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THE TWO VIRUSES: THE EFFECTS OF THEIR DIFFERENT EPIDEMIOLOGIES ON CONTROL STRATEGIES

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Summary
Surveys of the incidence of beet yellows virus (BYV) and beet mild yellowing virus (BMYV) in the English root crop from 1981 to 1984 showed that BMYV was the main cause of virus yellows.
Studies of virus movement within plants showed that field-grown plants inoculated with BYV rapidly became sources of infection whereas those inoculated with BMYV remained poor sources of infection until later in the season.
Strains of beet western yellows virus (BWYV) which infect sugar beet were found in oilseed rape, and Myzus persicae was shown to overwinter in this crop.
The implications of these findings for control strategies are discussed.

Sommaire
Des enquetes sur la distribution de la jaunisse grave de la betterave (BYV) et la jaunisse moderée (BMYV) dans les cultures de la betterave en Angleterre de 1981 a 1984 montraient que BMYV etaient la plus importante.
Des etudes du deplacement des virus au sein des betteraves poussant au champ, on a constate qu'apres l'inoculation les plantes deviennent rapidement sources de BYV, tandis que les plantes inoculees avec BMYV ne deviennent sources de virus que plus tard dans la saison.
Des souches de beet western yellows virus (BWYV) qui peuvent etre transmissible à la betterave etaient trouvees dans colza et Myzus persicae peut hiverner sur cette plante.
L'importance de ces resultats pour les methodes de lutte contre la jaunisse est discuté.

Zusammenfassung
Informationen über das Auftreten von BYV und BMYV in englischen Zuckerrübenkulturen von 1981 bis 1984 zeigten, dass der BMYV die vorwiegende Ursache der Vergilbungskrankheit war.
Untersuchungen über Virusbewegung innerhalb der Pflanzen zeigten, dass feldangebaute, mit BYV inokulierte Pflanzen sich rasch als Infektionsverbreiter erwiesen, während die mit BMYV inokulierten sich bis spät in der Wachstumsperiode als nur leicht aktiv bemerkbar machten.
Stämme des BWYV die Zuckerrüben befallen, wurden in Ölrettich gefunden und man stellte fest, dass Myzus persicae den Winter in dieser Kulter überlebte.
Die durch diese Feststellungen zu ziehenden Folgerungen für weitere Kontrollmassnahmen werden diskutiert.

INTRODUCTION

Two viruses, beet yellows virus (BYV) and beet mild yellowing virus (BMYV), cause virus yellows of sugar beet; both are carried to root crops in spring from their overwintering hosts by winged Myzus persicae. These winged aphids cause the initial foci of infection, and their wingless progeny then spread the viruses within the root crops. Chemical control of virus yellows relies on the use of aphicides, mainly to prevent the multiplication of wingless aphids.

The two viruses have different properties which affect their epidemiologies, and control strategies may need to be modified depending on which virus threatens to infect the crops.

BYV has a limited host range (1) and overwinters mainly in Beta spp. such as groundkeepers, sugar-beet seed crops, and mangold, fodder-beet or red-beet clamps. It is not usually carried very far from the over-wintering hosts because it is a semi-persistent virus and aphids lose the ability to transmit within three days of acquisition. BMYV has a much wider host range which includes several common weeds, such as Capsella bursa-pastoris (L.) Medic., Sinapis alba L. and Senecio vulgaris L. (2). It is a persistent virus, and M.persicae retain the ability to transmit for life (3), with the result that an individual aphid can potentially infect a larger number of plants and carry the virus greater distances from the numerous overwintering sources.

In the work described in this paper, enzyme-linked immunosorbent assay (ELISA) was used to determine the incidence of BYV and BMYV in the English sugar-beet root crops in the four years 1981-1984. The movement of the viruses within field-grown sugar-beet plants was monitored, and also the speed with which inoculated plants became sources of infection. Studies of oilseed rape as a potential overwintering host of the viruses and their vector showed that autumn-sown crops could be extensively infected with beet western yellows virus (BWYV), a virus closely related to BMYV, and that some strains of BWYV could infect sugar beet (4). M.persicae were found to overwinter on oilseed-rape crops, producing winged forms in spring.

