# The Influence of Aphid Natural Enemies on the Spread of Barley Yellow Dwarf Virus

Douglas W. Johnson*, Richard Harrington, Mark S. Taylor and Adam J. Burgess
*Department of Entomology, University of Kentucky, Research and Education Center, P.O. Box 469, Princeton, KY 42445 USA
and
Department of Entomology and Nematology, IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ. UK

Results are reported of a field and laboratory study on the influence of adult, coccinellid predators *Coccinella septempunctata* (L.) and braconid parasitoids *Aphidius rhopalosiphi* deStefani-Perez on the spread of barley yellow dwarf virus by bird cherry-oat aphid (Rhopalosiphum padi (L.)) and English grain aphid (Sitobion avenae (F.)).

**Introduction**:
Barley yellow dwarf (BYD) is the most widespread and economically important disease of cereals world wide (Plumb 1983). Though BYD virus produces a disease in cereals, the disease epidemiology is obligatorily dependent upon aphids for all movement and spatial development (Irwin and Thresh 1990). Among the most important of these aphid vectors are the bird cherry-oat aphid (*Rhopalosiphum padi* (L)) and grain aphid (*Sitobion avenae* (F.)) (Mann *et al.* 1996).

In the UK a 'Decision Support System' is being developed to assist rationalization of spraying of autumn sown crops for control of the main aphid vectors (Harrington *et. al.* 1994; Mann *et al.* 1996) It has been suggested that natural enemies of the aphid vectors may affect aphid movement (Sopp *et al.* 1987; Knaust 1996) thereby influencing disease development and thus would need to be accounted for in the Decision Support System. Two common natural enemies of cereal aphids, the adult, coccinellid predator (*Coccinella septempunctata* (L.)) and the braconid parasitoid (*Aphidius rhopalosiphi* deStefani-Perez) were chosen for examination. In this communication the authors outline the experiment and present a preliminary view of a portion of the resulting data.

**Methods and Materials**:
All experiments were conducted at IACR-Rothamsted, Harpenden, Hertfordshire, UK. A randomized complete block design with three replications was repeated in the field twice during the autumn of 1996 and once in the laboratory during the winter 1996-97. Individual treatments were applied as follows: no predator or parasitoid; a single predator; a single parasitoid and a single predator plus a single parasitoid. Crop plants used throughout this work were winter wheat *Triticum aestivum* L. variety "Beaufort" and winter barley *Hordeum vulgaris* L. variety "Puffin". Growth stages are reported in the format of Tottman and Broad (1987).

All insects were reared in controlled environment (CE) rooms under a 16 hours : 8 hours (light : dark) cycle at ca. 60% RH. Aphids and coccinellids were held at a constant 18$^{o}$C, while parasitoids were held at a constant 15$^{o}$C. Six aphid colonies were maintained. Each of the two aphid species was maintained separately on BYDV-infected wheat and barley and non-BYDV infected barley. Two parasitoid colonies were maintained one for each aphid species. Seven spot ladybirds (*C. septempunctata*) were collected during September 1996 at Rothamsted Experimental Station and nearby. They were fed for one week by allowing them free choice in an aphid colony of appropriate species. They were then held in 9 cm disposable petri dishes at 4$^{o}$C until needed. New aphids of the appropriate species were supplied to predator and parasitoid colonies from the aphid colonies reared on non-BYDV infected plants. In all predator and parasitoid colonies the aphid host was barley.

Forty eight, three-meter square plots of wheat and barley (24 plots each) were planted on 3 September 1996 utilizing standard agricultural practices with the exception that no insecticides were applied to either seed or plots. In each plot areas were identified for placement of cages. Ideally each area was two adjacent rows of 12 plants. Plants were checked for natural aphid infestation and any aphids were removed and the species, numbers and position of infested plants were recorded. Test plants were then covered with a 25 cm x 25 cm x 50 cm (length x width x height) mesh cage to prevent further colonization from wild aphid populations.

CE rooms were held at 10 hours : 14 hours (light : dark) and corresponding 14$^{o}$C : 9$^{o}$C temperature. Wheat and barley were sown in square, 25 plant grids at ca. 3 cm spacings in 53 cm x 53 cm x 5 cm

(length x width x depth) trays. Each tray held 4 grids. At GS 12, 20 individual grids were covered using the same cages utilized in the field study.

