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The incidence of *Turnip yellows virus* in oilseed rape crops (*Brassica napus* L.) in three different regions of England over three consecutive growing seasons and the relationship with the abundance of flying *Myzus persicae*.

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#### **Abstract**

Turnip yellows virus (TuYV) is the most important virus infecting oilseed rape in the UK. The incidence and spatial distribution of TuYV in winter oilseed rape (WOSR) crops in three regions of England was determined over three growing seasons. Leaf samples were collected from three fields in each region, in autumn (November-December) and spring (April) of the three crop seasons and tested for virus presence by enzyme-linked immunosorbent assay. Infection was detected in all fields except one. Higher TuYV incidences were recorded in the 2007-8 (<89%) and 2009-10 (<100%) crop seasons than in 2008-9 (<24%). Highest incidences were recorded in Lincolnshire (<100%), followed by Warwickshire (<88%), with lowest incidences in Yorkshire (1-74%). There was a significant increase in incidence detected between autumn and spring sampling in eight fields, a significant decrease in one field and no significant change in 18 fields. Rothamsted Insect Survey suction trap data for the aphid Myzus persicae in Lincolnshire, Warwickshire and Yorkshire revealed two peaks of flight activity in most years (2007-2009). The second peak (September-November) coincided with emergence of WOSR. The highest cumulative (August-November) trap catches in the three regions during the three crop seasons occurred in Lincolnshire and the lowest in Yorkshire; catches in autumn 2009 were highest and lowest in autumn 2008. Regression analysis revealed a highly significant association between the cumulative numbers of M. persicae caught in the suction traps closest to the crops between August and November each year and the incidence of TuYV detected in the WOSR crops in the autumn of each year. Results are discussed in the light of factors affecting the spread of TuYV and future possibilities for control.

**Keywords**: *Brassica napus* (oilseed rape), Turnip yellows virus incidence, aphids, *Myzus persicae* abundance

Turnip yellows virus in winter oilseed rape

# 1 INTRODUCTION

Turnip yellows virus (syn. Beet western yellows virus), a species in the Polerovirus genus of the Luteoviridae family is the most common virus infecting oilseed rape in the UK (Hardwick et al., 1994; Stevens et al., 2008). It has a wide host range and infects more than 150 species in 23 dicotyledonous families, including economically important crops such as lettuce, spinach, field beans, radish, cabbage and oilseed rape (Graichen, 1996).

TuYV infection of oilseed rape crops in England was first reported in 1980 (Gilligan et al., 1980) but the widespread incidence of the virus in the UK was first reported by (Smith & Hinckes, 1985). Results from a UK survey in 1983, covering 80 autumn-sown oilseed rape crops from Aberdeenshire to Essex showed that 97% were extensively infected with TuYV (Smith & Hinckes, 1985). Varying incidences of TuYV infection in oilseed rape crops have since been reported in the UK, ranging from less than 10% to 85% (Hardwick et al., 1994; Hill et al., 1989; Jay et al., 1999; Walsh et al., 1989). A survey conducted in 2009 covering oilseed rape crops on 80 farms from the south coast of England to Scotland showed TuYV infection of up to 70% (Clark & Stevens, 2009). Infection of oilseed rape crops by TuYV has also been reported in other countries including Germany (Schröder, 1994), France (Kerlan, 1991), the Czech Republic (Polak & Majkowa, 1992), Austria (Graichen et al., 2000), Iran (Shahraeen et al., 2003), Serbia (Jasnic & Bagi, 2007; Milošević et al. 2015) and Australia (Coutts et al., 2006). The virus has also been reported in lettuce in the UK (Walkey & Pink, 1990) and faba bean in Egypt and Morocco (Abraham et al., 2008).

Turnip yellows virus in winter oilseed rape

TuYV, like other members of the *Luteoviridae* is mostly restricted to the phloem tissue of host plants and is transmitted by aphid vectors from plant to plant in a persistent (acquisition times range from 30 min to hours, the vector cannot immediately transmit the virus and retains its ability to transmit through the moult and often for the remainder of its life), circulative (passes from the insect gut to the salivary glands via the haemolymph for transmission) and non-propagative (does not replicate in vector tissues) manner. The green peach aphid, *Myzus persicae* (Sulzer) is the principal vector in Europe (Schliephake et al., 2000) with a transmission efficiency of about 90% (Schliephake et al., 2000). Enzyme-linked immunosorbent assay (ELISA) on samples of *M. persicae* caught in yellow water traps and Rothamsted Insect Survey Suction traps have shown that 2-72% of *M. persicae* were carrying TuYV (Stevens et al., 2008).

TuYV infection is thought to be one of the major reasons why oilseed rape crops do not attain their potential yield in England (Stevens et al., 2008), estimated at 6.5 t/ha (Berry & Spink, 2006), compared to the current yield of 3.5 t/ha (Defra, 2018). TuYV infection seriously affects all components of yield, including the number of pods per plant, number of seeds per pod, and the oil content per seed. Plants infected with TuYV also have a reduced leaf area per plant and produce fewer primary branches (Jay et al., 1999). The effect of TuYV on the yield of oilseed rape depends on the incidence of virus infection and the crop variety (Walsh et al., 1989). One estimate of yield losses due to TuYV infection is up to 30% (Home-Grown Cereals Authority, 2009). Experimental plots of oilseed rape with 100% TuYV- infection yielded approximately 10% less seed and 13.4% less oil than plots with 18% virus infection (Smith and Hinckes, 1985). Plot experiments in Australia on spring oilseed rape, showed that a site with 96% infection suffered a yield loss of up to 46% (Jones et al., 2007) and in Germany, plots of winter oilseed rape with 90-100% TuYV infection yielded between 12% and 34% lower than plots that were

almost virus-free (Graichen & Schliephake, 1999). When oilseed rape plants were co-infected with a mix of TuYV, cauliflower mosaic virus (CaMV) and turnip mosaic virus (TuMV), the yields of plants with severe virus symptoms were reduced by an estimated 70-79% (Hardwick et al., 1994).

