Project title:	Tomato: Further optimisation of *Macrolophus* release and feeding strategies.
Project number:	PE 020a
Project leader:	Mr Paul Howlett, Wight Salads, Arreton, Isle of Wight, PO30 3AR
Report:	Final report [August, 2015]
Previous report:	Not applicable
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Location of project:	Wight Salads, Arreton, Isle of Wight RJC Ltd, Bramham, West Yorks Libraries at Fera and Lancaster, Leeds and York Universities Warwick University
Industry Representative:	Dr P Morley, (TGA Technical Officer) TGA, Pollards Nursery, Lake Lane, Barnham, W. Sussex, PO22 0AD
Date project commenced:	1 January 2015
Date project completed (or expected completion date):	31 December 2015

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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.

Dr R Jacobson

Director

RJC Ltd

Signature ..... Date .....

Report authorised by:

Mr P Howlett

Head of Agronomy

Wight Salads

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CONTENTS

GROWER SUMMARY

Headline

Establishment of *Macrolophus* in tomato crops can be enhanced by providing *Artemia* cysts as supplementary food. The effect can be further enhanced by maintaining the cysts in a hydrated state. A new feeding system was effective when used on a small scale but will require further development for commercial crops.

Background

In 2013, AHDB Horticulture (formerly HDC) funded project PC 302d developed a *Macrolophus*-based IPM strategy for the control of the leaf and fruit mining caterpillar, *Tuta absoluta.* The full details of the IPM programme can be found in AHDB Horticulture Factsheet 02/14. Where the predator established well, the programme was highly successful but delays elsewhere resulted in additional interventions with chemical pesticides. *Macrolophus* population growth appeared to be improved when the predator was provided with supplementary food in the form of eggs of *Artemia* (brine shrimp) and / or *Ephestia* (a stored product moth).

AHDB Horticulture project PE 020 allowed the team to conduct a more in depth study of these supplementary food materials during 2014. *Artemia* eggs are usually in an encysted form which is able to resist extreme adverse conditions for long periods of time. They are used by the fish farming industry either in the collected form or in a 'decapsulated' state with the outer coating removed by an industrial process. Upon delivery, the *Artemia* eggs are in a dehydrated state. Given that *Macrolophus* have 'piercing and sucking', rather than 'chewing', mouthparts we were concerned that the predators would have difficulty feeding on the desiccated eggs. It soon became obvious that both the encysted and decapsulated eggs would readily absorb moisture and could be rehydrated simply by placing them on a damp pad for about an hour. They would remain in that state for as long as sufficient moisture was available. To investigate whether the eggs would rehydrate naturally on leaves within a commercial tomato crop canopy, they were placed on leaves at various positions in the crop canopy and examined in situ after 24 hours and 7 days. At best, they were only partially hydrated. It was clear that there was normally insufficient moisture on the upper leaf surface to fully hydrate the eggs. *Ephestia* eggs are not dehydrated upon delivery

and are a suitable food material for *Macrolophus* in their delivered state. They are highly nutritious and are the preferred food material for raising *Macrolophus* in laboratory culture or in commercial breeding units. However, they are relatively expensive which restricts the quantity that can be distributed within a tomato crop. Suppliers sometimes dilute *Ephestia* eggs with the less expensive *Artemia* eggs in a 10-20% mix to aid distribution and thus reduce the overall cost of the product.

The overall objective of this project was to improve the reliability and efficacy of *Macrolophus pygmaeus* as a biological control agent on UK tomato crops. The specific technical objectives were to:

- evaluate a range of supplementary foods.
- develop a feeding system that will keep the food material in a hydrated state.
- evaluate a range of *Macrolophus* release and feeding strategies in a crop-scale trial.

Summary

This project was done in four stages. It began with laboratory-based experiments to determine whether it was beneficial to apply the supplementary food in the fully hydrated state. Three types of food (*i.e.* encysted *Artemia* eggs, decapsulated *Artemia* eggs, and a mixture of decapsulated *Artemia* and *Ephestia* eggs) were simultaneously tested in both desiccated and rehydrated states. Each treatment was replicated six times. The data showed a clear benefit from using rehydrated *Artemia* eggs and greater longevity of *Macrolophus* adults when fed on rehydrated encysted eggs rather than on rehydrated decapsulated eggs. The latter was improved by adding 10-20% *Ephestia* eggs but only to the same degree as rehydrated encysted eggs alone and the addition inevitably increased the cost of the material. As a consequence of these results, the subsequent studies focussed on rehydrated encysted eggs.

The second stage involved designing and developing a system to deliver hydrated food to the predators within a commercial crop. The final design comprised a lightweight biodegradable plant propagation module (60x60x60mm) with an internal base consisting of two 40x40mm layers of pre-soaked highly absorbant Green Blade gel liner. Approximately 1.2g of *Artemia* cysts were sprinkled over the upper surface of the gel liner in each module. The preliminary tests in commercial crops showed that the gel liners retained sufficient moisture to keep the cysts hydrated for at least 2-3 weeks. After 3 weeks a further 40x40mm layer of the pre-soaked gel liner was placed in the ‘basket’ and another 1.2g of

Artemia cysts were sprinkled over the surface. This design was taken forward to the main crop trial.

In the third stage, the most effective type / condition of supplementary food was tested in a commercial crop-scale trial to measure the impact on early-season establishment of *Macrolophus*. The tomato plants, cultivar Mecano, arrived from the propagator in week 50 2014. The treatments comprised two release rates of *Macrolophus* and two methods of providing supplementary food; *i.e.* four treatments in total. Each treatment was replicated on both the east and west side of the glasshouse unit. *Artemia* cysts were applied at a rate equivalent to 250g per hectare. There were 36 feeding points per plot (*i.e.* one point per 47m^2) giving approximately 1.2g of *Artemia* cysts per feeding point. Depending on the particular treatment, the feed was either loosely distributed on leaves or placed in hydrated baskets in the immediate area of *Macrolophus* release during week 51 2014. Feeding was repeated at three week intervals between week 51 2014 and week 8 2015. The predators were released as nymphs at commonly adopted rates (*i.e.* 0.5/m^2 or 1/m^2) in week 51 2014. On the first assessment date, which was three weeks after release of *Macrolophus*, there were significantly more *Macrolophus* where the predators had received rehydrated food in baskets than loose food on leaves. The assessments continued in the vicinity of the feeding stations for a further six weeks. At first, the population trends remained the same as the released nymphs developed into adults and then began to produce offspring. By the end of this period there was a very large increase in the number of first generation *Macrolophus* nymphs in all the treatments. Where hydrated food had been provided within baskets, an approximate 7 fold increase was recorded. However, the largest increases (30 fold) were seen in the treatments which had been fed with loose feed. This reversal in *Macrolophus* numbers between feeding methods towards the end of this period can be explained by examining the effect of several periods of heavy driving rain which penetrated the glasshouse roof panels. As a consequence of the leakage, the tomato leaves were often wet and loose food on the upper surfaces became hydrated. Given that both types of feeding system were now providing the predators with hydrated food, the loose system performed better because the food was spread over a much larger surface area and was therefore more accessible to the predatory nymphs. While this confounded the comparison of the basket feeder system with the loosely applied cysts, it did reinforce the evidence that significant benefits are to be gained from rehydrating the supplementary food.

