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COMPLEMENTARY METHODS FOR MONITORING SUGAR BEET APHIDS TO IMPROVE RISK MANAGEMENT OF VIRUS YELLOWS

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Harrington R., Stevens M., Alderson L., Cox D., Denholm I., Foster S., Hall M., Hallsworth P., Oliphant L., Parker S., Shortall C., Taylor M., Wright S. – Complementary methods for monitoring sugar beet aphids to improve risk management of virus yellows.

For many years the UK sugar beet industry has contributed to the funding of aphid monitoring in return for relevant information on the aphid vectors of viruses causing sugar beet yellows disease. This paper describes the two monitoring systems used and the application of data obtained. A network of suction traps provides data on the phenology and abundance of vector aphids on a regional basis. Yellow water traps give an indication of the degree of variability within a region and may eventually make it possible to link aphid incidence to field characteristics. Relationships between suction trap data and meteorological data enable provision of forecasts of aphid phenology and abundance, and of virus incidence, but not in time to influence the use of insecticide-treated seed. Individual *Myzus persicae* (Sulzer) from the suction traps are assayed for three insecticide-resistance mechanisms and for the presence of *Beet mild yellowing virus*. Latest methods used in these assessments are outlined. The information is collated into a package of advice to growers.

KEY WORDS: monitoring, forecasting, sugar beet, viruses, aphids, insecticide resistance.

INTRODUCTION

Aphid-borne viruses have the potential to cause major economic losses in the UK sugar beet crop. Sugar beet yellows disease is caused by three viruses. *Beet yellows virus* (BYV) is a closterovirus, which has a semi-persistent relationship with its vectors. It resides on the stylets of the vectors and in the phloem of host plants. *Beet mild yellowing virus* (BMVY) and *Beet chlorosis virus* (BChV) are luteoviruses. These have a persistent relationship with their vectors. All three viruses can only be transmitted by colonising species. *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas) are the most important vectors, although some clones of *M. euphorbiae* cannot transmit BChV (KOZLOWSKA-MAKULSKA *et al.*, 2009). This is interesting, as BChV is extremely closely related to BMYV, and warrants further investigation. BYV is the most damaging to plants which it infects, but is the least common of the three viruses. BMYV is the most prevalent but, in recent years, levels of BChV have increased and between 2004 and 2007 represented 43% of all yellowing viruses identified.

The virus threat is increased by the growing frequency of mild winters, which aid aphid survival and tend to lead to early and large migrations (HARRINGTON *et al.,* 1995), and by insecticide resistance, which makes *M. persicae* difficult to control. At least three resistance mechanisms are known in this aphid (FOSTER *et al*., 2007) and there are few insecticides to which all aphid clones are susceptible. Currently, neonicotinoids are effective and nearly 90% of the crop is planted with treated seed. However, there are the early signs of resistance developing in some populations and, although control has not thus far been compromised, the threat is clear (PUINEAN *et al*., 2009). All sugar beet in the UK is grown under contract to British Sugar plc and growers are required to pay a yield-based levy to the British Beet Research Organisation (BBRO), to fund marketing and research and development. For many years, the BBRO has contracted Rothamsted Research to provide a package of advice to the industry on aphid control. This includes forecasting the timing and size of aphid migrations into the crop and providing up-to-date information on the presence of aphid vectors, their insecticide resistance status and whether or not they are carrying BMYV. This paper outlines the techniques used and the dissemination and value of the information.

METHODS

APHID PHENOLOGY AND ABUNDANCE

A network of 16 suction traps has been operated throughout the UK for many years in order to monitor aphids (HARRINGTON and WOIWOD, 2007). Four of these (Rothamsted, Broom's Barn, Writtle and Kirton) are situated in the East of England, the main sugar beet growing area of the UK, and it is data from these that are currently used in the work for BBRO. The traps are emptied daily and the samples sent to Rothamsted where the aphids are identified.

Suction traps provide aphid data that are relevant to a wide region (TAYLOR, 1979; COCU *et al.,* 2005). However, there is much variation in the number of aphids arriving in individual fields according to a range of field characteristics. In order to assess this variation and provide more locally relevant information, a network of yellow water

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pan traps has been operated since 1994. In 2009, 29 such traps were operated. Samples are sent to Broom's Barn each week and the vector aphids identified, counted and then tested for the presence of virus.