On 18 September (GS 12, 20) the first field experiment was begun. Two plants, one each nearest the center of the two rows within a cage, were infested with 5 viruliferous fourth instar winged individuals of appropriate species. The aphids were confined to the plants using clip cages (Mann*et al*. 1995) and were held on the plants for five days. On 14 October (GS 12, 22-23) the second field experiment was begun. Aphid infestation was the same as the first experiment except that fourth instar non-winged individuals were used and confined on the plants in clip cages for only 24 hours. In the CE rooms caged plants (GS 12, 20) were infested with fourth instar apterae, using the same procedure as was utilized in the field trials except that all 10 aphids were in a single clip cage and only the center plant in each grid was infested. After 48 hours the clip cages were carefully removed.

One week before delivery into test cages ladybird beetles were removed from the cold storage and allowed "free choice" feeding on appropriate aphid species for 4 days, followed by 3 days of starvation. Three days prior to introduction into the test cages parasitoids were removed from the colony with an aspirator. They were held in 3 cm x 7.5 cm (diameter x length) glass bottles with net tops and fed a 50:50 mixture of honey : water on saturated Kimwipe®. On the day of introduction they were individually sexed and females were placed into small aspirators (Tamaki *et al.*1970).

On the day of clip cage removal, predators and parasitoids were released into the cages during the afternoon. Predators were introduced into the cages by placing a 1.3 cm x 5 cm (diameter x length) uncapped glass vial containing one beetle in the center of the caged area. Parasitoids were delivered near the volumetric center of the cage via an aspirator.

In all experiments the predator / parasitoid treatments were maintained for two weeks. At the end of this period the aphids were counted and recorded by plant location, the cages were removed, and the plants sprayed with an insecticide to prevent further aphid movement. In the field trials test areas were sprayed every two weeks until plant leaf samples were taken for BYDV assay. In the CE room trial plants were sprayed immediately after cage removal and moved to a room that did

not contain aphids and thereafter inspected to ensure that no aphids survived.

After the predator / parasitoid treatments were removed plants were allowed to grow for a further 5 weeks, after which a portion of the youngest completely unrolled leaf on the main stem was taken for analysis. The presence of BYDV MAV and PAV was confirmed by positive reaction with BYDV antiserum in enzyme-linked immunosorbent assay (ELISA).

Data were analyzed to test for differences in mean percent aphid infestation and mean percent virus infection resulting from the main effects 'Experiment' , 'Crop', 'Aphid Sp.' and 'Predator / Parasitoid Treatment'. Analysis was carried out using Statistical Analysis System (SAS Institute 1995). Percentages were analyzed by applying an analysis of variance (ANOVA) to square-root arcsin transformed data at the p= .05 level of significance. Results are reported as percentages.

**Results & Discussion:**

Tables 1, 2 and 3 summarize the mean percent aphid infestations for field experiment 1, 2 and CE room respectively. Preliminary statistical analysis indicated significant differences for all main effects as follows; Experiment P = 0.0004, Crop P = 0.0001, Aphid Sp. P = 0.0108, and Predator / Parasitoid Treatment P = 0.0017. However, several significant interactions were also indicated. They are; Experiment * Aphid Sp. P = 0.0001, Crop * Predator / Parasitoid Treatment P = 0.0179 and Aphid Sp. * Predator / Parasitoid Treatment P = 0.0328.

The first field experiment has greater overall values for infestation followed by the controlled environment experiment and then the second field experiment. This outcome might be expected solely on the basis of temperature. Temperatures in the first field experiment were warmer than in the second field experiment while the controlled environment room experiment was conducted at intermediate temperatures. It is also possible that plant size had an effect. The first field experiment and the controlled environment experiment were both started with "two leaf" stage plants while the second field experiment was at the "one to two tiller" stage. This later stage would have provided more leaf area per plant on which the aphids might settle and thus resulting in less need to move.

Barley plants tended to have higher infestation levels than wheat plants. This effect is constant across all three experiments, and both aphid species. Additionally, with one exception (See Table 2, second field experiment x *R. padi* x parasitoid), it is consistent within all predator / parasitoid treatments.

As of this writing all experimental plants have been subject to ELISA for detection of BYDV. However, analysis of the complete experiment is not yet available. The infection data reported here are from all three experiments, and include both barley and wheat but, only the aphid *S. avenae*, and the two natural enemies treatments: 'no predator or parasitoid', and 'single predator'. Initial ANOVA indicated significant differences between the three experiments. However, there was no significant difference between barley and wheat or between; 'no predator or parasitoid', and 'single predator'.