The final stage of the project was a natural progression from stage 3. The assessment procedures in the crop-scale trial changed to provide a more general indication of *Macrolophus* population growth throughout the crop. Where the predators had received

rehydrated food in baskets, numbers increased steadily over this sampling period with the rate of increase being roughly proportional to the initial release rate. Where *Macrolophus* received loose food on leaves, the numbers increased quite dramatically at week 15, with a very large proportion being in the nymphal stages, and then fell back again at week 19. The latter can be explained by migration away from the rows which had originally housed the release / feeding points as those nymphs developed into more mobile adults.

There was an anomaly in the data which was further investigated in the statistical analysis. The mean number of *Macrolophus* per sample point at week 15 for the loose application treatment at the higher release rate was 11.9, but this fell back to 9.3 when counts from rows under the metal roof gutters were removed. Similarly, for the loose application treatment at the lower release rate the respective numbers were 4.8 and 3.5. There was no apparent change for the other two treatments. This anomaly can be explained. Between weeks 11 and 15, the temporary thermal screen was removed from the glasshouse roof space thus exposing the roof which was relatively cold at night. Condensation formed on the metal roof gutters and moisture dripped onto the leaves below. This kept the remaining supplementary food in the hydrated state and once again provided evidence that this is advantageous to the predators.

Financial Benefits

Macrolophus pygmaeus is potentially one of the most useful biological control agents available to UK tomato growers but, at £70-80 per 1,000, it also one of the most expensive. Hence, it must be used to best effect. A saving in release of only 0.25 *Macrolophus* per m^2 across 66% of the UK tomato industry would equate to a saving of over £24k thus giving immediate payback on this project. *Tuta absoluta* is currently the most important pest of tomato crops in the UK. For example, at one nursery in 2012, 30% of fruit were damaged by the pest and graded out during June and July. This represented losses of approximately £50k per hectare to that grower for that period alone. Where successful, the *Macrolophus*-based control strategy has prevented such damage. Hence, avoiding the situation described in this single example would provide an immediate x2 payback on the cost of this project. *Macrolophus* also controls other pests such as *Liriomyza bryoniae.* These leafminers are currently the most expensive pests to control with biological control agents in UK tomato crops. Improved use of *Macrolophus* could provide savings of £400-£800 per hectare for growers who suffer this problem and so provide the industry with a payback on the cost of this project within a single year.

Action Points

- The combined results of the laboratory and fields studies confirm that it is beneficial to provide *Macrolophus* with supplementary food in the form of rehydrated *Artemia* cysts rather than the desiccated *Artemia* cysts as supplied.
- The prototype feeder basket developed in this project maintained *Artemia* cysts in the rehydrated state for 2-3 weeks and provided a suitable source of supplementary food for *Macrolophus* during that time.
- The use of the feeder baskets in commercial crops significantly increased the survival of the *Macrolophus* nymphs that were released adjacent to the feeder units at the start of those crops. However, the benefits were lost as those *Macrolophus* matured into adults and dispersed elsewhere in the crop.
- There were too few feeding baskets per hectare to influence the subsequent *Macrolophus* population growth and it would not be economically viable to greatly increase the number of feeder baskets within commercial crops.
- The feeder baskets as used in this project enhance *Macrolophus* population growth in small confined areas, such as rearing units, but a much greater number would be required to provide the same effect in a commercial crop. The solution will be to find an alternative means of keeping *Artemia* cysts moist on leaves without creating secondary problems from excessive moisture on leaves; eg making the plants susceptible to plant diseases such as Botrytis.

SCIENCE SECTION

Introduction

In 2013, HDC-funded project PC 302d developed a *Macrolophus*-based IPM strategy for the control of the leaf and fruit mining caterpillar, *Tuta absoluta.* The full details of the IPM programme can be found in HDC Factsheet 02/14 (Jacobson & Howlett, 2014). Where the predator established well, the programme was highly successful but delays elsewhere resulted in additional interventions with chemical pesticides. *Macrolophus* spp. population growth appeared to be improved when the predator was provided with supplementary food in the form of eggs of *Artemia* spp. (brine shrimp) and / or *Ephestia* spp. (a stored product moth). HDC Project PE 020 allowed the team to conduct a more in depth study of these supplementary food materials during 2014.

Artemia eggs are collected in bulk from the surface of salt lakes in various parts of the world. They are usually in an encysted form which is able to resist extreme adverse conditions for long periods of time. They are used by the fish farming industry either in the collected form or in a 'decapsulated' state with the outer coating removed by an industrial process. The quality of the material apparently varies according to the geographical origin and the extent of the industrial processing.

Upon delivery, the *Artemia* eggs are in a dehydrated state (Figure 1). Given that *Macrolophus* spp. have 'piercing and sucking', rather than 'chewing', mouthparts there was concern that the predators would have difficulty feeding on the desiccated eggs. It soon became obvious that both the encysted and decapsulated eggs would readily absorb moisture and could be rehydrated simply by placing them on a damp pad for about an hour (Figure 2). They would remain in that state for as long as sufficient moisture was available. To investigate whether the eggs would rehydrate naturally on leaves within a commercial tomato crop canopy, they were placed on leaves at various positions in the crop canopy and examined them in situ after 24 hours and 7 days. At best, they were only partially hydrated. There was clearly insufficient moisture on the upper leaf surface to fully hydrate the eggs (Jacobson & Howlett, 2014a).

