THE EPIDEMIOLOGY OF BARLEY YELLOW DWARF VIRUS IN CEREALS AND GRASSES

by

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SUMMARY

The transmission rates of the RPV, PAV and MAV strains of barley yellow dwarf virus (BYDV) by *Rhopalosiphum padi* and *Sitobion avenae* were examined. The transmission of virus was investigated between perennial ryegrass cv. Talbot (*Lolium perenne* L.) and several winter barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.) cultivars (cv.).

The incidence of BYDV in cereal test plants was identified by visual symptoms of the disease, i.e. yellow or red leaves and stunting. The strains of BYDV in infected plants were confirmed by enzyme-linked immunisorbent assay (ELISA). The incidence of BYDV in perennial ryegrass was always identified by ELISA as ryegrass test plants did not exhibit symptoms of the disease.

In general, *R. padi* was a more efficient vector of BYDV than *S. avenae* from source leaves infected with either one, two, or three strains of BYDV.

The three strains of BYDV were transmitted at different rates to each plant cultivar. Generally, the transmission of RPV and PAV by *R. padi* to oat test plants was similar from oat source leaves infected with either one or other of the strains. However, the transmission of PAV was greater than RPV by *R. padi* from both ryegrass and oat source leaves to winter barley plants. In contrast, *R. padi* transmitted the RPV strain at a higher frequency than PAV from oats and barley to ryegrass test plants.

S. avenae transmitted MAV at a higher frequency than PAV from barley or oat source leaves to oat test plants. However, the transmission rate of PAV by *S. avenae* was affected by the age of the source leaves. The vector was more likely to transmit PAV from young leaves than from old ones. The transmission rate of either PAV or MAV by *S. avenae* was poor to winter barley. *S. avenae* rarely inoculated any cereal plant with RPV, while *R. padi* was unable to transmit the MAV strain to cereals from leaves infected with that strain alone.

R. padi was, however, able to transmit MAV together with RPV from cereal leaves infected with both strains. Similarly, the same species infected oat plants with PAV + MAV, although less readily, from source leaves containing both strains. *S. avenae* succeeded in transmitting MAV at a high frequency from the former combination, but did not transmit either strain from the latter, which was unusual as it is a vector of both PAV and MAV.

S. avenae transmitted PAV and MAV together from a mixture containing RPV, PAV and MAV from oat cv. M. Tabard to oat cv. Pennalt. Transmission of BYDV by this vector from such mixtures was variable however. For example, *S. avenae* did transmit BYDV (PAV and MAV either alone or in combination) from perennial ryegrass cv. Talbot to oat cv. Dula, from cv. Dula to winter barley cv. Igri and from cv. Igri to cv. Dula. Transmission did not take place between the latter cultivars in two repeat experiments, or from ryegrass to cv. Igri, or between cv. Dula. *R. padi*, on

the other hand, did transmit BYDV in all experiments from source leaves containing the three strains. Interestingly, RPV + MAV was not transmitted by *R. padi* from triple-infected source leaves, although PAV + MAV was.

The transmission by *R. padi* of PAV, either alone or in combination, was greater than that of RPV (alone or in a mixture) to cereals from plants containing more than one strain of BYDV. *S. avenae* rarely transmitted RPV and transmitted PAV and MAV at similar frequencies.

In contrast, no PAV alone was transmitted by either vector from winter barley to perennial ryegrass, while MAV either alone, or often in combination with RPV, was detected in the majority of ryegrass plants fed upon by both aphid species. This was unusual as *S. avenae* is not an efficient vector of RPV + MAV. Similarly, *R. padi* does not transmit MAV alone at a high frequency.

Generally, the transmission of BYDV by both vectors was greater from cereal to grass than from cereal to cereal, which in turn was greater than from grass to cereal.

PAV alone and in combination with RPV and/or MAV caused more severe stunting than RPV or MAV alone in winter barley cv. Igri and oat cv. Dula. Further observations revealed that oat plants inoculated at an early age were more readily infected than older plants. A similar trend was not observed with the ryegrass plants.

The incidence and fluctuation in incidence of BYDV detected in ryegrass crops in four areas of south-west and central Scotland, namely Ayrshire, Dumfriesshire, Stirlingshire and Wigtownshire was investigated between March 1988 and February 1989.

BYDV was detected in 93.8% of the grass swards. Most ryegrass samples taken from these swards were shown to contain a mixture of all three strains together. However, the individual strains, both alone and in various combinations, were also detected, although less frequently, in all areas. The exceptions were PAV alone and PAV + MAV which were detected in only a few ryegrass samples from Wigtownshire.

The incidence of BYDV detected in ryegrass crops in south-west and central Scotland declined between March and July 1988, before gradually rising in August. Detection increased sharply in September, especially of the RPV and MAV strains. The incidence gradually decreased over the winter months, before rising (RPV and PAV), or falling slightly (MAV) in February 1989. The percentage detection of all strains in February 1989 was similar to those 12 months earlier in March 1988, with the exception of MAV in Dumfriesshire and Wigtownshire which was higher at the end of 12 months' sampling.

The incidence of the strains of BYDV in ryegrass leys varied between their geographical locations and also between fields within districts. RPV (either alone or in

combination) was the most prevalent strain in Ayrshire, while the incidence of PAV was highest in Wigtownshire and that of MAV was greatest in Dumfriesshire and Stirlingshire. Levels of BYDV detected increased with the age of the sward.

Results from glasshouse experiments, especially those obtained from mixed infections, combined with data obtained from the field survey gave an insight into the role of ryegrass swards and cereal crops in the epidemiology of BYDV.

PLATE 1. Symptoms of BYDV on winter barley in the field.

CHAPTER 1

GENERAL INTRODUCTION

1.1 THE VIRUS

Plant viruses are obligate parasites and few can survive for long outside living tissues. The associations between viruses and their hosts are therefore particularly complex (Thresh, 1978). One such virus is barley yellow dwarf virus (BYDV).

Barley yellow dwarf (BYD) is the most economically significant virus disease of cereals worldwide (Rochow, 1970b; Plumb 1983; Burnett, 1984). This disease affects over 100 species in the family Gramineae, including barley, oats, wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), rice (*Oryza sativa* L.), triticale (*X Triticosecale* Wittmack), rye (*Secale cereale* L.), sorghum (*Sorghum vulgare* Pers.) and many wild grasses (Oswald & Houston, 1953b; Bruehl, 1961; Slykhuis, 1967; Slykhuis *et al.*, 1967).

BYDV consists of isometric particles which have been reported to be from 20 nm to 30 nm in diameter (Rochow & Brakke, 1964; Jensen, 1969b; Paliwal & Sinha, 1970; Gill & Chong, 1976; Rochow & Israel, 1977), with sedimentation coefficients of 115-118 S (Rochow & Brakke, 1964). The virions contain a single component of single-stranded ribonucleic acid (RNA) of molecular weight 2.0×10^{-6} d. The virus is restricted to the phloem tissue of infected plants (Esau, 1957a; Bruehl, 1961). The degeneration of this conductive tissue is a characteristic feature of luteovirus infections (Rochow & Duffus, 1981).

1.2 CLASSIFICATION OF BYDV

BYDV is a member of the luteovirus group (Shepherd *et al.*, 1976) of which the MAV strain (see below) is the type member (Matthews, 1982). Barley yellow dwarf itself is caused by five strains of the virus (Rochow, 1969a; Rochow & Muller, 1971) which share a common host range and induce similar symptoms of chlorosis and stunting in graminaceous hosts (Gildow, 1990). The strains of BYDV are transmitted by over 20 species of aphids (A'Brook, 1981; Irwin & Thresh, 1990) in a circulative, persistent manner, i.e. the aphid is still able to transmit the virus following a moult and remains infective for most of its life.

The different isolates of BYDV were classified into five strains according to their vector specificities (Rochow, 1969a; Rochow & Muller, 1971). The groups were designated acronyms from the initial letters of their principal vector species:

RPV - transmitted specifically by *Rhopalosiphum padi* L., the bird cherry-oat aphid (Plate 2).

MAV - transmitted specifically by *Sitobion avenae* Fabr., the grain aphid (previously placed in the genus *Macrosiphum*, and alternatively referred to as *M. granarium* Kby. (Kloet & Hincks, 1964), Plate 3).

RMV - transmitted specifically by *Rhopalosiphum maidis* Fitch., the cornleaf aphid.

SGV - transmitted specifically by *Schizaphis graminum* Rondani.

PLATE 2. *Rhopalosiphum padi*, adult and nymphs.

PLATE 3. *Sitobion avenae*, adults and nymphs.

PAV - transmitted nonspecifically by *R. padi* and S. avenae.

