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Time course studies of BYDV concentration in roots and leaves of oats.

Enzyme-Linked Immunosorbent Assay (ELISA) was used to follow the development of the concentration of a BYDV isolate (39/78) in roots and leaves of a susceptible variety of oats (cv. Sol II). 39/78 is a severe isolate transmitted much more efficiently by Rhopalosiphum padi (L.) than by Sitobion avenae (Fab.). Two groups of oat plants were infected with this isolate. The first group was inoculated at the 1-2 leaf stage (Zadoks 11) and the second group was inoculated at the 4-5 leaf stage (Zadoks 13). 5-10 viruliferous R. padi were placed on each plant. The ELISA testing was started 20 h after the end of the inoculation access period which was 36 h, and continued for 21 days. The virus could be detected after 20 h in roots and 48 h in leaves in the first group. In the second group virus was first detected after 72 h, and 5 days in the roots and leaves, respectively. The virus concentration reached its highest level in the roots 10 days after inoculation of the plants at the 1-2 leaf stage. At this time the concentration of the virus in the roots was 2-3 times higher than in the leaves. When the 4-5 leaf stage plants became infected the maximum concentration of the virus in the leaves and the roots was reached 12 days and 10 days after inoculation, respectively. In this case the concentration of the virus was 2-3 times higher in leaves than in roots. There was no direct proportion between the BYDV concentration and the time for symptom expression and symptom severity in infected oats. It was also shown that it is possible to detect BYDV by ELISA in fresh green seeds as well as in old dried seeds of infected oats.

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Influence of the growth stage of rice plants on the transmission of Barley Yellow Dwarf Virus, Rice "Giallume" isolate.

INTRODUCTION

Rice "giallume" (RG) is a disease correlated with Barley Yellow Dwarf Virus (BYDV) (AMICI et al., 1978; ROCHOW and DUFFUS, 1981; OSLER, 1984). The virus is transmitted by at least three known different aphid species, i.e. Rhopalosiphum padi (L.), Sitobion avenae (F.) and Metopolophium dirhodum (Wlk) (OSLER, 1980). In a previous work it was demonstrated that the percentage pf infected plants and the mean length of the incubation period of the disease depends on the number and species of aphid used in the inoculation (OSLER and MOLETTI, 1982; OSLER, 1984).

In nature the disease usually spreads in characteristic patches (MOLETTI et al., 1979). In general, they are larger expanded when inoculations occur early in the season and for the more susceptible cvs. to the virus. It was also observed that symptoms were more severe in the plants located in the middle of the patches than toward the periphery. This supports the assumption that delayed inoculations induce less severe damages, as observed for oat and wheat by ENDO and BROWN (1983) and by GILL (1980), and a lower percentage of infected plants.

This paper reports the results obtained on the effect of inoculation with BYDV on 12 rice cvs. having various degrees of susceptibility to the virus, conducted at three different growth stages of the plants. The main effects investigated were lenght of the incubation period (IP) of the disease and percentage of infected plants.

MATERIALS and METHODS

The virus isolate (RGV - 1976) used in the trials had been originally obtained from naturally infected rice plants, was maintained in controlled environmental conditions, and alternated between the rice and Avena byzantina C. Kock cv. Coast Black, by serial transmissions with R.padi. It was non specifically transmitted by three different aphids species (PLUMB, 1974; ROCHOW and DUFFUS, 1981; OSLER, 1984).

For the transmission trials, apterous females of a virus-free colony of R. padi, reared on A. byzantina were used as sole vector. Source of inoculum were rice plants cv. Balilla, artificially infected with R. padi. Test plants of the 12 cvs. of rice inoculated were obtained from seeds germinated at 28° C, in the dark, and than transferred to boxes with peat and sand in the greenhouse (temperature 22-25° C; U.R. 60-80 % and supplementary light).

The three-days-acquisition period was completed in a grow-chamber (at 20° C, 5.000 lux and U.R. 70-80 %) feeding the aphids on the entire plant source of inoculum. The exposed aphids were transferred to the test plants (three per plant) and fed for a period of two days. The plants were than treated with an insecticide, moved outdoors and maintained free of aphid infestations by periodical spraying. For each cultivar an average of 60 test plants were inoculated, at one leaf (stage I), four leaves (stage II) and second tillering (stage III) (see Table 1). Sowing was performed respectivelly eight, 23 and 38 days before inoculation, in order to have contemporaneously the 3 chosen growth stages for each trial of plants. Starting from the 12th day after inoculation, the plants were periodically checked for symptoms (Fig. 1).

RESULTS

Table 1 reports the total number of infected plants for each of the 11 susceptible cvs., in the three different growth stages that were inoculated. No visible symptoms were obtained on Veneria, a resistant variety to the aspecific isolate of the virus used in the trials.

It can be noticed that the percentage of infected plants decreses, in or

Table 1. Response of 12 rice cvs. - to RGV inoculation with R.padi (*) - at three different growth stages.

Cultivar	Plant stage when inoculated(**)	Plants inoculated No.	Plants infected %	Plants infected average incubation period of RG
Balilla	I	60	87	14
	II	60	83	16
	III	60	57	20
Cripto	I	60	95	15
	II	60	75	18
	III	60	62	21
Europa	I	58	76	15
	II	60	52	20
	III	60	62	23
Monticelli	I	60	92	20
	II	60	82	22
	III	60	63	24
Padano	I	59	100	12
	II	60	62	16
	III	60	67	19
Radon	I	60	95	14
	II	60	80	16
	III	60	55	22
Ribe	I	58	86	16
	II	60	57	20
	III	39	56	21
Ringo	I	60	78	17
	II	60	45	19
	III	60	68	22
R.Marchetti	I	59	92	15
	II	60	83	15
	III	59	66	23
Roma	I	60	93	17
	II	57	68	16
	III	60	63	22
S.Andrea	I	60	88	17
	II	60	83	19
	III	59	58	23
Veneria	I	60	--	--
	II	60	--	--
	III	60	--	--

(*) three aphids for test plant

(**) I=one leaf stage; II=four leaves; III=second tiller

der, from plants inoculated in stage I to those inoculated in stage III. Table 1 reports also the average length of IP of the disease in the different cvs. and for each of the three growth stages considered. The IP length ranges from 12 to 24 days.

The shortest incubation period was noticed for the cv Padano inoculated at stage I, and the longest one for the cv Monticelli inoculated at stage III.

Among the 11 susceptible cvs. (Table 1 and Fig. 1), the trend of the IP mean length of RG is generally inversely proportional to the increase of the growth stage of plants when inoculated, and to the degree of susceptibility of the cvs. previously determined by the single transmission by R. padi (MOLETTI et al., 1979). Within the same variety, data related to the reaction of the plants inoculated at stage II, are usually half-way between those achieved in the other stages of inoculation.

DISCUSSION

On the basis of the results achieved it appears that age of the plant when inoculated has a determinant influence, both on length of the incubation period of rice "giallume" and on the percentage of plants reacting positively to inoculation with the viral agent (BYDV).

More particulary IP of RG tends to become longer as age of the plant when inoculated is more advanced confirming the data obtained by GILL (1980) for BYDV on wheat plants. The highest percentage of infected plants has been attained in the inoculation made at phase I and the lowest one at phase III. The data gained in the inoculation on plants in phase II are mid-way for both factors.

Length of IP on rice plants is influenced also by the cultivar. In fact the more susceptible ones (MOLETTI et al., 1979) have shown to have the shortest IP.

In the trials discussed in the present paper, we have used for the transmissions three vector aphids per plant, instead of one as in the past. As a result the differences in the percentage of positive transmis

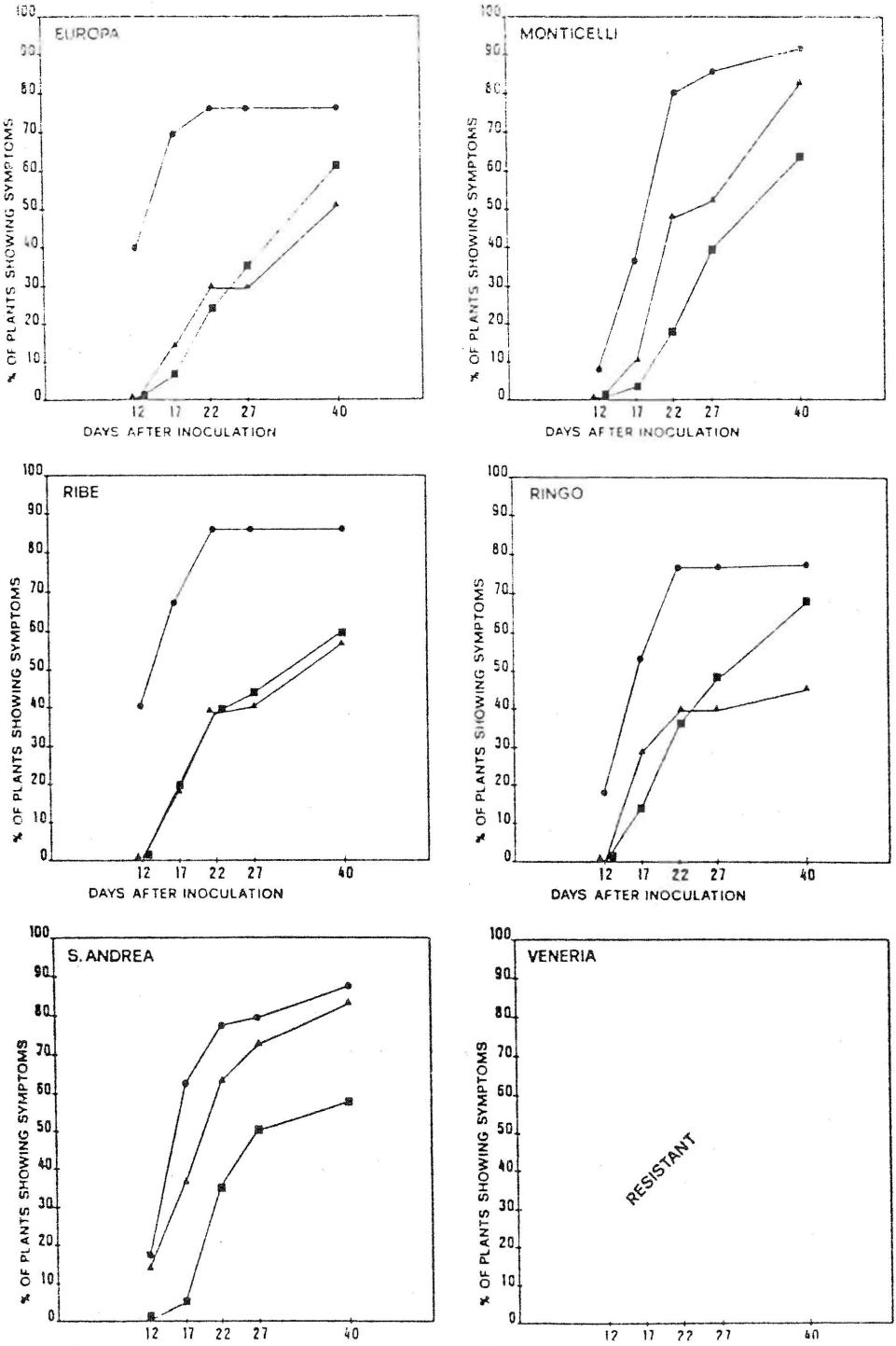
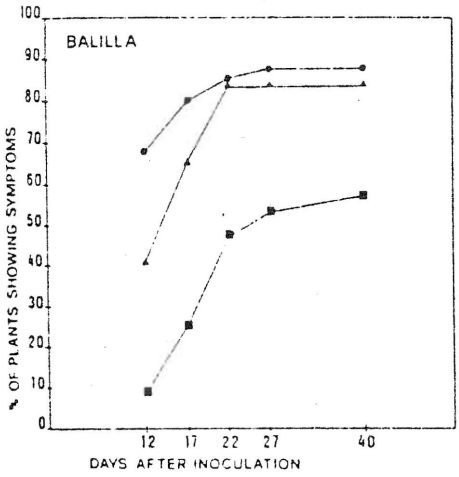
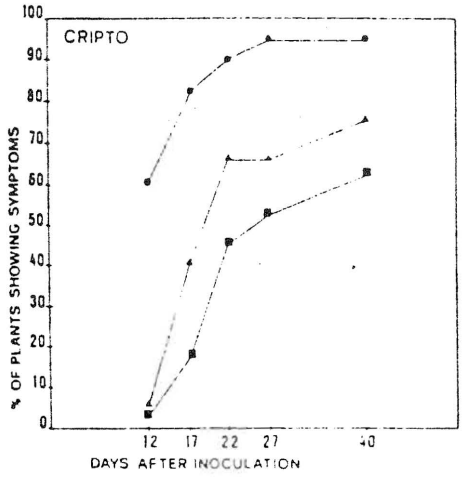
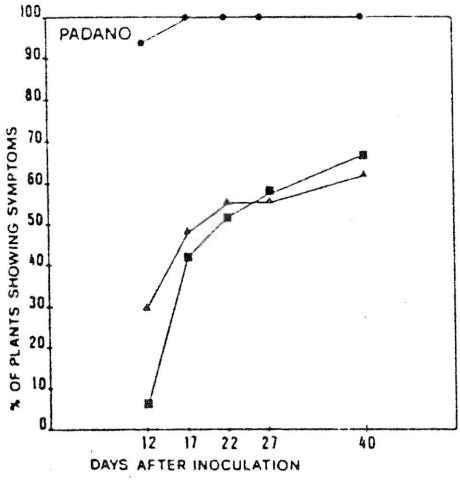
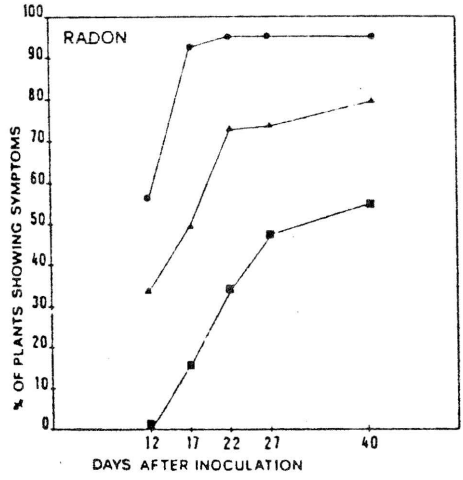


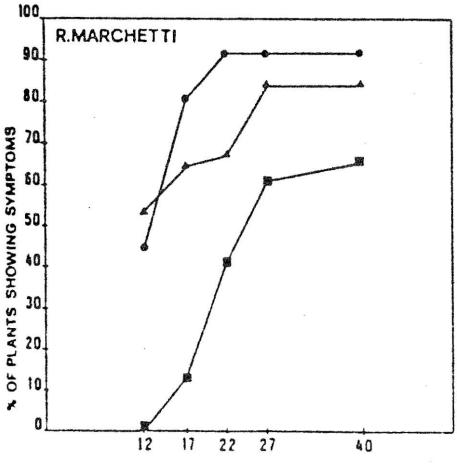
Fig. 1. Effects of plant growth stages at time of inoculation on the length of incubation period of RG determined in 12 rice varieties having different susceptibility to the virus (●—●= one leaf stage; ▲—▲= four leaves; ■—■ =second tillering).

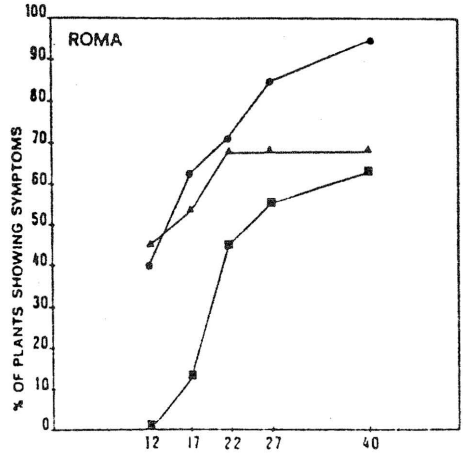

BALILLA
% OF PLANTS SHOWING SYMPTOMS
DAYS AFTER INOCULATION
12 17 22 27 40


CRIPTO
% OF PLANTS SHOWING SYMPTOMS
DAYS AFTER INOCULATION
12 17 22 27 40


PADANO
% OF PLANTS SHOWING SYMPTOMS
DAYS AFTER INOCULATION
12 17 22 27 40


RADON
% OF PLANTS SHOWING SYMPTOMS
DAYS AFTER INOCULATION
12 17 22 27 40


R.MARCHETTI
% OF PLANTS SHOWING SYMPTOMS
12 17 22 27 40


ROMA
% OF PLANTS SHOWING SYMPTOMS
12 17 22 27 40

sions we had previously noticed among varieties having a different susceptibility to BYDV (MOLETTI et al., 1979) have been reduced.