The results obtained are discussed in relation to control strategies.

MATERIALS AND METHODS

ELISA The ELISA procedure was based on the method of Clark and Adams (5). Microtitre plates were coated with BYV or BMYV globulin at 1 µg/ml. Leaf tissue extracts were prepared by cutting a 26mm disc (approximate weight 0.2g) from a yellow part of each leaf near the apex including vascular tissue. Each disc was placed in a small polythene bag with buffer and macerated by passing between a pair of hand-rotated steel rollers (gap 0.25mm). The tissue extracts were diluted 1/10 with buffer for BMYV detection and 1/20 for BYV detection. After leaving the tissue extracts in the wells for 18h at 4°C, globulin conjugated to alkaline phosphatase was added at a dilution of 1/500 for BMYV, and 1/1000 for BYV. Freshly prepared substrate was added and absorbance values at 405nm recorded after 30 and 60 min. Uninfected sugar beet were used as controls; leaf extracts from 8 of these virus-free plants were included on each microtitre plate, and their mean absorbance value plus three times the standard deviation of this value was used as the threshold level for virus infection.

To detect BWYV in oilseed rape, leaf extracts were prepared by macerating a leaf disc from each plant being tested; uninfected oilseed-rape plants were used as controls. The leaf extracts were added to microtitre plates coated with BMYV globulin, using the same ELISA procedure as that used to detect BMYV in sugar-beet leaf tissue.

Field survey of BYV and BMYV incidence. Each year British Sugar factory fieldmen from the 13 sugar factories operate a Specific Field Survey in which data concerning all aspects of the growth of the sugar-beet root crop are collected from selected fields (6). In a proportion of these fields they count virus yellows infected plants in 10 counts of 100 plants per field at the end of June, July and August. To provide information on the incidence and distribution of BYV and BMYV in the root crop from 1981 to 1984, fieldmen were asked to send to Broom's Barn Experimental Station a leaf showing symptoms from a plant they counted as infected with virus yellows in each of the 10 random counts in a proportion of the fields in which they were estimating incidence of virus yellows. ELISA was then used to detect BYV and BMYV in these leaves, and the distribution of the two viruses was mapped.

Field-inoculation studies. Two hundred field-grown sugar-beet seedlings (cv Nomo) were inoculated at the 2-6 leaf stage, and a further 200 at the 10-20 leaf stage, with either BYV or BMYV. At intervals after inoculation, M.persicae in clip-on cages (diameter 18mm) were placed for 48h on every other leaf of five plants from each inoculation treatment, and on one uninoculated plant; they were then transferred to Montia perfoliata seedlings in the glasshouse for 72 hours before being killed by nicotine fumigation. Discs were then taken from the part of the sugar-beet leaves on which the aphids had been feeding, and ELISA was used to detect BYV or BMYV in this tissue. The M.perfoliata test plants were grown in aphid-proof glasshouses at temperatures of 20-25°C in a 16h photoperiod; symptoms indicating infection with BYV or BMYV were recorded 4 to 6 weeks after inoculation.

Field sampling oilseed rape for assessing M.persicae infestation and BWYV infection. From October 1982 until April 1983 random samples of plants were collected from a commercial crop of oilseed rape adjoining Broom's Barn; these samples were searched for M.persicae, and tested by ELISA using antiserum to BMYV. An experimental 0.2ha plot of oilseed rape at Broom's Barn Experimental Station was drilled in August 1983. Starting one week after emergence, random samples of plants were collected at weekly intervals until July 1984, searched for M.persicae, and tested by ELISA for BWYV infection.

RESULTS

Field survey of BYV and BMYV incidence. In 1981 leaves from plants with symptoms of virus yellows were obtained from 4 British Sugar factory areas in East Anglia and the West Midlands. In 1982 the survey was extended to include 11 factory areas, all in the eastern part of England, and in 1983 and 1984 all 13 factory areas were included in the survey. A summary of the results is given in Table 1.

Table 1 Virus yellows survey 1981-1984: summary of ELISA determination of virus content of plants diagnosed by fieldmen as showing symptoms of virus yellows.