Effective control of TuYV in oilseed rape is necessary to improve yields. However, for the most part, farmers growing oilseed rape in the UK seem not to be aware of the presence, let alone the incidence of TuYV in their crops. For this reason and as an important prerequisite for developing effective approaches to controlling the disease it is important to investigate the incidence of TuYV infection of oilseed rape crops at various locations, in different years and the type of disease pattern and spread in the fields. The quantitative relationship between the flight activities of the aphid vectors is also important for the timing and development of control strategies.

Here the incidence and distribution of TuYV in fields of winter oilseed rape in three regions of England in the autumn and spring of three consecutive crop seasons are described. It has been said that the spread of brassica viruses is related to the abundance and movement of aphid vectors (Walsh & Tomlinson, 1985). In Germany, high levels of TuYV infection were detected in winter oilseed rape crops during 1995-96 following high levels of flight activity of aphids during the autumn of 1995 (Graichen & Schliephake, 1999). We have investigated these relationships in the context of *M. persicae* (said to be the most important vector of TuYV in Europe) and quantified them by relating the number of flying *M. persicae* caught in the Rothamsted Insect Survey suction traps located nearest to sampling sites to the incidences of TuYV at the sampling sites in the three regions, over the three consecutive growing seasons.

 Turnip yellows virus in winter oilseed rape

## 2 MATERIALS AND METHODS

# 2.1 Surveys of winter oilseed rape crops

Using a line transect sampling method (Buckland et al., 2001), upper leaves from 100 plants were sampled from three crop fields in each of three oilseed rape-growing regions of England, Eastern (Lincolnshire), Northern (Yorkshire) and Midlands (Warwickshire) in autumn (November-December; 2-6 true leaves) and the following spring (April; starting to flower) during the 2007-8, 2008-9 and 2009-10 crop seasons (27 fields in all). Aphids were very rarely seen on the sampled plants, consequently no records were taken. Samples from each plant were placed in separate polythene bags to register sampling positions within fields. The Lincolnshire fields were near Long Sutton, the Warwickshire fields were near Gaydon, and the Yorkshire sites were near Allerton, Little Ouseburn, Aberford, Green Hammerton and Whixley. The locations of the fields were determined using a Global Positioning System (Garmin E-Trex HGPS Receiver, Garmin Corporation Olathe, KS, USA) (Supporting information, Table S1).

The sampling procedure involved estimating the length and breadth of each field in order to divide the field into ten equally-spaced transects with ten equally-spaced-samples collected per transect. Where fields were exceptionally large, only a proportion of the fields were sampled. The mean field size was 20.8 hectares (range from 4.3 to 40.0 ha).

# 2.2 Sampling sites

Farms sampled rotate oilseed rape crops every 3-6 years with crops such as wheat, potato, sugar beet, peas and may also practice land fallowing. Winter oilseed rape is normally sown in England between late August and early September, overwinters, flowers in the spring (April-May) and is harvested in July/August. Seed sowing in field three in Warwickshire during the 2008-9 crop

season was delayed by the prevailing weather conditions until late September. Where the information was available, the cultivars in the fields sampled are given in Table S1.

# 2.3 Detection of TuYV in the samples

The presence of TuYV was tested in all sampled plants by standard triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) using paired wells in microtitre plates (96-well Nunc Maxisorp; Nunc, Roskilde, Denmark) as described by Hunter et al. (2002).

Absorbance values ( $A_{405nm}$ ) were measured with a Biochrom Anthos 2010 microplate reader (Biochrom Ltd., Cambridge, U.K.). Values for 10 uninfected leaf samples were also measured. A sample was deemed to be positive when the absorbance was greater than the mean absorbance of ten healthy samples on each ELISA plate, plus 2.262 x standard deviation of the mean of the ten healthy samples (where 2.262 = Inverse of Student's t-distribution at 5% probability level with 9 degrees of freedom).

#### 2.4 Data analysis

TuYV incidence data were analysed using a generalised linear model (GLM) (Nelder & Wedderburn, 1972). Differences between county and crop season means and their interactions were compared using the approximate least significant difference (LSD) calculated from the analyses.

A two-sample binomial test (Armitage & Berry, 1994) was used to determine whether there was a significant change in TuYV incidences in each field between autumn and spring. The overall correlation between autumn and spring incidences of TuYV infections was assessed using the

Pearson correlation coefficient. All statistical analyses were carried out using GenStat (GenStat Release version 12.1) (Payne et al., 2009).

The cumulative numbers of *M. persicae* caught monthly in the Rothamsted Insect Survey suction traps closest to the sampling sites (Kirton in Lincolnshire [22-26 km from fields sampled], Askham Bryan in Yorkshire [13-17 km from fields sampled] and Wellesbourne in Warwickshire [10-11 km from fields sampled]) were determined from the weekly suction trap aphid catches obtained between August (crop sowing) and November for each year. A non-linear regression analysis fitting an exponential function was used to explore the relationship between mean TuYV incidences in autumn and the cumulative aphid counts.