In previous research we had stated that length of IP of RG in rice plants is related to the number of vector aphids per plant used in the transmissions (OSLER and MOLETTI, 1982), as well as to the species of aphid adopted in the transmission trials (OSLER et al.,in press). Now, we have to emphasize also the important role played by the growth stage of the plants when inoculated.

It was also possible to establish preliminarly that domage is more severe as IP tends to become shorter. Does mean that length of IP can be considered as an indicative parameter for valuating tolerance of the plant to the virus (unpublished data).

From the epidemiological point of view it is now possible to explain the different extension of the "giallume" patches in the rice fields noticed in various years and for different cvs. The more extended patches correspond to the more susceptible varieties to BYDV and appear in the years when natural inoculations occur early in the season when rice plants are still very young.

SUMMARY

Using the aphid Rhopalosiphum padi L. as vector and Rice "Giallume" Virus (RGV) - an isolate of Barley Yellow Dwarf Virus (BYDV) - 12 rice varieties with different susceptibility to the virus were experimentally inoculated. For each variety, inoculations were performed at three different growth stages of the plants, i.e. one leaf,four leaves and second tiller.

All the one-leaf inoculations induced more infected plants compared with the four leaves ones, and the latter more than those exposed to the vector at the second tiller stage.

Length of incubation period of the disease was shorter in plants of the most susceptible varieties, i.e. Balilla, Padano and Ribe. Moreover it

was observed that incubation period tends to increase as age of the test plants at the inoculation is more advanced.

The main factors affecting length of incubation period of the disease are discussed.

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Ultrastructure and X-ray microanalysis on maize leaves infected with Barley Yellow Dwarf Virus.

Introduction.Barley Yellow Dwarf Virus (BYDV),typical member of the luteovirus group,causes severe economic losses in several cereal grains.The different strains of BYDV show aphid vector specificity (I2) and are transmitted by about I5 aphid species.BYDV appears to be primarily confined to the phloem cells (7,8 and 9).In maize,the disease is characterized by stunting of the plant and by dark red colouration of the leaves,starting from the tip and the margin of the lowest leaves. The effects of BYDV infection in maize and the epidemiology of the disease have been studied (I3),but only a small amount of information has been acquired for what concerns the cellular alterations and the element distribution in healthy and infected tissues(5).Therefore,we carried out this study to check if there was a relationship between the ultrastructural changes caused by BYDV and the different infected lines.Moreover,we tried to do a semi-quantitative analysis of the element distribution in red infected tissues,compared with the green ones,in order to know if the lack(or excess)of some particular elements could be correlated with the disease.

Materials and Methods.Natural and artificial infection on 4 pure lines of Zea mays L.(W64A,FRI8,B84 and F33.I6)have been obtained by the aphid Rhopalosiphum padi L.Symptoms appeared 4-6 weeks after inoculation.Leaf samples,cut either from green or red tissues,were double fixed in glutaraldehyde-osmium tetroxide,dehydrated and embedded as previously described(3). Ultrathin sections were stained with lead citrate and examined with a Hitachi 300 electron microscope.

X-ray microanalysis.Some specimens were fixed in O.IM phosphate-buffered 3% glutaraldehyde,pH6.9,for 2h at 4°C;then prepared according to the critical-point drying technique.Other

samples,unfixed,were dried for 72h at 40°C(IO).Then allspecimens were processed for X-ray microanalysis as described(4) using a SEM Cambridge Stereoscan 250,equipped with an energy-dispersive spectrometer (EDS) system.Counts were accumulated for 500sec for all spectra.

Results.The ultrastructural study gave us the possibility to analyse the cellular alterations at progressive stages of infection.Initially,when the green leaves turned to a light red colour,in the line W64A,we observed a large amount of starch granules in the plastids and several crystals in the phloem cells(fig.I);in the line FRI8,many vesicles occurred in the cytoplasm;while in the lines B84 and F33.I6 the most prominent alteration was the disorganization of the thylakoids in the plastids of the bundle sheath cells(fig.2) and in the phloem cells of F33.I6 the presence of fibrillar material surrounded by a membrane(fig.3).The virus particles were identified only in phloem cells of reddish tissues.They were associated with amorphous material and small quantities of remnant components. At this late stage of infection in all the lines,the cytoplasmic organelles and membranes were altered.Moreover,in the line F33.I6 a deeply abnormal accumulation of starch granules in the plastids of the bundle sheath cells were observed(fig.4).

SEM X-ray microanalysis.No consistent differences were noticed by analysing leaf samples prepared according to the two different methods used,therefore,we report the data obtained on dried leaves of the line F33.I6,artificially infected.The elements and their intensities as detected during the analysis of the lower epidermis of dried red leaves are reported in table I,while in table 2 the data of the control green tissues were reported.Comparable results were obtained by analysing the leaves of the other lines.Comparing the data related to the line F33.I6,it is clear and evident thet the most significant difference between the element distribution in infected and healthy tissues,concerns the intensity of K:this element in red areas has values much lower then in green ones .

Table 1 X-ray microanalysis performed on dried red leaves.

ELEMENT	INTENSITY CPS	ERROR %	RATIO I/I(Ca)	INTENSITY %
Al	2.9511	4.2098	0.9154	15.3104
Si	5.4863	2.8774	1.7017	28.4629
P	1.5081	7.6338	0.4678	7.8242
S	1.6490	7.9857	0.5115	8.5548
Cl	1.4425	9.0670	0.4474	7.4837
K	3.0142	5.2116	0.9349	15.6378
Ca	3.2240	5.3658	1.0000	16.7262

Table 2 X-ray microanalysis performed on dried green leaves.

ELEMENT	INTENSITY CPS	ERROR %	RATIO I/I(Ca)	INTENSITY %
Al	3.1640	4.5517	0.4347	3.9764
Si	9.4962	2.1785	1.3048	11.9346
P	6.8185	2.7119	0.9369	8.5693
S	1.8675	9.2446	0.2566	2.3470
Cl	6.1897	3.3223	0.8505	7.7791
K	44.7551	0.8242	6.1497	56.2472
Ca	7.2777	3.0519	1.0000	9.1464

Discussion. From our results it seems that the ultrastructural alterations observed in the different maize lines infected with BYDV, could depend, to a certain extent, on the particular line tested. In some lines the carbohydrate metabolism seems more affected: BYDV causes substantial accumulation of starch specially in bundle sheath cells chloroplasts. Also in barley diseased leaves an accumulation of both soluble carbohydrates and starch was noticed (9). This starch accumulation may be due to reduced permeability of chloroplast membrane, or to changes in enzyme activities within the chloroplasts. In other lines the BYDV causes a direct effect on the photosynthetic apparatus: this is the most obvious and perhaps the most common way by which infection reduces plant growth. In our case the alteration of the thylakoid membranes and the reduction of the chlorophyll a and b in the infected tissues, suggests that the initial cause that led to the reduction of carbon fixation seemed to be the chlorophyll degradation as observed in Chinese cabbage leaves infected with TYMV (2). Regarding the breaking down of the cellular membranes as noticed in all the lines at the late stage of infection and, obviously, the loss of their integrity, it is known that K^+ is the prevalent cation in plant cells and it is responsible for the maintenance of the ionic balance in the cells. Moreover, K^+ is necessary in large amounts as it is essential for the respiration process and for the carbohydrate metabolism. As a consequence of a lack of this element, the plants become stunted and the leaves curl from the tip and the margins of the blade. This is the first report of a loss of K^+ in the case of virus plant disease, while the alteration of intracellular monovalent cation levels is a common result of infection by several lytic animal viruses. For instance, in chick cells infected with Sindbis virus, where the potassium tracer $^{86}Rb^+$ was used to measure K^+ transport, a reduction of intracellular levels of K^+ was observed (II and 6). It has been proposed (I) that alterations in membrane permeability during virus infection would account for

the altered intracellular monovalent cation levels.

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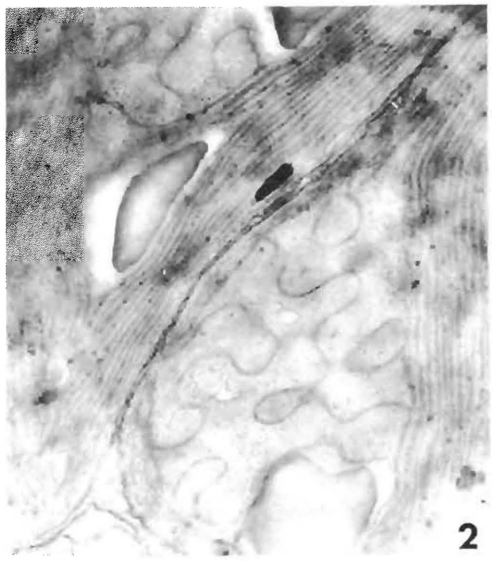

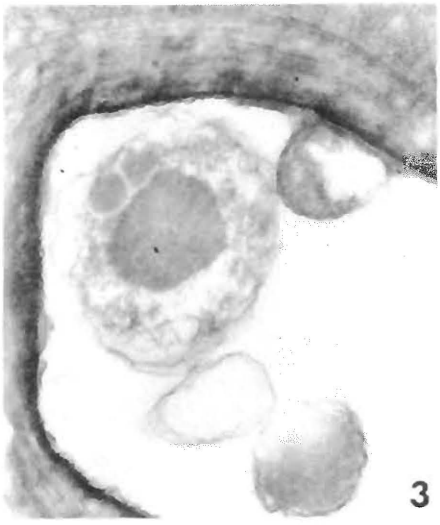

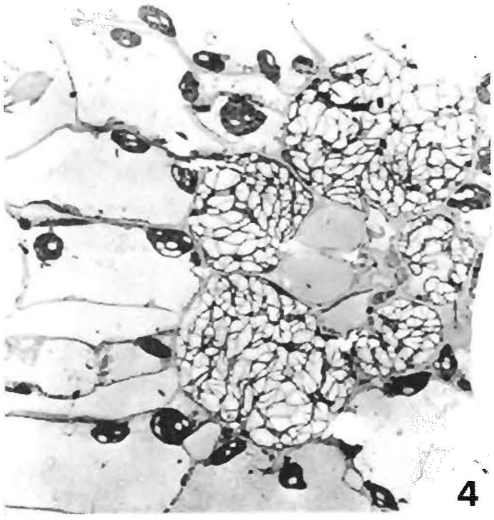

Figs. 1 to 4: Micrographs of maize leaves infected with BYDV. In the line W64A(Fig.1) several crystals are present in the phloem cells. At the beginning of the infection, in the line F33.16, the thylakoids of the bundle sheath(BS) chloroplasts are disorganized(Fig.2) and in the phloem cells fibrillar material is visible(Fig.3). At the late stages of infection, an abnormal accumulation of starch granules is noticed in the BS-chloroplasts.
(Fig.1: x20,000; Fig.2: x36,000; Fig.3: x30,000; Fig.4: x13,000)

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Characterization of Barley Yellow Dwarf Virus Strains Transmitted in Autumn by Rhopalosiphum padi

ABSTRACT

The Barley Yellow Dwarf Virus (BYDV) potential infectivity of the winged forms of Rhopalosiphum padi landing in autumn on young winter barley seedlings in the Paris region, has been evaluated by means of biological and immuno-enzymatical (ELISA) techniques. For this purpose 98 Rhopalosiphum padi winged individuals caught in October 1983 on winter barley seedlings have been placed individually on young barley plants (var. CAPRI) grown in the greenhouse.

The aphids are killed nine days later after checking for their identity and counting of their progeny; plants are tested with ELISA against a BYDV non specific strain.
These plants are kept under observation for the possible coming out of yellow"s symptoms.

The results indicate that 50 % of the captured Rh. padi are vectors of one or more strains of BYDV. Ninety-eight percent of these strains are ELISA detectable in an intermediate host by means of an antiserum characterizing non specific strains of BYDV vectors (PAV according to Rochow).

This strain is thus the most frequent. This situation is rather common in France and Belgium.

No relation has been found between the fecundity of the winged aphids and their ability to convey the BYDV.

These results corroborate the essential part taken by Rh. padi as a vector of BYDV in these region during the fall. The ELISA test enables the direct or indirect detection of winged aphids as possible vectors of BYDV; however it does not informs about the pathogenicity of the aphid-borne strains.

1. INTRODUCTION

The importance of cereal aphids as vectors of the Barley Yellow Dwarf Virus (BYDV) is still difficult to evaluate and the forecast of virus hazards not yet clearly defined. Three main factors determine the importance of this disease in its sensitive phase :

- the number of infectious winged aphids present;
- the pathogenicity of the aphid-borne strains;
- the activity of the aphids and of their progenity.

We have studied in natural conditions and in the Paris region the first stage of an outbreak of BYDV disease in the three following bearings :

- frequency of infectious winged aphids;
- types of vector;
- pathogenicity of the strains borne by Rhopalosiphum padi considered as the main vector of BYDV in this region.

2. MATERIALS AND METHODS

Ninety-eight Rh. padi winged individuals captured between the 7^{th} and the 13^{th} of October 1983 on one-blade stage winter barley seedlings have been placed individually on young barley plants (var. CAPRI) (One non-developed blade stage) grown in the greenhouse.

Aphids are killed nine days after inoculation by means of a contact insecticide (Isathrine) after checking for their identity and counting of their progeny; plants are tested with ELISA against a non-specific (NS) BYDV strain (probably equivalent to PAV strains according to Rochow) by using the second blade of each plant. Then, these plants are kept under observation at 18°C under 6,000 lux (14-h photoperiod), for 5 weeks, for the possible coming out of yellow's symptoms.

3. RESULTS

3.1. Classification of the plants according to their ELISA reaction and intensity of the developed BYDV symptoms

Out of 98 analysed plants, 49 show a very low optical density (OD), similar to that of the healthy controls ($0.04 \leq OD \leq 0.10$). Five weeks later, however, 2 of these plants show typical symptoms of BYDV infection.

Then other plants show few of any symptoms but have OD values 1.5 to 3

times higher than that of the healthy controls ($0.10 < OD < 0.25$).

The 39 remaining plants react more or less in ELISA with the NS - BYDV antiserum ($0.25 < OD < 1.25$), but present a great variability as far as the intensity of the symptoms is concerned. Indeed 26 plants exhibit typical symptoms 15 days after inoculation as compared to 13 plants showing some very weak symptoms after 5 weeks.

The great variability in the ELISA OD values shown by the PAV - type isolates can be explained by their fluctuating concentration in strains more of less seriologically related to the NS strain used for the preparation of the antiserum on one hand, by the presence of strains of other vectorial types on the other hand.

3.2. Vectorial analysis of the strains

Twelve isolates were studied for their ability to be transmitted by the two kinds of aphid vectors of the specific strains of BYDV : Rhopalosiphum padi (RPV) and Macrosiphum avenae (MAV).

Two of these isolates originate from plants which have shown typical symptoms of BYDV and no reaction in ELISA in the first group; the others have been found on plants of the second group which show weak symptoms of BYDV and a medium reaction in ELISA (OD values 1.5 to 3 times higher than that of the healthy controls).

The 12 isolates have been transmitted in equivalent amounts to young seedlings of winter barley (var. CAPRI), by the above-mentioned aphids. All have also reacted positively to a second ELISA test performed with the NS PAV antiserum after a 5-week observation period in the greenhouse. Each isolate involves so at least one PAV NS strain associated at one time with the one, at another time with the other specific RPV and MAV strains and, occasionally, with both at the same time. These phenomena of late appearance of BYDV symptoms on some of the plants or the temporary lack of reaction in ELISA can also be explained by the simultaneous presence in variable concentrations of several strains of virus in a plant. These strains induce, between themselves in the host, complex reactions similar to premunition.

3.3. Relation between aphid activity and virus infection

No relation between the fecundity of the winged aphids captured and their ability to convey the BYDV can be demonstrated from this experience.

The observations show that the precise number of infected plant in a plot cannot be calculated from the counting of plants infested with the apterous

progeny of a winged aphid even when the percent virus-bearing aphids is known.

4. DISCUSSION AND CONCLUSION

The total number of plants infected with BYDV in this trial amounts to 51 out of 98. These plantes are thus related to the same number of healthy or infected aphids. It can be assumed that the BYDV - NS antiserum detects mosts of the isolates conveyed by Rhopalosiphum padi as only 2 of them out of 51 are not detected in an ELISA test. This NS strain is thus the most frequent and this situation can be considered as common in France and Belgium.

