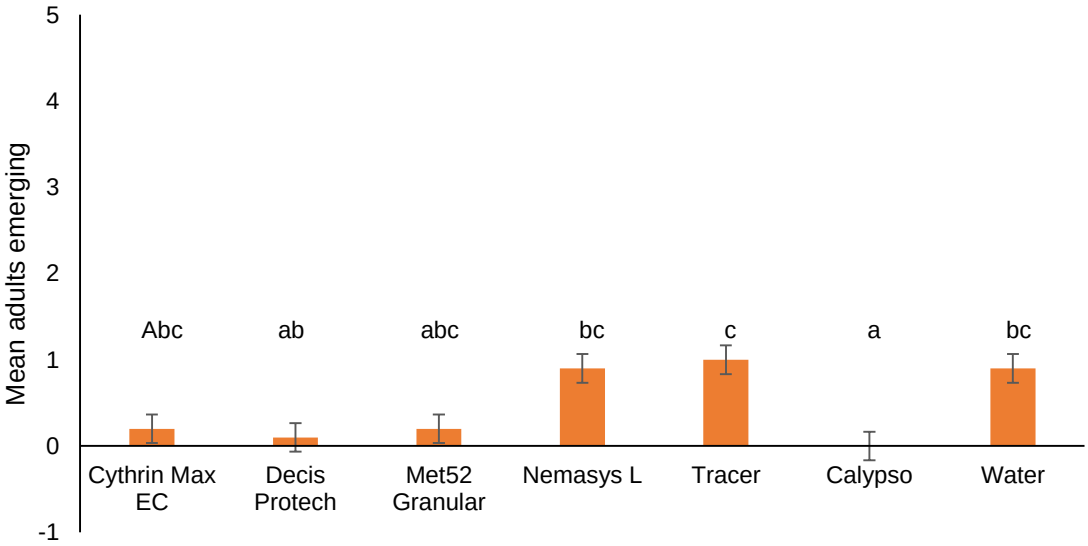

Figure 16. Mean number of agapanthus gall midge adults successfully emerging from growing media treatments. Ten larvae were added to each pot. Means with the same letter are not significantly different from each other.

Test against flower-dwelling larvae

Eight biological and chemical controls were tested for their effectiveness as foliar sprays against the flower-dwelling larval stage of the midge using cut stems of infested *agapanthus*. The number of larvae inside flower heads was unexpectedly high (mean = 719, min = 12, max = 3465). As a result flower dissections took a long time, and only a subsection of the replicates were able to be fully sampled.

Because the number of larvae in a flower head can be so variable, the percentages of live larvae were used to assess the effectiveness of treatments (Table 3).

Table 3. Mean values ± standard error of ratio of percentage live larvae within flowers treated with nine different chemical and biological control treatments.

	Row Labels	**Percentage live larvae ± standard error**	**Number of replicates sampled**
1	Nemasys L	83.29 ± 4.95	6
2	Nemasys (date 2)	73.38 ± 6.18	6
3	Met52 OD (date 2)	66.39 ± 9.45	6
4	Tracer	82.05 ± 10.82	6
5	Calypso	89.90 ± 1.68	5
6	Decis Protech	85.48 ± 6.04	6
7	Cythrin Max EC	84.00 ± 5.58	6
8	Water (date 1)	86.81 ± 4.08	5
8	Water (date 2)	67.25 ± 7.38	5

ANOVA was carried out in Genstat to test for significant differences between the treatments in the following measures:

- Larvae in saucer - mature
- Larvae in saucer – immature
- Total larvae in saucer
- Percentage mature larvae in saucer
- Larvae in flower head - alive
- Larvae in flower head - dead
- Total larvae in flower
- Percentage live larvae in flowers

Separate analyses were carried out for treatments tested on the two different application dates. There were no statistical differences between treatments for any of the measures tested.

Discussion

Objective 1. Determine and describe the life cycle of agapanthus gall midge.

Observations of midge activity in the garden demonstrate that the midge has a very long active season, with the pest available to infest *Agapanthus* flowers as soon as they develop. It continues to be active as long as the latest flowering *Agapanthus* cultivars. This, combined with an established infestation in the north of England means there is unlikely to be any climatic barriers to the midge becoming established throughout England and Wales.

The consistent presence of the midge in flowers in the garden, with no real peaks and troughs in infestation levels indicates multiple overlapping generations of the midge. The relatively stable numbers of flower heads infested, and inverse relationship between number of flower heads available and symptom severity point toward infestation pressure being reasonably constant throughout the season. This means the key time to focus control product application on the adult life stage would be first generation adults at the beginning of the season. The development of a pheromone trap to help identify first generation adult activity would benefit the timing of potential management strategies and pheromone traps may even contribute to control. It may be possible to target the overwintering life stage in the ground, and thereby minimise the number of adults available to reproduce the following season.

Dissections of infested agapanthus showed that there can be a wide variety of symptoms, both in severity and location of infestation in the plant tissues of the flower head. Infestation in senesced flowers is externally symptomless. This means that close and regular monitoring is necessary to detect an outbreak.