A Black-White (BW) join-count statistic (Cliff & Ord, 1969) was calculated using GenStat to assess spatial autocorrelation within each field for each sampling occasion, comparing a null hypothesis of random distribution of infected samples with an alternative hypothesis of spatial clustering. The statistic measures the number of neighbour pairs containing both an infected and a healthy plant, where neighbours were defined here to be adjacent samples in vertical or horizontal directions (each non-edge sample has four neighbours, i.e. "rook case"). The significance of the join-count statistic is achieved by computing a standard normal deviate, called a Z-score which is given by the formula:

$$Z(BW) = \frac{Observed (BW) - Expected (BW)}{\sigma_{BW}}$$

where  $\sigma_{BW}$  is the standard deviation for BW joins. The expected number of BW neighbours is calculated based on the overall proportion of infected plants and represents the likely pattern under a random distribution. As the aim was to detect evidence of clustering, a one-sided test for

Turnip yellows virus in winter oilseed rape negative values of the BW join-count statistic was appropriate, negative values indicating a positive spatial autocorrelation between infected plants and probability levels of  $P \le 0.05$  indicating significant spatial autocorrelation.

#### **3 RESULTS**

# 3.1 Incidence of TuYV infection in winter oilseed rape crops

The overall mean TuYV incidences recorded in the autumns of 2007 (36.67  $\pm$  4.56%) and 2009 (48.67  $\pm$  3.62%) were not significantly different from each other but were significantly higher (P < 0.001) than recorded in the autumn of 2008 (6.11  $\pm$  2.33%) (Table 1). Highly significant differences (P < 0.001) in the mean percentage autumn virus incidences were found between the counties (Table 1). Lincolnshire had the highest mean TuYV incidence (55.00  $\pm$  3.69%), followed by Warwickshire (23.78  $\pm$  3.94%) whilst Yorkshire had the lowest (12.67  $\pm$  3.18%) (Table 1; Fig 2b). The interaction effects between the counties and crop seasons for autumn virus incidences were significant (P < 0.05). The highest mean incidence (94.00  $\pm$  4.08%) was recorded in Lincolnshire during the autumn of 2009, whilst the lowest (2.33  $\pm$  2.60%) was recorded in Yorkshire during the autumn of 2008.

The highest overall mean TuYV incidence in spring was recorded in the 2007-8 crop (55.67  $\pm$  6.91%), followed by the 2009-10 crop (53.78  $\pm$  5.86%); the 2008-9 crop had the lowest (8.67  $\pm$  4.11%) (Table 2). Lincolnshire had the highest overall mean spring incidence (56.67  $\pm$  5.17%), followed by Warwickshire (42.00  $\pm$  6.43%) and Yorkshire had the lowest (19.44  $\pm$  5.55%). The GLM analysis did not indicate a significant interaction between the regions and crop seasons for the spring incidences of TuYV (P = 0.163). However, the highest mean incidence (94.00  $\pm$ 

Turnip yellows virus in winter oilseed rape 6.04%) was recorded in Lincolnshire in spring of the 2009-10 crop, whilst the lowest mean incidence  $(3.67 \pm 4.79\%)$  was recorded in Yorkshire during 2008-9 crop season (Table 2).

The comparisons of autumn and spring incidences of TuYV in the individual 27 fields in the three regions over the three crop seasons using the two-sample binomial test are shown in Table S1. Large differences in the incidences of TuYV in the various fields, counties and crop seasons surveyed, ranging from 0% (recorded in autumn and spring in Warwickshire in 2008-9) to 100% (recorded in Lincolnshire in the autumn of 2009) were found. There were significant (P < 0.05) changes in the proportions of plants infected with TuYV between autumn and spring in five of the nine fields in 2007-8 (all 3 fields in Warwickshire and one each in Lincolnshire and Yorkshire), three fields in 2008-9 (two in Lincolnshire and one in Warwickshire) and only one field in 2009-10 (Yorkshire) (Table S1). With the exception of one of the fields in Lincolnshire in 2008-9, all significant changes involved an increase in TuYV incidence. Overall, there was a significantly high correlation (r = 0.89, P < 0.001, d.f. = 25) between autumn and spring incidences of TuYV in the oilseed rape crops surveyed.

#### 3.2 Within field virus distribution

The results of the analysis of spatial distribution of TuYV-infected plants are given in Table S1. Where possible, Black-White join-count statistics were calculated for each of the 27 fields surveyed in the three-crop seasons and then tested as standard normal deviates (Z-scores). Most of the fields (17 of the 25 analysed) showed positive, but non-significant spatial autocorrelation (negative Z-scores, P > 0.05) when sampled in autumn, indicating that most of the infected plants showed a slightly aggregated pattern of distribution. For a one-sided test at a probability of 0.05, values less than -1.645 (large negative Z-score) indicated that the number of observed join-counts

Turnip yellows virus in winter oilseed rape was significantly less than expected, an indication of clustering of the infected plants. Lincolnshire field 1 was the only field showing significant positive autocorrelation (Z = -1.922, P < 0.05) between the infected plants in autumn (2007), indicating significant clustering (Fig. 1a). Spring sampling revealed a slightly more random pattern with half of the fields (13 of the 26 analysed) showing negative spatial autocorrelation (positive Z-scores) (e.g. Warwickshire field 2 in spring 2010, Z = 0.561, P = 0.288, Fig. 1b). Warwickshire field 1 showed a significant positive autocorrelation (Z = -2.177, Z = 0.05) between the infected plants in spring 2008, indicating clustering.

# 3.3 Numbers of Myzus persicae caught in Rothamsted Insect Survey suction traps

Rothamsted Insect Survey suction trap catches showed two peaks of flight activity of *M. persicae* in Lincolnshire, Warwickshire and Yorkshire in most years (2007-2009) (Fig. 2a). The first peak occurred between June and July and the second occurred between September and November in each year, the latter coinciding with the emergence of oilseed rape crops. Crops were sown in August or September. The highest cumulative (August to November) trap catches of *M. persicae* during the three crop seasons occurred in Lincolnshire and the lowest in Yorkshire; catches in autumn 2009 were highest and those in autumn 2008 were lowest.