Ephestia eggs are not dehydrated and are a suitable food material for *Macrolophus* spp. in their delivered state. They are highly nutritious and are the preferred food material for

raising *Macrolophus* spp. in laboratory culture or in commercial breeding units. However, they are relatively expensive which restricts the quantity that can be distributed within a tomato crop. Suppliers sometimes dilute *Ephestia* eggs with the less expensive *Artemia* eggs in a 10-20% mix to aid distribution and reduce the overall cost of the product (Figure 3).

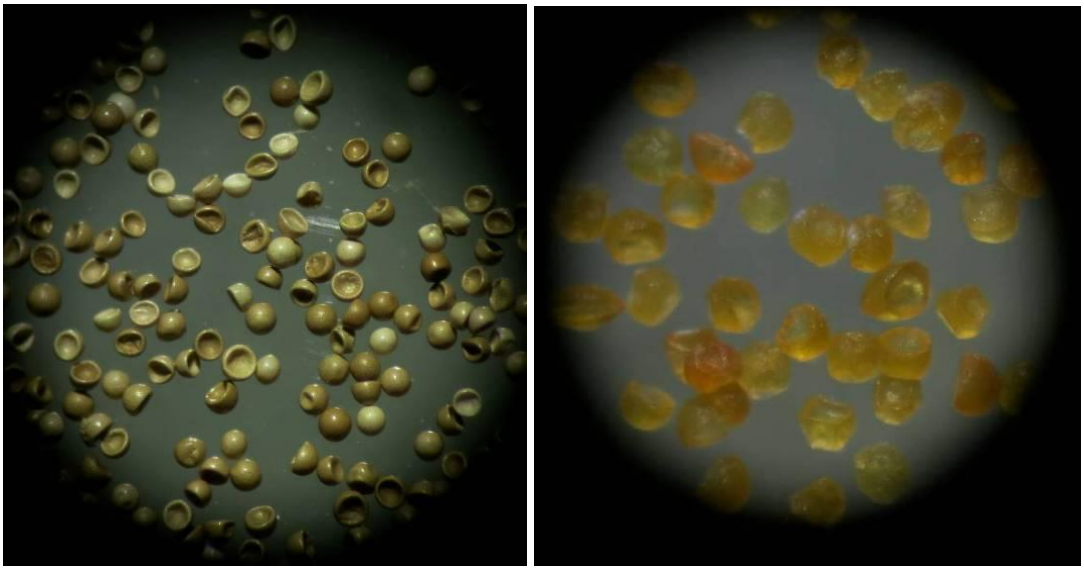

Figure 1. *Artemia* cysts (left) and decapsulated eggs (right) in their desiccated state as delivered

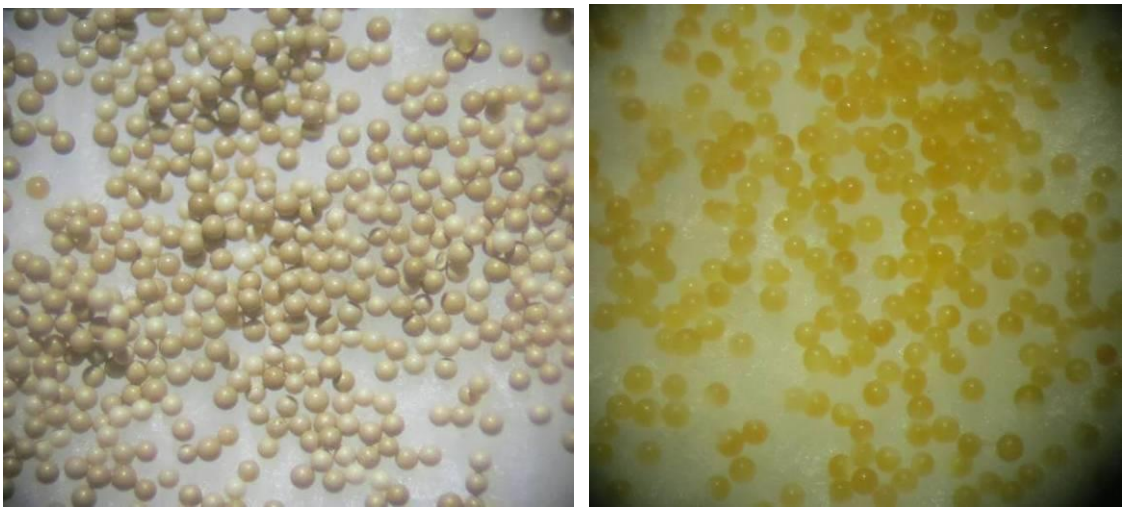

Figure 2. *Artemia* cysts (left) and decapsulated eggs (right) in their re-hydrated state

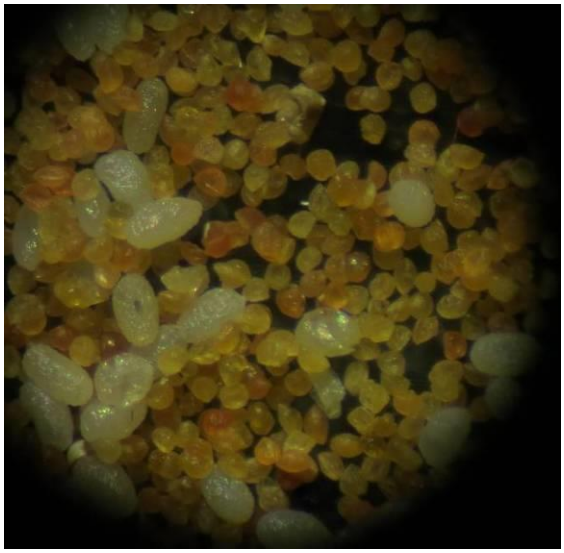

Figure 3. *Ephestis* eggs mixed with desiccated *Artemia* decapsulated eggs

The present study was done in four stages. It began with laboratory-based experiments to determine whether it was beneficial to apply the supplementary food in the fully hydrated state. The second stage involved designing and developing a suitable system to deliver hydrated food to the predators within a commercial crop. In the third stage, the most effective type / condition of supplementary food was taken forward into a commercial crop-scale trial to measure the impact on early-season establishment of *M. pygmaeus*. In the final stage of the work, the assessment procedures in the crop-scale trial changed to provide a more general indication of *M. pygmaeus* population growth throughout the crop. The crop-scale trials followed the general approach that had been successfully developed in HDC project PC 240 (Jacobson & Morley, 2009) and more recently used in HDC projects PC 251 (Jacobson, 2008), PC 295 (Jacobson, 2009), PC 302/b/d (Jacobson & Morley, 2010; Jacobson & Howlett, 2012; Jacobson & Howlett, 2013) and PE 020 (Jacobson & Howlett, 2014a). This approach immediately identified any important interactions with current agronomic practice and eliminated the need for an additional exploitation phase to transfer the technology to the commercial situation.