Research into the properties of these five strains has demonstrated that they form two major groups (Matthews, 1982). Group 1 contains PAV, MAV and SGV variants, and Group 2 contains RPV and RMV variants. These divisions are based on cytopathology, i.e. the ultrastuctural changes found in the tissue of oat plants infected by the different isolates of BYDV (Gill & Chong, 1976, 1979); serology (enzyme immunoassays) using polyclonal (Rochow & Carmichael, 1979) and monoclonal (Hsu *et al.*, 1984) antisera; electrophoretic patterns of double-stranded RNA (dsRNA) extracts from infected plants (Gildow *et al.*, 1983); cloned complementary deoxyribonucleic acid (cDNA) probe studies (Lister *et al.*, 1990); cross-protection studies (Aapola & Rochow, 1971), and serological blocking of virus transmission by aphids (Rochow *et al.*, 1971).

It has been demonstrated using monoclonal antibodies in serologically specific electron microscopy that the RPV, PAV and MAV isolates share a common epitope, i.e. antigen binding site (Diaco *et al.*, 1986). Other serological evidence indicates that BYDV is related to several other luteoviruses such as beet western yellows (BWYV) and soybean dwarf viruses (Duffus, 1977; D'Arcy, 1986; D'Arcy & Hewings, 1986). The RPV, PAV and MAV isolates of BYDV were found to be closely related to BWYV

(Duffus & Rochow, 1978; Rochow & Duffus, 1978). It may be that barley yellow dwarf should be regarded as an overall name for diseases with similar symptoms and effects that are caused by persistently aphid-transmitted viruses, only some of which are serologically related (Plumb, 1983). However, at present BYD is still regarded as separate to the other 'yellows' diseases in the luteovirus group.

1.3 SYMPTOMS OF BYDV INFECTION

The symptoms of BYD vary with the plant species, with the crop variety, the age and physiological condition of the plant at the time infection occurs, the strain of virus and environmental conditions (Rochow & Duffus, 1981; Burnett, 1984).

Barley yellow dwarf was first identified as an aphid-transmitted virus disease by Oswald & Houston in 1951. It was named as such due to the brilliant yellowing of the barley leaves accompanied by moderate to severe stunting of the plants (Oswald & Houston, 1951).

The symptoms of BYDV on cereal crops were first described in detail by Oswald & Houston (1953a). In **barley** plants at the seedling stage, a yellow discoloration begins at the leaf tips within 12 to 15 days following inoculation, and progresses down the whole blade starting at the leaf margins. The colour is not the chlorotic yellow associated with conditions of nitrogen deficiency, cold weather, or saturated soil, but is a

golden/orange yellow. In a few varieties, yellowing may begin as irregular blotches midway in the leaf blade as well as at the tip. These areas later coalesce and leaves become totally yellow. Areas of the plants that remain green are often a darker green than normal, almost a blue-green. A few varieties exhibit a marked serration on the leaf margins. Infected plants can become severely stunted, especially varieties with extreme susceptibility (Plate 4). The tillering process is stimulated, but elongation of internodes at the jointing stage can be completely inhibited with no production of seed heads. Growth ceases in such plants, but they remain alive for surprisingly long periods when not crowded out by adjacent healthy plants. The root system of an infected plant is equally as retarded as the top growth.

New growth produced by plants infected at intermediate growth stages (tillering to jointing) becomes typically yellow, but stunting is less severe. Such plants manage to head but the number of seeds produced per plant is invariably reduced and individual grain weight is lower compared to healthy plants.

Infection of plants at still later stages of growth produces little visual evidence of the disease, except a bright yellowing of the uppermost leaves, particularly the flag leaf.

In **oats** 2 to 3 weeks following inoculation, yellowish-green blotches form near the leaf tip. These

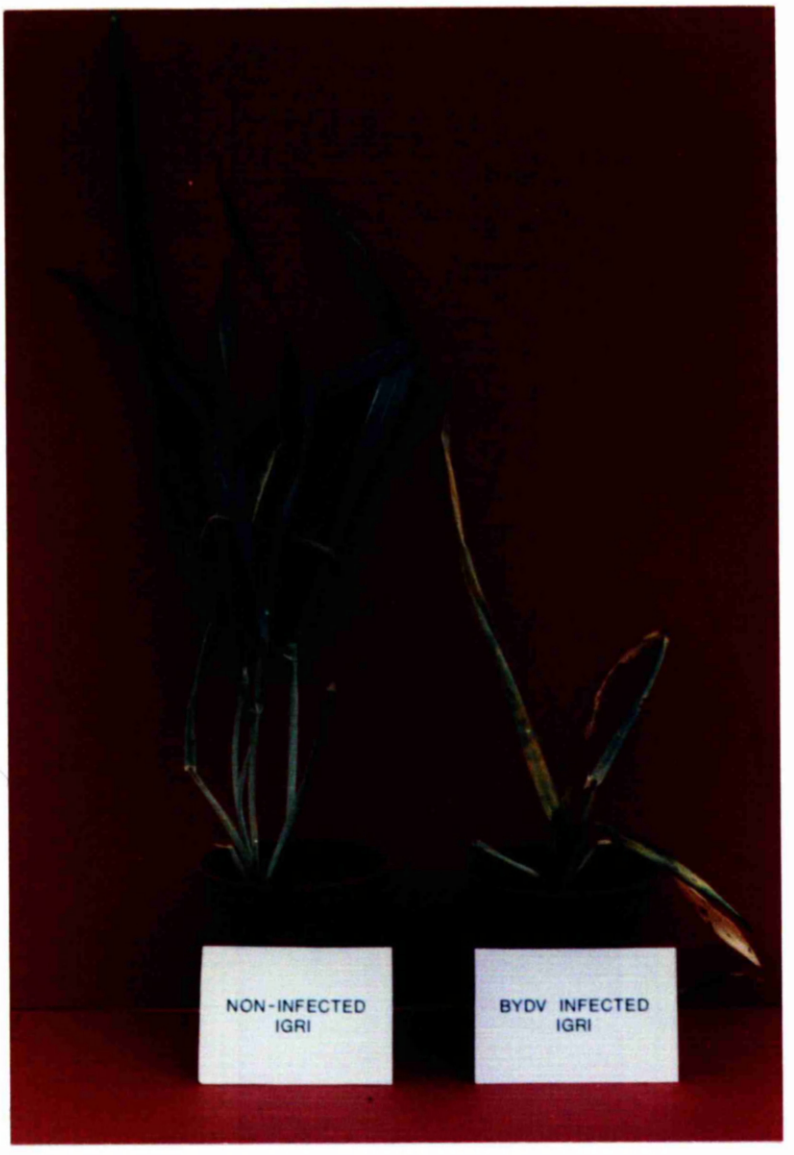


PLATE 4. Symptoms of BYDV on winter barley cv. Igri exhibiting severe stunting and chlorosis (right) in comparison to a healthy plant (left).

blotches soon turn reddish-purple and coalesce leaving the entire leaf tip reddish-purple. The yellow islands in the green tissues (often blue-green) develop in advance of the red discoloration as it progresses down the leaves. Deep serrations of the margins of newly emerging leaves is common in most susceptible oat varieties. It is often so extreme that tips of leaves and sometimes as much as half the blade are completely severed before they emerge from the sheaths (Plate 5).

Depending on the age and variety of the oat plant at inoculation, stunting can be severe (Plate 5). Late infection of oats can be recognised only by the characteristic reddening of the late emerging leaves.

Compared to the previous two plant species, **wheat** is the most severely damaged by BYDV when it is infected at the seedling stage. The first indication of infection is a darker than normal green colour of the outer leaves, a chlorosis of new growth and an overall stunting. Gradually the whole plant becomes chlorotic as growth stops. A suppression of tillering occurs in wheat. Heading is sparse, and the yield from such plants is negligible.