# 3.4 Relationship between flying Myzus persicae and TuYV incidence

There was a clear relationship between TuYV incidence and aphid numbers (Fig. 2). Regression analysis revealed a highly significant association between the numbers of M. persicae caught in the Rothamsted Insect Survey suction traps closest to the crops between August and November each year and the incidence of TuYV in the oilseed rape crops (d.f. = 2, 8; F = 24.2; P < 0.001) in autumn of each year (Fig. 3).

#### **4 DISCUSSION**

In this study. TuYV was prevalent in winter oilseed rape fields in three regions of England over a three year period with incidences within individual fields ranging from 0-100%. The virus was detected in 26 of the 27 oilseed rape fields sampled from three regions (Lincolnshire, Warwickshire and Yorkshire). This finding corroborates the previous reports of widespread occurrence of TuYV in oilseed rape crops in the UK (Smith and Hinckes 1985; Walsh et al., 1989; Hill et al., 1989; Hardwick et al., 1994; Jay et al., 1999; Stevens et al., 2008) where incidences of 0-100% were also reported. The only field where no infection was recorded (Warwickshire field three in the 2008-9 crop season) was sown late (27th September, 2008) relative to the other two Warwickshire fields (sown 26-27th August, 2008) and the very few plants that had emerged were very small when sampled on 15<sup>th</sup> December, 2008. It is clear from Fig. 2a that aphid numbers in Warwickshire were very low by late September 2008 and this, combined with the late planting date and the sparsity of plants, is very likely to be the reason for the lack of infection. We have not done any detailed assessments of TuYV incidence in winter oilseed rape in the UK since 2010, however, the limited testing of commercial oilseed rape crops that we have done, has revealed incidences of 0-100% in the 2018-19 crop, as for the 2007-2010 study reported here. That said, many crops tested early in 2019 had incidences of 100%, reflecting the very high numbers of *M. persicae* caught in Rothamsted Insect Survey suction traps between August and November 2018.

The analysis of the autumn incidences of TuYV in the oilseed rape crops in the different regions sampled were clearly associated with the cumulative numbers of *M. persicae* caught in the suction traps in these regions between August and November. This indicates that the significantly higher incidences of TuYV recorded in the 2007-8 and 2009-10 crops compared with those in the 2008-9 crops were due to the increased flight activity of *M. persicae* between August and

Turnip yellows virus in winter oilseed rape November in 2007 and 2009, relative to 2008 (Fig. 2). Graichen and Schliephake (1999) also demonstrated that a high incidence of TuYV in winter oilseed rape appeared to be closely related to the flight activity of M. persicae vectors in Germany. Clark and Stevens (2011) indicated a close correlation between aphid numbers caught in the autumn and the amount of virus present in oilseed rape in the UK. The timing and intensity of the spring and summer M. persicae aphid flights in the Columbia basin in the USA were associated with heat unit accumulation (day degrees) (Thomas et al., 1997). The differences between the accumulated day degrees in the first part (January to May) of 2007, 2008 and 2009 for the site of the Warwickshire Rothamsted Insect Survey suction trap do not appear to account for the abundance of M. persicae caught in this suction trap between August and November in these years and hence the incidence of TuYV. It is possible that the accumulated day degrees later in these years might account for the differences. however other factors including rainfall, predation, parasites, pathogens, availability of suitable host plants etc. are also likely to have an effect. Mild autumn conditions favour the development of the aphid vectors and encourage TuYV spread (Stevens et al., 2008). The low numbers of aphids in 2008 accounting for the low incidence of TuYV, were said to be due to wet and windy weather and an abundance of natural enemies.

In general, the incidence of TuYV within oilseed rape crops is considered to increase from initial autumn infection to a maximum level in the following spring (Stevens et al., 2008). Our data showed significant increases in the incidence of TuYV in eight fields between autumn and spring, but no significant change in 18 fields and a significant decrease in only one field. Most of the increases in incidence between autumn and spring occurred in the 2007/8 crop. All the fields in Warwickshire showed a significant increase in TuYV incidence between autumn 2007 and spring 2008 and the cumulative day degrees at our site in Warwickshire (10-11km from the Warwickshire fields sampled) were greater between January and May 2007 than for the same

Turnip yellows virus in winter oilseed rape months in 2008 and 2009. This indicates that in warmer winters, there could be yield/financial benefits from applying control measures such as effective insecticides at some point between November and April, depending up on regulations (numbers of spray and spray intervals permitted) and costs.

The significant regional differences in TuYV incidence, where highest levels of infection were observed in Lincolnshire and lowest in Yorkshire are likely to be attributable to a number of factors. There was much increased flight activity of *M. persicae* in Lincolnshire in the autumns of 2007 and 2009 relative to Yorkshire and Warwickshire. Also, the large area of vegetable brassicas grown in Lincolnshire is likely to be a reservoir of TuYV and source for aphids over the summer months, when no oilseed rape crops are present. Between 1986 and 1989, surveys in England and Wales detected lower TuYV incidences in the north and east (Hill et al. 1989). In 1992 and 1993, the incidence of TuYV was higher in Wales and the midlands, western and south western regions of England than in the eastern, south eastern or northern regions of England (Hardwick et al. 1994). Our data over three years and that of others (Blake, 2009; Clark and Stevens, 2009) suggest that there has been a change in prevalence, in that the highest levels of TuYV have been in Lincolnshire, close to the Wash and on the south coast.