Year	Total number of plants tested	Percentage containing: BMYV	BYV	BMYV+BYV	No virus
1981	1043	60	8	7	25
1982	1112	54	5	3	38
1983	1388	53	11	8	28
1984	703	47	9	1	43

In each year of the survey BMYV was found to be the main cause of virus yellowing. When the entire root-growing area was surveyed in 1983 and 1984 BMYV was widely distributed throughout the root-growing area; BYV occurred less frequently and was rarely found in 4 of the northern factory areas. In each year a high proportion of yellowed leaves, which had been diagnosed by fieldmen as infected with virus yellows, contained no virus.

Field inoculation studies. When plants were inoculated in the field at the 2-6 leaf stage with BYV, the virus could be detected by ELISA in the youngest leaves (leaves 5 and 7) one week after inoculation (Fig 1a), and was acquired and transmitted from these leaves to the indicator host plant M.perfoliata by M.persicae. Three weeks after inoculation, and thereafter for the rest of the growing season, BYV was detected in and transmitted by M.persicae from all tested leaves on inoculated plants. When plants were inoculated at the 10-20 leaf stage, BYV was detected in and transmitted from the youngest leaves (leaves 15-23) after three weeks (Fig 1b), and all leaves younger than the inoculated leaf after four weeks; it was never transmitted from a leaf older than the inoculated leaf (7).

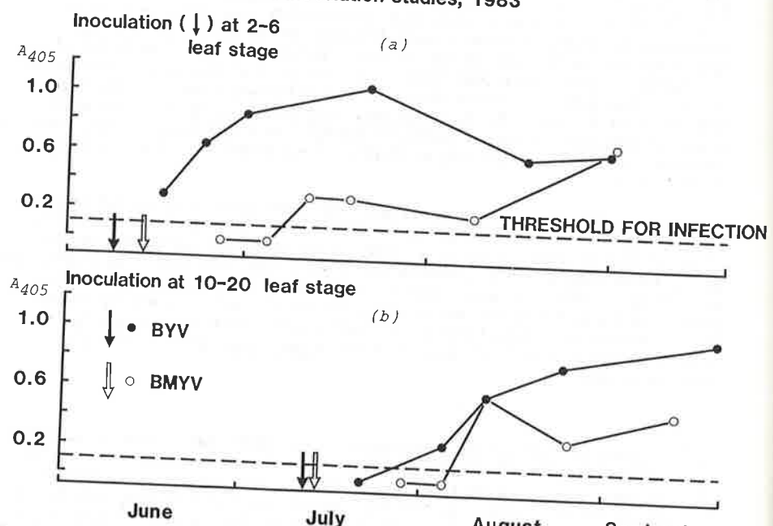

Fig.1. Mean A_{405} values obtained at intervals after inoculation of sugar-beet plants at (a) the 2-6 leaf stage and (b) the 10-20 leaf stage with BYV (●) and BMYV (○). Arrows denote inoculation date for BYV (↓) and BMYV (⇓), and the dotted line the threshold level for infection.

BMYV was not detected in leaves until four weeks after inoculation at the 2-6 leaf stage, and the virus was not consistently transmitted from leaves which gave positive absorbance values in ELISA; as the season progressed the efficiency of acquisition and transmission improved, but remained erratic. Plants inoculated at the 10-20 leaf stage behaved in a similar way to those inoculated at the 2-6 leaf stage.

BWYV infection of oilseed rape and sugar beet. In March 1982 plants collected from nine commercial crops of oilseed rape were found to contain a virus which reacted positively in ELISA with antiserum to BMYV. Sap from these plants contained isometric virus particles 27nm in diameter. Host range studies showed that the virus also infected Lactuca sativa L., which is not a host of BMYV (8); it was identified as BWYV on the basis of particle shape, serological properties and host range.

Five BWYV-infected plants were collected from a crop of oilseed rape and used as sources of infection for sugar-beet seedlings in glasshouse tests. M.persicae transmitted BWYV from two of these five plants to between 10% and 50% of inoculated seedlings.