Percent virus infection for field experiments 1, 2 and CE room respectively were (mean ± standard error) 46.8 ± 4.1, 23.3 ± 3.7 and 30.3 ± 4.5 (n=12, F=8.92, P = 0.0013 ). Percent infection by crop was 37.0 ± 3.9 for barley and 29.9 ± 4.1 for wheat (n=18, P = 0.1250). Percent infections by treatments was; 'no predator or parasitoid' 31.1 ± 3.7 and 'single predator' at 35.9 ± 4.4 (n=18, P =0.3248). There were no significant three way interactions or two way interactions involving the factor 'Experiment'. However, there may be a two way interaction involving the factors 'Crop' and 'Treatment' (P = 0.0512).

**Summary:**
There was a significant different between the overall mean percent virus infection in the three experiments. The virus infection levels follow the same pattern as the aphid infestation levels with the first field experiment having the greatest percent virus infection, followed by the CE room experiment then the second field experiment. There was no significant difference between crop type, however the barley plots did produce a greater mean infection. The was no difference between the two predator / parasitoid treatments.

Currently, the available percent aphid infestation and percent virus infection data do not provide evidence to indicate that natural enemies either reduce or enhance the level of BYDV. However, the reader is reminded that this is a very preliminary analysis. We suggest for example that the number of aphids present at the time of cage removal may prove to be a significant co-variate. Though two cages might have

quite similar percent infestations they may be infested with very different numbers of aphids. Additionally this communication does not report any analysis of the spatial distribution of either aphid infestation or virus infections, which will be examined later.

**References:**

Harrington, R., Mann, J.A., Plumb, R.T., Smith, A.J., Taylor, M.S., Foster, G.N., Holmes, S.J., Masterman, A.J., Tones, S.J., Knight, J.D., Oakley, J.N., Barker, I., & Walters, K.F.A., 1994. Monitoring and forecasting for BYDV control - the way forward? Aspects Applied Biology. 37:197-206.

Irwin, M. & Thresh, J. M., 1990. Epidemiology of barley yellow dwarf: A study in ecological complexity. Annual Review Phytopathology 28:393-424.

Knaust, H.J., 1996.Untersuchungen zur sekundaren ausbreitung von getreideblattlausen und deren bedeutung fur die epidemiologie des BYD-virus. Hannover Univ. Ph. D. Diss. Cuvilier Verlag, Gottingen. 205pp

Mann, J.A., Tatchell, G.M., Dupuch, M.J., Harrington, R., Clark, S.J. & McCartney, H.A., 1995. Movement of apterous *Sitobion avenae*(Homoptera: Aphididae) in response to leaf disturbances caused by wind and rain. Annals Applied Biology 125:417-427.

Mann, J.A., Harrington, R., Morgan, D., Walters, K.F.A., Barker, I., Tones, S. & Foster, G.N., 1996. Towards decision support for control of barley yellow dwarf vectors. Proceedings Brighton Crop Protection Conf - Pests & Diseases. 1995, 179-184.

Plumb, R.T., 1983. Barley yellow dwarf virus - a global problem. In *Plant Virus Epidemiology*. ed. R.T. Plumb, J.M Thresh, pp. 185-98. London: Blackwell Sci 377 pp.

SAS Institute. 1995. SAS procedure guide for personal computers Ver. 6, ed. SAS Institute, Cary, NC. 1996.

Sopp, P.I., Sunderland, K.D. & Combes, D.S., 1987. Observations on the number of cereal aphids on the soil in relation to aphid density in winter wheat. Annals Applied Biology 111:53-57.

Tamaki, G., Halfhill, J.M. & Hathaway, D.O., 1970. Dispersal and Reduction of Colonies of Pea Aphids by *Aphidius smithi* (Hymenoptera: Aphidiidae). Annals Entomological Society America 63:973-980.

Tottman, D.R. & Broad, H., 1987. Decimal code for the growth stages of cereals. Annals Applied Biology 110:683-687.

**Acknowledgements:**
We thank all colleagues in the Rothamsted Insect Survey who braved the foul weather conditions while they lay prostrate counting aphids. IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. The senior author thanks the University of Kentucky, the Kentucky Small Grain Growers Association, Zenica Ag Products and Gustafson Inc. for support during my sabbatical year.