The spatial autocorrelation analysis revealed that TuYV-infected oilseed rape plants showed either random or slightly aggregated pattern of distribution within individual fields, with most fields showing slightly aggregated patterns during autumn. This finding agrees with that of Bourdon (1987) who reported that crop plants infected with viruses can show random or aggregated distributions, with aggregated distributions more common in vector-borne viruses. There is very little data on the spatial incidence of TuYV, however Raybould et al. (1999) also reported plants infected by TuYV and other viruses (CaMV, TuMV and turnip yellow mosaic

Turnip yellows virus in winter oilseed rape virus) were distributed randomly, or were very weakly aggregated within wild populations of Brassica oleracea. Most of the oilseed crops showed positive autocorrelation (slight aggregated pattern) during autumn but a more random pattern during the following spring. Data on a nonpersistently transmitted virus showed that aggregation of infected plants appears where there is limited spread of the virus from the initial (primary) foci of infection (Eckel and Lampert, 1993). This suggests that autumn infection (tending towards aggregation) is mostly due to primary infection with some, probably limited, secondary infection. The reduction in aggregation between autumn (18 out of 25 had negative Z-scores and only seven had positive Z-scores) and the following spring (13 out of 26 had negative Z scores and 13 had positive Z-scores) indicates that the infection of plants between the autumn sampling dates and May of the following years was mostly due to secondary spread of the virus within the fields, rather than further primary infection coming from outside the fields. This is consistent with the lack of flying aphid vectors caught in suction traps during this period (Anon, 2011) and the characteristic spatial spread of persistently transmitted viruses (Thresh, 1976). All the crops we sampled were in arable crop growing vicinities and the most common crops surrounding the fields sampled were winter wheat and winter barley, neither of which are known to be important hosts for TuYV.

Oilseed rape is not reaching its full yield potential in the UK. No doubt there are a number of reasons for this, however, there is compelling evidence for TuYV being a major contributor to the shortfalls. TuYV is probably the most widespread and common disease of oilseed rape in the UK. The high levels of infection found, in years where there have been wet and not particularly warm summers and autumns, indicates that in warmer, drier years predicted under climate change scenarios (Parry, 1990), TuYV is likely to be an even greater problem. Higher incidences of TuYV were recorded for the major OSR growing countries of Europe (Poland, Germany and France) (Newbert 2016), than those outlined here for a three year period in England. This

Turnip yellows virus in winter oilseed rape indicates that losses across parts of mainland Europe must be much higher than in the UK and control measures are needed. The most effective insecticides for *M. persicae* control, the neonicotinoids, are now banned in the EU due to adverse effects on pollinating insects (Dewar, 2017). Varieties of oilseed rape with partial resistance to TuYV are now available, however, all varieties possess the same resistance source. This has created high selection pressure for resistance-breaking strains of TuYV. A further alternative source of resistance has been reported recently (Hackenberg et al. 2019).

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#### REFERENCES

Abraham A. D., Varrelmann M., Vetten H. J. (2008) Molecular evidence for the occurrence of two new luteoviruses in cool season food legumes in Northeast Africa. *African Journal of Biotechnology*, 7, 414-420.

Anon (2011) Aphid bulletin: Bulletin Archives. *Rothamsted Insect Survey* [Online]. Available: <a href="http://www.rothamsted.bbsrc.ac.uk/insect-survey/STAphidBulletinArchive.php?Year=2011">http://www.rothamsted.bbsrc.ac.uk/insect-survey/STAphidBulletinArchive.php?Year=2011</a> [Accessed 26 July, 2019].

- Armitage P., Berry G. (1994) *Statistical methods in medical research*, Oxford: Blackwell Science.
- Berry P. M., Spink J. (2006) A physiological analysis of oilseed rape yields: Past and future. *Journal of Agricultural Science*, **144**, 381-392.
- Blake A. (2009) *Turnip yellows virus threatens oilseed rape* [Online]. Reed Business Information. Available: <a href="http://www.fwi.co.uk/Articles/2009/06/04/115896/Turnip-yellow-virus-threatens-oilseed-rape.htm">http://www.fwi.co.uk/Articles/2009/06/04/115896/Turnip-yellow-virus-threatens-oilseed-rape.htm</a> [Accessed 26 July, 2019].
- Bourdon J. J. (ed.) (1987) *Diseases and plant population biology*, Cambridge, UK: Cambridge University Press.
- Buckland S. T., Anderson D. R., Burnham K. P., Laake J. L., Borchers D. L., Thomas L. (2001) *Introduction to Distance Sampling*, Oxford: Oxford University Press.
- Clark B., Stevens M. (2009) Strategies to reduce the impact of *Turnip yellows virus* on oilseed rape production in the UK. *In: HGCA Research and Development, Annual Report, 2009 results*. Stoneleigh Park, Kenilworth, UK: Home-Grown Cereal Authority. Available: <a href="https://cereals.ahdb.org.uk/media/160420/3498-annual-project-report-2009.pdf">https://cereals.ahdb.org.uk/media/160420/3498-annual-project-report-2009.pdf</a>
- Clark B., Stevens M. (2011) Strategies to reduce the impact of Turnip yellows virus on oilseed rape production in the UK. In: HGCA Research and Development, Annual Report, 2011 results. Stoneleigh Park, Kenilworth, UK: Home-Grown Cereal Authority. Available: <a href="https://cereals.ahdb.org.uk/media/160414/3498-annual-project-report-2011.pdf">https://cereals.ahdb.org.uk/media/160414/3498-annual-project-report-2011.pdf</a>
- Cliff A. D., Ord J. K. (eds.) (1969) The problem of spatial autocorrelation, London: Pion.
- Coutts B. A., Hawkes J. R., Jones R. A. C. (2006) Occurrence of *Beet western yellows virus* and its aphid vectors in over-summering broad-leafed weeds and volunteer crop plants in the grainbelt region of south-western Australia. *Australian Journal of Agricultural Research*, **57**, 975-982.
- Department for Environment Food and Rural Affairs (2018) National Statistics. Farming Statistics, Provisional crop areas, yields and livestock populations, At June 2018 United Kingdom. Available:

  <a href="https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\_data/file/747210/structure-jun2018prov-UK-11oct18.pdf">https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\_data/file/747210/structure-jun2018prov-UK-11oct18.pdf</a> (Accessed 26.7.19).
- Dewar, A. M. (2017) The adverse impact of the neonicotinoid seed treatment ban on crop protection in oilseed rape in the United Kingdom. *Pest Management Science* **73**, 1305-1309.
- Eckel R. V. W., Lampert E. P. (1993) Spatial and temporal analysis of tobacco etch virus distribution and its relationship to aphid (Homoptera: Aphidae) vectors in flue-cured tobacco. *Journal of Economic Entomology*, **86**, 1534-1545.
- Gilligan C. A., Pechan P. M., Day R., Hill S. A. (1980) *Beet western yellows virus* on oilseed rape (*Brassica napus* L). *Plant Pathology*, **29**, 53-53.
- Graichen K. (1996) Rape is not a source of infection of *Beet western yellows virus*. *L'Informatore Agrario*, **52**, 79-81.
- Graichen K., Rabeinstein F., Kurtz E. (2000) The occurrence of *Turnip yellows virus* in winter oilseed rape in Austria. *Pflanzenschutzberichte*, **59**, 35-46.
- Graichen K., Schliephake E. (1999) Infestation of winter oilseed rape by *Turnip yellows luteovirus* and its effect on yield in Germany. *In: 10th International Rapeseed Congress*, pp. 31-136. Canberra, ACT: International Consultaive Group for Rapeseed Research.
- Hackenberg, D., Asare-Bediako, E., Baker, A., Walley, P., Jenner, C., Greer, S., Bramham, L., Batley, J., Edwards, D. Delourme, R., Barker, G., Teakle, G., Walsh, J. (2019) Identification and QTL mapping of resistance to Turnip yellows virus (TuYV) in oilseed rape, *Brassica napus. Theoretical and Applied Genetics* <a href="https://doi.org/10.1007/s00122-019-03469-z">https://doi.org/10.1007/s00122-019-03469-z</a>

- Hardwick N. V., Davies J. M., Wright D. M. (1994) The incidence of three virus diseases in England and Wales in the 1991/92 and 1992/93 seasons. *Plant Pathology*, **43**, 1045-1049.
- Hill S. A., Lane A., Hardwick N. V. (1989) The incidence and importance of *Beet western* yellows virus in oilseed rape. Aspects of Applied Biology, **23**, 311-318.
- Hunter P. J., Jones J. E., Walsh J. A. (2002) Involvement of *Beet western yellows virus*, *Cauliflower mosaic virus* and *Turnip mosaic virus* in internal disorders of stored white cabbage. *Phytopathology*, **92**, 816-826.
- Jasnic S., Bagi F. (2007) Viral diseases of oilseed rape. *Plant Doctor*, **35**, 458-461.
- Jay C. N., Rossall S., Smith H. G. (1999) Effects of *Beet western yellows virus* on growth and yield of oilseed rape (*Brassica napus*). *Journal of Agricultural Science, Cambridge*, **133**, 131-139.
- Jones R. A. C., Coutts B. A., Hawkes J. (2007) Yield-limiting potential of *Beet western yellows virus* in *Brassica napus*. *Australian Journal of Agricultural Research*, **58**, 788-801.
- Kerlan C. (1991) Les viroses, une etude de longue haleine. *Le dossier, Oleoscop no.5, Septembre*, **91**, 6-7.
- Milošević, D., Marjanović-Jeromela, A., Ignjatov, M., Jovičić, D., Stanković, I., Bulajić, A., Krstić, B. (2015) First Report of Turnip yellows virus on Oilseed Rape in Serbia. *Plant Disease* **99**, 1869.
- Nelder J. A., Wedderburn R. W. M. (1972) Generalised linear models. *Journal of Royal Statistical Society*, **Series A, 135**, 370-384.
- Newbert, M. J. (2016) The genetic diversity of Turnip yellows virus in oilseed rape (*Brassica napus*) in Europe, pathogenic determinants, new sources of resistance and host range. PhD thesis, University of Warwick, 241pp.
- Parry M. L. (1990) Climate change and world agriculture, London: Earthscan Publications.
- Payne R. W., Murray D. A., Harding S. A., Baird D. B., Soutar D. M. (eds.) 2009. *GenStat for Windows: Introduction*, Hemel Hempstead: VSN International.
- Polak J., Majkowa L. (1992) Winter oilseed rape as a likely source and reservoir of *Beet western yellows virus*. *Ochrana Rostlin-UVTIZ*, **28**, 191-196.
- Raybould A. F., Maskell L. C., Edwards M. L., Cooper J. I., Gray A. J. (1999) The prevalence and spatial distribution of viruses in natural populations of *Brassica oleracea*. *New Phytologist*, **141**, 265-275.
- Schliephake E., Graichen K., Rabenstein F. (2000) Investigations on the vector transmission of the *Beet mild yellowing virus* (BMYV) and the *Turnip yellows virus* (TuYV). *Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection*, **107**, 81-87.
- Schroder M. (1994) Investigations on the susceptibility of oilseed rape (*Brassica napus* L. ssp *napus*) to different virus diseases. *Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection*, **101**, 576-589.
- Shahraeen N., Farzadfar S., Lesemann D. E. (2003) Incidence of viruses infecting winter oilseed rape (*Brassica napus* ssp. *oleifera*) in Iran. *Journal of Phytopathology*, **151**, 614-616.
- Smith H. G., Hinckes J. A. (1985) Studies on *Beet western yellows virus* in oilseed rape (*Brassica napus* ssp *oleifera*) and sugar beet (*Beta vulgaris*). *Annals of Applied Biology*, **107**, 473-484.
- Stevens M., McGrann G., Clark B. (2008) *Turnip yellows virus* (syn *Beet western yellows virus*): an emerging threat to European oilseed rape production? *HGCA Research Review*, 69, 1-36. Retrieved from https://cereals.ahdb.org.uk/media/269200/rr69.pdf
- Thomas P. E., Pike, K.S. and Reed, G.L. (1997) Role of Green peach aphid flights in the epidemiology of *Potato leaf roll* disease in the Columbia Basin. *Plant Disease*, **81**, 1311-1316.