As these observations are related to winged aphid forms caught in the beginning of October on young winter barley seedlings on the one hand, and considering the great number infected individuals among them on the other hand, the percentage of infected plants in a field in these conditions could very soon exceed the threshold of tolerance corresponding to an optimal crop production.

One of us working nearly at the same moment in other environmental conditions - namely the Sambre and Meuse valleys - has shown that 34 % of the winged forms of Rhopalosiphum padi were vectors. These results corroborate the essential part taken by this aphid as a vector of BYDV in this region during the fall.

Finally, the ELISA test enables the direct or indirect detection on winged aphids vectors of BYDV after a nine-day plant inoculation period; however it does not inform about the pathogenicity of the borne strains. This type of biological diagnosis requires a very long time, which is at variance with the agricultural requirements.

5. ACKNOWLEDGEMENTS

The assistance of A. RIGA of the Agricultural Centre of Gembloux in the correction of the English version of this work is acknowledged.

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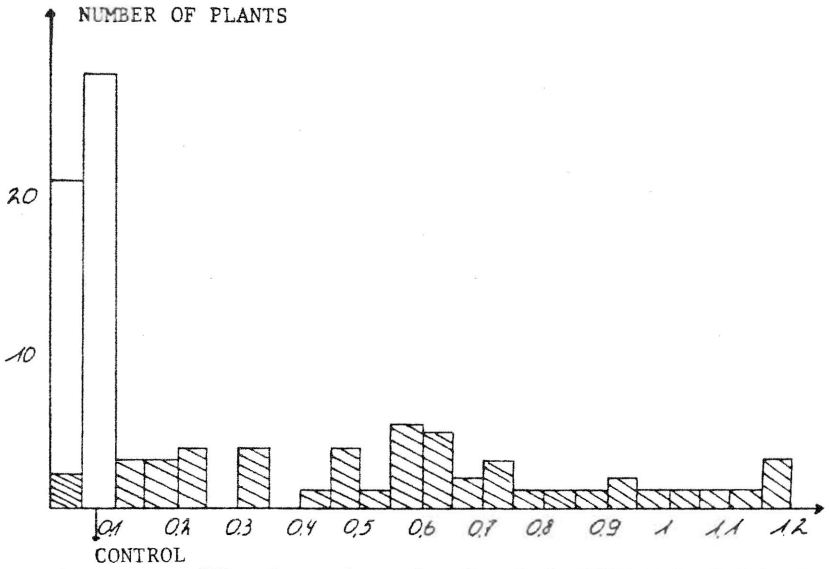

Figure 1 - *Visual symptoms developed in BYDV infected barley seedlings versus their ELISA reaction.*

uninfected plants

infected plants with BYDV symptoms

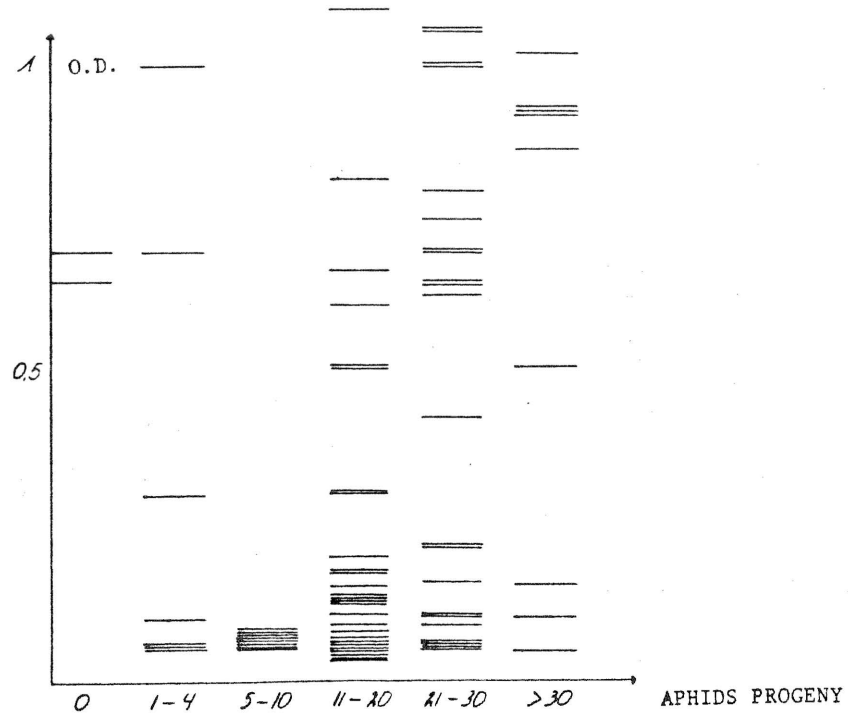

Figure 2 - *Filing of BYDV barley infected plants as related to their reaction in ELISA and to the progeny of the winged RH. padi vector*

Occurence and characteristics of barley yellow dwarf virus in Switzerland

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Experiments were made to determine whether different strains of barley yellow dwarf virus (BYDV) occur in Switzerland. This is important when immuno assays are used for BYDV identification because different strains may have differing serlogical properties.

Material and Methods. 19 isolates were collected from arable land in the french and german speaking parts of Switzerland at altitudes from 300 to 1500 m. The original host plants were oat, barley, rye and wheat with obvious symptoms typical for each species.
Two successive transmission cycles were made. For most isolates all of the following aphid species were investigated *Rhopalosiphum padi*, *Sitobion avenae*, *Metopolophium dirhodum*, *Metopolophium festucae* and *Rhopalosiphum maidis*. For the other isolates, only the first two species were used. Testplants were barley varieties, either Blackhulles or Gerbel.

Results and discussion. No significant differences in transmission rates were observed between the isolates. The aphid species used gave the following average percentages of infected plants: *Rh. padi* 90%, *S. avenae* 62% and *M. dirhodum* 29%, respectively.With *M. festucae* and *Rh. maidis*, positive transmission occured only erratically.
We found that all our isolates were transmitted non specifically and reacted with the available antisera (Gugerli and Derron 1981). In conclusion we expect that virus identification in Switzerland can be done by immuno assays with our antiserum.

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Infectivity testing and barley yellow dwarf virus epidemiology

Introduction

Since 1969 alate aphids that are potential vectors of barley yellow dwarf virus (BYDV) have been tested for their infectivity by catching them alive and allowing them to feed on oat test plants. Results have allowed the development of a system for forecasting the need to control BYDV in autumn-sown cereals, as well as increasing our knowledge of virus epidemiology and aphid phenology.

Materials and Methods

Aphids were trapped, identified and tested as described in Plumb (1976). The trap was sited 10m from the trap of the Rothamsted Insect Survey (RIS) operating at 12.2m above ground and was surrounded by arable crops. The catching jar was modified by drilling holes in its base and inserting fibre wicks to conduct away any moisture from rain or mist. The trap was emptied twice daily at approximately 9.00h and 16.00h. The following aphid species were identified and tested. Rhopalosiphum insertum, R. maidis, R. padi, Sitobion avenae, S. fragariae, Metopolophium dirhodum, and M. festucae. The sex of each aphid was determined, the number of progeny that it produced, and whether it survived the 2-3 day test feeding period.

Virus infection was identified in several ways; a) by symptoms in an oat test plant b) by a serological test of the test plant using enzyme-linked immunosorbent assay or immuno-specific electron microscopy c) by a test of the aphid using the fluorescent modification of ELISA (Torrance & Jones, 1982).

Results and Discussion

There are usually three distinct migrations of cereal aphids each year. A small migration in spring, presumably from overwintering to summer hosts, usually of R. padi but often M. festucae is relatively common; a larger migration in July, principally of aphids leaving the ripening crop, the

numbers of each species reflecting their relative abundance on the crops; and often the largest migration in September and October of aphids returning to their winter hosts; this migration is almost exclusively of R. padi and R. insertum.

Spring. The dates of first capture of cereal feeding aphids in the traps of the RIS and the date of first capture of an infective individual of each species gave a similar pattern each year, even though the time of year differed.

The mean date (1969-83) of capture of the first and the first infective R. padi, S. avenae and M. dirhodum is given in Table 1.

Table 1

Date of first capture and first infective R. padi, S. avenae and M. dirhodum (1969-83)

	First Capture	First Infective	Interval (days)
R. padi	19 May	30 May	11
S. avenae	23 May	19 June	27
M. dirhodum	3 June	6 July	33

The time of occurrence of infective aphids was reflected in the extent of infection of crops sown at different times in the Spring. Crops sown in late April or May had 2-4 times more infection by BYDV than crops sown in early March and also generally had larger aphid populations (Jenkyn & Plumb, 1983).

Summer. The weekly proportion of infective aphids can be a guide to the proportion of crop infected, but it is impossible to attribute the origin of the infective aphids to spring or autumn-sown cereals.

The relative importance of the different aphid vectors can be assessed and shows wide variations from year to year. This usually relates to the most prevalent virus strains which differ in the severity of the damage they cause as well as in the efficiency with which they are transmitted by different vectors. (Table 2).

Autumn. A comparison of the results of testing aphids by the modified ELISA or the feeding test in 1982 gave the results shown in Table 3. There are large discrepancies in the proportion of aphids designated as infective but

Table 2

Percentage transmission of BYDV by aphid vectors in 1981-3

	1981	1982	1983
Rhopalosiphum spp.	4.8	2.7	1.1
Sitobion spp.	9.7	4.1	2.7
M. dirhodum	0	4.9	5.2
M. festucae	0	2.5	5.3

the largest differences, as might be expected, were when fewest aphids were caught.

Table 3

Comparison of infectivity measured by fluorogenic ELISA and direct feeding tests.

Week beginning	% infective			
	Feeding	ELISA*		
		2X	P<0.01	P<0.001
30 Aug	8.7	0	20.5	0
6 Sept	0	0	0	0
13 "	10.0	3.5	26.3	14.0
20 "	3.0	2.9	8.6	5.7
27 "	2.6	2.9	13.2	7.4
4 Oct	3.1	2.8	8.3	2.8
11 "	8.0	21.1	31.6	15.8
18 "	2.6	4.0	4.0	4.0
25 "	11.5	1.8	12.7	3.8
1 Nov	0	0	50.0	30.8

* The figures given are based on the definition of a positive reaction as either twice the background (2X) or significantly greater than it at P<0.01 or P<0.001.

In 1980 the concept of an Infectivity Index was introduced. This integrated three of the principal influences on BYDV infection of the autumn-sown crop.

(i) Aphid numbers
(ii) Aphid infectivity
(iii) Crop growth stage

Each week from 1 September the number of aphids caught in the RIS trap is multiplied by the proportion found to be infective. Few species other than *Rhopalosiphum* spp. are caught. For this genus infectivity is determined

for all species but only numbers of R. padi are used in the calculation of the Index as this species has the greatest potential for spreading BYDV in the crop. A total weekly Index is obtained by adding the separate species indices and a cumulative index is produced as in Table 4.

Table 4

Cumulative weekly Infectivity Index in autumn 1982

Crop sown in	Infectivity Index on								
week beginning	5 Sept	12	19	26	3 Oct	10	17	24	31
1 Sept	2	2	86	93	102	117	129	134	145
6 "		0	84	91	100	115	127	132	143
13 "			84	91	100	115	127	132	143
20 "				7	16	31	43	48	59
27 "					9	24	36	41	52
4 Oct						15	27	32	43
11 "							12	17	28
18 "								5	16
25 "									11

Field experiments that tested the effects of pesticides applied in the autumn to wheat, oats and barley crops sown at different times from September - December, showed that crops which gave an economic response had an Infectivity Index of 50 or more. This figure is now used as a guide to which crops will need a pesticide treatment and provides a rational basis for pesticide use. The threshold of 50 applies to Rothamsted and seems generally applicable to East Anglia. However, it was clear that other regions would need to establish their own thresholds and in 1983 nine sites obtained data for the calculation of the Index.

The method has demonstrated its value for BYDV infection of cereal crops in Britain but has potential for use with other virus/vector/crop combinations.

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Barley yellow dwarf virus in ryegrass and winter barley in the west of Scotland

INTRODUCTION

Perennial ryegrass (*Lolium perenne*) is the predominant crop in the west of Scotland, where it grows vigorously in the mild, wet climate. However, winter barley has assumed a significant hectarage in the past five years, and continues to increase rapidly, being sown largely into ploughed grassland. On the evidence of the experience of other areas with a similar agriculture (e.g. south west England) this is a setting for a BYDV problem.

In the work reported here the enzyme-linked immunosorbent assay technique (ELISA) was used extensively to investigate the incidence of BYDV in ryegrass and cereals. Some of the results obtained since 1981 are described.

MATERIALS AND METHODS

Enzyme-linked immunosorbent assay

The technique used was essentially that described by Clark & Adams (1977). The coating immuno-globulin was used at 1 µg/ml, and conjugate (with alkaline phosphatase) was diluted 1/500 to 1/1000. Substrate incubation (p-nitrophenyl phosphate) was for 40-60 min. at 20°C.

The two antisera used were kindly supplied by Dr. M.F. Clark of East Malling Research Station. One of these had been prepared against a strain of the virus which had a severe effect on cereal growth, and the other against a strain which had a mild effect on cereal growth.

As the effect which the majority of strains identified in grass had on cereal growth is unknown, they are referred to as severe-type and mild-type on the basis of the antiserum with which they reacted.

BYDV infection of ryegrass plots

In May and August 1982 plots of grass sown in 1980, and measuring 5m x 1m, were divided into 10 equal sub-areas of 1m x 0.5m and from each were collected 10 symptomless leaves. These were tested by ELISA.

BYDV infection of farm crops

In May 1982 a 2 ha area in each of 13 farm crops of *L. perenne* was divided into 15 sub-areas and from each were collected 10 widely spaced symptomless leaves. In July a 20m x 20m area in six of the crops was divided into 16 sub-areas and 10 symptomless leaves were collected from each. All the samples were tested by ELISA.

RESULTS

Foliar symptoms and BYDV infection

In 1981 and 1982 foliar symptoms in 1980-sown plots were most frequently seen on tillers which were apparantly growing vigorously.

In 1981 mild-type BYDV was detected in 18 of the 20 samples of *L. perenne* with foliar symptoms which were tested and found to be infected. Severe-type BYDV was found in only five of these. In *L. multiflorum* all 24 infected samples with symptoms contained mild-type BYDV, whilst seven were infected with severe-type BYDV. Approximately 40% of symptomless plants were infected, mild and severe-type strains occurring with roughly equal frequency.

In 1982 all but two of the 81 samples of *L. perenne* and *L. multiflorum* with foliar symptoms which were tested contained mild-type BYDV. Severe-type BYDV was found in 30 of the samples, but this increase in incidence compared to 1981 was only in proportion to the increase in the general level of infection in the ryegrass plots. Of the 120 symptomless samples tested, 53 were infected with severe-type and 37 mild-type BYDV, giving an overall infection of 61%.

Extent of BYDV infection in 1982 in plots sown in 1980

By May 1982, about 18 months after the plots were sown, cultivars of all three *Lolium* spp were extensively infected with BYDV. Infection had further increased by August (Table 1). There was limited evidence of a difference in the susceptibility of grass species to virus infection with *L. multiflorum* being less severely affected than *L. perenne* or *L. perenne* x *hybridum*.

Table 1. BYDV infection of 1980-sown plots in August 1982

Grass species	Cultivar	Number of sub-areas (max. 10) Severe -type	Mild -type	Total
L. perenne	Fortis	6	10	10
L. perenne x *hybridum*	Augusta	8	4	9
	004/6	6	6	7
L. multiflorum	Tetila	4	0	4
	008/7	4	3	5

BYDV infection of farm crops

BYDV was detected in samples of *L. perenne* from all 13 fields examined in May 1983. Between one and 12 of the 15 samples per field were infected, but there was no consistent association between sward age and infection level. When six of the crops were re-examined in July the presence of BYDV was confirmed, and, on average, 50% of the samples were infected (Table 2). At both sample dates severe-type BYDV was the predominant strain.

Table 2. 1983 field survey - secondary sampling - July 1983

Year sown	Number of sub-areas (max. 16) Severe -type	Mild -type	Total	%
1982	4	0	4	25
1980	10	1	10	63
1979	8	0	8	50
1979	9	1	9	56
1978	8	5	9	56
1977	3	3	6	38
Mean	7.0	1.7	7.7	48

BYDV in winter barley

Typical symptoms of BYDV appeared in late October 1983 in many crops sown directly after grass in late August and early September. Grass turves left on the soil surface were extensively infected with severe-type BYDV (19/25 turves examined), and they also provide a source of viruliferous alate *Rhopalosiphum padi*. In one crop infection reached 100% by late November and 15-20% of the plants died in November and December. By March 1984 it was evident that several crops would not produce satisfactory crops, and the decision was made to plough-in.