Oswald & Houston (1953a) reported that infection of wheat after tillering is recognised only by bright yellowing starting at the tips of newly-formed leaves with no serration of leaf edges. By contrast, Holmes (1983a) described leaves of infected wheat plants turning red and

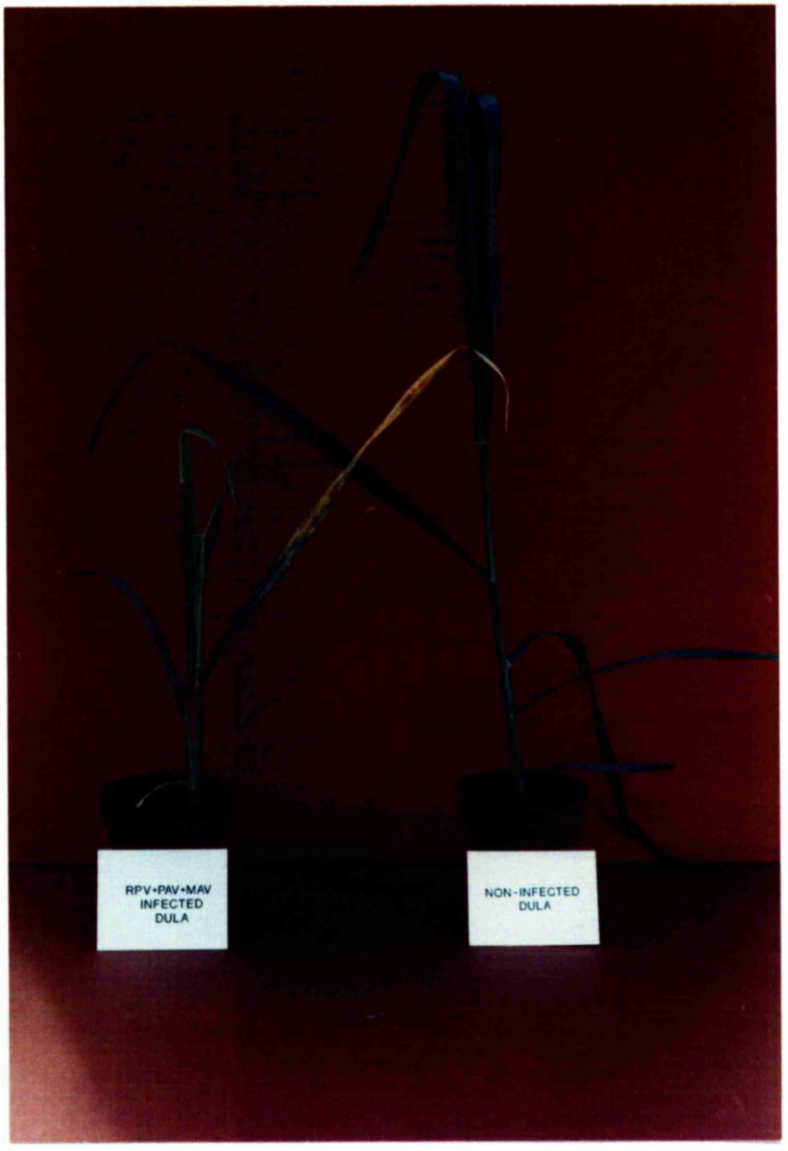


PLATE 5. Symptoms of BYDV on oat cv. Dula exhibiting leaf serration, severed leaf and stunting (left) in comparison to a healthy plant (right).

yellow, while Burnett (1984) commented that leaves of wheat may be serrated.

Oswald & Houston (1953a) only observed one variety of **rye**, which exhibited no discoloration and little if any stunting, when infected with BYDV.

Burnett (1984) described symptoms of BYD in rye and **triticale** as yellow leaves, occasionally showing a little reddening.

Symptoms of BYDV in **maize** are expressed as red-purple discoloration of the lower leaves (Refatti et al., 1990).

In **rice**, infected leaves turn from yellow to orange: the discoloration beginning at the tips and edges and progressing down the leaf (Burnett, 1984).

Foliar symptoms in perennial and Italian (*L. multiflorum* Lam.) **ryegrass** range from brilliant yellow through various shades of yellow/orange and orange/red to orange and crimson (Holmes, 1983b). This colouring gradually extends downwards until 50% of the leaf blade is discolored while the lower portion of the leaf often remains green (Catherall, 1966). Symptoms are vague and can be easily confused with chlorosis and browning caused by adverse environmental or nutritional factors (Catherall, 1966; Catherall & Wilkins, 1977). Moreover, symptoms only appear after defoliation has ceased and the leaf tips are allowed to develop (Catherall & Parry, 1987). Consequently, ryegrass infected with BYDV is

frequently symptomless (Catherall, 1963; Doodson, 1967; Lindsten & Gerhardson, 1969). Infected ryegrass produces more tillers and gives an abnormally higher ratio of vegetative to fertile tillers (Catherall, 1966) compared to uninfected plants.

1.3.1 Factors affecting the development of symptoms

1.3.1.1 CEREALS

(1) TEMPERATURE:

Oats infected with BYDV are much more likely to develop characteristic red-leaf symptoms when cool, rather than when warm temperatures prevail (Rochow, 1969a; Jensen, 1973; Rochow, 1979a; Rochow & Duffus, 1981; Yount & Carroll, 1983). Studies by Jensen (1968b) showed that at 10^{o}C symptoms developed more slowly but eventually were the most severe. At 27^{o}C, symptoms were either very mild or completely masked. At 15^{o}C and 21^{o}C, chlorosis developed rapidly, but dwarfing was not as severe as at 10^{o}C.

(2) LIGHT:

High light intensity is critical for symptom development, not only for test plants in the glasshouse, but also for infected plants in the field (Rochow & Duffus, 1981).

(3) VARIETY OF CEREAL PLANT:

Different varieties of cereal vary in their susceptibility to BYDV. Thus more severe symptoms are observed in some cultivars than in others inoculated with

the same isolate of BYDV (Rochow, 1969a).

The variation in susceptibility is referred to as degrees of tolerance, and several studies have found numerous varieties and species of cereals with various levels of tolerance (Endo & Brown, 1963; Jones & Catherall, 1970; Jedlinski, 1972; Baltenberger *et al.*, 1987). For example, Jenkins (1966) observed a marked stunting of growth in the less tolerant varieties which was associated with extensive leaf yellowing in barley cv. Proctor and with characteristic reddish-purpling of the leaves of oat cv. Blenda. The tolerant oat cvs. Albion and CI7488 displayed some leaf discoloration, while the two Ethiopian barley cvs. CI2325 and CI7224 showed little effect other than slight yellowing of the leaf tips.

(4) AGE OF PLANT:

As mentioned earlier, Oswald & Houston (1953a) noted that symptoms were less severe in plants infected at a late growth stage. Generally, plants inoculated at the 3-leaf stage (GS 13, Zadoks *et al.*, 1974) were damaged most; each delay of inoculation caused successively less damage, i.e. inoculating cereals at early joint, late joint, and early heading stages (Endo & Brown, 1963).

Panayotou (1979) reported that symptom expression in barley, oats and wheat seedlings inoculated 1 week after germination was more severe than in plants inoculated 4 weeks later. Occasionally, plants severely damaged by early infection with BYDV may recover sufficiently to fill

the few kernels produced, whereas late-infected and therefore less severely damaged plants may produce more kernels that can later be adequately filled (Endo & Brown, 1963).

(5) STRAIN OF BYDV:

Although the symptoms expressed by plants infected with the various strains of BYDV differ with the host cultivar, generally the virus strains transmitted nonspecifically by *R. padi* (PAV) cause more severe symptoms in plants than do virus strains transmitted specifically by one aphid species (Gill, 1967; Aapola & Rochow, 1971; Slykhuis, 1976).

Plants infected with two strains of BYDV develop more severe symptoms than those infected with either strain alone (Rochow 1969b; Aapola & Rochow, 1971; Baltenberger *et al.*, 1987). The severity of the symptoms is increased in plants doubly infected with a mixture that includes RPV (Aapola & Rochow, 1971).

In cross-protection tests, prior inoculation with MAV (which generally causes mild to moderate symptoms in cereals) usually prevented the establishment of PAV (which causes severe symptoms) when the challenge inoculation was done 4 or 16 days after the first (Aapola & Rochow, 1971). Similarly, most plants infected with PAV were protected from infection by MAV when the interval between inoculations was 4 or 16 days. No protection was found in any test with RPV and either MAV, or PAV using the same

time interval. Protection was also never detected between strains when the interval between inoculations was 1 day.

1.3.1.2 GRASSES

(1) MANAGEMENT:

As mentioned earlier, symptoms in grasses begin at the leaf tip and rarely extend downwards to cover the entire leaf blade. Symptoms, when they appear, are therefore best seen in ryegrass crops which remain uncut, for seed or conservation (Catherall & Parry, 1987).

(2) SEASON:

Symptoms of BYDV in ryegrass crops left uncut are clearest from April to June (Catherall & Wilkins, 1977; Holmes, 1983b; Catherall & Parry, 1987). Any foliar discoloration occurring later in the year is generally less severe and more easily confused with premature senescence due to unfavourable weather or nutritional imbalance (Catherall & Parry, 1987).

(3) SPECIES OF GRASS PLANTS:

Holmes (1983b) found that the incidence of tillers with symptoms in May, before the first cut, was greatest in perennial ryegrass, least in Italian ryegrass, and intermediate in hybrid ryegrass. However, Italian ryegrass generally had more tillers with symptoms by mid-August compared to the other two grass species.