There were extremely high numbers of larvae found inside dissected flower heads, which means a single infested flower head could potentially provide the source of a huge infestation in the following year. This again shows that close monitoring is important to prevent an outbreak. The unexpectedly high numbers of larvae also made the experiment unpractically time-consuming, so it would be useful to consider better methods for studying infestation level and treatment success. It would be useful to test if external symptoms can be used to predict the number of larvae inside a flower head, but current observations indicate this is unlikely to be straightforward. Another possibility is to test treatment success by keeping infested plants in sealed rearing cages, introducing a clean plant and assessing whether that plant becomes infested.

Rearing tubes confirmed that pupation takes around ten days throughout the summer, and that larvae can overwinter underground. To discern more detail about the pupation process further in depth techniques and experiments would be necessary, for example developing tubes where pupation can be observed, or having very large numbers of tubes so that the contents of some can be regularly examined.

Attempted observations of mating and oviposition were unsuccessful. Other cecidomyiid species are known to have most activity at dawn or dusk times so future observations could be scheduled for these time periods. It would also be beneficial to rear insects in separate tubes, as mating may already have occurred inside tubes which had multiple individuals.

Objective 2. Confirm the distribution and host range.

The distribution mapping of the midge confirms that while the pest is currently mostly restricted to the South of England, there are isolated cases in the north of England, which have successfully overwintered. The midge is therefore a potential pest throughout England and Wales. The widespread nature of the midge, combined with reliable anecdotal reports means that it is likely the midge has been present in the UK for at least two years prior to its discovery in 2014.

The study of susceptibility of six *Agapanthus* cultivars highlighted 'Northern Star' as hosting the highest numbers of larvae out of the cultivars tested. This is unfortunate as 'Northern star' is very widely grown. It does mean that this cultivar may be useful as an indicator plant, as a means of focussed monitoring for the midge. This study confirms that there are differences in cultivars in terms of their attractiveness or susceptibility to the midge. Future work should try to ascertain whether some cultivars have resistance to the midge, even in the absence of other cultivars that they might prefer.

Objective 3. Quantify the effectiveness of potential chemical and biological controls.

Of the treatments tested against larvae dropping to the ground to pupate, only a drench of Calypso caused a statistically significant reduction in midge emergence. However very high mortality in the water-treated controls mean that this result needs validating in a repeated experiment before Calypso can be recommended to growers for control of agapanthus gall midge. The high natural mortality may have been due to excess moisture in the pots, this could be addressed in a repeat experiment by reducing the liquid added (both in the treatment drenches and the water controls).

None of the chemical and biological controls applied as foliar sprays had a significant effect on percentage survival of midge larvae. Gall-inhabiting organisms are often difficult to treat with foliar sprays as contact-acting products may not penetrate far enough into the plant tissue

to reach the target pests. Future tests of foliar sprays could include additional potential pesticides, ideally with systemic action, and the addition of wetters to contact-acting treatments to aid their distribution in the flower.

Conclusions

This project has revealed details about the life cycle of the midge that highlight the need for regular and close inspection of Agapanthus plants to detect infestations early. The pest is likely to become more problematic for growers as it is already widespread in the UK, has a long and consistent active season and can infest many stages of flower development, including senesced flowers which may be symptomless.

Until effective control products are identifies by further study, no recommendations of chemical and biological control can be made to growers. The advice is still to prevent infestation by sourcing incoming material from uninfested sources, monitoring for infestation and destroying infested material.

This work has uncovered useful information about the life cycle and biology of the agapanthus gall midge, and has provided some experience of experimental methodologies that should help to progress any future work.

Knowledge and Technology Transfer

- Social media posts by RHS in January, July and August 2016
- Talk to International Congress of Entomology, Florida, 26 September 2016. Title: Agapanthus gall midge – a new pest affecting Agapanthus in the UK
- Talk to RHS Plant Societies at workshop on 17 November 2016 Title: Horticultural gall midges
- Poster presentation at Innovations in Plant Biosecurity, Fera, 15 and 16 March 2017. Title: The agapanthus gall midge – an emerging risk not recognised previously
- Article in The Garden magazine – to be published July 2017. Title: Agapanthus gall midge: Update on a new UK pest.
- Webpages developed/ updated:
    - RHS web profile: https://www.rhs.org.uk/advice/profile?PID=901
    - Species description now available to the public: https://www.rhs.org.uk/science/pdf/plant-health/Agapanthus-gall-midge-species-description.pdf
    - Science project page updated: https://www.rhs.org.uk/science/plant-health-in-gardens/entomology/rhs-projects-on-plant-pests/agapanthus-gall-midge
    - AHDB project page: https://www.rhs.org.uk/science/plant-health-in-gardens/entomology/rhs-projects-on-plant-pests/Biology-and-control-of-agapanthus-gall-midge

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Harris KM, Salisbury A, Jones H (2016) Enigmadiplosis agapanthi*, a new genus and species of gall midge (Diptera, Cecidomyiidae) damaging Agapanthus flowers in England.* Cecidology 31, 17-25.