Little damage was seen in crops sown after cereals, and the infectivity of migratory aphids in the autumn was low.

DISCUSSION

The effect of the rapid and widespread infection of *L. perenne* by BYDV on grass productivity and longevity is undetermined, although reports elsewhere (Catherall, 1963) suggest that damage can be severe under certain conditions. However, the damage seen in winter cereals resulting from BYDV infection leaves no doubt about the importance of *L. perenne* as a source of those strains of the virus which can seriously affect cereal growth, and their principal vector *R. padi*. Damage to winter cereals in 1983/84 was not confined to the west of Scotland but occurred for the first time in eastern and northern areas of the country, serving a timely reminder that even at northern latitudes BYDV is not a disease to be ignored.

The ELISA technique is proving an invaluable tool for the study of the epidemiology of BYDV in reygrass, but must be regarded as a useful adjunct to biological tests and not a complete replacement for them.

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The differential response of some small grain varieties to some isolates of barley yellow dwarf virus (BYDV).

The differential interaction between small grain varieties to isolates of BYDV is probably not an uncommon phenomenon. It has been mentioned for varieties or breeding lines of barley and oats by, among others, Rochow (1969), Jones and Catherall (1970) and Gill and Buchannon (1972), and Eweida et al. (1983) demonstrated similar relationships with oats and maize.

In my material it was sufficiently common to cause difficulties in early work on screening breeding lines of barley and oats for toleranse to BYDV. This made it necessary to attempt a more active search for and selection of virus strains suitable for a particular use. Some excperiences and results from this work will be presented here.

Table 1 gives the results of an experiment where barley cv Lise and oats cv Blenda were inoculated on the 1-2 leaf stage with three virus isolates that were all most efficiently transmitted by the aphid Rhopalosiphum padi. The plants were harvested 5 weeks after inoculation, and the results are given as fresh weight of plants in per cent of uninoculated control plants.

Table 1. Fresh weight of plants inoculated with three isolates of BYDV in % of uninoculated plants.

	Isolates		
	636	637	638
Barley cv Lise	67	46	30
Oats cv Blenda	44	57	75

This experiment demonstrates a relationship between virus isolates and host plant varieties which was very common among the isolates tested: Increasing severety to barley is related to decreasing severity in oats and vice versa. As severe isolates are necessary to distinguish between high and moderate tolerance, these figures will also indicate that an isolate which is suitable for screening for BYDV-tolerance in barley may not be equally efficient in oats.

In the experiment referred in table 2 the same virus isolates, together with a fourth isolate (isolate 74), which was previously rated as mild on all varieties tested, were inoculated to four varieties each of barley and oats. The varieties were chosen for varying levels of toleranse based on earlier experience and on information from the litterature. The experiment was performed in the same way as the beforementioned and the results are presented in relative numbers.

Table 2. Fresh weight of plants inoculated with four isolates of BYDV in % of uninoculated plants.

	Isolates			
	636	637	638	74
Barley:				
Lise	63	53	41	80
Møyar	73	70	56	82
CI 9654	105	96	98	106
CI 666	9	12	20	69
Oats:				
Blenda	47	58	60	98
Titus	72	92	94	106
Pol	94	98	99	86
Albion	86	98	84	106

The reactions of the varieties Lise, Møyar, Blenda, Titus and Pol on infection with isolates 636, 637, 638 followed the same pattern as for Lise and Blenda in table 1, although the reactions of Pol and partly of Titus were mild with only insignificant differences. CI 9654 was highly tolerant to all three isolates. CI 666 was much damaged by all three isolates but the rating of the isolates for severety on this variety was opposite that on the two other barley varieties. This pattern of reaction has been veryfied several times with similar virus strains, and is thus an example of differential interaction between virus isolates and varieties of barley.

Although milder in Blenda than any of the other isolates, isolate 74 was not severe in any of the barley varieties, and thus did not follow the trend from the other three isolates in this experiment. Similar isolates giving only mild or no reactions in all varieties tested were isolated several times. Pairs of strains which were moderately severe in both Lise barley and Blenda oats and for which no differential interaction could be demonstrated were also found, but an isolate being very severe on both Blenda and Lise was not encountered during this work.

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Studies on field variants of barley yellow dwarf virus in Sweden

Barley yellow dwarf disease is frequently a great problem in spring sown cereals in Sweden. The most important aphid species which transmits the disease is Rhopalosiphum padi (L.). Some years, however, Sitobion avenae (Fabr.) has the main role as a vector. The size of populations and behaviour of R. padi in Sweden are rather well known, but similar information about S. avenae is lacking.

In 1982 and 1983 experiments were carried out to establish the existence and frequency of different isolates of barley yellow dwarf virus (BYDV) in Sweden by using ELISA and aphid transmission tests. Samples of cereal plants with symptoms of BYDV were collected randomly from different parts of the country. Antisera against two known isolates of BYDV, one mild and one severe, were used. The former isolate (27/77) is transmitted specifically by S. avenae and the latter (39/78) is transmitted much more efficiently by R. padi than by S. avenae. R. padi and S. avenae were used in the transmission tests.

In 1982 the dominating aphid species on cereals was R. padi. It was shown by both ELISA and aphid transmission tests that virus isolates similar to 39/78 were common and that the frequency of isolates similar to 27/77 was very low.

The results obtained in 1983 were in contrast to those in 1982. The dominating aphid species in the fields was S. avenae and the most common virus isolates were similar to 27/77. This situation resembles that of 1977 when mainly S. avenae specific isolates occurred.

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Economical importance of Barley yellow mosaic virus in Germany

In some regions of the Federal Republic of Germany barley yellow mosaic caused by Barley yellow mosaic virus (BaYMV) is the most important disease on cereals. It has been identified 1978 (Huth and Lesemann, 1978) and was found at first especially in areas where predominantly barley or cereals has been grown. In these areas BaYMV occured probably since at least 20 years or more. Since nearly 1960 farmers observed the appearance of yellow patches in fields of autumn sown barley. These patches appeared again at the same positions in fields when years later barley has been grown repeatedly. Whereas 1978 only a few fields were known showing some patches of diseased plants now in some regions large areas are entirely infested by BaYMV and we are afraid that in these areas barley could never be cultivated any longer. From observations in Lower Saxony and Northrhine Westphalia, it has been established, that more than one third of the arable land are infested by BaYMV (Huth, 1984). From the discovery of the virus in North-Germany as well as in South-Germany, which are situated outside the main spread area, we assume that nearly one third of the arable land in Germany is endangered to become infested. Rapid spread of the virus is enabled by soil-transmissibility. Vectors are Polymyxa graminis (Toyama and Kusaba, 1970, Macfarlane, 1982), and as Zerlick supposes (pers.communicat.) possibly some further soil fungy. Spread inside fields is facilitated by machinery and consequently virus-contaminated patches are at first elongated along the line of cultivation (Huth, 1984). Machines also easily carry virus-contaminated soil from field to field. If the transmitted soil contained only a defined small number of virus-bearing spores, it lasts maybe 5 to 10 years or more until small virus infested patches will be recognisable in barley fields.

One of the most important carrier seems to be the wind. It has not been proved, but we suppose that soil dust contaminated by virus-bearing spores, blown by high winds, can be drifted not only to neighbouring fields but also over long

distances. Sometimes a spontaneous appearance of large virus-infested patches in barley fields have been observed which have been enriched by waste soil from sugar refineries. It shows that virus-bearing resting spores of the vector obviously survive some years in waste soil.

It is unlikely that the virus is spread by strow because viruses are rapidly inactivated outside the vectors. Seed-transmissions have not been observed but it might be possible that a very few kernels could be contaminated by soil-particles contaminated by virus-bearing spores. This mode of transmission should be unimportant.

Symptom development depends on several factors from which the temperatures seem to be one of the most important. In general low temperatures promote the appearance of mosaic symptoms and especially temperatures below 5° C lead to yellow discolotations of leaves of infected plants. Symptom development is discontinued at temperatures of more than 15 to 18°C. Temperatures influence also the growth of infected plants. Under mild climatic conditions cultivars like Sonja, Augusta or Dura in general are less damaged and often appear symptomless in later development stages. During relatively low temperatures in the whole growth period, sometimes plants reach only half the length of healthy plants. Therefore in the eastern part of the Federal Republic of Germany infected plants are mostly more damaged than plants growing in the western part. The relatively high temperatures in England might also be the reason for the fact that there the cultivar Sonja does not show symptoms. The degree of damage is probably also influenced by the number of virus-bearing spores of the vector in the soil (Zerlick, pers.commun.). If the fields are infested for a longer period of time, than the more virus-bearing spores occur in soil and the more often barley roots can be contaminated by them. According to the number of contaminations, plants produce more or less strong symptoms. Obviously because of the low rate of spores in sandy soil and the resulting low rate of root contaminations plants are less damaged than if they are growing in clay containing heavy soil. Furthermore the sowing time also seems to be important for the strengths of symptoms: the

earlier barley is sown in autumn the stronger the damage and the more often roots can repeatedly be contaminated by spores over a long period which results in a high level of viruses already in young plants.

Besides of growth reductions, also losses of kernel yield are mostly consequences of infections by BaYMV. Yield reductions depend on several factors such as temperatures during the whole plant development, level of virus-bearing resting spores in soil, as well as barley cultivars. In some areas also type of soil and sowing time seem to be important factors.

Virus-infected plants, especially of highly susceptible cultivars, are sometimes killed during long lasting frosty periods and the yield of the surviving plants can be reduced to 20 % of such of healthy plants. Otherwise during long lasting high temperatures beginning in April also the virus-infected plants mostly regenerate well, symptoms disappear and yield reaches nearly the hight of healthy plants. The table (Table 1) summarize the relative yields of some cultivars growing in areas with different climatic conditions: in Mellrich and Paderborn, in areas with in general low temperatures the yield is more reduced than in Geldern and Kleve in the western part of Germany near the border to The Netherlands with averagely mild climatic conditions.

Table 1

Relative yields of some cultivars of winter barley growing in different areas in Germany in 1983. Fields were situated in the west (1), middle (2) and east part (3) of North Germany.

	Geldern[1)]	Kleve[1)]	Mellrich[2)]	Paderborn[2)]	Sunstedt[3)]
Birgit(=Stand)	100	100	100	100	100
Tapir			41	54	56
Dura				35	36
Sigra			16	31	52
Mammut			18	28	40
Gerbel	72	73	10	29	
Vogels.Gold					27
Sonja	62	60			58
Augusta	76	79			

Yield losses caused on fields entirely infested by BaYMV can be avoided only by growing resistant cultivars. At present there are nine cultivars known to be resistant. It is to be expected that in the near future some more resistant cultivars will be available. Recently a lot of sources of resistance to BaYMV have been found in barley samples from some gene banks (Huth, unpubl.). Furthermore in the meantime Friedt et al. (1983) were able to produce a lot of new barley lines which are resistant to this virus. From these results we conclude that BaYMV in future would not longer be a problem in Germany.

According to last informations we are sure that in Germany there are more than two types of soil-borne barley-infecting viruses which produce the same symptoms but are different in some respects (Huth, Lesemann and Paul, 1984). We are afraid that at least some of the cultivars resistant to BaYMV are not resistant to the others.

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The effects of barley yellow mosaic virus on the components of yield and interactions between virus infection and infection by Polymyxa graminis

Introduction

Barley yellow mosaic virus (BaYMV) is now known to be widespread in Britain and causes obvious damage to many of the commonly grown autumn-sown cultivars. However, measurements of the losses that infection causes are difficult to determine because of interactions between soil, virus and the fungus vector and because infection within a field is often patchy. The effect of virus alone can be measured by comparing the yields of plants manually inoculated with or without virus but plants are not naturally infected this way and the infection and movement of virus from roots to foliage may be integral to how it has its effect. The results reported here compare the effects of BaYMV and P. graminis on cvs Sonja and Maris Otter.

Materials and Methods

Field experiments were made near Rothamsted on private farms that were known to have BaYMV infected soil. Plants for measurements were collected from these experimental sites or from commercial crops found to be infected with BaYMV. Spring barley cultivars were tested by sowing in October in boxes containing soil from a diseased site. Plants were scored for symptoms and the components of yield measured on all infected shoots and an equal number of randomly selected symptomless shoots.

Results and Discussion

Experiment 1. Winter barley cv Sonja was sown on 23 September and cv Maris Otter on 2 October and 3 November on a field that had grown a uniformly infected crop of Maris Otter the previous season. BaYMV was recorded on a random collection of plants at monthly intervals until April and Polymyxa graminis was scored on washed roots in February, March and April. A 0-3 scale was used to score P. graminis; 0 = no cystosori present; 1 = one or two cystosori; 2 = several clumps of cystosori, 3 = many cystosori, sometimes merging to form black areas on roots. The results are given in Tables 1 and 2.

Table 1

Infection by BaYMV in cvs Sonja and Maris Otter sown at different times

	% BaYMV		
	Sonja	M. Otter (2 Oct)	M. Otter (3 Nov)
22 Oct	<1	<1	-
6 Nov	<1	0	-
4 Dec	0	25	0
6 Jan	4	27	0
9 Feb	4	88	32
10 Mar	6	96	48
9 Apr	4	92	20

The difference in susceptibility of cvs Sonja and Maris Otter usually found in British conditions is clearly shown, as is the effect of sowing date on infection of the very susceptible cv Maris Otter. There was little difference in the proportion of infected and symptomless plants showing cystosori of P. graminis. However, it was more likely that an infected Sonja plant would be infected with P. graminis and would be more severely infected than a symptomless plant.

Table 2

Infection by Polymyxa graminis of BaYMV-infected and symptomless plants of cvs Sonja and Maris Otter

	% infection with P. graminis						Severity of P. graminis					
	Sonja		M. Otter (2 Oct)		M. Otter (3 Nov)		Sonja		M. Otter (2 Oct)		M. Otter (3 Nov)	
	BYMV	0	BYMV	0	BYMV	0	BYMV	0	BYMV	0	BYMV	0
9 Feb	100	67	100	75	-	4	1.0	0.8	1.0	0.9	-	0.1
10 Mar	92	84	84	100	9	0	1.3	1.2	0.6	0.6	0.1	-
9 Apr	100	92	92	100	35	20	2.0	1.2	1.0	1.1	0.4	0.4

In February 100 plants of cv Sonja and the 2 October sown Maris Otter were dug up, their roots washed free of soil and potted up in a soilless compost and grown to maturity in an unheated glasshouse. Too few plants of cv Sonja

showed symptoms to allow valid comparisons but they were possible for cv Maris Otter as 76% showed symptoms (Table 3).

Table 3

The effects of BaYMV on the components of yield of cv Maris Otter

	Infected	Healthy	X100 $\frac{\text{Healthy - infected}}{\text{Healthy}}$	
Shoot Number	10.0	10.2	- 2.0	NS
Height (cm)	65.2	85.7	-24.0	***
Grain Number	187.3	200.4	- 6.5	***
Grains/ear	18.7	19.7	- 5.1	***
1000 g wt (g)	27.7	30.4	- 8.9	*
Yield (g)	5.2	6.2	-16.1	*

NS - not significant, * $p<0.05$, *** $p<0.001$

All components of yield were decreased except shoot number and the largest effect was on height which is superficially the easiest symptom to see at or near harvest.

Experiment 2. Because no measurements were possible on cv Sonja in experiment 1 the effects of BaYMV were examined on a different site two years later when scattered infection of cv Sonja was seen in March. 100 plants showing symptoms and 100 apparently healthy plants were dug up from the same area, their roots washed, and then grown to maturity in the glasshouse as before. Components of yield were eventually measured on 95 BaYMV infected plants and 98 without symptoms (Table 4).

Table 4

The effects of BaYMV on the components of yield of cv Sonja

	Infected	Healthy	X100 $\frac{\text{Healthy - infected}}{\text{Healthy}}$	
Shoot Number	3.8	4.9	-22.4	***
Height	46.9	67.3	-30.3	***
Grains/ear	16.2	18.4	-12.0	***
1000 g wt	48.6	58.7	-17.2	***
Yield	3.0	5.2	-42.3	***

*** $P<0.001$

In this experiment all components of yield were significantly decreased by BaYMV infection and all by a greater proportion than in cv Maris Otter. The conditions between the experiments may have differed too much to make valid comparisons possible but it does seem that when infected cv Sonja is damaged by BaYMV as much as is the much more susceptible cv Maris Otter.