Materials and methods

Stage 1. Laboratory studies

In a preliminary study, a laboratory-based bioassay was developed to test the suitability of the various food materials. Each test arena consisted of a ventilated 90mm Petri dish containing a damp pad and a crumpled tissue refuge (Figure 4). The food was placed on the damp pad and no further action was taken for two hours to allow the *Artemia* eggs to become fully hydrated. Ten *M. pygmaeus* adults were then placed in the arena and their survival recorded daily over 18 days. The relative longevity of the insects on the various types of food was recorded.

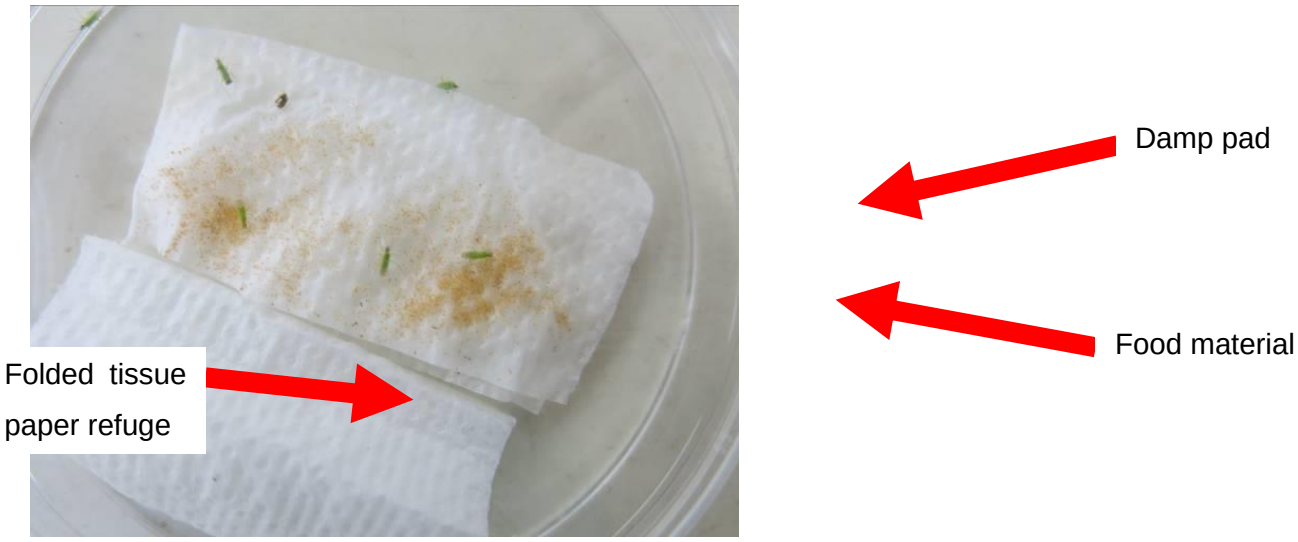

Figure 4. An individual test arena as used in stage 1 of the project

In the trial presented here, three types of food (*i.e.* encysted *Artemia* eggs, decapsulated *Artemia* eggs, and a mixture of decapsulated *Artemia* and *Ephestia* eggs) were simultaneously tested in both desiccated and rehydrated states giving a 2 x 3 factorial of six treatments. Each treatment was replicated six times.

Macrolophus pygmaeus were sourced from Koppert UK. Upon the day of delivery, ten adults were placed in each replicate dish. They were examined for mortality each day for 18 days and dead insects were removed on each occasion. An LT_{50} was calculated straight from the mortality data for each replicate (*i.e.* the day on which mortality of the test insects reached 50%). Also determined was the mean time to death, estimated as the mean of the

day on which each insect died. For both variables, the means and standard deviations were calculated, and each was subject to analysis of variance.

Stage 2. Supplementary food delivery system

A 'hydration basket' was developed by trial and error. A large number of different materials and designs were tested in the laboratory to determine how well the *Artemia* cysts / decapsulated eggs became rehydrated and how long they remained in that hydrated state. It was important that the chosen design was simple and required minimal labour for construction, placement and maintenance. The basket also had to be lightweight and easy to hang within the crop canopy. The prototypes were tested for longevity in a commercial crop during August and September 2014 before final selection for the main crop trial. The selected baskets were monitored throughout the main crop-scale trial so that the design could be further fine-tuned as necessary.

Stage 3. Early-season establishment of *M. pygmaeus* in a crop-scale trial

The trial was done in four identical 3,400m^2 glasshouses at Lane End Nursery, Arreton, Isle of Wight. Each glasshouse was divided into two by a central roadway thereby providing a total of eight large plots. All glasshouses contained tomato cv Mecano which arrived from the plant propagators on 13 December 2014 (week 50, 2014). The glasshouses housed four Treatments, the details of which are shown in Figure 5. The Treatments comprised two release rates of *M. pygmaeus* and two methods of providing supplementary food. Each Treatment was replicated on both the east and west side of the glasshouse unit.

Artemia cysts were purchased from BCP Certis and used at a rate equivalent to 250g per hectare. There were 36 feeding points per main plot (Figure 6) giving 1.2g of *Artemia* cysts per feeding point. This equated to about three quarters of a level teaspoon for ease of application. The feed was either distributed on leaves in the immediate area of *M. pygmaeus* release or placed in hydrated baskets during week 51 2014 depending on the particular Treatment. Feeding was repeated at three week intervals between week 51 2014 and week 8 2015.

Macrolophus pygmaeus nymphs were purchased from Koppert UK and arrived in 16 containers, each stated to contain 500 individuals, during week 51 2014. Four containers were taken as sub-samples and the contents accurately counted. There were found to be an average of 710 nymphs per container. Release rates were subsequently based on this

figure rather than the claimed content. The appropriate numbers of *M. pygmaeus* were released into 'bug boxes' which were either hung on the plant stem or on the side of the basket depending on the treatment (Figure 10). In addition, six batches of 10 nymphs were tested to determine the proportion that survived to adulthood. They were placed in test arenas identical to those described for the laboratory-based bioassays (above). The insects were checked daily for 14 days and adults were removed as they developed to reduce the risk of cannibalism.