Incidence of aphids and virus in oilseed rape. Sampling in 1982 in the crop of oilseed rape adjoining Broom's Barn showed that by mid-October 33% of the plants were infested with M.persicae and 5% were infected with BWYV. During October and November the numbers of plants infested with M.persicae increased to 50% but then declined during December. By the end of December, more than 30% of plants were infected with BWYV (Fig 2).

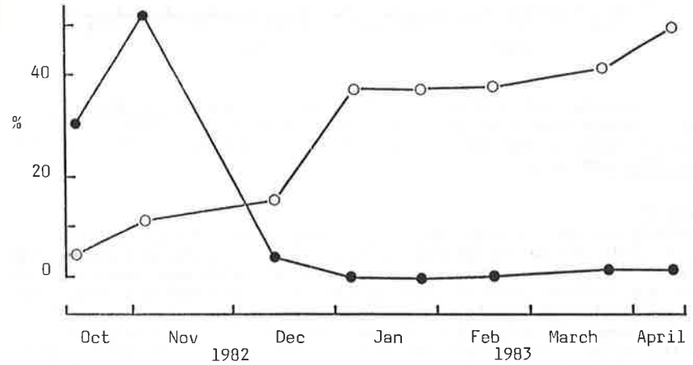

Fig.2. The percentage of oilseed-rape plants infested with Myzus persicae (●) and infected with BWYV (○) in a commercial crop adjoining Broom's Barn Experimental Station, winter, 1982/83.

In a more detailed study on an unsprayed oilseed-rape plot at Broom's Barn Experimental Station, starting in September 1983, winged M.persicae were found on seedlings at emergence and by November 100% plants were infested with wingless M.persicae. The proportions of plants infected with BWYV increased during the autumn, to 96% by January. Following a decrease during the rest of the winter, the numbers of M.persicae and proportions of plants infested increased during June and July, winged nymphs then being produced (Fig 3).

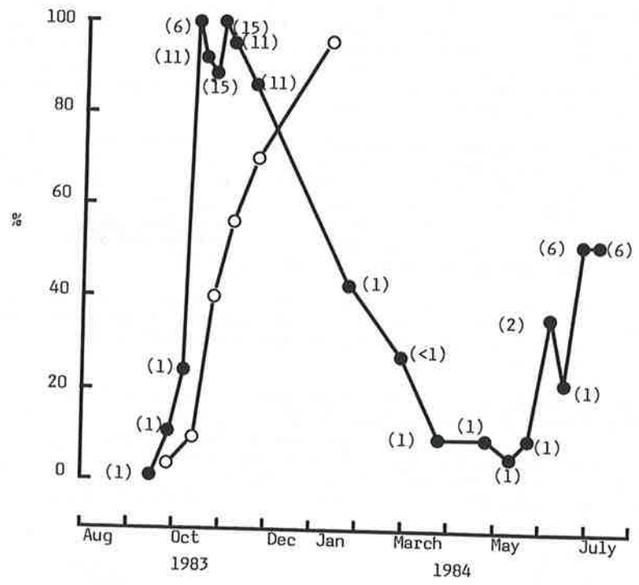

Fig.3 The percentage of oilseed-rape plants infested with Myzus persicae (●) and infected with BWYV (○) in a 0.2ha plot at Broom's Barn Experimental Station, 1983/84. Figures in parentheses are the mean numbers of M.persicae per plant.

DISCUSSION

The surveys carried out from 1981 to 1984 showed that BMYV was the main cause of virus yellows in the English root crop, and could be found through the entire root-growing area. A small number of fields were found in which BYV was the dominant cause of virus yellows; this information implies that infection originated locally, and could be used to advise growers on the increased danger of outbreaks of the more damaging BYV in these areas the following season. More trials are needed to determine the effect of BMYV alone on yield, because in areas in which this virus is consistently the only cause of virus yellows it may be uneconomic to apply sprays.