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Barley yellow mosaic virus - reactions of U.K. varieties of winter barley

Introduction

Since the first outbreaks were confirmed in U.K. in 1980 (Hill, S. A. & Evans, E. J. 1980) barley yellow mosaic virus (BaYMV) has spread progressively into all the major winter barley growing areas. Appearing first in East Anglia and Oxfordshire it spread in 1981 to the South West of England, in 1982 into the North and Scotland and latterly has been confirmed in the South-East. Each year similar numbers of new outbreaks are confirmed as the virus invades new areas and becomes established. Whilst the means by which the virus was first introduced to U.K. (probably three or four years prior to 1980) is uncertain, its subsequent spread over long distances into new localities is clearly due to the movement of viruliferous cystosori of the vector fungus Polymyxa graminis.

Control

Following the discovery of the disease a number of unsuccessful attempts to control it have been made. Deep ploughing as opposed to minimal cultivation reduced the spread of the disease but had no affect on its severity. Pre-cropping soil sterilants did not reduce disease incidence, nor did seed dressings and sprays of fungicide. Whilst late-sown (November) plots initially had less severe virus than early-sown (September) plots, by March all were equally badly affected. Thus the only remaining means of avoidance of BaYMV appears to be the use of resistant varieties.

Varietal resistance - initial findings

In trials in East Anglia on land with a viruliferous Polymyxa population, the BaYMV resistance of popular winter barley varieties, some reputedly resistant German varieties and some which were newly released, has been determined. Initial results from these trials suggested that the varieties tested could be grouped according to their BaYMV reaction and that such groups appeared to relate to the breeding parentage (origin) of the variety (see table 1). Further observation showed that as varieties differed in response to BaYMV infection, they differed in the same way in their

Table 1: The reaction of winter barley varieties in relation to their origin

Variety	BaYMV	Origin
Maris Otter Halcyon Tipper Triumph	very susceptible	UK
Pirate Gerbel Hexa	moderately resistant	France Holland
Igri Sonja Birgit Athene	resistant	Germany

tolerance of low growing temperatures. Thus the U.K. malting varieties which are all virus susceptible, are not cold tolerant, and conversely the more virus resistant German bred varieties can grow better in the cooler conditions of Northern Europe.

In three years of trials it became evident that in colder winters the susceptibility of some varieties was increased. In particular, Igri was much more severely affected in a cold year and could not be regarded as resistant. This observation relates to that reported by Huth (personal communication) in which varieties were less severely affected when grown in the West of Germany than when grown in the North.

Varietal behaviour after different precropping

Maris Otter has been the variety traditionally grown in East Anglia for its use in malting for beer making. In contrast, in other parts of England varieties grown for animal feeding have been more widely grown. In contrast to earlier observations, field experience in these different situations appears to suggest that the reaction of varieties to BaYMV

infection may differ according to the varietal pre-cropping. Varieties may appear resistant when grown on land previously cropped with infected Maris Otter, but susceptible after infected Igri. This difference in behaviour was first seen in farmers crops, but has since been confirmed in experimental sites. In table 2 the mean percentage of plants showing BaYMV symptoms during early spring is given to illustrate varietal reactions. Results from two sites are shown, one following many Maris Otter crops (Clacton) and the other following a series of Igri crops (Reading). The varieties apparently react differently at the two sites, with the differences between the assessments following a pattern which relates to the breeding of the varieties in each group. This contrasting behaviour is evident in an increasing number of commercial fields and affects many other varieties.

Table 2: Varietal reaction to BaYMV at two sites with different varietal pre-cropping

Variety	mean % plants infected		
	Reading+	Clacton°	Difference
Maris Otter	34	97	+63
Halcyon	26	75	+49
Metro	46	73	+27
Igri	49	28	-21
Sonja	35	1	-34
Gerbel	55	18	-37
Panda	37	6	-31
Monix	52	2	-50
Athene	4	0	- 4

+ Igri precropping ° Maris Otter precropping

In some fields in which Maris Otter was infected in 1980, Sonja has been grown in the last four years. Symptoms of BaYMV have been absent from these fields despite quite cold winters, until 1984. Thus it would appear that the character of a virus or vector population can be changed by selection following repeated cropping with specific varieties over a period of only three years.

Polymyxa and virus levels

Plant samples from the trial sites have been examined and the incidence of Polymyxa in the roots and virus in the roots and shoots determined. Predictably, total virus levels in roots and shoots correlated well with the expression of BaYMV symptoms. Polymyxa levels, in contrast, appear to bear no relation to varietal reaction to virus (see table 3).

Table 3: Polymyxa incidence in roots (Clacton site)

	cystosori per cm. root				
Date	6/3	21/3	4/4	17/4	Mean
M. Otter	16	12	1	16	11
Halcyon	14	21	1	29	16
Igri	38	60	5	53	39
Sonja	1	1	0	2	1
Athene	5	37	0	20	16

The assessments presented are those for the trial at Clacton, those from the Reading trial differed only in overall magnitude, fewer cystosori being found, but the relative varietal incidence was similar.

Discussion

Preliminary experience suggests that in U.K. grown winter barley varieties there is a spectrum of resistance. As described by Huth (1982) some appear very susceptible, some more resistant and a small proportion completely resistant. However further observations suggest that no single definition of response seems possible since varietal behaviour varies according to varietal pre-cropping regime. This phenomenon does not appear to have been reported from other countries which have the disease, but equally the U.K. type malting varieties have not been grown elsewhere. Further, it appears that the character of a site may be changed following repeated cropping with a 'resistant' variety. This would suggest that repeated cropping is reselecting from existing vector or virus populations rather than that there is genetical adaptation within them. Thus it seems more likely that selection within the fungal vector population may be taking place, although preliminary observations of Polymyxa incidence did not confirm this.

From the limited testing so far undertaken it appears that U.K. BaYMV isolates contain both the virus strains defined by Huth (personal communication). Thus more detailed study is required of both virus and vector before realistic control strategies can be defined.

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Purification and some particle properties of barley yellow mosaic virus isolated in Germany

Barley yellow mosaic virus (BaYMV) and the disease caused by it occurs since many years in Japan and since some years also in Europe and is gaining increasing economical importance. Data on BaYMV particle properties, however, are still incomplete as the virus is difficult to multiply and to purify in sufficient quantity.

At first our experiments were impeded by the fact that field-infected plants showing clear symptoms were available only during a few weeks per year and that plants grown in infected soil in greenhouses did not yield enough virus. In 1983 FRIEDT succeeded in mechanically infecting barley with BaYMV in conditioned rooms on a larger scale, thus making available infected plants continuously.

Several purification methods were tried, including the one published by USUGI & SAITO (1976). The experiments evidenced that two things are important: i) rapid working, and ii) reducing the number of purification steps as much as possible. BaYMV has a tendency to aggregate and to get insoluble so that virus is easily lost during extensive purification.

The purification protocol (Table 1) does not contain new techniques, and it is similar to the protocol of USUGI &

SAITO, but it is much shorter. We omitted repeated differential centrifugations and the sucrose density gradient centrifugation that did not work well. Instead a centrifugation through a sucrose cushion early in the purification was used. Sometimes even the medium-speed centrifugations were not done. Step 6 was sometimes used to eliminate ferritin-like material, but it reduced also virus yield. Most effective was the overnight centrifugation in CsCl. It allowed to separate the majority of impurities from the virus, and to concentrate it. BaYMV treated in this way is only partly purified, but can be used to study some particle properties and to produce antisera.

The centrifugations in CsCl had a surprising result. USUGI & SAITO (1976) have found one virus band in CsCl gradients containing the long as well as the short particles of BaYMV. Both types have, therefore, identical buoyant densities. When we prepared BaYMV from plants infected through soil we obtained, however, two bands of varying thicknesses, both containing always short and long particles. With antiserum supplied from Japan decoration tests showed that the majority of particles in the lower band was decorated, whereas the majority of particles in the upper band was not, indicating a clear serological difference.

When BaYMV was purified from mechanically infected barley, only one band in the position of the upper band was present. The particles of this band reacted exclusively with the antiserum made to the mechanically transmitted BaYMV. For a distinction of both virus types the me-

Table 1 PURIFICATION PROTOCOL

1. Homogenize tissue in 0.1M K-citrate, adjusted to pH 7 with citric acid, 3g/1ml, squeeze pulp;
2. stir sap with 1/5vol CCl_4, separate phases (6,000rpm, 10min);
3. filter and clarify aqueous phase (20,000rpm, 10min);
4. sediment virus through 30% sucrose cushion (35,000rpm, 120min);
5. dissolve pellets in citrate soln. by gently stirring with a glass rod, centrifuge 6,000rpm, 10min);
6. supernate may be frozen and thawed;
7. centrifuge at 20,000rpm for 10min;
8. mix supernate with CsCl to give a 28% (w/w) soln., run overnight in a swinging bucket rotor (35,000rpm, 10°C);
9. remove virus bands;
10. remove CsCl by diluting and subsequent ultracentrifugation;
11. dissolve pellets in citrate soln.

Longer runs in CsCl may be used, the temperature must not exceed 12°C.
Steps 3, 6, and 7 can be omitted.

chanically transmissible type was labelled BaYMV-M, and the other BYMV-NM. The naturally infected plants found up till now in Germany contained a mixture of -M and -NM.

In decoration tests always some particles of the respective other type were found in both bands obtained in CsCl gradients using virus from naturally infected plants. One possible explanation may be the tendency of BaYMV to aggregate during purification. The ratio of BaYMV-M to BaYMV-NM seemed to change during the seasons. In wintertime (up to February) BaYMV-NM prevailed, later on BaYMV-M tended to increase in quantity, but the variability of the results was great.

Recent results indicate that BaYMV may occur in more than the two types mentioned. We found barley plants with typical symptoms that contained a virus strongly reacting with a Japanese antiserum to wheat yellow mosaic virus, but poorly with the Japanese antiserum to BaYMV.

The protein subunits of BaYMV-M and of the mixture BaYMV-M and -NM were tested in continuous as well as in discountinuous SDS-PAGE. At least two bands were seen, corresponding to a M_r of 35-36,000d and 28-30,000d. Within the lower range mostly two closely spaced bands were found, they were well separated only by the discontinuous system. Recent unpublished experiments of EHLERS in this institute showed that with BaYMV-M the uppermost band was strongest, followed by the lowest band, whereas the middle band was faint. Using a mixture of BaYMV-M and -NM, but containing predominantly -NM there was only a very faint upper band, but the two lower bands were strongly visible. More experiments are

needed and under way to learn the meaning of these differences and the origine of the multiple bands.

The isolated nucleic acids of both BaYMV types formed in PAGE under nondenaturing conditions two bands each, corresponding to a M_r of 2.8 and 1.4 Md. Thus the M_r ratio is similar to the length ratio of the particles. It remains to be tested, whether BaYMV is a two-component virus or whether the two particles are only broken or aggregated products.

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Electron Microscopy and Identification of Barley Yellow Mosaic Virus in Germany and Differentiations of Two Virus Types Occuring in field-infected Barley

Field-infected plants of barley with mosaic symptoms revealed elongated particles with two predominant lengths, 270 and 568 nm when stained in phosphotungstate. In ultrathin sections of affected leaf tissues conspicuous cytoplasmic inclusions of the pinwheel type with laminated aggregates, as well as dense regularly arranged membrane aggregates were found. The latter were continuous with the endoplasmic reticulum and were found in one variant resembling structurally a coat of mail, and in a more condensed form attaining almost crystal-like regularity. Virus particles were only rarely found in densely packed bundles.

In winter and early spring, particles from these plants could be trapped using grids coated with Japanese antiserum to barley yellow mosaic virus (BaYMV) and to wheat yellow mosaic virus (WYMV). However, only antiserum to BaYMV strongly decorated trapped particles, but not antiserum to WYMV. Thus, particle morphology, cytopathology, and serological reactions indicate a close similarity of the virus with BaYMV, which is serologically related to WYMV.

However, in purification experiments on leaf material grown in late spring two virus bands were obtained after isopycnic density gradient centrifugation in CsCl. Only the lower but not the upper of these bands reacted with Japanese antisera to BaYMV and to WYMV. Particles of either band showed identical length distributions.

Later in early summer the band predominated that did not react with BaYMV antiserum. Mechanical transmission from field material resulted always in infection with particles not reacting with BaYMV antiserum. Antisera to the mechanically trans-

mitted virus did not react with particles reacting with BaYMV antiserum. Thus, two types of virus exist in barley in the field, which are identical in morphology, cytopathology, symptomatology, and soil transmissibility, but they differ in buoyant density, serological reactivity, and mechanical transmissibility.

A detailed report on the presented results will be published in Phytopathologische Zeitschrift (Huth, W., D.-E.Lesemann, and H.L. Paul, 1984: Barley yellow mosaic virus; Purification, Electron Microscopy, Serology, and other Properties of two types of the virus. Phytopath.Z.111, 37-54).

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Genetics of Resistance to Barley Yellow Mosaic Virus

Introduction

Winter barley crops in central and northern Europe have frequently been damaged by the soil-borne barley yellow mosaic virus (BaYMV) in recent years, and increasing growing areas have been infested with this new pathogen (HILL and EVANS 1980, HUTH 1984). BaYMV is only multiplied in the barley plant when temperatures are continuously below 15°C, and the rapid spread of the disease was certainly initiated by the considerable extension of winter barley cultivation in the last years (Fig. 1).

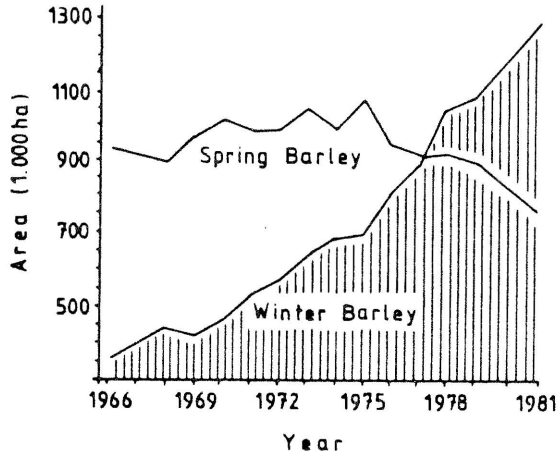

Fig. 1. Trends of barley cultivation in the Federal Republic of Germany (adapted from FRIEDT 1984).

Most of our winter barley cultivars suffer considerable yield losses when grown on BaYMV-infested fields. The grain yield of such susceptible varieties can be reduced by more than 70% as compared to a resistant cultivar like 'Birgit' (Fig. 2, FRIEDT 1984). Such yield losses can only be prevented by growing resistant cultivars, since the virus cannot be controlled chemi-

cally. Unfortunately, the presently recommended resistant varieties are not fully satisfactory in yield at non-virus places. Therefore, about 97% of the total winter barley crop are still susceptible varieties (ANONYMUS 1984). Consequently, it is one of the major breeding goals to combine resistance to BaYMV with superior grain quality and yield. Besides that, diverse sources of resistance should be introduced in order to improve its stability. Conventional breeding procedures as well as haploid techniques are applied simultaneously to accelerate the breeding process.

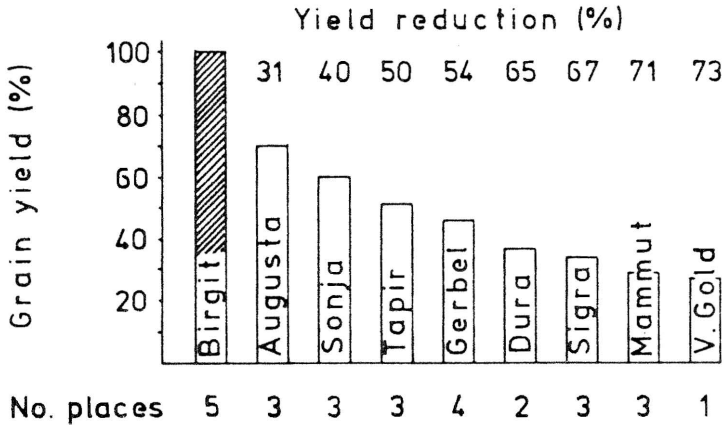

Fig. 2. Average grain yield of BaYMV-susceptible cultivars at 1 to 5 places as related to the resistant cv. 'Birgit' (rearranged and summarized from HUTH 1984).