**Table 1. Mean ± SE percent plants infested with *S. avenae* or *R. padi* from plots planted with wheat or barley, and subjected to various levels of predators and/or parasitoids in the first field experiment.**

| Crop | Barley | | Wheat | |
|---|---|---|---|---|
| Aphid | *R. padi.* | *S. avenae* | *R. padi* | *S. avenae* |
| Treatment* | | | | |
| None | 35.0 ±12.0 | 51.0 ±12.0 | 31.6 ± 10 | 44.0 ± 9.0 |
| Predator | 17.0 ± 0.0 | 60.0 ±16.0 | 12.5 ± 3.0 | 16.0 ± 9.0 |
| Parasitoid | 38.0 ± 16.0 | 56.0 ± 17.0 | 33.0 ± 17.0 | 57.0 ± 14.0 |
| Both | 25.0 ± 5.0 | 73.0 ± 2.0 | 6 .4 ± 5.0 | 39.0 ± 9.0 |
| Aphid Sp. | 30.3 ± 6.0 | 59.3 ± 6.5 | 18.6 ± 5.0 | 39.6 ± 6.3 |
| Crop | 45.5 ± 5.4 | | 30.1 ± 4.6 | |
| Experiment | 37.6 ± 3.7 | | | |

***None = no predator or parasitoid, Predator = one predator, Parasitoid = one parasitoid, Both = one predator plus one parasitoid.**

**Table 2. Mean ± SE percent plans infested with *S. avenae* or *R. padi* from plots planted with wheat or barley, and subjected to various levels of predators and/or parasitoids in the second field experiment.**

| Crop | Barley | | Wheat | |
|---|---|---|---|---|
| Aphid | *R. padi.* | *S. avenae* | *R. padi* | *S. avenae* |
| Treatment* | | | | |
| None | 39.8 ± 11.8 | 34.2 ± 4.4 | 38.4 ± 8.2 | 18.3 ± 2.5 |
| Predator | 48.6 ± 6.4 | 31.0 ± 8.0 | 9.1 ± 6.9 | 2.8 ±1.3 |
| Parasitoid | 36.0 ± 4.2 | 31.3 ± 6.3 | 38.1 ± 10.6 | 22.5 ± 4.5 |
| Both | 35.8 ± 16.7 | 25.5 ± 3.9 | 17.3 ± 9.1 | 15.6 ± 4.2 |
| Aphid Sp. | 40.1 ± 4.9 | 30.5 ± 2.7 | 25.7 ± 5.4 | 14.5 ± 2.8 |
| Crop | 35.1 ± 2.9 | | 20.3 ± 3.3 | |
| Experiment | 27.9 ± 2.4 | | | |

***None = no predator or parasitoid, Predator = one predator, Parasitoid = one parasitoid, Both = one predator plus one parasitoid.**

**Table 3. Mean ± SE percent plants infested with *S. avenae* or *R. padi* from plots planted with wheat or barley, and subjected to various levels of predators and/or parasitoids in the controlled environment experiment.**

| Crop | Barley | | Wheat | |
|---|---|---|---|---|
| Aphid | *R. padi.* | *S. avenae* | *R. padi* | *S. avenae* |
| Treatment* | | | | |
| None | 40.0 ± 4.6 | 37.3 ± 2.7 | 28.0 ± 8.3 | 30.7 ± 10.9 |
| Predator | 47.0 ± 4.4 | 40.5 ± 3.8 | 46.7 ± 4.8 | 36.0 ± 10.1 |
| Parasitoid | 48.0 ± 6.1 | 51.9 ± 11.6 | 39.7 ± 13.9 | 61.3 ± 1.3 |
| Both | 28.7 ± 13.4 | 44.0 ± 10.0 | 17.8 ± 3.4 | 4.8 ± 8.4 |
| Aphid Sp. | 41.0 ± 4.1 | 43.4 ± 3.8 | 33.0 ± 5.9 | 43.2 ± 5.1 |
| Crop | 42.2 ± 2.7 | | 38.1 ± 3.6 | |
| Experiment | 40.1 ± 2.5 | | | |

***None = no predator or parasitoid, Predator = one predator, Parasitoid = one parasitoid, Both = one predator plus one parasitoid .**