Panayotou (1985) showed that different varieties of herbage grasses differed in their tolerance to BYDV.

(4) STRAIN OF VIRUS:

Studies on ryegrass crops in the west of Scotland established that symptoms of BYDV were more frequently caused by MAV-like strains of BYDV than by PAV-like strains (Holmes, 1985). Of the ryegrass samples exhibiting foliar symptoms, on average 20% contained MAV, and 1% PAV from 1981 to 1982.

1.4 THE PHYSIOLOGICAL AND BIOLOGICAL EFFECTS OF BYDV INFECTION

1.4.1 Cereals

The primary effect of BYDV is the disruption of translocation through the phloem (Esau, 1957a, 1957b). This results in the leaf chlorosis and stunting described earlier.

Infection by BYDV has been reported to reduce the photosynthetic efficiency of barley by 44-80% (Orlob & Arny, 1961; Jensen, 1968a, 1969a), and by 45% in wheat (Jensen, 1972), and to reduce the chlorophyll content of barley by approximately 50% (Jensen, 1968a) and of wheat by 80% (Jensen, 1972). Soluble carbohydrates and starches have been reported to accumulate in BYDV-infected barley (Orlob & Arny, 1961; Goodman et al., 1965).

BYDV has been found to induce changes in respiration rates in infected cereals. Orlob & Arny (1961) reported that respiration rates expressed in terms of dry weight increased initially in infected barley, but subsequently

decreased as the plants aged. Jensen (1968b) found that levels of respiration were affected by illumination and temperature. Respiration, based on fresh or dry weight, was 60-80% above normal in infected barley plants after 10 h of darkness, but much less than healthy tissues after 5 h of illumination. When plants were grown at various temperatures, the respiration per unit fresh weight of infected plants was generally increased. However, respiration per unit dry weight was above normal at 21°C and 27°C, and below at 10°C and 15°C.

Depending on the variety of cereal and its degree of tolerance, BYDV reduces height, number of spike-bearing tillers, total dry weight, grain yield and seed size, especially in susceptible varieties (Grafton *et al.*, 1982; Baltenberger *et al.*, 1987). Seed from infected barley plants produce seedlings of poor vigour in germination tests (Gill, 1970).

BYDV infection also reduces the tolerance of oat, barley and, to a lesser extent, wheat to low temperatures and contributes to winter kill in cold, temperate regions (Grafton *et al.*, 1982; Paliwal & Andrews, 1990).

1.4.2 Grasses

Infection by BYDV does not decrease plant survival, in fact, infected ryegrass plants produce more tillers, giving a higher ratio of vegetative to fertile tillers than healthy plants (Catherall, 1966; Catherall & Wilkins,

1977). BYDV infection often modifies the pattern of seasonal productivity (Catherall 1966; Catherall & Wilkins, 1977). Upward growth is permitted, even promoted in spring but suppressed in autumn.

Catherall (1966) found that the virus caused a greater loss of yield in simulated ryegrass swards cut twice a year (17.4%) than in those cut four times (8.4%). Decreased yield was due to the stunting of the grass plants.

BYDV infection markedly reduces root growth which inevitably reduces water and mineral uptake (Catherall & Parry, 1987). In areas prone to drought with no irrigation, this may result in plant death (Irwin & Thresh, 1990). The decreased root to shoot ratios may also be unable to sustain the aggressive growth necessary to combat weed invasion (Catherall & Parry, 1987). Catherall (1987) showed that BYDV reduces the competitiveness of perennial ryegrass more so than of Italian ryegrass.

The production of vegetative tillers and dwarfing combined with the initial aggressiveness and persistence may retard or inhibit compensation from healthy individuals and, under certain managements (frequent cutting), the diseased plants may dominate the sward (Catherall, 1966).

1.5 TRANSMISSION OF BYDV

As BYDV is restricted to the phloem tissue of infected plants (Esau, 1957a; Bruehl, 1961), and cannot be mechanically transferred or transmitted through seed (Oswald & Houston, 1953a), it is entirely dependent on aphid vectors for its dissemination to new plant hosts.

1.5.1 Process of virus acquisition

To transmit BYDV, the aphid must successfully penetrate the phloem cells with its stylet. Once the virus is ingested into the food canal within the stylet, the virions suspended in phloem sap flow through the aphid's foregut and midgut to the hindgut (Gildow, 1990). Once in the hindgut, specific virions can be actively transported through the gut wall by endocytosis into the body cavity (haemocoel) of the aphid (Gildow, 1985). Unacquired virions remain in the hindgut lumen, pass out of the aphid in the honeydew, and cannot be transmitted (Gildow, 1990). The virus does not multiply in the gut (Paliwal & Sinha, 1970).

1.5.2 Process of virus transmission

Once in the haemocoel, virions suspended in the aphid's blood (haemolymph) circulate throughout the haemocoel until they come into contact with the accessory salivary gland. This form of mechanism is referred to as circulative (Gildow, 1990). Once in contact with the

salivary gland, the virions pass through the gland cells, and are released into the salivary duct. The duct drains the salivary gland and is connected directly to the salivary canal in the stylet. Virions are excreted, along with salivary cell secretions, into plants during feeding (Gildow, 1990).

Infection of the host plant with BYDV is only successful when the virus is introduced into the phloem (Gill, 1968). Once the virus has reached the conducting cells of the phloem, transport is relatively rapid (Bennett, 1956). The time interval found by Gill (1968) for BYDV to move out of inoculated leaves agreed with suggestions by Schneider (1965) that virus may be introduced into parenchymatous tissue, possibly in the phloem, and then has to move varying distances through this tissue (parenchyma) before it reaches the sieve tubes.

1.5.3 Mechanisms of virus selection

The gut wall acts as a barrier that prevents some BYDV isolates from entering the haemocoel of non-vectors (Gildow, 1990). This virus-membrane interaction is very specific. However, there is a higher degree of specificity regulating the virus-membrane interaction on the salivary gland (Gildow, 1990), since some non-vector aphid species can acquire certain BYDV isolates that they cannot transmit (Rochow & Pang, 1961).

R. padi fed on leaves containing the MGV (MAV) strain could not transmit the strain to test plants. Virus-free *M. granarium (S. avenae)*, injected with haemolymph from these non-vector aphids could transmit the virus to test plants as the species is an efficient vector of MGV. This indicated that the *R. padi* (6%) had been able to acquire the virus, i.e. the MGV virions had passed through the hindgut wall into the haemocoel (Rochow & Pang 1961). However, the virions had not been able to pass through the salivary gland cell wall in *R. padi*, a non-vector, but had succeeded in doing so in *M. granarium,* a specific vector (Rochow & Pang, 1961).

Several other studies (Rochow, 1969a, 1969b; Gildow & Rochow, 1980a, 1980b) have indicated that the ability of an isolate to be transmitted is dependent upon the recognition of that isolate by regulatory mechanisms associated with the aphid salivary gland. Non-vector aphid species micro-injected with highly concentrated purified preparations of BYDV isolates did not transmit BYDV as the particles of the isolates were not recognised and transported through the salivary gland (Rochow, 1969b).

The method of recognition was postulated to involve the protein capsid of the virus (Rochow, 1969b). This was supported by later work on transmission interference by the MAV and PAV strains in their common vector, *S. avenae* (Gildow & Rochow, 1980a).

The transmission of PAV by *S. avenae* was reduced if it had first acquired the MAV strain. In addition, when purified PAV and MAV were injected simultaneously into the haemocoel of *S. avenae*, transmission of PAV decreased as the MAV concentration increased. It was suggested that cell receptors on the salivary gland recognised both PAV and MAV due to a similarity in coat-protein structure. Consequently, MAV and PAV may have been competing for recognition, attachment and transmission through the salivary gland by common cell receptors. If most receptors were saturated with MAV first, attachment and penetration by PAV would be inhibited and expressed as decreased PAV transmission (Gildow & Rochow, 1980a). Transmission interference did not occur in *R. padi* between RPV and PAV. It was assumed that RPV and PAV did not share similar coat-protein structures, and so were recognised by independent receptors. Thus competition for sites would not occur (Gildow & Rochow, 1980a).

Further support for the virus-specific receptor concept came from studies where the protein capsid of MAV was altered by UV-irradiation. As a result, MAV did not interfere with PAV transmission, as altered MAV could not compete with PAV for receptor sites (Gildow & Rochow, 1980b).

The importance of the virus capsid protein and the regulatory receptor mechanism was demonstrated by the phenomenon of heterologous encapsidation.