Materials and methods

The winter barley (Hordeum vulgare L.) cultivars listed in table 1 and their hybrids were used (cf. FRIEDT 1984, see text). Parent varieties and hybrid plants were all tested for their reaction to BaYMV by mechanical inoculation as described by FRIEDT (1983) and FRIEDT and FOROUGHI-WEHR (1984). Part of the material was also tested in a BaYMV-infested field at Sunstedt near Braunschweig. Hybrid plants (F_1) were either multiplied for subsequent genetic analyses and pedigree selection, or were used as donor plants for anther culture as described by FOROUGHI-WEHR et al. (1976) and FOROUGHI-WEHR and FRIEDT (1984).

Table 1

	BaYMV-resistant	BaYMV-susceptible
6-row:	Barbo, Birgit, Franka, Ogra, Dea, Bulgarian, Esaw, Wigo, Mokusekko 3	Arma, Bosquet, Corona, Freya, Gerbel, Hasso, Largo, Leuta, Illia, LP 61444, Mammut, Marko, Robur, Thibaut
2-row:	Diana, Sonate, Resistant Ym No.1	Alpha, Danilo, Igri

F_2-populations were mechanically inoculated in the 4-leaves stage to test their reaction to BaYMV. Resistant plants were potted and grown to maturity for further field selection in subsequent generations.

Results and discussion

The tested cultivars and hybrids showed corresponding, resistant or susceptible reactions to BaYMV in the field- and greenhouse-tests. The F_1-plants from crosses of resistant German cultivars to susceptible varieties were all susceptible to BaYMV, whereas the respective F_2-populations segregated into about 75% susceptible and 25% resistant individuals (Tab. 2).

Table 2
BaYMV-reactions of crosses of resistant parents to the susceptible cv. 'Igri' (from FRIEDT 1984)

Hybrid	F_1	F_2					
Igri x			Total	Suscep.	Resist.	χ^2	P
x Birgit(♀)	Susc.	Obs.	125	89	36	0.77	0.3-0.5
		Exp.		93.75	31.25		
x Franka(♂)	Susc.	Obs.	129	95	34	0.12	0.7-0.8
		Exp.		96.75	32.25		
x Ogra (♀)	Susc.	Obs.	124	90	34	0.39	0.5-0.7
		Exp.		93	31		
x Sonate(♂)	Susc.	Obs.	128	92	36	0.67	0.3-0.5
		Exp.		96	32		

Expected values are based on a single recessive gene

These results indicate, that resistance of modern German varieties is most probably inherited by a single recessive gene.

Besides that, numerous resistant stocks have been identified among materials stored in different genebanks (e.g. TAKAHASHI et al. 1973, HUTH 1982). Most of these resistant samples originate in China, Korea, Japan or Turkey. For example the Japanese naked cultivar 'Mihori hadaka' carries a partially dominant gene Ym 2 on chromosome 1, whereas the Chinese six-rowed spring barley 'Mokusekko 3' carries a dominant resistance gene Ym 1 on chromosome 4 (TAKAHASHI et al. 1973).
Hybrid plants from crosses of 'Mokusekko 3' to susceptible cultivars like 'Igri' were all resistant to BaYMV and the resulting F_2-populations contained 25% susceptible and 75% resistant plants (Tab. 3), which demonstrates that gene Ym 1 is completely dominant in this case.

Table 3
BaYMV-reactions of crosses segregating for gene Ym 1
(from FRIEDT 1984)

Hybrid	F_1	F_2					
			Total	Suscep.	Resist.	χ^2	P
Mokusekko x Igri	Resist.	Obs.	69	15	54	0.39	>0.5
		Exp.		17.25	51.75		
Igri x Resist.Ym No.1	Resist.	Obs.	94	13	81	6.25	<0.05
		Exp.		23.5	70.5		

Expected values are based on a single dominant gene

This gene Ym 1 was also transferred into the two-rowed Japanese barley 'Resistant Ym No.1' (MURAMATSU 1976). An F_2-population from a cross of this strain to the susceptible cultivar 'Igri' showed an excess of resistant plants over the expected 75% (Tab. 3). When this line was crossed to German winter barley cultivars carrying a recessive resistance gene, no susceptible plants were found in the F_2-generation. However, segregation into 13 resistant to 3 susceptible individuals would be expected, if the genes were independent. Therefore, the two genes mentioned are either allelic, or 'Resistant Ym No.1' carries

an additional recessive gene which would be allelic to that of the German varieties, e.g. 'Birgit', 'Franka' or 'Sonate'.

Hybrid plants from crosses of different resistant parents, including German cultivars (e.g. 'Barbo', 'Birgit', 'Franka', 'Sonate'), as well as 'Mokusekko 3' and 'Resistant Ym No.1' were all resistant to BaYMV and in the derived F_2-populations no one susceptible individual was observed among more than 2,000 plants tested.
It is intended to introduce additional germplasm into our native breeding materials to broaden their genetic basis of BaYMV-resistance. The incorporation of new genes is most rapidly achieved via haploids. About 2,000 androgenetic lines have been recovered meanwhile from crosses involving resistant cultivars like 'Franka' or 'Diana'. About 65% of the lines tested for their BaYMV-reaction proved to be resistant (FOROUGHI-WEHR and FRIEDT 1984), while 50% resistant individuals were expected.

Summary

The soil-borne barley yellow mosaic virus (BaYMV) causes increasing damage in European winter barley crop. Such yield losses can only be prevented by growing resistant varieties. Various resistant cultivars were used for crosses to study the inheritance of their BaYMV-resistance. The results of F_1, F_2, and doubled-haploid populations demonstrate, that the resistance of all the presently used German breeding materials is most probably identically controlled by a single recessive gene. Besides that, various genetic stocks and breeders' strains have been identified, e.g. in Japan. Materials carrying the dominant resistance gene Ym 1 are used in a breeding programme to broaden the genetic basis of BaYMV-resistance in our native winter barley via conventional pedigree selection and haploidy techniques, respectively.

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Purification and serological properties of Soil-borne Oat Stripe Virus

A virus morphologically similar to Soil-borne Wheat Mosaic Virus(SBWMV) has been identified on oat since 1982 in France. In the field first symptoms on winter oat are observed in March-April. In June leaves develop golden stripes similar to those described in Great Britain for a virus probably closely related.

The purification of this virus has been realized in three steps:

1 - Grinding in borat buffer 0,5 M, pH 9, followed by clarification of the plant extract.
2 - Ultracentrifugation of the aqueous phase through a sucrose cushion containing Triton X 100 and solubilization of the pellet in destilled water.
3 - Isopycnic ultracentrifugation in cesium chloride. A monodisperse viral fraction is obtained.

Rabbit antisera are prepared by 4 successive intamuscular injections of purified virus particles (500 to 1000 µg per injection). These antisera react relatively weakly in precipitation tests (1/300 in Ring tests) but in Sandwich ELISA specific reactions are obtained with infected plant extracts diluted 1 : 5000 times. In ELISA an English isolate received from Dr. Plumb (Rothamsted) reacts strongly with the French antiserum. No reactions are observed with two isolates of SBWMV from France and Nebraska.

With indirect ELISA no serological relationships have been demonstrated between this virus and TMV, ORSV, CGMMV and BNYVV. However, with this technique first results show that the virus of oat is serologically related to SBWMV. The best name for this virus seems to be Soil-borne Oat Stripe Virus by analogy to SBWMV the virus firstly isolated from wheat.

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Presence of Soil-borne Wheat Mosaic Virus in France

Soil-borne Wheat Mosaic Virus (SBWMV) has been observed since 1975 in the west part of France. However, at the present time, this virus is responsible of a serious disease only in the south-west part of Paris-Basin. Symptomatologically confusion is possible between SBWMV and Wheat Yellow Mosaic Virus which occurs in the same area. SBWMV has been successfully purified and an antiserum has been prepared. In ELISA French and Nebraska antisera detect specifically different French isolates of this virus. Susceptibility of wheat cultivars has been tested during these last four years in different places of Loir et Cher department. 18 out of 27 cultivars are observed to be resistant or weakly susceptible in field tests. Most of these resistant cultivars multiply the virus in their roots and in their leaves (detected by ELISA or by electron-microscopy). These observations may explain at least patially the benefic effect of soil fumigation with methyl-bromine on wheat cultivars considered as resistant. No transmission by seeds of French isolates of SBWMV has been observed.

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OCCURRENCE OF MAIZE ROUGH DWARF VIRUS IN FRANCE AND SPAIN

In June 1983 a disease survey was made on maize fields in Spain mainly in Aragon and Castilla. A great number of plants with virus like symptoms were observed. A mosaic was frequently found due to Maize Dwarf Mosaic Virus. But, an other kind of reaction was present : a dwarfing and dark green color of some maize plants. A close observation allowed us to observe enations on the lower surfaces of the leaves. Some days later the same kind of symptoms was also found in southern France near Perpignan. In September, in the same area, plants attacked later showed only a shortening of the upper internodes and enations on leaves (Fig. 1), leaf sheaths and husks. The ears were very small with kernels only at the base ; the brace roots were short or absent.

At the same period some dwarfed plants with enations were also present in the south-west of France in the department of Landes.

In both places, Spain and Perpignan area a great number of the planthopper species *Laodelphax striatellus* were detected in and around maize fields.

Purifications trials from leaves or roots material using LOVISOLO (1971 a) procedure with P.E.G. 6000 precipitation and ultracentrifugation led us to obtain a great number of particles. The purified preparation obtained was checked by electron microscopy after staining with 2 % potassium phosphotungstate : spherical particles around 57 nm in diameter were found, probably subviral particles i. e. particles devoided of the outer shell (Fig. 2) ; the particles in sections of virus infected plants being isometric c. 70 nm in diameter (LOVISOLO, 1971 b).

Using the planthopper *Loadelphax striatellus* collected on grasses (*Digitaria sanguinalis, Echinochloa crusgalli*) around maize fields in Perpignan area or healthy insects after transfer on virus infected plants we obtained enations on healthy maize c. v. OH570 after 2 weeks of incubation period.

Agar-gel diffusion tests with crude and purified preparations were positive using antisera obtained from E. LUISONI (Torino, Italy).

According to symptomatology, particle size and serological reactions we could conclude that the disease observed was due to Maize Rough Dwarf Virus, the presence of which in France and Spain is so demonstrated for the first time.

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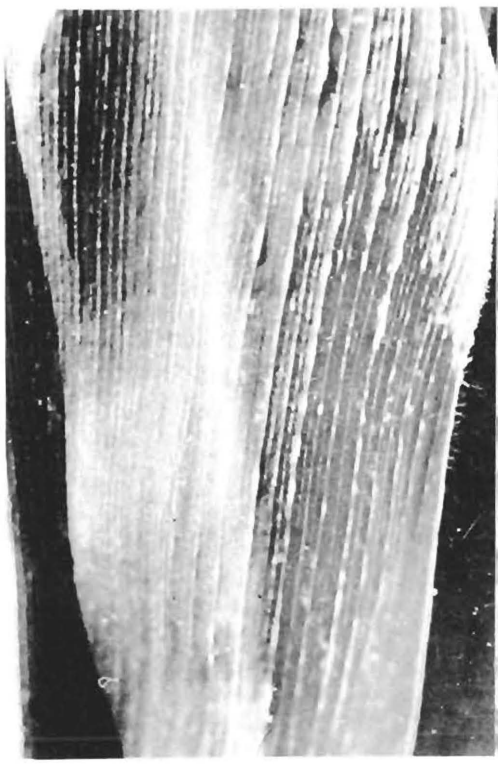

Fig. 1. Part of infected leaf with enations .

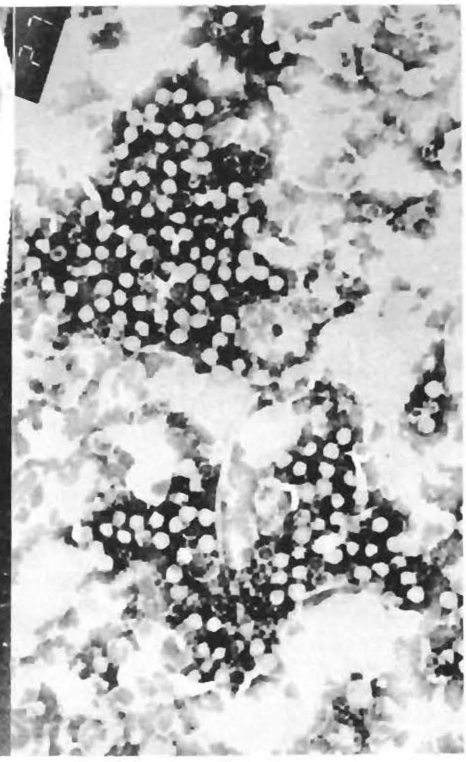

Fig. 2. Particles c. 57 nm from a purified preparation in P.T.A.

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FIRST REPORT OF BROME MOSAIC VIRUS IN FRANCE

During our annual surveys in southern France to follow Maize Viruses situation, since 1980, we had collected in the ditches along Maize fields, wild grasses mainly *Dactylis glomerata* and *Festuca arundinacea* showing mosaic symptoms.

We, later on, tried to isolate and identify the causal agents occuring in these gramineae after aphid and mechanical transfers.

So, the aphid *Rhopalosiphum padi* used as vestor was maintained during two days on these virus source, then transfered onto Maize inbred line F7, Oat cultivar Coast Black (*Avena byzantina*)and Barley cultivar Hudson (*Hordeum sativum*), and finally killed two or three days later. After one (on Maize) or two weeks (on Oat, Barley) typical symptoms of Barley Yellow dwarf virus were obtained similar to those previously described (SIGNORET and ALLIOT, 1980).

By mechanical inoculation on to several gramineae we equally obtained mosaic symptoms and we decided to study in details this other virus (BMV - F) able to produce a bright mosaic on barley plant cultivar Rika to compare the results with those produced by the ATCC strain of Brome Mosaic Virus (BMV - A) obtained from M. BRAKKE (Lincoln, Nebraska).

Host plants

Several gramineae and dicotyledones species were tested. Mechanical inoculations assays were done on seedlings 10-15 days old using extracts of diseased leaves of Rika Barley ground in 0.1 M phosphate buffer pH 7.0 (1 : 10, W/V) celite 545 being added to the sap extract (50 mg/ml) symptoms of mosaic developped on numerous cultivars of wheat, Barley,

ryegrass in about one week whatever the isolate used. Sweet maize seedlings (*Zea mays*) Cv/Golden giant showed primary local lesions in five days, followed rapidly by necrosis and plant death. The mosaic took longer to appear on several grasses (*Lolium perenne*, *L. multiflorum*, *Bromus wildenowii*, *B. carinatus* and *B. sitchensis*). These two last *Bromus* being new hosts for BMV. Only the French isolate induced symptoms on *Dactylis glomerata* and *Festuca arundinacea*.

On bean (*Phaseolus vulgaris* c.v. Top Crop, Red Kidney, Black valentine) and *Vigna unguiculata* (Black and Monarch) small black pin-prick like lesions were obtained in ten days more numerous with our isolate. *Chenopodium amaranticolor* formed pale green lesions turning yellow then white with a red halo.

Stability in sap

We observed a great variation of the properties of the isolates in sap, due to the test plants used and also to the host plant from which the virus was obtained :

Dilution and point on :	ATCC strain	French isolate
Vigna unguiculata	10^{-3}	10^{-4}
Chenopodium amaranticolor	10^{-4}	10^{-5}
Values similar to those published	(10^{-4} - 10^{-5})	

Temperature inactivation point :

For the two strain, results corresponding to the lowest temperature published for B.M.V. (FRANCKI, 1980).

Purification

Several methods were checked ; the one of CHIU and SILL (1963) using a butanol clarification was not satisfactory, the final suspension being slightly green, we have modified the time and speed of the ultracentrifugation to obtain a good yield of 2 g by kilogramme of fresh leaves. The PFEIFFER and HIRTH method (1974) need also two days of work for a similar yield. Finally for routine work we used the LANE (1977) simple method after the following modifications : the leaves were ground with two instead of one volume buffer and only one precipitation of the virus with polyethylene glycol M. wt 6 000 was done. The method is fast (half a day) giving a yield higher than 2 g by kg of leaves. In general with the U.S. strain we have always obtained a lower yield average 1.3 g/kg instead

2.1. g/kg with isolate.

Serology

In order to prepare an antisera the CHIU and SILL (1963) method with our modification was used followed by a density gradient centrifugation : this method being very close to that one used by VON WECHMAR and VAN REGENMORTEL (1968) in studying BMV serology. Antisera with dilution and points of at least 1/512 were obtained against the two strains. Immuno-diffusion tests in Petri dishes (Fig. 1) with 1 p. cent Agar showed that the antigens (BMV - A and BMV F) combine with the same antibodies showing us that our French virus isolate is serologically identical to the ATCC BMV - A Strain.