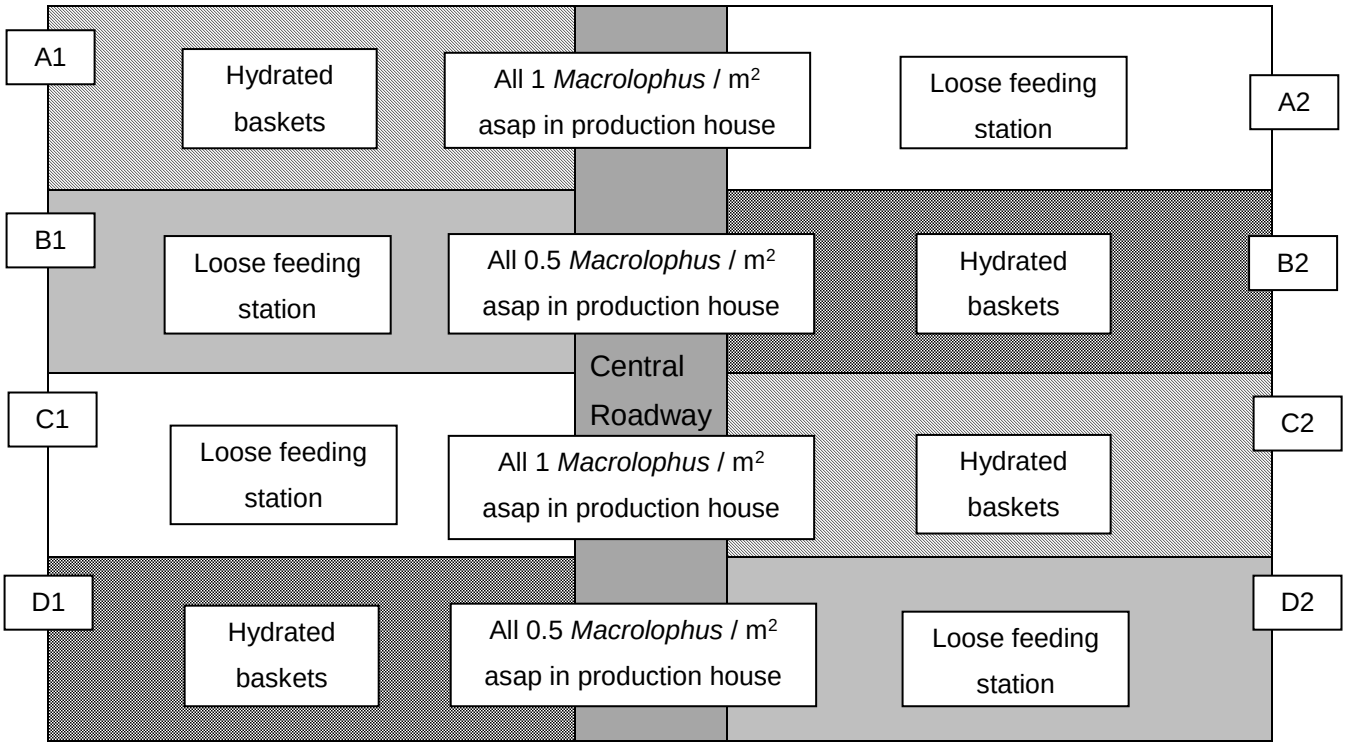

Figure 5. Layout of four Treatments in eight main plots at Lane End Nursery

Initial establishment of *M. pygmaeus* was determined in four assessments over nine weeks between week 51 2014 and week 8 2015. These assessments focused on the plants in the immediate vicinity of the feeding stations. The tops of six plant heads around each feeding station were tapped over a white tray and four fully expanded leaves per plant head were examined for presence of *M. pygmaeus* adults and nymphs. The total numbers of *M. pygmaeus* found around each feeding station were recorded.

The sampling points formed a regular grid, with four sections of (nine) alternate rows sampled in a 'cascading' grid (see Figure 6). The data were analysed as for a split-plot analysis, with each half-house treated as an experimental unit. The sample data were

treated as extra strata in the analysis, looking at rows within half-blocks, and sections within rows.

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Road with pathway numbers																

Figure 6. Layout of each of the main plots on the west side of the glasshouse unit (those on east side were mirror images with even numbered rows). The shaded squares contained the *M. pygmaeus* release points, feeding stations and initial assessment sites.

Stage 4. Population growth of M. pygmaeus throughout the crops after week 8 2015.

Stage 4 was a natural progression from stage 3 in the same crops. From week 8 2015, due to crop growth and plant layering, it was no longer possible or appropriate to sample immediately above the feeding stations. Subsequent sample points were within the same rows but now chosen at random down those rows. Hence, the data began to provide a more general indication of population growth throughout the crop. Otherwise, the sampling procedure and data collection at weeks 11, 15 and 19 was the same as in stage 3. Statistical analysis of data was also the same as in stage 3 but with an additional analysis of covariance splitting the rows into two groups: *i.e.* rows 17 or 18 *versus* all other rows.

Results and Discussion

Stage 1. Laboratory studies

Macrolophus pygmaeus were observed feeding on all hydrated forms of the food materials (*e.g.* Figure 7). The results of the mortality assessments are summarised in Figure 8. The survival of *M. pygmaeus* adults on the three types of desiccated food was not significantly different. The mean time to death was 2 days and the LT_{50} was 2.5 days. Survival of *M. pygmaeus* adults on each of the three rehydrated types of food was significantly greater ($p<0.05$) than on the desiccated food from day 2 onwards. The results for the three rehydrated treatments were similar until day 5 but then diverged with survival on the rehydrated decapsulated eggs decreasing more rapidly than on the other two types of rehydrated food. The mean time to death of the predators fed on rehydrated cysts or rehydrated decapsulated *Artemia* / *Ephestia* eggs was 7.8 days, while it was 5.7 days on rehydrated decapsulated eggs alone ($p<0.05$). The LT_{50} was 7.1 days for *M. pygmaeus* adults fed on rehydrated cysts or rehydrated decapsulated / *Ephestia* eggs against 5.8 days when fed on rehydrated decapsulated eggs alone ($p<0.05$).