1.5.4 Heterologous encapsidation

Rochow (1977) defined heterologous encapsidation as "the general phenomenon of nucleic acid of one virus becoming enclosed within a capsid derived wholly, or in part, from synthesis of a second virus". This describes the phenomenon of dependent transmission of BYDV from mixed infections. Other terms used to describe this phenomenon are 'genomic masking', 'transcapsidation', and 'phenotypic mixing'. Transcapsidation is when "the nucleic acid of one virus becomes enclosed in a complete coat of the second virus", i.e. when two different BYDV strains multiply simultaneously in the same plant, the RNA of one strain becomes encapsidated in the coat protein of the other. Phenotypic mixing describes "virus particles that contain nucleic acid of one virus enclosed in a protein capsid synthesised under direction of two viruses", i.e. the RNA of one virus is encapsidated by protein subunits from two viruses (Rochow, 1977).

The most studied form of dependent transmission is the interaction between the RPV and MAV strains of BYDV within oats (Rochow, 1965, 1970a, 1972, 1973, 1977; Rochow & Gill, 1978; Gildow & Rochow, 1980a; Rochow, 1982a). *R. padi* generally does not transmit the MAV strain of BYDV when fed on MAV-infected plants, but frequently transmits MAV from plants infected with both MAV and RPV. The RNA of MAV becomes incorporated into a capsid of RPV coat protein during simultaneous replication of the two viruses. This form of dependent transmission is not

reciprocal as *S. avenae* can only transmit MAV from a mixed infection of RPV and MAV (Rochow, 1969a, 1982a).

This combined transmission by *R. padi* was described as transcapsidation, or genomic masking (Rochow 1970a, 1972). Neutralizing studies by Rochow (1970a) suggested that genomic masking was a function of the RPV coat protein. These studies involved blocking MAV with MAV antiserum in preparations made from plants doubly infected with MAV and RPV. *S. avenae* subsequently could not transmit MAV to oats, but *R. padi* could. The MAV transmitted by *R. padi* was neutralized by RPV antiserum.

R. padi, fed through Parafilm on concentrated mixed preparations of RPV and MAV, could only transmit the RPV strain (Rochow, 1970a). This indicated that transcapsidation occurred in the source plant and not within the vector (Rochow, 1973). The cell receptors on the salivary gland would recognise and transmit all particles with RPV capsids (including 'masked' MAV strains) regardless of their RNA content (Gildow & Rochow, 1980a). It appears that the transcapsidated virus particles function in *R. padi* as RPV because of the protein capsid, but as MAV in the plant due to the MAV nucleic acid (Rochow, 1970a, 1973, 1977).

Other forms of dependent transmission from doubly infected plants have been found:

(1) *R. maidis* rarely transmits the MAV strain from MAV-infected plants, but often transmits MAV together

with the RMV strain from double-infected plants (Rochow, 1975).

(2) *R. maidis* transmits RPV in the presence of RMV at a low percentage of transmission (Rochow, 1982a).

(3) *R. padi* is able to transmit RMV and SGV in the presence of RPV (Rochow, 1982a).

(4) The PAV strain is effective in enabling *R. padi* to transmit MAV, but somewhat less effective in enabling *R. padi* to transmit RMV (Rochow, 1982a).

R. padi readily maintains mixed infections of RMV and RPV, SGV and RPV, and MAV and RPV through successive serial transfers. However, successive serial transmissions of RPV and RMV, or MAV and RMV by *R. maidis* usually results in the loss of the dependent virus (RPV or MAV), so that only RMV (helper virus) remains (Rochow, 1975, 1982a). It was postulated that a different kind of heterologous encapsidation (phenotypic mixing rather than transcapsidation) occurred in the reciprocal dependent virus transmission of the serologically related RPV and RMV isolates (Rochow, 1982a).

1.5.5 Factors affecting transmission and vector specificity

(1) LENGTH OF ACQUISITION FEEDING PERIOD:

In general, the longer the acquisition feeding period, the greater the probability of virus transmission by a 'non-vector'. For example, *R. padi* which had been allowed acquisition feeds of 3 to 5 days on MAV-infected plants,

succeeded in transmitting MAV to 7% of test plants (Rochow, 1961).

The MAV isolate transmitted by *R. padi* was not a variant or mutation as *S. avenae* could subsequently transmit it to 100% of test plants. This occasional loss of specificity was neither due to a variation in the aphid colony as progeny of *R. padi* that had transmitted the MAV were no more able to transmit MAV in subsequent tests than were progeny of individuals that had failed to transmit the virus (Price *et al.*, 1971).

Paliwal & Sinha (1970) demonstrated that the proportion of aphids (*S. avenae*) that transmitted BYDV (MAV) was in a linear relationship with the length of the acquisition feed over a 24 h period.

(2) SPECIES OF APHID:

The time required for aphids to locate phloem and begin feeding varies with the aphid species. *S. avenae* requires approximately 30 min to locate the phloem, however, after 90 min only 65% of aphids successfully initiate feeding (Scheller & Shukle, 1986). *R. padi* requires 60 min, while *S. graminum* requires 200 min to successfully penetrate phloem (Montllor & Gildow, 1986).

The median latent period (LP50) also varies with the species of aphid (Broek & Gill, 1980). The latent period is "the period beginning when the virus is first ingested and lasting until the virus enters the salivary duct of the stylet and is transmitted to plants during feeding"

(Gildow, 1990). The LP50 is the time required for 50% of an aphid test population to transmit virus (Gildow, 1990). The latent period varies with the strain of BYDV and temperature as well as the species of aphid. At 15°C and 20°C, the LP50 for *S. avenae* transmitting an MAV-like isolate is 65.5 h and 44.5 h, respectively; for *R. padi*, the transmission times for an RPV-like isolate is 50.1 h and 35.0 h, respectively, and 62.4 h and 35.2 h, respectively, for a PAV-like isolate.

It has been noted that, irrespective of the conditions under which transmission takes place, *S. avenae* is generally a less efficient vector of PAV than *R. padi* (Rochow, 1969a; Halbert & Pike, 1985).

(3) CLONES OF APHIDS:

Variations among clones of the same aphid species cause alterations in the strain specificity (Rochow, 1969b; Irwin & Thresh, 1990).

A clone of *R. padi* from Kansas, U.S.A., regularly transmitted the RMV strain of virus that was rarely transmitted in parallel tests by the New York clone of *R. padi* (Rochow & Eastop, 1966).

(4) TEMPERATURE:

Transmission of RMV by *R. padi* and *S. avenae* is rare in tests at low temperatures, but the incidence of transmission increases with increasing temperatures, reaching maxima (70% and 83%, respectively) at 30°C (Rochow, 1969a). Transmission of PAV by *S. avenae* and of

RPV, or PAV by *S. graminum* occurred more often at low temperatures than at high ones. The transmission, or vector specificity of other strains or aphid species were not affected over a temperature range of 15-30^{o}C (Rochow, 1969a).

(5) AGE OF SOURCE LEAF:

The age of leaf from which aphids acquired virus influenced virus transmission of some BYDV isolates with certain aphid species (Rochow, 1974; Foxe & Rochow, 1975).

R. padi was more likely to transmit MAV occasionally from young than from old leaves. Similarly, *S. avenae* transmitted PAV regularly only from young leaves. The vector selectivity of the other virus-vector relationships was not affected (Rochow, 1974; Foxe & Rochow, 1975).

1.6 METHODS OF DETECTING BYDV

1.6.1 Symptoms

Diagnosis of BYDV based solely on symptoms is unreliable. As mentioned earlier, several factors can affect the development of symptoms. Moreover, the identification of mixed BYDV strains is impossible, on the basis of symptoms alone.

1.6.2 Aphid transmission tests

The presence and identity of virus can be verified using specific aphid vectors to transmit the virus to

susceptible test plants (Rochow, 1969a). This is complicated when more than one strain of BYDV is present in test samples, e.g. field samples (Rochow & Muller, 1974), and by the phenomenon of dependent transmission (Rochow, 1982a). Differentiating the strains in a mixture requires repeated acquisition and transmission tests which are time-consuming and laborious (Rochow, 1979a).

Other disadvantages are that few samples can be tested during one growing season and much growth chamber and greenhouse space is required (Rochow, 1979b).

1.6.3 Enzyme-linked immunosorbent assay (ELISA)

The ELISA technique uses specific antisera adsorbed to the wells of microtitre plates which selectively trap virus particles. Trapped virus then in turn reacts with a specific enzyme-labelled antibody. The addition of an enzyme substrate produces a colour change in the presence of bound antibody, which can be assessed visually, or quantitatively using a photometer (Clark & Adams, 1977; Torrance & Jones, 1981).

There are several forms of ELISA which are used to detect virus in plant tissue. One is the direct form of ELISA, or double antibody sandwich (DAS), which employs virus-specific antiserum globulin (Ig) conjugated with an enzyme. Another is the indirect form which uses anti-Ig antibodies labelled with an enzyme (Torrance & Jones, 1981). The antibodies used can be either polyclonal or

monoclonal.