ELISA did not detect virus in a high proportion of leaves diagnosed visually by fieldmen as being infected with virus yellows. The reliability of ELISA in detecting BYV and BMYV in field-grown plants was assessed each year by inoculating plants at different stages of growth with one or other virus, and then using ELISA to detect virus in leaf discs taken at intervals through the season from leaves showing yellowing symptoms. Absorbance values indicating infection were consistently obtained from infected plants, and it was concluded that the yellowing shown in the leaves from the field survey which gave negative ELISA values was due to other causes, such as drought, downy mildew or nutrient deficiency. This emphasises the difficulties in diagnosing virus yellows in the field from leaf symptoms, particularly in years of low general incidence of the disease when fieldmen are less familiar with the symptoms and will probably tend to overestimate incidence. Henceforth, courses in field identification of disease and deficiency symptoms will be held more frequently than previously.

The field inoculation studies showed differences in the speed with which the two viruses moved within the inoculated plants. In 1983, when these studies were carried out, BYV was found to move rapidly from the inoculated leaf to all parts of growing plants; these could therefore quickly become sources of infection for neighbouring plants, and wingless aphids could spread BYV within a field from a few initial foci of infection. However, plants infected with BMYV early in the season were found to be poor sources of infection, and it is possible that BMYV is spread mainly by the winged aphids which bring the virus into the crops from the more numerous alternative hosts. Whereas the application of sprays to prevent the multiplication of wingless aphids within the crops may effectively control spread of BYV, control of BMYV may need to be directed at the winged aphids by, for example, applying repellents to discourage them from feeding.

Overwintered oilseed rape was found to be commonly infected with BWYV, a virus closely related to BMYV. Some strains of BWYV from oilseed rape were found to infect sugar beet, although the poor rate of transmission implies that there is little danger of aphids carrying it directly from oilseed rape to sugar beet. However, these beet-infecting strains of BWYV can also be transmitted to weeds such as C.bursa-pastoris from which they can infect beet and, therefore, are likely to contribute to the outbreaks of virus yellows that occur each year in variable amounts in most sugar-beet crops.

M.persicae has been found to overwinter on oilseed rape crops, and the importance of this for infestation of sugar-beet and other crops in the spring needs to be established. The insecticides used to control other pests, such as flea beetle, in oilseed-rape crops in autumn and spring may also control M.persicae, or influence the level of aphid's resistance to insecticides.

The studies have shown differences in the incidence of the two viruses, in the rate at which field-grown plants inoculated with BYV become sources of infection compared with those inoculated with BMYV, and in their host ranges. To be effective, control measures may need to be directed at the individual virus diseases rather than at "virus yellows".

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PHYTOSANITAERE MASSNAHMEN ZUR BEKAEMPFUNG DER VERGILBUNGSKRANKHEIT AN ZUCKERRUEBEN

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Zusammenfassung

Zuckerrüben in der Umgebung von Glashäusern mit Winteranbau von Stielmangold (*Beta vulgaris* L. var. *flavescens*) wiesen regelmässig einen starken Befall durch die Vergilbungskrankheit auf. Stielmangold kann als Virusquelle für das Milde Vergilbungsvirus (BMYV) und gleichzeitig als Wirtspflanze zur Ueberwinterung des Vektors *Myzus persicae* dienen. Durch eine sorgfältige Blattlausbekämpfung konnte die Funktion von Stielmangold als Virusqulle ausgeschaltet werden. In der Folge war der Vergilbungsbefall in der Umgebung der Glashäuser stark reduziert

Summary

Sugar beets were always heavily infested with beet mild yellowing virus (BMYV) when planted near glasshouses where Swiss chard was grown during the winter. Swiss chard may both serve as virus source for BMYV and as overwintering host for *Myzus persicae*. The exclusion of Swiss chard in the function as a virus source was achieved by an efficient aphid control on Swiss chard, resulting in much less virus yellows in beets near the glasshouses.

Sommaire

Les betteraves situées près des serres contenant des bettes à cardes pendant l'hiver étaient régulièrement atteintes fortement par la jaunisse des betteraves. Les bettes à cardes peuvent servir comme source pour le virus de la jaunisse modérée en étant en même temps une plante hôte du vecteur *Myzus persicae*. Des traitements efficaces contre les pucerons ont permis d'exclure les bettes à cardes dans leur fonction comme source de virus. Aux alentours des serres, l'attaque par la jaunisse était fortement réduite par la suite.