- Thresh J. M. (1976) Gradients of plant virus diseases. *Annals of Applied Biology*, **82**, 381-406.
- Walkey D. G. A., Pink D. A. C. (1990) Studies on resistance to *Beet western yellows virus* in lettuce (*Lactuca sativa*) and the occurrence of field sources of the virus. *Plant Pathology*, **39**, 141-155.
- Walsh J. A., Perrin R. M., Miller A., Laycock D. S. (1989) Studies on beet western yellows virus in winter oilseed rape (*Brassica napus* ssp. *oleifera*) and the effect of insecticidal treatment on its spread. *Crop Protection*, **8**, 137-143.
- Walsh J. A., Tomlinson J. A. (1985) Viruses infecting winter oilseed rape (*Brassica napus* ssp. *oleifera*). *Annals of Applied Biology*, **107**, 485-495.



# TABLE TITLES AND FIGURE LEGENDS

**TABLE 1** Mean autumn percentage incidence of turnip yellows virus (TuYV) in winter oilseed rape crops in Lincolnshire, Warwickshire and Yorkshire in the 2007-8, 2008-9 and 2009-10 crop seasons.

**TABLE 2** Mean spring percentage incidence of turnip yellows virus (TuYV) in winter oilseed rape crops in Lincolnshire, Warwickshire and Yorkshire in the 2007-8, 2008-9 and 2009-10 crop seasons.

**TABLE S1** Incidence and spatial analysis of turnip yellows virus (TuYV) in oilseed rape crops sampled in Lincolnshire, Warwickshire and Yorkshire in the 2007-8, 2008-9 and 2009-10 crop seasons.

**FIGURE 1a** Spatial distribution of turnip yellows virus-infected plants in oilseed rape field 1 in Lincolnshire in autumn of the 2007-8 crop season showing significant clustering (Rook's case connection of Black-White join-count statistic was used to test for the spatial autocorrelation of the TuYV-infected plants. In this field Z = -1.922, P < 0.05).

**FIGURE 1b** Spatial distribution of turnip yellows virus-infected plants in oilseed rape field 2 in Warwickshire in spring of the 2009-10 crop season showing random distribution (Rook's case connection of Black-White join-count statistic was used to test for the spatial autocorrelation of the TuYV-infected plants. In this field Z = 0.561, P = 0.288).

**FIGURE 2.** (a) Rothamsted Insect Survey catches of *Myzus persicae* in suction traps located in Lincolnshire, Warwickshire and Yorkshire between June 2007 and April 2010 (Source: Aphid Bulletin, Rothamsted Insect Survey, Rothamsted Research, U.K.). (b) Mean incidences of turnip yellows virus (TuYV) from three fields in each of Lincolnshire, Warwickshire and Yorkshire in the autumns of 2007, 2008 and 2009.

FIGURE 3 Relationship between cumulative numbers of *Myzus persicae* caught in the Rothamsted Insect Survey suction traps located in Lincolnshire, Warwickshire and Yorkshire between August and November in 2007, 2008 and 2009 and mean percentage turnip yellows virus incidence in oilseed rape crops in the autumn of each year in the three regions (d.f. = 2, 8; F = 24.2; P < 0.001).

**TABLE 1** Mean autumn percentage incidence of turnip yellows virus (TuYV) in winter oilseed rape crops in Lincolnshire, Warwickshire and Yorkshire in the 2007-8, 2008-9 and 2009-10 crop seasons

Region	Mean TuYV incidence (%) in autumn of crop season			Means <sup>1</sup>
	2007-8	2008-9	2009-10	_
Lincolnshire	$58.00 \pm 8.51 \text{ b}^{2,3}$	$13.00 \pm 5.80 \mathrm{d, e}$	94.00 ± 4.08 a	$55.00 \pm 3.69 \text{ a}$
Warwickshire	$27.33 \pm 7.69  c, d$	$3.00 \pm 2.94$ e	$41.00 \pm 8.48 \text{ b, c}$	$23.78 \pm 3.94 b$
Yorkshire	$24.67 \pm 7.43  c, d$	$2.33 \pm 2.60$ e	$11.00 \pm 5.40 \mathrm{d, e}$	$12.67 \pm 3.18$ c
Means <sup>4</sup>	36.67± 4.56 a	6.11±2.33 b	48.67±3.62 a	

<sup>&</sup>lt;sup>1</sup> Means in the same column followed by different letters are significantly different from each other (P < 0.001).

Analysis of the TuYV incidence data was carried out using a generalised linear model (GLM).

<sup>&</sup>lt;sup>2</sup> Mean and standard error (mean  $\pm$  SE).