Particle structure

Particles are isolmetric (Fig. 2) having an average diameter of 26 nm with hollow centres roughly 8 nm in diameter values identical to those published by ANDREREGG *and al.* (1963), CHAUVIN *and al.* (1978). Comparative electrophoresis of the two strains on 2.5 p. cent polyacrylamide gel (PAGE) gave in each case four species of nucleic acid ; corresponding RNA having identical molecular weight.

Conclusion

According to test plant reactions, physical properties, serological tests, type and size of particles, number and size of RNA we could conclude that a strain of Brome mosaic virus was isolated in souther France.

In 1982 several *Bromus* species with mosaic symptoms had been sent to our laboratory from the plant breeding station of INRA (Lusignan) ; we could identify from theses grasses an other strain of BMV showing slight differences with the first one.

The occurrence of BMV in France is so reported for the first time, studies are in progress on the distribution and the transmission of this virus.

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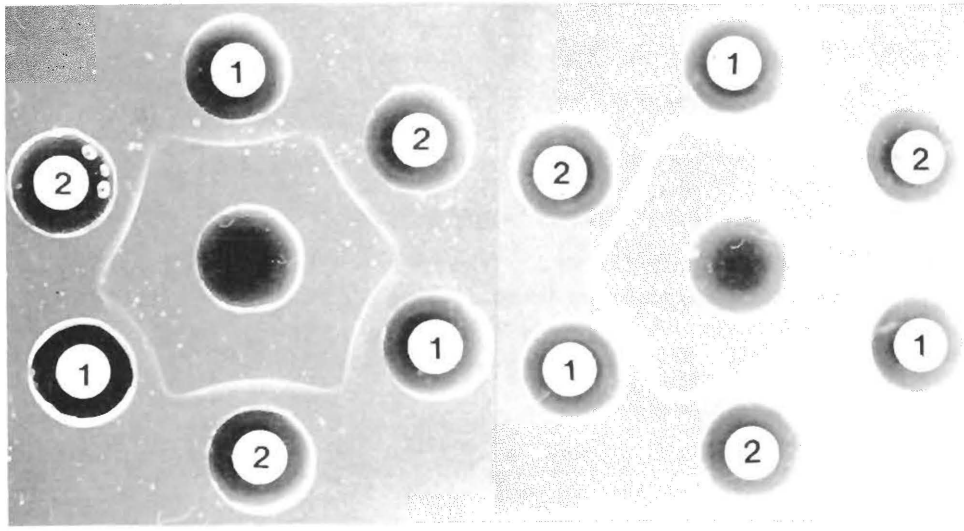

Fig. 1. Reaction of antiserum to Brome mosaic virus strain A (left), strain F (right) to different antigen sources in gel-diffusion plate.
Wells 1 : Purified American strain Wells 2 : Purified French isolate.

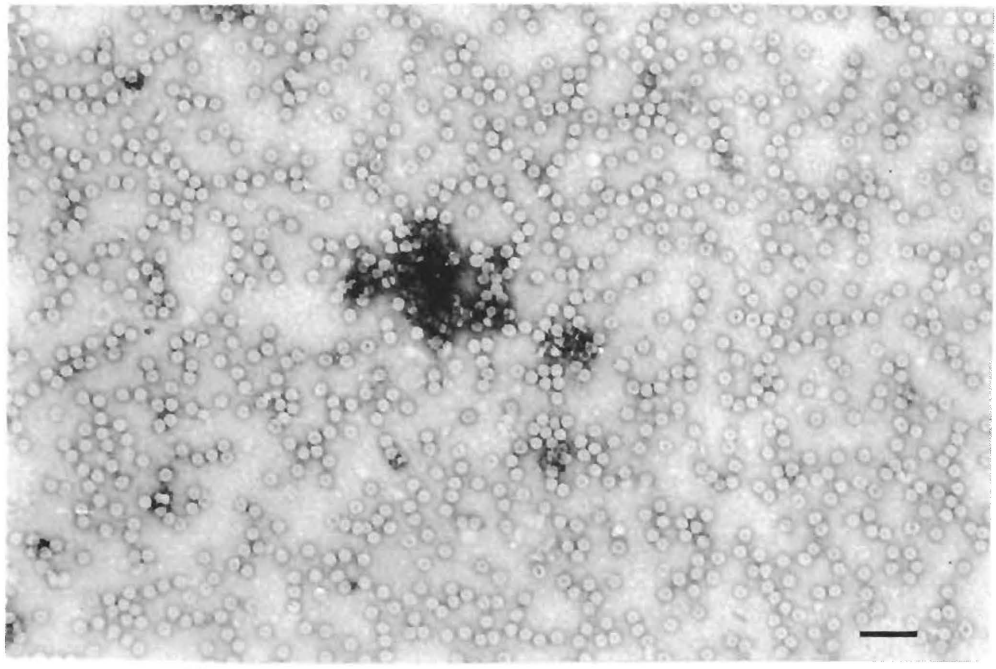

Fig. 2. Virus particles from a purified preparations in 1 p. cent pH 7.0 P.T.A. (Bar = 130 nm).

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Occurrrence and development of hopper-borne cereal diseases in Sweden

Altogether six hopper-borne cereal diseases have been recognized in Sweden (Lindsten 1979a, 1979b, 1980a). Three of these, oat sterile dwarf, cereal tillering disease and European wheat striate mosaic, are transmitted by planthoppers (Fulgoromorpha) and three, wheat dwarf, oat blue dwarf and aster yellows, by leafhoppers (Cicadomorpha). Oat sterile dwarf is for many years the most important one of these diseases and it is mainly this which will be dealt with here.

Planthopper-borne diseases

Oat sterile dwarf (OSD) which is mainly an oat disease causes severe stunting, increased tillering and infected plants will give very poor or no yield. It has Javesella pellucida (Fabr.) as natural vector and is closely connected with its distribution and development. J. pellucida is usually the most common and frequent hopper in cereal fields and occurs all over Sweden but OSD was at first restricted to the central parts of the arable country (mainly X, W and S in Fig. 1).

Damages similar to those of OSD are known from the 1920s but the cause was established first in the late 1950s. Severe outbreaks of OSD seem to come about every 10th year and then continue for 1-4 years ahead depending on the control measures undertaken. Changes in the vector density due to biotic and climatic factors will also influence.

The severe outbreaks of OSD in 1961-62 were mainly limited to the mentioned counties but in following years a spread southwards to C, U, and T counties took place. See Fig. 1. The new outbreaks in the early 1970s were mainly limited to western-

Sweden (R, P, O and S counties) and only slight spread southwards was noticed. The disease disappeared in the middle of the 1970s. However, it returned again in 1980 (Lindsten 1980b) and caused severe damage in certain localities (in C county) but the most severe damage in 1981-82 occurred again in S, P and O counties. A spread southwards was observed and in addition a more long distance spread to F and H counties took place. Severe damages were observed in these latter counties for the first time in 1982. In 1983, on the other hand, very low frequencies of OSD occurred owing to successful control but also owing to low density of the vector caused by climatic factors and predators, especially spiders.

European wheat striate mosaic (EWSM) causes elongated chlorotic streaks not only on wheat but also on barley and oats. Young infected plants often die prematurely. It is spread by the same vector as OSD. However, J. pellucida all over the country is infective with EWSM (often >10% of the hoppers) in contrast to OSD. In spite of this the frequence infected plants is low in the fields and even in years with very high hopper densities no serious damage is caused.

Cereal tillering disease (CTD) which is rather similar to OSD damages barley more than oats in contrast to this . It is transmitted by Laodelphax striatellus (Fallén) which with few exceptions is much less frequent than J. pellucida and has a more southern and limited distribution. Occasionally, e.g. in 1971 and some other years in the 1970s, severe damage by CTD occurred in some localities in E county but usually lack or in any case a too low density of L. striatellus is limiting this disease.

Leafhopper-borne diseases

Of the leafhopper-borne diseases only wheat dwarf (WD), transmitted by Psammotettix alienus (Dahlb), is known to have been of practical importance. In the beginning of this cen-

tury (e.g. 1912, 1915 and 1918) a disease most probably identical to Wd caused disastrous damage to winter wheat in especially E and D counties. The latest severe outbreak seems to have taken place in the 1940s.

High density of P. alienus occurred some years in the 1950s but no damage was reported. During the 1960s and 1970s viruliferous P. alienus and WD-plants were found in various places in C, D and E counties but usually only in low frequencies.

The other two leafhopper-borne diseases, oat blue dwarf and aster yellows, are both transmitted by Macrosteles spp. They have only been found occasionally and seem to be of no importance in Sweden.

Discussion

OSD can easily be controlled by replacing oats with barley, especialy when used as a cover crop for the undersowings. However, to eradicate the virus in an infested area is difficult. The gradual spread of OSD southwards in Sweden is therefore considered as a serious problem (Lindsten, 1984). On the other hand it is surprising how restricted the disease earlier has been to certain counties, and how slowly the virus has spread southwards, in spite of high densities of the vector also in adjacent counties. Sometimes a sudden decrease of the hopper population due to biotic or climatic factors may contribute to stop a further spread (Lindsten, 1984).

The other disease agent (it is still not known if EWSM is caused by a virus) transmitted by J. pellucida is often suppressed by the OSDV both in plants and in the vector. This may be one explanation why EWSM occurs in rather low frequencies in OSD-infected fields. However, it is surprising and difficult to explain why EWSM is not more common and severe in fields with high frequencies of J. pellucida without OSDV, as this species seems to be carrying the disease

agent of EWSM to a very high extent. Apparently J. pellucida is much less efficient as a vector for this disease under natural conditions than in the greenhouse.

First recently the wheat dwarf was shown to be caused by a geminivirus (Lindsten et al. 1980). However, its importance as a disease is almost historic. One reason why WD nowadays seems to be of little importance is that the frequence of the vector in general will be too low. A change in the agricultural practices may have contributed to this but also to a decrease of the availability of virus sources (Lindsten, 1979b).

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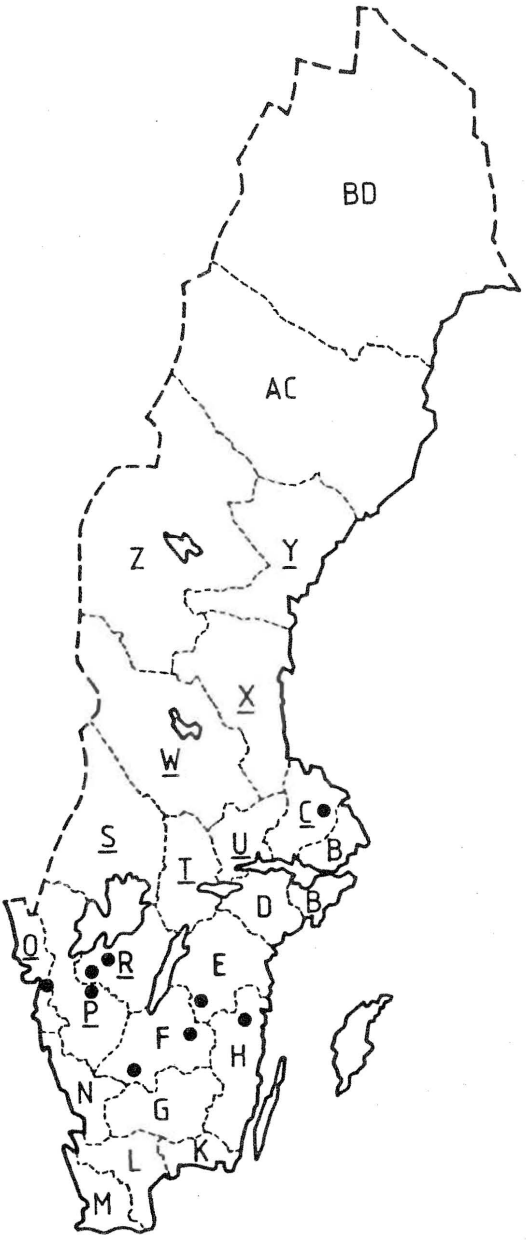

Fig. 1. Map of Sweden showing earlier distribution of oat sterile dwarf (= underlined county letters) and new southern findings during the 1980s (= ●).

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Properties of Virus-Like Particles Isolated from Brachypodium sylvaticum (Huds.) Beauv.

During a survey of amenity grasses, spherical virus-like particles (VLPs) ≈ 32nm in diameter were found in leaf squashes of a botanic garden specimen of Brachypodium sylvaticum (Huds.) Beauv. Symptoms in the abnormal plant consisted of yellow streaks in young leaves. Electron microscopy of ultrathin sections revealed VLPs in the cytoplasm of foliar mesophyll cells. When diseased foliage was the inoculum no agent was manually transmitted to a range of monocotyledonous and dicotyledonous test plants. The particles were purified from plants which were propagated vegetatively from the original diseased plant. Purification was accomplished by acid (pH 4.5) precipitation of the plant protein followed by differential and rate zonal sucrose density gradient centrifugation. In addition to the expected ≈ 32nm particles, smaller, spherical VLPs (≈ 16nm in diameter) were present in the crude leaf homogenate. The smaller particles were separated from the more numerous larger particles during sucrose density gradient centrifugation. An antiserum made against the 32nm VLPs had an homologous titre of 1/512-1/1024 in gel diffusion tests and was observed, with the electron microscope, to clump and decorate the large particles but did not react with particles of a known virus (cherry leaf roll virus) nor did it trap, on antiserum coated grids, VLPs from homogenates of healthy B.sylvaticum leaves.

In heterologous serological tests, neither the purified VLP (32nm) nor sap from diseased leaves reacted with antisera to the following viruses: arabis mosaic[1, 10], barley yellow dwarf[11], bean pod mottle[10], belladonna mottle[3], broad bean mottle[2], broad bean stain[7], brome mosaic[1, 3], carnation ringspot[3], cherry leaf roll[1], chicory yellow mottle[7], cocksfoot mild mosaic[7], cocksfoot mottle[4, 10], cowpea mosaic-yellow strain[10], cymbidium ringspot[10], cynosurus mottle[4], cymbidium ringspot[10], Dinebra retroflexa[8], Echtes Ackerbohnenmosaik[7], elderberry latent[7], galinsago mosaic[5], lucerne transient streak[7], maize white line mosaic[6], melandrium yellow fleck[10], molinia streak[4], narcissus tip necrosis[10], pelargonium leaf curl[7], pelargonium flower break[10], pelargonium line pattern[10], pelargonium ring pattern[10], pelargonium ringspot[10], Peru corn[7], phleum

mottle[10], radish mosaic[10], raspberry ringspot[9], red clover mottle[10], red clover necrotic mosaic[10], rice yellow mottle[7], saguaro cactus[3], solanum nodiflorum mottle[10], southern bean mosaic[3], sowbane mosaic[3], squash mosaic[10], strawberry latent ringspot[9], tobacco necrosis[9], tobacco necrosis satellite[9], tobacco ringspot[3], tomato blackring[9], tomato bushy stunt[3], tomato ringspot[3], turnip crinkle[10], turnip rosette[10], and turnip yellow mosaic[10].

Purified VLPs had an adsorption spectrum typical of nucleoprotein with A_{260}/A_{280} of 1.5. The sedimentation coefficient, determined by centrifugation in a Beckman Model E analytical ultracentrifuge with Schleiren optics was 122S extrapolated to infinite dilution. Buoyant density measurements, calculated from the refractive indices of VLP-containing fractions of Cs_2SO_4 and CsCl gradients after isopycnic centrifugation, were 1.33 and 1.35g cm^{-3}. The estimated RNA content of the particles based on their density in CsCl was 19-20%.

In SDS-polyacrylamide gels, the VLP (structural) protein migrated as one major band with a MW of ≈ 37,600 although one or sometimes two minor bands with MWs ≈ 35,000 and 32,600 were seen in some preparations. Nucleic acid extracted from the VLP was degraded by RNase but not DNase. Under non-denaturing conditions, one major species of RNA with a MW of 1.67×10^6 was detected on agarose gels. Purified RNA acted as a messenger, incorporating ^{35}S-methionine into protein, in a rabbit reticulocyte system.