1.6.3.1 PRODUCTION OF ANTIBODIES

(1) POLYCLONAL:

Purified virus is emulsified with Freund's complete adjuvant (stimulates the immune response) before being injected into a rabbit. The rabbit produces antibodies specific to the viral antigen. These antibodies are obtained when the animal is bled. The globulin in the antiserum obtained is purified through a series of dialyses (Clark & Adams, 1977; Rochow & Carmichael, 1979; Halk & De Boer, 1985).

(2) MONOCLONAL:

The production of monoclonal antibodies is described in many papers (De St. Groth & Scheidegger, 1980; Kennett *et al.*, 1980; Oi & Herzenberg, 1980; Galfré & Milstein, 1981; Schonherr & Houwink, 1984), which are summarized by Halk & De Boer (1985).

Rats are immunized with purified virus in Freund's adjuvant. After a suitable length of time the rats are killed and their spleens are removed. B-lymphocyte cells (antibody-producing cells) are then fused to myeloma cells (cancer cells) to produce a hybridoma (somatic cell hybrid). The hybridoma acquires from the B-lymphocyte the ability to produce a monospecific antibody (monoclonal antibody), and from the myeloma cell the ability to be cultured indefinitely *in vitro*.

High concentrations of the monoclonal antibodies can be produced when hybridoma cells are injected into the peritoneum of a rabbit where they form tumours (ascites tumours). The fluid secreted from these tumours contains the monoclonal antibodies. Antibodies produced by a single hybridoma clone are identical and are specific for a single antigen binding site.

1.6.3.2 ADVANTAGES OF ELISA

(1) SPEED:

Results can be available within 2 days compared to 3-5 weeks with aphid transmission tests (Rochow, 1979a, 1982b; Clark *et al.*, 1986).

(2) SIMPLICITY:

Strains of BYDV can be identified at the end of each experiment, unlike aphid transmission tests where several serial transfers may be necessary to determine the strain/s present (Rochow, 1979a).

(3) SENSITIVITY:

High sensitivity to low amounts (1-10 ng/ml) of virus (Clark & Adams, 1977; Lister & Rochow, 1979; Rochow, 1979a, 1982b; Clark *et al.*, 1986). This is especially suited to BYDV which naturally occurs at low concentrations in plants (Lister & Rochow, 1979).

(4) SPECIFICITY:

Individual strains can be identified, which is particularly important when analysing leaf samples

containing a mixture of isolates (Rochow, 1979a, 1982b).

(5) STRAIN RELATIONSHIPS:

The reactions in ELISA tests determined the relationship between the five strains of BYDV. Such reactions determined the division of the viruses into two groups (Aapola & Rochow, 1971; Rochow & Carmichael, 1979).

(6) RELIABILITY:

The ELISA procedure allows identification of BYDV in plants where symptoms are masked (Rochow, 1979a, 1982b).

(7) SCALE:

The procedure is particularly suited to the large scale testing of field samples (Clark & Adams, 1977; Torrance & Jones, 1981; Clark *et al.*, 1986).

(8) VERSATILITY:

Virus can be detected in crude extracts or purified preparations (Clark & Adams, 1977; Clark *et al.*, 1986).

(9) QUANTITATIVE:

The amount of virus present in a leaf can be quantified in an ELISA test using a photometer (Pereira *et al.*, 1989).

(10) LEAF CONDITION:

ELISA can detect virus in leaf samples that are in such poor condition as to preclude an aphid transmission test (Rochow, 1982b).

1.6.3.3 DISADVANTAGES OF ELISA

The pronounced specificity of the antibody to the isolates against which it has been produced, may mean the presence of new/mutated strains or isolates would be missed (Rochow, 1979a).

1.6.3.4 COMPARISON OF DIRECT AND INDIRECT ELISA

The direct ELISA requires the preparation of a specific enzyme conjugate for each antibody used, whereas the indirect ELISA uses a single conjugate based on a species antiglobulin antibody (Clark *et al.*, 1986). As a result, the extreme specificity of the direct ELISA can preclude detection of even closely related strains of the same virus (Koenig, 1978; Bar-Joseph & Salomon, 1980). The indirect ELISA can give quantitative evaluation of strain relationships (Hsu *et al.*, 1984).

The introduction of the hybridoma technology by Köhler & Milstein (1975) and the production of monoclonal antibodies has offered several other advantages over polyclonal antiserum (Halk & De Boer, 1985; Clark *et al.*, 1986):

(1) An unlimited quantity of antibody can be produced from a small quantity of antigen.
(2) Hybridomas can be preserved by freezing in liquid nitrogen, thereby assuring a continuous supply of antibody over time.

(3) The use of monoclonal antibodies eliminates the qualitative and quantitative variability in specific antibody content in different batches of polyclonal serum obtained from different animals, or different bleedings of the same animal.

A disadvantage of monoclonal over polyclonal antibodies is that each monoclonal antibody requires testing against a range of BYDV isolates to determine which, or how many serotypes of the virus the antiserum will recognise (Van Regenmortel, 1986). The process can be costly and time-consuming before antisera to a suitably wide range of BYDV isolates can be produced (Halk & De Boer, 1985; Van Regenmortel, 1986).

1.6.4 Other examples of the use of labelled antibodies to detect BYDV

Serologically specific electron microscopy has been used to detect strains of BYDV within an aphid using polyclonal antisera (Paliwal, 1982a), and within plants using monoclonal antisera (Diaco *et al.*, 1986).

BYDV has also been detected in oats and aphids using amplified ELISA; a form of increased sensitivity for the detection of virus (Torrance, 1987). Individual aphids have also been tested for BYDV by a modification of ELISA using a fluorogenic substrate known as MUP-ELISA (Torrance *et al.*, 1986b).

Recently the use of labelled cDNAs as probes has been utilized for detecting virus by dot-blot hybridization (Lister *et al.*, 1990).

Other modifications of ELISA use Clq (a component of complement obtained from bovine serum) to trap virus antibody aggregates (Torrance, 1980), or $F(ab')_2$ fragments of the antibody (Barbara & Clark, 1982; Koenig & Paul, 1982).

1.7 EPIDEMIOLOGY

BYD is a serious disease because it causes significant losses in grain yield (Conti *et al.*, 1990) in many species of the Gramineae (Oswald & Houston, 1953b; Bruehl, 1961; Slykhuis, 1967; Slykhuis *et al.*, 1967), and is very widespread. Indeed, BYDV has been found on six continents (Catherall & Wilkins, 1977).

BYDV was first recorded in Britain in 1954 (Watson & Mulligan, 1957), and has been recognized as the most important virus disease of cereals in Britain (Barker, 1990). Up to 90% of cereal crops have been reported to be infected in England and Wales (King, 1977). A high proportion of perennial ryegrass fields has also been reported to be infected; 93% in England and Wales (Doodson, 1967) and 70% in the west of Scotland (Holmes, 1977).

The strains of virus detected in Britain (Plumb, 1974; Holmes, 1991) are similar to the RPV, PAV and MAV

isolates designated by Rochow (1969a). The most common aphid species capable of transmitting these strains of BYDV are *R. padi, S. avenae* and *Metopolophium dirhodum* Walker (Plumb, 1974).

As the virus is dependent on vectors for transmission, the epidemiology of BYD is also dependent on the life cycle of the aphid species involved.

1.7.1 Life cycle of aphids

1.7.1.1 *Rhopalosiphum padi*

R. padi is a host-alternating (heteroecious) aphid, i.e. its life cycle is divided between two different families of host plant, in this case bird cherry tree (*Prunus padus* L.) and several species of grasses and cereals (Leather, 1988).

R. padi is mainly holocyclic, i.e. it overwinters as an egg, on its primary host, bird cherry (Tatchell *et al.*, 1988). Date of egg hatch is dependent on latitude and is closely synchronised with bud burst. Thus, egg hatch has been recorded as early as 3rd March in East Anglia, whereas in Scotland it can be as late as 25th March (Wiktelius, 1984).

The aphids that hatch from these eggs are termed the fundatrices (Leather, 1988). These are apterous (wingless) and feed on the bursting buds (Leather, 1988; Tatchell *et al.*, 1988). After one or two further

generations, dense colonies soon develop (Leather, 1988; Tatchell *et al.*, 1988). In May and June, winged (alate) forms called emigrants are produced, in response to crowding and the reduced nutritional status of the leaf (Dixon, 1971; Leather & Lehti, 1981; Wiktelius, 1984). These leave the bird cherry tree and migrate to their secondary hosts, grasses and cereals (Leather, 1988; Tatchell *et al.*, 1988). During the summer they produce alternate generations of winged (alate exules) and wingless (apterous exules) forms which exploit the most nutritive stages of their host plant. They are often difficult to enumerate as they prefer to feed on the lower leaves and stem (Dean, 1973; Dedryver & Robert, 1977; Hand, 1986). In response to shortening daylength and declining temperatures, they begin to produce gynoparae and males (Dixon & Glen, 1971).