The data shows a clear benefit from using rehydrated *Artemia* eggs and greater longevity of *M. pygmaeus* adults when fed on rehydrated cysts than on rehydrated decapsulated eggs. The latter can be improved by adding 10-20% *Ephestia* eggs though this will inevitably increase the cost of the material.

Figure 7. *Macrolophus pygmaeus* feeding on a rehydrated decapsulated *Artemia* egg

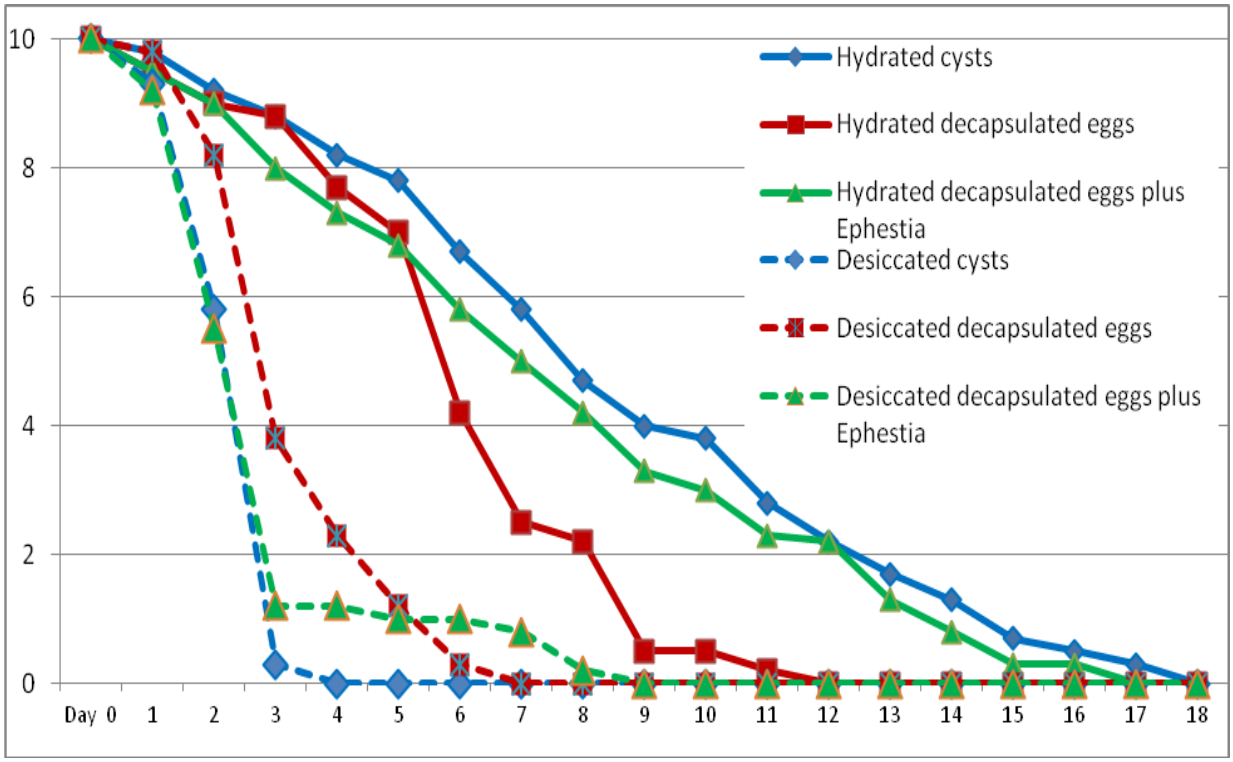

Figure 8. Mean numbers of live *Macrolophus pygmaeus* adults present in experimental arenas over 18 days when fed on six types of food material

Stage 2. Supplementary food delivery system

The final design of the 'hydrated basket' comprised a lightweight biodegradable plant propagation module (60x60x60mm) with an internal base consisting of two 40x40mm layers of pre-soaked highly absorbant 'Green Blade' gel liner (Figure 9). Approximately 1.2g of *Artemia* cysts were sprinkled over the upper surface of the gel liner in each basket. The preliminary tests in commercial crops in August-September 2014 showed that the gel liners retained sufficient moisture to keep the cysts hydrated for at least 2-3 weeks. After 3 weeks a further 40x40mm layer of the pre-soaked gel liner was placed in the basket and another 1.2g of Artemia cysts were sprinkled over the surface. This design was taken forward to the main crop trial. The baskets were stapled over the plant support string so that they could be slid up the string as the plant grew, thus keeping them within the top of the plant canopy (Figure 9).

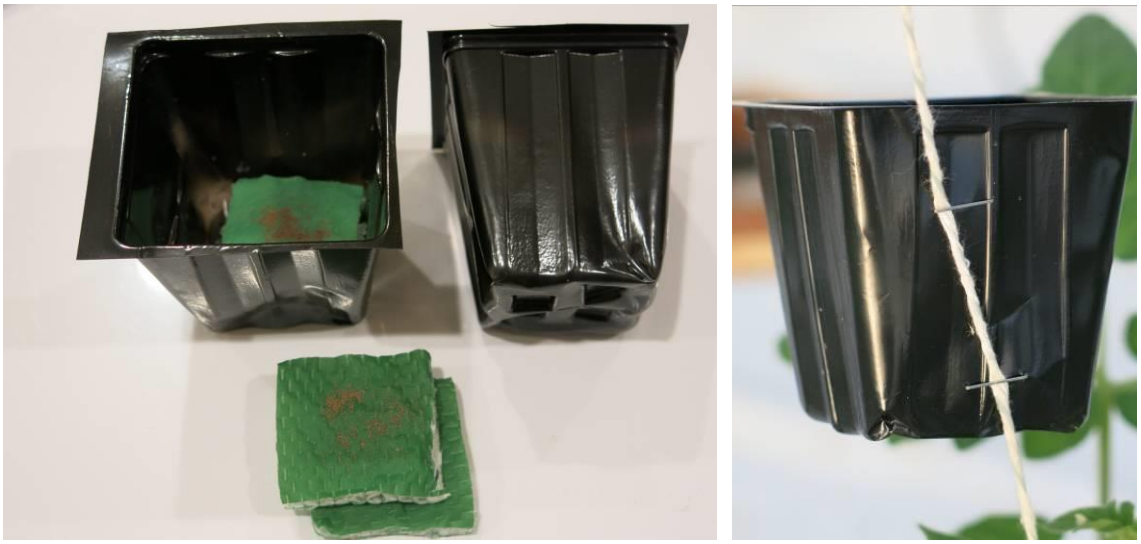

Figure 9. Components of the 'hydrated basket' (left) as selected for use in the crop-scale trial and the method of attaching to the plant support string (right).