Forecasting virus incidence

The suction traps at Rothamsted and Broom's Barn began operating in 1965, at Writtle in 1975 and at Kirton in 1980. The long runs of data facilitate analyses of aphid phenology and abundance in relation to weather data. January and February temperature have been found to be especially closely correlated with aphid phenology and abundance during the time that the sugar beet crop is susceptible to virus acquisition (Harrington and Woiwod, 2007). Early and large aphid flights lead to increased risk from virus, but the timing of crop planting and whether or not neonicotinoid-treated seed is used clearly have a major influence on virus incidence. These factors are brought together in forecasting algorithms described by Qi *et al.* (2004). Forecasts of timing and size of aphid migrations and of expected virus incidence at the end of August in four sugar beet factory areas are issued in early March.

Insecticide resistance status of *Myzus persicae*

All *M. persicae*, *M. euphorbiae* and *Aulacorthum solani* (Kaltenbach) from the suction trap samples are blotted on tissue, placed individually in wells of microtitre plates in 50ul PBS-Tween and then stored at -20ºC prior to assay for insecticide resistance status (*M. persicae* only) and/or virus content (see next section).

Three insecticide resistance mechanisms are present in *M. persicae*. Elevated carboxylesterases sequester insecticide and confer resistance to organophosphates and, to some extent, pyrethroids. Modified acetylcholinesterase (MACE) involves a mutation that prevents insecticides binding to their target site and confers resistance specifically to pirimicarb. Another mutation known as knock-down resistance (kdr, and closely related super-kdr), confers resistance to pyrethroids. Elevated carboxylesterase-based insecticide resistance is assayed using the total esterase test of Grant *et al.* (1989). This is now only done for aphids from the Rothamsted trap and is not relevant to growers as organophosphates are no longer used in the UK. Testing for MACE resistance until 2005 was done using a biochemical kinetic enzyme assay in the absence and presence of a diagnostic concentration of pirimicarb according to the method of Moores *et al.* (1994). New DNA-based assays for the kdr, super-kdr and MACE mutations (Anstead *et al.* 2004) are now used for all the traps except Rothamsted (in order to allow carboxylesterase testing at this site, which requires a different collecting medium). This latter method provides the additional benefit of distinguishing between resistance genotypes.

Viruses present in aphid

A maximum of 100 *M. persicae* from any one site are tested for virus content in any one week. Aphids are ground in 50 l PBS Tween in the microtitre plates and the solution is made up to 210 l, with 200 l of the aphid extract used for the assessment of viruses. This fraction is divided into two equal parts to determine the numbers of aphids containing *Beet mild yellowing virus* (BMYV) and *Turnip yellows virus* (TuYV) (formerly known as *Beet western yellows virus*). In Britain only a small proportion of TuYV isolates infect sugar beet (Smith *et al.*, 1991), but serologically these two viruses are very similar and it is necessary to identify both before the proportion of aphids carrying the more important BMYV can be determined. Molecular methods have been devised to test the aphids for BMYV and BChV (Vigano and Stevens, 2007), and work is ongoing to test a single aphid for all the yellowing viruses simultaneously. The method involves the use of monoclonal antibodies in an amplified enzyme linked immunosorbent assay (ELISA) (Smith *et al.*, 1991).

Dissemination to the industry

Weekly bulletins and maps are issued by Broom's Barn to the industry via the internet, by email and by post throughout the sugar beet growing season.

RESULTS AND DISCUSSION

Forecasting virus incidence

Fig. I shows the performance of the virus forecast. The actual levels of virus yellows at the end of August follow closely the forecast levels with pest management, as the majority of the sugar beet crop has used neonicotinoid-treated seed since 1994. In 2001, control plots with no insecticide usage were monitored. Average virus incidence in these plots was found to be 29%, against a forecast of 33%. Prior to 1994, most crops were sprayed only when forecasts indicated high levels of virus. Unfortunately, seed has to be treated well before a forecast can be provided. With around 90% of crops coming from treated seed, the forecast is not as relevant as it used to be, as sprays are not required in such crops. However, widespread use of neonicotinoids, particularly their increasing application as foliar sprays on other crops, is likely to hasten the onset of resistance to this class of insecticides, the use of which is also threatened because of perceived damage to bee populations. If neonicotinoids are lost for either of these reasons, aphid control will become very much more difficult, as there is already considerable resistance to pyrethroids and pirimicarb, and the forecast will become more important again.