In autumn, the winged gynoparae fly to the bird cherry tree (Leather, 1981), and reproduce viviparously producing sexual egg-laying females known as oviparae. The males arrive on the host tree once the oviparae are mature and mate, after which the oviparae lay eggs (Dixon, 1971; Leather, 1981).

1.7.1.2 *Sitobion avenae* AND *Metopolophium dirhodum*

S. avenae is considered to be monoecious on Gramineae (Hand, 1989), while *M. dirhodum* is heteroecious on *Rosa* spp. (Hand & Williams, 1981). *S. avenae* has a sexual cycle, i.e. holocyclic (Phillips, 1916), but is also able

to overwinter anholocyclically, i.e. without sexual reproduction or an egg stage (George 1974; Dedryver, 1978). Anholocyclic populations in Gramineae have been recorded for *M. dirhodum* (Turl, 1980; Hand, 1989).

Recordable numbers of *S. avenae* begin to fly in early May from their winter hosts (grasses) to cereals. *S. avenae* and *M. dirhodum* are most common during crop growth and cereal ripening in July (Hand, 1989).

S. avenae prefers to feed on the ears of plants, while *M. dirhodum* prefers to feed on the flag and lower leaves (Dean, 1973; Dedryver & Robert, 1977; Hand, 1986).

1.7.2 Disease etiology

1.7.2.1 AUTUMN-SOWN CEREALS

In Britain, most cereals are now autumn-sown (Plumb, 1990), and therefore the autumn migration of vectors is most important (Tatchell *et al.*, 1988). *Rhopalosiphum* spp. is one of the most numerous viruliferous species recorded in suction traps at this time (A'Brook & Dewar, 1980).

Primary infection of autumn-sown cereals depends on the numbers of migrant vectors, the proportion that carry and transmit BYDV, and the period for which crops are available for infection (Tatchell *et al.*, 1988).

Autumn-sown crops are planted as early as practically possible in autumn (Plumb, 1989), often in early to mid-

September (Holmes, 1983a). The migratory flights of *R. padi* usually peak in mid to late September (Holmes, 1983a). Subsequently, many of these aphids may land on autumn-sown cereals, however A'Brook & Dewar (1980) found that the infectivity of *R. padi* (in west Wales) was relatively low, ranging between 7.3% and 14.3%. Primary infection, therefore, often consists of scattered symptomless plants (Holmes, 1983a; Conti *et al.*, 1990) as only a few viruliferous aphids enter the fields in the autumn, and symptoms do not normally develop until the spring.

Mild weather can result in the development of numerous vectors during crop emergence from September to November, which can cause serious yield losses in cereals infected at an early growth stage (Doodson & Saunders, 1970). In addition, continued mild weather can lead to secondary spread by the movement of wingless progeny in late autumn (A'Brook & Dewar, 1980; Holmes, 1983a).

The important secondary spread appears as yellow patches in spring when symptoms develop, accompanied by severe stunting (Holmes, 1983a). In Britain, most of the patchy virus spread is due to *R. padi* (Plumb, 1990) which transmits the strains of BYDV (RPV and PAV) that cause the most damage to cereals (A'Brook & Dewar, 1980). Weather is the most important factor governing the extent of secondary spread and in an 'average' winter it may be limited to November and December (Holmes, 1983a).

In Scotland, important secondary spread is more likely to be restricted to spring or early summer, because the overwintering host for *R. padi*, the bird cherry, is present in greater numbers than in England and Wales and therefore available for gynoparae to land on (Holmes, 1983a). Moreover, the temperature is too low during most winters to allow the survival of aphids in the Gramineae (Holmes, 1983a; Plumb, 1990).

The major migrations of *S. avenae* and *M. dirhodum* occur in June and July when such late infection by BYDV has little effect on yield (Doodson & Saunders, 1970). In addition, these vectors normally transmit MAV which causes milder symptoms of infection in plants, although *S. avenae* has been reported to transmit PAV (A'Brook & Dewar, 1980). The aphids may, however, bring about further spread of BYDV from patches of plants infected in the previous autumn (Holmes, 1983a).

1.7.2.2 SPRING-SOWN CEREALS

Crops sown in March usually avoid serious infection as subsequent aphid migrations are normally too late to cause any significant damage (Doodson & Saunders, 1970; A'Brook & Dewar, 1980; Holmes, 1983a; Plumb, 1990).

The late sowing of spring cereals can, however, increase the risk of infection as the crops are still young when exposed to infective vectors (alate emigrants) migrating in May and June (Plumb, 1990).

Generally, infection appears as a scattering of diseased plants which is often associated with transmission by *S. avenae* (Holmes, 1983a; Plumb, 1990).

1.7.3 Source of inoculum

Infection is caused by spread of the virus by a vector from one or more reservoirs (Irwin & Thresh, 1990). The primary inoculum sources may be local, regional, or distant (Irwin & Thresh, 1988). Aphid vectors move the virus from the former reservoir by walking, or short host-seeking flights; from a regional source by moderately long flights; and from distant reservoirs by long-distance migratory flights (Irwin & Thresh, 1990).

1.7.3.1 LOCAL

(1) GRASS CROPS:

Owing to the interval between harvest and the emergence of autumn-sown crops, all morphs of cereal aphids flying during this interval develop on grasses, except where maize is grown (A'Brook & Dewar, 1980). Perennial ryegrass has been shown to be a widespread reservoir of BYDV (Catherall, 1963; Doodson, 1967; Holmes, 1977, 1985). However, several studies have shown that the predominating strain of BYDV in cereal crops differs from that present in the adjacent grass crops (Gill, 1970; Rochow & Muller, 1976; Plumb, 1977; Fargette *et al.*, 1982; Paliwal, 1982b).

(2) CEREAL CROPS:

Winter cereals are thought to be an important source of BYDV infection to spring-sown crops (Slykhuis *et al.*, 1967; Rochow & Muller, 1976).

(3) PLOUGHED GRASS AND VOLUNTEER CEREALS:

Ploughed grass that has not been destroyed by chemicals or cultivation can be a source of the direct transfer of potentially viruliferous aphids to emerging cereals (Holmes, 1983a; Kendall, 1986; Plumb, 1989). If the weedy stubble left after the harvest of a cereal crop is not immediately destroyed it can serve as a suitable habitat for the influx and reproduction of aphids leaving ripening crops. Moreover, virus infection in the subsequent crop can be widespread from the incorporation of such weedy stubbles. This is often referred to as the "green bridge" (Plumb, 1990). The regrowth of plants from shed grain or combine spillage after harvest may provide an additional source of inoculum within the crop (Kendall, 1986).

1.7.3.2 REGIONAL

Grass and cereal crops may or may not be a source of BYDV infection in the same manner as described for the local source of inoculum. In areas where maize is cultivated, this bridging crop has been considered a source of infection to autumn-sown crops (Stoner, 1977; Brown *et al.*, 1984; Henry *et al.*, 1989).

Spread by aphids would have to be solely by alatae and may be by alate exules which tend to fly low and not for long distances (Tatchell *et al.*, 1988).

1.7.3.3 DISTANT

Gill (1970) and Paliwal (1982b) postulated that strong winds carried viruliferous aphids over long distances from BYDV-infected areas, and these acted as a primary source of inoculum in cereal crops; introducing new strains into an area, rather than causing significant secondary spread (G.N. Foster, personal communication). Gynoparae and males fly higher and would therefore migrate further on the stronger winds found at greater altitudes than the lower flying alate exules (Tatchell *et al.*, 1988).

1.7.4 Effect of BYDV on aphid epidemiology

Greater proportions of alate *R. padi* and *S. avenae* are produced on BYDV-infected barley and oats than on healthy plants (Gildow, 1980, 1983). *S. graminum* showed no such response (Montllor & Gildow, 1986). The increased alate production of viruliferous *R. padi* and *S. avenae* would cause further dissemination of the disease as these winged forms migrate to other hosts.

Kieckhefer *et al.* (1976) found that generally alate *S. graminum, S. avenae* and *R. padi* did not discriminate between green leaves and leaves yellowed by BYDV. However, Ajayi & Dewar (1983) noted that more alate

emigrant *S. avenae* and *M. dirhodum* collected in BYDV-infected oat and barley fields showing severe yellowing than in healthy fields. Similar differences did not occur in alate populations between infected or healthy wheat fields where the former did not develop severe BYDV symptoms.