Figure 10 . *Macrolophus pygmaeus* were released into 'bug boxes' hung either on the plant (left) or on the side of the hydrated basket (right) depending on the Treatment.

Stage 3. Early-season establishment of M. pygmaeus in a crop-scale trial

The mean percentage of delivered *M. pygmaeus* nymphs which successfully developed into adults was 80.4%. This was consistent with previous quality control checks and therefore considered to be acceptable.

The mean numbers of *M. pygmaeus* per assessment point in the four Treatments over the four assessment dates are shown in Figure 11. On the first assessment date, which was three weeks after release of the *M. pygmaeus*, there were significantly more (p<0.05) *M. pygmaeus* where the predators had received rehydrated food in baskets than loose food on leaves. At 1.7 *M. pygmaeus* per sample point, the higher release rate of predators with hydrated food in baskets was 54% greater (p<0.05) than the lower release rate fed by the same method, which was, in turn, 22% greater (p<0.05) than the higher release rate fed with loose food. Most of the predators found had now developed from nymphs to adults, which was consistent with the development rate observed in the laboratory-based survival tests. The leaves in the crop canopy were dry at this stage and the loose food remained dehydrated (Figure 12). These early results were entirely consistent with the improved survival recorded in stage 1 of the project.

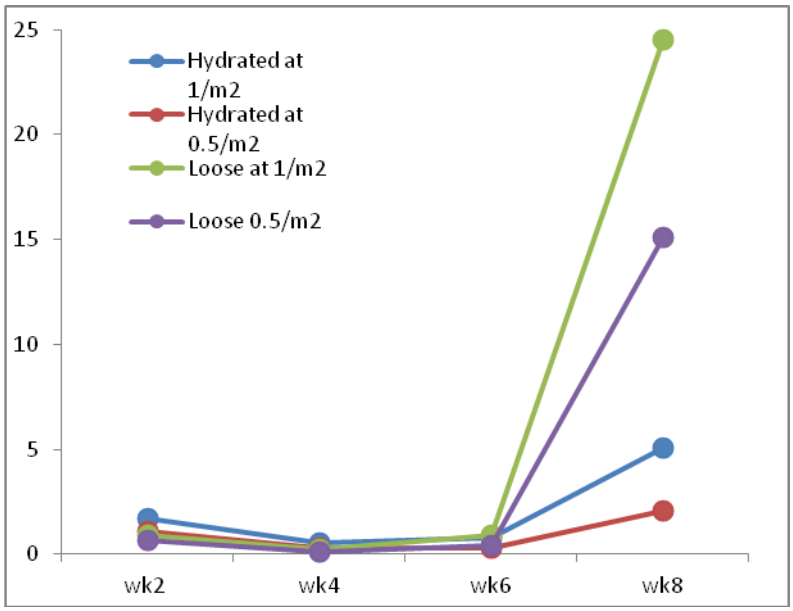

Figure 11. Mean number of *M. pygmaeus* per assessment point in the four Treatments over four assessment dates in 2015.

Figure 12. Condition of non-hydrated cysts on open leaves on 7 January (week 2) 2015

On the second assessment date, all *M. pygmaeus* collected were adults. Overall numbers declined by about 75% which could be explained by natural mortality and dispersal. The results still followed the same trend as the first assessment but the differences were no longer significant. On the third assessment date, numbers of adults continued to decline but the overall numbers of *M. pygmaeus* doubled due to the appearance of the first nymphs produced within the crop. These nymphs were all very small and it was clear that this was just the start of that generation of offspring.

At the fourth assessment, there was a very large increase in numbers of nymphs with all life cycle stages being present. However, the largest increases were seen in the treatments which had been fed with loose feed (about 30 fold increase overall). Where hydrated food had been provided within baskets, an approximate 7 fold increase was recorded. This was confirmed by the analysis of variance which showed significant effects of both release rate (*p=0.013*) and feeding system (*p=0.003*), with a suggestion of an interaction between them ((*p=0.065*). This complete reversal between the second and third assessments may be explained by two factors:

- There were several periods of heavy driving rain between weeks 2 and 8 2015, which led to leakage between many glasshouse roof panels. As a consequence, the upper surfaces of leaves in some areas of the crop were often wet, resulting in the loose food becoming hydrated.
- Given that both types of feeding system were now providing the predators with hydrated food, the loose system performed better because the food was spread over a much larger surface area and was therefore more accessible to the predatory nymphs.

Stage 4. Population growth of M. pygmaeus throughout the crops after week 8 2015.

The mean numbers of *M. pygmaeus* per assessment point in the four Treatments over the three assessment dates between weeks 11 and 19 2015 are shown in Figure 13. Where the predators had received rehydrated food in baskets numbers increased steadily over the sampling period, with the rate of increase being roughly proportional to the initial release rate. Where *M. pygmaeus* received loose food on leaves, the numbers increased quite dramatically at week 15, with a very large proportion being in the nymphal stages, and then fell back again at week 19. This can be explained by migration away from the rows which had originally housed the release / feeding points as those nymphs developed into more mobile adults. It is important to reiterate that there had been no supplementary food applied since week 8 to keep the predators in those rows.

More formal analysis of variance showed significant effects of both rate ($p<0.05$) and treatment ($p<0.01$), and a significant interaction between them ($p<0.05$) for all three sampling times. The interaction is simply an acknowledgement that the difference between the low and the high rates is larger for the loose application treatment than for the hydrated basket treatment. An analysis of the logarithmic count cause the interaction to disappear, essentially confirming the interpretation above.