Aphid phenology and abundance

There is considerable inter-annual variation in the phenology and abundance of aphid vectors of sugar beet

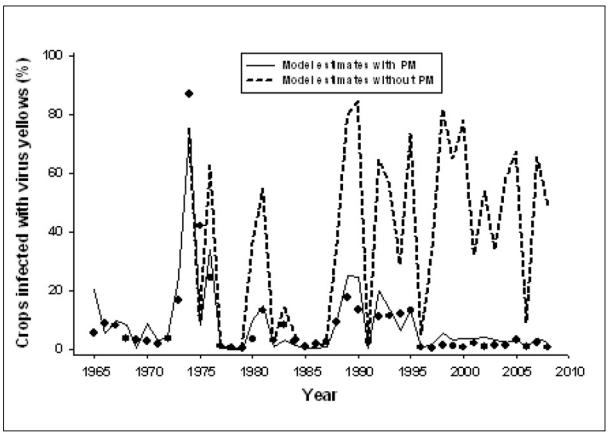

Fig. I – Incidence of virus yellows in sugar beet since 1965 in eastern England.

viruses, and in abundance between suction traps and yellow water pan traps. However, the patterns of phenology and abundance of vectors in suction traps and the means for yellow water pan traps are very similar, especially for yellow water pan traps 40km of less distant from the comparator suction trap.

Insecticide resistance status of *M. persicae*

The loss or organosphosphates to the insecticide armoury has been mirrored by a reduction in levels of high and extreme resistance (R_2 and R_3) through elevated carboxylesterases (Fig. II). On the other hand, increasing levels of MACE resistance, specifically to pirimicarb, reflect the high use of this compound to control *M. persicae* in a range of crops. Kdr resistance is now widespread, with about 25% of aphids possessing this mutation is its heterozygous form. Homozygotes are very rare in the UK and are more resistant than heterozygotes, but heterozygotes cannot be controlled by pyrethroids used at field doses. Super-kdr remains extremely rare.

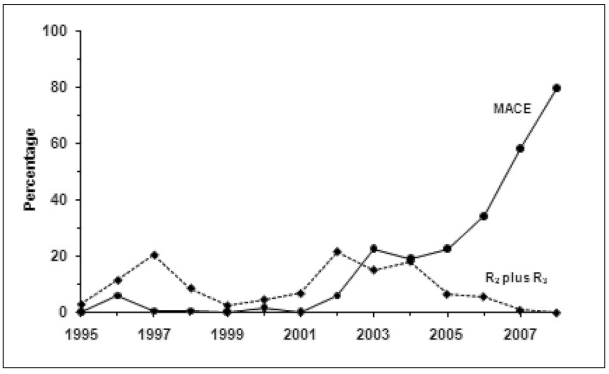

Fig. II – Percentage of R_2 plus R_3 and MACE *Myzus persicae* at Rothamsted, Broom's Barn, Kirton and Writtle combined.

Viruses present in aphids

In total, 3678 aphids (2705 *M. persicae*, 738 *M. euphorbiae* and 235 *A. solani*) were tested for BMYV over the four year period 2005-2008. Only 8 *M. persicae* (0.26% of the total) and 3 *M. euphorbiae* (0.41%) were carrying BMYV. No *A.solani* tested positive for BMYV. The percentage of aphids carrying BMYV may appear low. However, when seen in terms of the number that will have infested the crop, it ensures a high potential number of primary virus foci. It should be remembered that single aphids from the trap samples cannot be tested using ELISA for BYV. However, this virus is not usually significant compared to BMYV. It is also impossible to distinguish BMYV and BChV by ELISA. However, a new RT-PCR method for detecting both BMYV and BChV has recently been developed at Broom's Barn (Vigano and Stevens, 2007).

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