<sup>&</sup>lt;sup>3</sup> Region-crop season incidence interaction means followed by different letters are significantly different from each other (P < 0.05).

<sup>&</sup>lt;sup>4</sup> Means in the same row followed by different letters are significantly different from each other (P < 0.001).

**TABLE 2** Mean spring percentage incidence of turnip yellows virus (TuYV) in winter oilseed rape crops in Lincolnshire, Warwickshire and Yorkshire in the 2007-8, 2008-9 and 2009-10 crop seasons

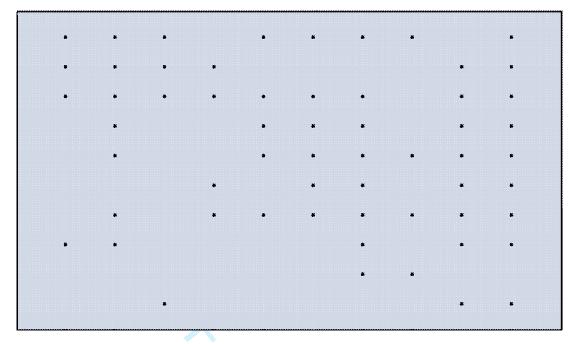
Region	Mean TuYV incidence (%) in spring of crop season			Means <sup>1</sup>
	2007-8	2008-9	2009-10	-
Lincolnshire	$66.00 \pm 12.08^{2,3}$	$10.00 \pm 7.65$	$94.00 \pm 6.04$	56.67 ± 5.17 a
Warwickshire	$68.33 \pm 11.86$	12.33±8.38	45.33±12.69	$42.00 \pm 6.43$ a
Yorkshire	32.67 ± 11.96	$3.67 \pm 4.79$	$22.00 \pm 10.56$	19.44 ± 5.55 b
Meansd	55.67 ± 6.91 a	8.67 ± 4.11 b	53.78 ± 5.86 a	_

Means in the same column followed by different letters are significantly different from each other (P = 0.002).

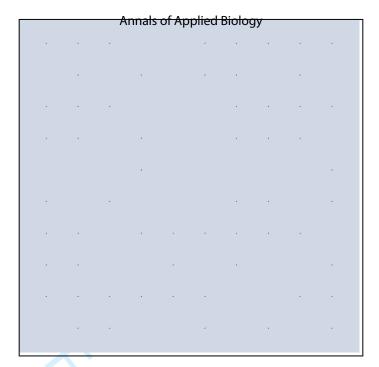
Analysis of the TuYV incidence data was carried out using a generalised linear model (GLM).

<sup>&</sup>lt;sup>2</sup> Mean and standard error (mean  $\pm$  SE).

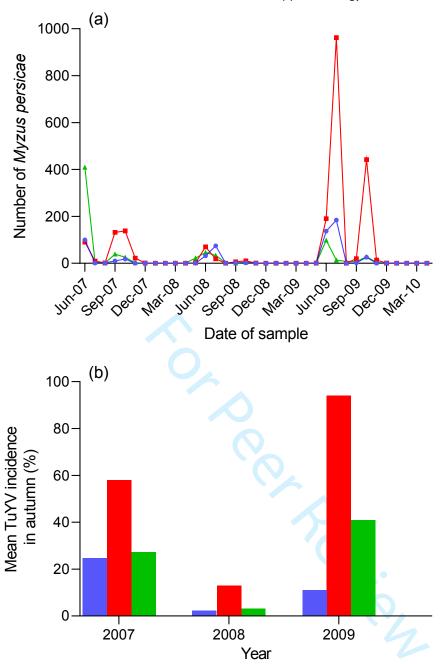
<sup>&</sup>lt;sup>3</sup> The interaction effect between county and crop season is not significant (P = 0.163).



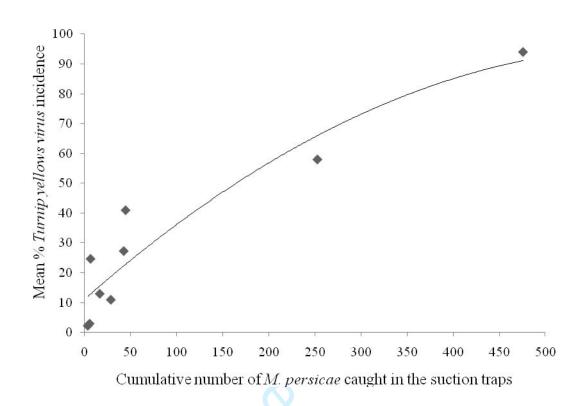
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**FIGURE 1b** Spatial distribution of turnip yellows virus-infected plants in oilseed rape field 2 in Warwickshire in spring of the 2009-10 crop season showing random distribution (Rook's case connection of Black-White join-count statistic was used to test for the spatial autocorrelation of the TuYV-infected plants. In this field Z = 0.561, P = 0.288).



**FIGURE 2.** (a) Rothamsted Insect Survey catches of *Myzus persicae* in suction traps located in Lincolnshire, Warwickshire and Yorkshire between June 2007 and April 2010 (Source: Aphid Bulletin, Rothamsted Insect Survey, Rothamsted Research, U.K.). (b) Mean incidences of turnip yellows virus (TuYV) from three fields in each of Lincolnshire, Warwickshire and Yorkshire in the autumns of 2007, 2008 and 2009.



**FIGURE 3** Relationship between cumulative numbers of *Myzus persicae* caught in the Rothamsted Insect Survey suction traps located in Lincolnshire, Warwickshire and Yorkshire between August and November in 2007, 2008 and 2009 and mean percentage turnip yellows virus incidence in oilseed rape crops in the autumn of each year in the three regions (d.f. = 2, 8; F = 24.2; P < 0.001).