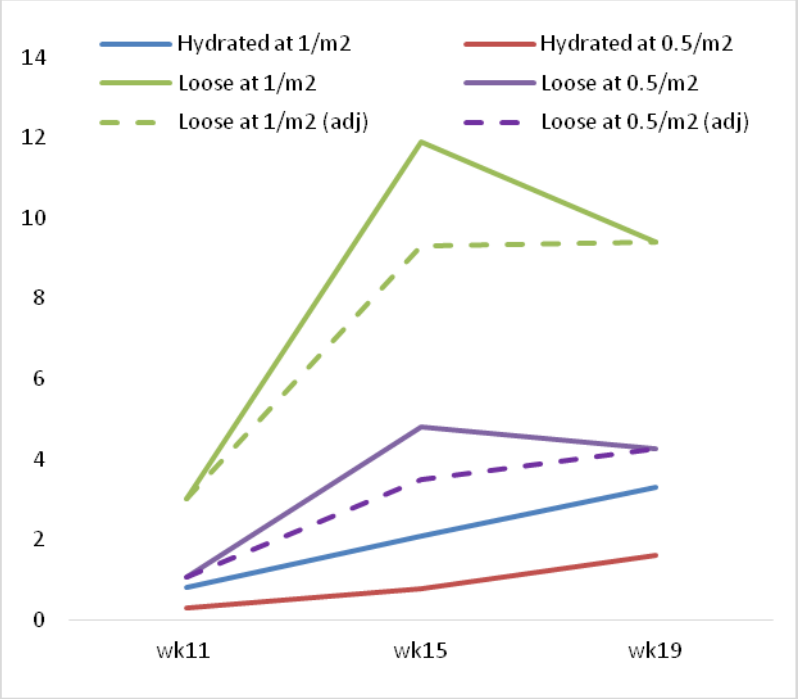

Figure 13. Mean number of *Macrolophus pygmaeus* per assessment point in the 4 Treatments for weeks 11 to 19 2015. The dashed lines show results with data from rows 17 and 18 removed.

There was an anomaly in the data which was further investigated in the statistical analysis. The mean number of *M. pygmaeus* at week 15 for the loose application treatment at the higher release rate was 11.9, but this fell back to 9.3 when counts from rows 17 and 18 were removed. Similarly, for the loose application treatment at the lower release rate, the respective numbers were 4.8 and 3.5. There was no apparent change for the other two treatments, nor was there any material difference for rows 17 and 18 at the other two sample times. The changes in the loose application treatments are reflected in the dashed lines in Figure 13. Examination of the variances showed that the between row and within row variances were quite similar for week 11, which possibly reflects the fact that the sample points within a row were randomly rather than regularly spaced. At week 15, between row variance was much larger, and this was moderated when an analysis of

covariance was used to distinguish between rows 17 and 18 and the other rows. At week 19, the variances were closer although the within row variance was approximately half that between rows. These differences are rather subtle, and do not detract from the headline story that the loose application treatment out-performed the hydrated basket treatment, with the higher rate in both treatments being superior.

Figure 14 shows a 'heat' diagram for week 15 which clearly illustrates the greater counts in rows 17 and 18 of the two loose application treatments. This anomaly can be explained. Between weeks 11 and 15, the temporary thermal screen was removed from the glasshouse roof space thus exposing the roof which was relatively cold at night. Condensation formed on the central gutter above rows 17 and 18 (Figure 15) and dripped onto the leaves below. This kept the remaining supplementary food in the hydrated state and was therefore advantageous to predators. By week 19, the leaves which had received the supplementary food had been removed and so the benefit to the predators was lost.

Week 15

row		1/2	5/6	9/10	13/14	17/18	21/22	25/26	29/30	33/34
block	half									
A	1	2.75	1.25	1.25	1.25	0.75	2.25	1.25	2	1.5
	2	8.25	8.75	6.25	9	33.5	9.5	6.25	14	9.75
B	1	2.75	2	1.5	3	15.75	2	4.5	4.75	4
	2	1.5	0.5	0.5	0.75	1	0.5	0.25	0.75	0.5
C	1	8.25	10	7.5	14.25	31.25	8.25	6.75	14.5	7.5
	2	2.5	2	3	3	5	3	1.5	2	1.5
D	1	1.75	0.5	1	1	1.25	0.5	1.25	0	1
	2	2.5	3.25	5.5	3.25	15.5	2.25	7	3.75	3.75

Hydrated at 1/m2
Loose at 1/m2
Loose 0.5/m2
Hydrated at 0.5/m2
Loose at 1/m2
Hydrated at 1/m2
Hydrated at 0.5/m2
Loose 0.5/m2

0 to 4.5
5 to 9.5
10 to 14.5
15 to 19.5
>20

Figure 14. 'Heat' diagram, showing the average numbers of *Macrolophus pygmaeus* sampled in each row of the experiment during week 15

Figure 15. Condensation on the central gutter resulting in water dripping onto the foliage and the *Artemia* cysts immediately below week 15**.**

Conclusions:

- The combined results of the laboratory and fields studies confirm that it is beneficial to provide *M. pygmaeus* with supplementary food in the form of rehydrated *Artemia* cysts rather than desiccated *Artemia* cysts (as supplied).
- The prototype feeder basket maintained the *Artemia* cysts in the rehydrated state for 2-3 weeks and provided a suitable source of supplementary food for *M. pygmaeus* during that time.
- The use of the feeder baskets in commercial crops significantly increased the survival of the *M. pygmaeus* nymphs that were released adjacent to the feeder units at the start of those crops. However, the benefits were lost as those *M. pygmaeus* matured into adults and dispersed elsewhere in the crop.
- We believe that there were too few feeding baskets per hectare to influence the subsequent *M. pygmaeus* population growth. Unfortunately, we do not believe it would be economically viable to greatly increase the number of feeder baskets within commercial crops.
- Unusual weather conditions between weeks 2-8 2015 resulted in the upper surfaces of leaves in some areas of the crop being wet, which resulted in the loose food becoming rehydrated. Given that both types of feeding system were then providing the predators with hydrated food, the loose system performed better because the food was spread over a much larger surface area and was more accessible to the predatory nymphs. While this confounded the comparison of hydrated baskets with loosely applied desiccated cysts, it did reinforce the evidence that significant benefits could be gained from rehydrating the supplementary food.
- We believe that the feeder baskets as used in this project would enhance *M. pygmaeus* population growth in small confined areas, such as rearing units, but a much greater number would be required to provide the same effect in a commercial crop.
- The solution may be to find an alternative means of keeping *Artemia* cysts moist on leaves without creating secondary problems from excessive moisture on leaves; e.g. making the plants susceptible to plant diseases such as Botrytis.

Knowledge and Technology Transfer

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