The VLP from B.sylvaticum did not fit neatly into any accepted group of plant viruses and was serologically unrelated to a diverse array of viruses having spherical particles and RNA genomes. However, the size, rough looking surface and outline of the particles resembled those of tombusviruses. Although the MW of the major polypeptide (≈ 37,600) and

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[1] Our antisera; [2] Supplied by Dr W.P. Mowat, Scottish Crops Research Institute (SCRI), Invergowrie, Dundee, UK; [3] American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, U.S.A.
[4] Dr W. Huth, Institut für Viruskrankheiten der Pflanzen, Braunschweig, F.R. Germany; [5] Dr R.I.B. Francki, Dept of Plant Pathology, Waite Agricultural Research Institute, Glen Osmond, South Australia; [6] Dr A.R. Gotlieb, Botany Dept, University of Vermont, Burlington, VT, USA; [7] Tested by Dr T. Jones, SCRI; [8] Tested by Dr R.S. Greber, Dept of Primary Industries, Plant Pathology Branch, Indooroopilly, Queensland, Australia; [9] Dr A.F. Murant, SCRI; [10] Tested by Dr O.M. Stone, Glasshouse Crops Research Institute, Littlehampton, Sussex, UK; [11] Tested by Dr C. Lyons, Long Ashton Research Station, Bristol, UK.

RNA species (1.67 x 10^6) of the VLP was near to that of tomato bushy stunt (41,000 and 1.5-1.65 x 10^6), the sedimentation coefficient (≈ 122S) was less than those for definitive tombusviruses (132-140S) but near to that of saguaro cactus virus (≈ 118S), a tentative tombus group member (Martelli, 1981). Recently, a non-sap transmissible spherical virus with a protein MW (≈ 40,000) was reported in the grass species Dinebra retroflexa (Vahl.) Pang. and Panicum maximum var. trichoglume Robyns in Australia, but it did not react with our antiserum.

This is a brief report of work which will be described in detail elsewhere.

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Viruses Found in a Survey of Amenity Grasses in the UK

Species of graminae which are not involved in intensive agriculture cover about 10% of the land area of the UK, yet little is known about the viruses which infect them. Here we report the results of a survey in which grasses/sedges from 11 sites were tested for viruses. Material was tested from semi-natural habitats such as meadows and upland pastures, specimen collections in botanic gardens and trial plots of turf grasses from commercial breeders.

Samples (850) representing 281 species in 91 genera were tested by one or more of the following methods (see Edwards, Cooper and Massalski, 1983, for details): (1) manual inoculation of homogenized leaves to test plants, (2) examination of leaf squashes in the electron microscope and (3) enzyme-linked immunosorbent assay (ELISA) using the following antisera: arabis mosaic (AMV), raspberry ringspot virus (RRV), strawberry latent ringspot virus (SLRV), tomato blackring virus (TBRV) from the Scottish Crop Research Institute (SCRI), Invergowrie, Dundee, UK; brome mosaic virus (BMV) PVAS 178, American Type Culture Collection (ATCC); cocksfoot mild mosaic virus (CMMV), Dr W. Huth, Institut für Viruskrankheiten der Pflanzen, Braunschweig, F.R. Germany; and cockfoot streak (CSV), Dr L. Torrance, Ministry of Agriculture, Fisheries and Food, Plant Pathology Laboratory, Hatching Green, Harpenden, Herts, UK.

Evidence for virus infection was found in about 4% of the samples. Eight agents were identified serologically in test plants as TBRV and tobacco necrosis virus (TNV) (antiserum against strain D, from SCRI). TNV was transmitted from seven samples of Festuca rubra L. and F.tenuifolia (Sibth.) and TBRV from one plant of Molinia caerulea (L.) Moench. var. breviramosa to test plants of Chenopodium quinoa Willd. However, we did not exclude the possibility that TNV was contaminating Festuca foliage. Furthermore, the apparent infection of Molinia with TBRV was not confirmed when ELISA tests were done using frozen foliage of the Molinia plant as a source of antigen. Indeed, of the 572 samples of 242 species tested by ELISA for the nepoviruses TBRV, AMV, RRV and SLRV, none proved to be positive. By contrast, BMV was identified by ELISA and/or immunosorbent electron microscopy in eight grass species/cultivars; one isolate, from M.caerulea was indistinguishable from isolate 178 (ATCC)

when compared in gel diffusion tests using antisera to either isolate (Edwards, Cooper and Massalski, 1983). Infection by CMMV and CSV/CCV was inferred from positive ELISA tests on 2.5% of 281 samples. These included one Chloridoid, one Panicoid and five Pooid species (Table). All were from botanic gardens or breeders collections; Andropogon scoparius, Dactylis maritima and Uniola paniculata are North American species. CMMV-positive Melica nutans and U.paniculata also reacted with an antiserum (Dr L. Torrance) to a Scottish isolate of CMMV from Phleum pratense L.

A range of virus isolates closely related to CMMV are known from several Pooid species in the UK and the F.R. Germany (see Torrance and Harrison, 1981) but the virus has not been reported previously from Melica nutans. This is the first record of CMMV naturally infecting a Chloridoid species, U.paniculata. We did not distinguish whether both CSV and CCV occurred together or separately. However, CSV which is common in the UK and continental Europe (Catherall, 1971) and which experimentally infected Cynosurus cristatus (Schumann, 1969) has not been reported previously in natural infections of D.maritima, C.cristatus, C.echinatus and a Panicoid species, A.scoparius.

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Table. Species which were ELISA positive for CMMV and CSV*

Grasses	Test results	
	ELISA positives**	EM
Chloridoideae		
Uniola paniculata L. (1) site 3	CMMV	negative
Panicoideae		
Andropogon scoparius Michx. (3) site 4	CSV	negative
Pooidae		
Cynosurus cristatus L. (8) sites 7	CSV	negative
Cynosurus echinatus L. (4) site 3	CSV	filamentous VLPs
Dactylis glomerata L. (17) site 5	CSV	filamentous VLPs
Dactylis maritima Walt. (1) site 3	CSV	negative
Melica nutans L. (4) site 3	CMMV	spherical VLPs

* The serum also reacted against a spherical virus-like particle that has not been characterized.

** A_{405} values > 0.5.

In parentheses, no. of specimens tested.

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Resistances of grasses to two Sobemoviruses, cocksfoot mottle and cynosurus mottle

Two Sobemoviruses infect grasses in Britain and in several other European countries. They are cocksfoot mottle virus (CfMV) which attacks Dactylis glomerata (cocksfoot) and cynosurus mottle virus (CyMV) which attacks Cynosurus cristatus (crested dog's-tail). Each virus is lethal in a large proportion of plants of its natural host species.

However, some genotypes of each species were found to survive, usually with reduced vigour. Thus, fewer plants (60%) from a Belgian sample of C.cristatus developed the usual severe, lethal mottling than from a Portugese sample (94%). Infected plants from a CfMV-resistant cocksfoot (cv. Cambria) were found to be generally much more aggressive in the competitive environment of the sward than infected plants from the susceptible cv. S.37. Thus, Cambria populations outyielded comparable populations of S.37 by only 7% when healthy, but by 40% when infected with CfMV. Mortality in Cambria was only 32% compared with 65% in S.37. Presumably, it is its greater survival rate and aggressiveness when it is infected which enables Cambria to maintain a close-knit and productive sward during CfMV epidemics. With S.37, the high death rate will obviously permit an ingress of weeds, and it is probably the competition of these against the surviving, but seriously weakened, remnants of cocksfoot which degenerates swards of this cv. to extinction.

A few genotypes of Cambria cocksfoot were found which recovered completely from infection with CfMV: likewise, a few genotypes of C.cristatus were found which recovered from infection with CyMV. Table 1 summarises the results of studies on the disease status of cocksfoot and C.cristatus genotypes when first inoculated, following their recovery, and following re-inoculation. Recovered cocksfoot genotypes appeared to acquire immunity from further infection, but recovered genotypes of C.cristatus remained susceptible to re-infection. Possibly, CfMV and CyMV infections trigger the production of some antiviral agent(s) which blocks virus replication. In the case of recovered cocksfoots, production of the agent(s) would seem to continue even in the absence of the inducing virus, but in the case of recovered C.cristatus, production of the agent(s) would appear to cease when it is no longer required.

Table 1. Disease status of genotypes of cocksfoot and Cynosurus cristatus capable of recovering from virus infection, when first inoculated, following recovery, and following re-inoculation

	Detection of virus by:			
	Foliar symptoms	Transmission to indicator hosts	Electron microscopy	Gel-diffusion serology
Cocksfoot plants inoculated with CfMV				
1st inoculation	+	+	0	++
Recovered plant	-	-	-	-
2nd inoculation	-	-	-	-
C.cristatus plants inoculated with CyMV				
1st inoculation	++	+++	+++	+++
Recovered plant	-	-	-	-
2nd inoculation	++	+++	+++	+++

- nothing detected; +, ++, +++ increasing ease of detection
0 no information

Unlike in the case of ryegrasses (Lolium spp.) which resist infection by ryegrass mosaic virus (Salehuzzaman and Wilkins, 1983), any antiviral agent(s) present in resistant cocksfoot and C.cristatus plants were ineffective in vitro and failed to reduce the infectivity of CfMV- or CyMV-inocula when added to them.

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Evaluation of barley for resistance to barley stripe mosaic virus

Using the host-virus combination barley - barley stripe mosaic virus (BSMV), we determinated the resistance of different host genotypes and compared the disease indices with the actual presence of the virus by serological methods.

Material and Methods

In our investigations we used the defined virus strains "Russia", "Norwich", "Type" and later "Rothamstedt". In initial experiments the valuation-method by KUHN and SMITH (1977) for our virus-host combination had proved to be more suitable than that one by HAGEDORN and HAMPTON (1975). With the former method the disease-indices, in the following defined as D I, were found out as follows. 6, 11, 15 and 21 d pi we detected the percentage figures of infected plants and put them in the following formula:

$$D\ I = 4W + 3X + 2Y + Z$$

These valuation dates were choosen, because only plants of susceptible varieties showed symptoms before the 6th day, and after 21st day no additional plants with symptoms were added. The tests for the resistance behaviour of the varieties and origins of barley were performed in the months of April till September in 3 repetitions in the greenhouse. In dependence on the ability of germination 50 to 100 single plants per repetition and varieties could be tested.

For examining whether the symptoms, and therewith the D I-

values, reflected the real virus affect, this one was qualitatively and quantitatively determined. For the quantitative verification of virus by the radial-immuno-diffussion test (RIDT) according to MANCINI and others (1965), for the qualitative one, respectively, with the simplified RIDT after SLACK and SHEPHERD (1975), we used antisera in each case against the dissociated virus proteins (D-proteins).
For the qualitative verification of BSMV in single plants leaf pieces of symptom-carrying and symptomless plants from a selected spectrum of varieties were put in agar and incubated for one week at room temperature. After removing the leaf pieces the precipitates around the recess spot were valued.

For the quantitative determination of BSMV leaf sap, after adding the same volume of 5 per cent pyrrolidine, in each case 10 µl, was instilled into the depots of the agar plates, after one week's incubation at room temperature it was evaluated by means of a cytoplast and eyepiece micrometer. For the determination of a calibration curve we prepared dilution series from 0.7 to 0.1 mg virus per ml. On representing graphically the relationship between antigen concentration and size of precipitates we used radius-squares of the precipitates. The antigen amounts per ml reached values up to 1.8 mg.

Results:

By utilizing the valuation procedure by KUHN and SMITH (1977) values between 0 and 1000 for the varieties or origins, in each case, can be determined. For classifying these with regard to the degree of resistance we undertook putting them in categories. A variety and origin, respectively, is marked us as resistant, if the D I-value lies under 100, and as susceptible, if the value exeeds the value of 500. D I-values between 100 and 500 then reflect a medium resistance.

Not all results being represented here we would like to give you, as an example some values obtained with the strain "Russia" in the following table representing, in each case, medium values from 3 single experiments:

D I for the resistance of different host genotypes to BSMV

host genotypes	disease index D I $\bar{x}$	s.d.	LSD (TUCKEY) (α = 5 %)	category
C. I. 4998	0	0		resistant
C. I. 1119	0	0		"
C. I. 4199	32,67	23,86		"
C. I. 4219	217,00	175,35		middle resistant
C. I. 4505	375,67	198,61		"
C. I. 4385	391,00	111,70		"
Xenia	745,33	180,27		susceptible
C. I. 666	899,33	94,11		"

By reason of a two-factorial analysis of variance it could be shown that the factors strain and variety had a significant influence on the size of the D I-values (α = 5 %). The limiting differences were determined by Tuckey test. The varieties and origins connected with a line in the table do not differ significantly. The differences between the categories "resistant" and "susceptible" are always significant, whereas this is only partly the case between medium-resistant, susceptible and resistant varieties and origins, respectively. The justification putting these categories of the varieties and origins of barley examined in resistance-classes is also obvious, if the per cent of symptom-carrying plants of all varieties and origins of barley in one disease-category at the different valuation-dates is determined and represented in curves. Already 6 days after inoculation, in dependence on the strain, 50 to 70 % of the plants of the category "susceptible" showed symptoms. The plants of the

origins classified as "resistant" were only infected, on the other hand, at 1 to 2 % at that date, the "medium-resistant" ones at 4 to 18 %. By the end of the valuation, on the 21st day, 9 % of the plants in maximum showed symptoms in the resistant varieties, whereas there were 40 to 60 % in the medium-resistant varieties and over 80 % in the susceptible ones. Alltogether, 17 origins could be put in categories as "resistant" to one or several strains of BSMV.

It was to be examined by the simplified RIDT, whether the symptom-formation reflected the real virus affect. This test on latency was performed on single plants of a selected varieties-spectrum. The results of the virus-identification by serology and the valuations of symptoms were compared with the Mc NEMAR-sign test and examined on significance as well. It appeared that there exists a good coincidence between the occurrence of symptoms on the host plants and the detectability of the virus by this serological test after infection with the strains "Russia", "Type" and "Rothamsted". After "Norwich"-infection in some origins of barley there are symptomless virus carriers to be found increasingly. They are concentrated beside a sporadic occurrence above all on certain varieties. Therefore, it can be concluded that symptomless plants generally are not infected and a latent virus affect does not play a great role. The different portion of sympton-free plants consequently reflects a different frequency of infection.

The variations of the virus concentration determined by the quantitative RIDT depending on the time and the plant section distinctly show that, as a rule, the virus concentration rises in the newly-developing leaves. The concentration is at the lowest in the inoculated leaf and increases to the rest above the third leaf. It was striking that in most of the cases a rapid drop of the virus concentration took place in the second and third leaves after a quick rise. Alltogether, it comes to be obvious that the higher D I of the susceptible varieties were also connected with a higher virus concentration than in

the medium-resistant origins. That seems remarkable, because the D I-value was calculated from the number of symptom-carrying plants, and the symptom intensity was not taken into account. Having only utilized symptom-carrying plants for the serological tests less individuals were not only infected with medium-resistant varieties, but in these a less virus multiplication took place, too.

Discussion

Extensive investigations on the resistance of barley against BSMV were already carried out on a big assortment with many virus isolates (TIMIAN, 1975), however, searching for resistant genotypes it was only examined, whether symptoms were present or not. By selecting the origins of barley from the world assortment in Beltsville we took TIMIAN'S results into consideration. In experiments of many years he had determined from nearly 5000 varieties and origins of barley 45 as being resistant. By all means, he utilized a field isolate of BSMV for his screening test and he only valued on a presence (susceptible) and non-presence (resistant) of symptoms, respectively. In the following greenhouse experiments with 12 to 20 other BSMV isolates he found out differences in the resistance behaviour of these 45 origins against the different isolates. In extending his studies we included defined BSMV strains in our investigations. Beyond that we were able to prove the great multiplicity in the resistance behaviour of the varieties and origins of barley by means of calculating the disease indices.

A good agreement was found to exist between the appearance of symptoms and the actual presence of BSMV, as evidenced by RIDT. Virus concentrations were higher in susceptible genotypes with high D I values than in genotypes with medium resistance.

Analysis of the D I values revealed generally a constant ranking of the genotypes to the three virus pathotypes (strains) tested. This fact together with the virus behaviour in the

host suggest the presence of horizontal resistance.

Summary

Using the host-virus combination barley - barley stripe mosaic virus (BSMV), we applied the disease index (D I) system of KUHN and SMITH (1977) for evaluation of the resistance of different host genotypes to three strains of BSMV after mechanical inoculation. By means of these indices we were able determining three categories of host reactions: "Resistant" (D I = 0 to 100), "middle resistant" (D I = 101 to 500) and "susceptible" (D I = 501 to 1000).
Good agreement was established between the appearance of symptoms on barley plants and the actual presence of BSMV, as evidenced by a simplified radial immunodiffusion technique (RIDT).
Virus concentration were higher in susceptible genotypes with high D I values than in genotypes with medium resistance. Thus, the latter ones are characterized not only by a lower infection rate but also by reduced virus multiplication.
According to the D I values and the results of serological tests, the resistance type of the virus-host combination under review is classified as resistance to infection (horizontal resistance).

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