S. graminum was found to feed better on infected oats than on healthy oats, as indicated by fewer number of probes to the phloem and longer periods ingesting (Montllor & Gildow, 1986).

Fecundity, longevity and developmental rate are affected by feeding on BYDV-infected plants. However, authors differ in their assessments of the effects. Markkula & Laurema (1964), Miller & Coon (1964), Ajayi & Dewar (1983), and Araya & Foster (1987) found that fecundity of *S. avenae* and *R. padi* increased on BYDV-infected plants, although Araya & Foster (1987) found that fecundity of *R. padi* did not increase on infected oats compared to on wheat. Miller & Coon (1964) also stated that longevity and developmental rate was increased in viruliferous aphids, while Araya & Foster (1987) demonstrated that longevity decreased when *R. padi* fed on BYDV-infected wheat.

Generally, improved feeding efficiency favours acquisition of virus, while increased population size and wing development favour aphid survival and virus spread (Gildow, 1990). Gildow (1990) proposed that BYDV and

cereal aphids had co-evolved into a mutually beneficial association.

Irwin & Thresh (1990) commented that a viruliferous aphid is not necessarily infective; to become infective, the virus isolate in the vector must be compatible with the genetic make-up of that aphid species. Therefore, an aphid enters the disease cycle only after overcoming many ecological, behavioural, physiological, and genetic barriers. This suggests that there are a number of possibilities for breaking the BYDV cycle.

1.8 CONTROL OF BYDV INFECTION

Different strategies have been developed to reduce the incidence of BYDV.

1.8.1 Biological

The most common natural enemies of the vector aphid species are coccinellid beetles, anthocorid bugs, and syrphid and chrysopid larvae (Leather, 1988). Current work is being undertaken in the southern cone of South America to utilize five coccinellid species as a biological control system (Zúñiga, 1990). A substantial decline in aphid population has been recorded.

1.8.2 Husbandry

The incidence of BYDV can be reduced by:

(1) the destruction of old grass, cereal stubbles and volunteer cereals prior to the sowing of cereals (Holmes, 1983a; Plumb, 1990; Kendall, 1986);

(2) rotation of BYDV-susceptible crops with non-host crops (Irwin & Thresh, 1990);

(3) sowing autumn cereals after the major aphid flights, and early sowing of spring cereals so that the plants are established before the aphid migration in early summer (Plumb, 1990). In U.K., generally, sowing winter cereals after the end of September, and spring cereals in March avoids serious infection of BYDV and subsequent crop loss (A'Brook, 1974; McGrath *et al.*, 1987; Plumb, 1990).

1.8.3 Chemical

(1) Conventional ploughing of old grass, cereal stubbles and volunteer cereals is an unsatisfactory method of destroying these sources of inoculum since aphid vectors can live for some time on the buried plant matter (Kendall, 1986). In such a situation, an aphicide spray should be applied soon after crop emergence and again in spring if the winter is mild (Holmes, 1983a; 1984b). Kendall (1986) found that significantly less virus infection occurred in plots of young

cereals where the previous stubble or grass had been sprayed with herbicides containing paraquat or glyphosate. However, sprays are only justified where virus occurs (Kendall, 1986).

(2) When weather conditions and vector densities suggest high risk of infection in cereal crops, aphicides can be very efficient in controlling virus spread (Conti *et al.*, 1990). Whilst the spray does not prevent the influx of migrating aphids, it does reduce the damaging, secondary spread from primary foci (Holmes, 1984b).

The efficiency of aphicide applications depends on their timing. A spray applied too early (late September) allows aphid reinfestation of winter cereals to occur. If applied too late (early December) then secondary spread of virus prior to treatment causes crop damage. Late October is recommended for optimum aphid/virus control in a single spray (McGrath *et al.*, 1987). Timing also depends on the conditions prevailing in the area.

Brain & Hewson (1984) demonstrated that deltamethrin, a synthetic pyrethroid, gave effective control of aphid vectors and reduced BYDV incidence in winter cereals.

1.8.4 Resistance

The Yd_2 gene, transferred to spring and winter barley cultivars from Ethiopian land races, confers a degree of resistance to BYDV in cereals, manifest by mild symptoms

and limited virus replication (Herrera & Plumb, 1988; Irwin & thresh, 1990).

The Yd_2 gene is very effective against PAV- and MAV-like isolates but much less effective, or ineffective against RPV-like isolates (Herrera & Plumb, 1988, 1989). The successful use of such varieties (e.g. winter barley cv. Vixen and spring barley cv. Atlas 68) will depend upon the incidence of the BYDV strains (Herrera & Plumb, 1988).

1.8.5 Forecasting

Since 1980, one of the bases for deciding whether BYDV should be controlled in autumn-sown cereal crops has been the Infectivity Index (II). This is calculated by multiplying the number of *R. padi*, *S. avenae* and *M. dirhodum* caught in a suction trap 12 m above the ground by the proportion of *Rhopalosiphum* spp., *S. avenae* and *M. dirhodum*, respectively, found to be infective. The latter is determined by trapping aphids alive in a suction trap 1.7 m above the ground, and placing them individually on susceptible oat seedlings (Plumb, 1976, 1986; Plumb & Carter, 1988). An Infectivity Index of 50 or more in the September to October period is considered to indicate a high risk of significant infection by BYDV.

The object of the Infectivity Index is to assess the risk of BYDV being introduced into autumn-sown cereals, and thus act as a guide for the timely application of an aphicide. Conversely, it can also be used to avoid

unnecessary routine insecticide treatments if the risk from BYDV is considered to be low (Plumb *et al.*, 1990).

However, the Infectivity Indexing Scheme does not assess:

(1) the risk of virus spread from aphids walking from weed grasses or cereal stubbles to following cereal crops;
(2) secondary spread by aphids walking within cereal crops in the autumn;
(3) aphids overwintering in exceptionally mild winters;
(4) the population of *S. avenae* (Holmes, 1989).

1.9 OBJECTIVES OF THE PRESENT STUDY

The objectives of the research undertaken by the author were to determine:

(1) the relative efficiency of BYDV transmission by *R. padi* and *S. avenae* using susceptible oat cultivars;
(2) the comparative rates of BYDV transmission by *R. padi* and *S. avenae* from perennial ryegrass and oats to a range of commercially grown winter barley cultivars;
(3) the rate of transmission of BYDV by the two aphid vectors from cereals to perennial ryegrass;
(4) whether individual strains were transmitted from triple-infected source leaves in proportions different from those transmitted from double- or single-infected leaves;

(5) the strains of virus present in perennial ryegrass crops and their distribution in south-west and central Scotland.

Such data would provide a relative assessment of the threat which several strains and mixtures of strains of BYDV, transmitted by each aphid species, pose to a range of cereal cultivars. In addition, the research would ascertain whether this threat was greater from a grass or a cereal source.

This thesis is a contribution to the development of a comprehensive forecasting system to enable farmers to combat BYDV.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 INTRODUCTION

RPV, PAV and MAV were the only strains of BYDV studied in this work as the SGV and RMV strains had not been found in ryegrass in Scotland (Holmes, 1991).

Studies have shown that BYD appears to be caused by two separate virus subgroups, RPV being in one, and PAV and MAV in the other (Rochow & Carmichael, 1979). As they have not been renamed to date, RPV, PAV and MAV were, throughout this research, all referred to as strains of BYDV.

Two aphid vectors, *R. padi* and *S. avenae* were used as they are the most common species associated with autumn-sown cereals in Britain (Kendall, 1986).

2.2 PRODUCTION AND MAINTENANCE OF APHIDS

Virus-free aphids used as vectors in the studies were produced in stock colonies in an insectory maintained at 16^{o}C under cool white fluorescent light (Dual tubes (Thorn), 85 W and 125 W) with a 16 h photoperiod in each 24 h cycle.

Stock cultures were started from non-viruliferous, apterous, viviparous females confined on winter barely cv. Igri by covers on 12.5 cm diameter (diam.) pots. Clear, plastic propagator covers (diam. 13 cm, height 18 cm) were used for *R. padi*. *S. avenae* was slower to reproduce and consequently it took longer for the

colony to build up. As the height of the barley plants increased, taller covers were required. These were made of insect-proof netting (diam. 10.5 cm, height 31.5 cm).

2.3 MAINTENANCE OF BYDV ISOLATES

All the isolates of BYDV used in the present research were obtained from perennial ryegrass crops and maintained either in oat plants, or perennial ryegrass plants.

In the winter of 1987, turves were collected from perennial ryegrass leys in the west of Scotland, potted in Levington M3 potting compost (Fisons Horticulture, PLC) in 12.5 cm diam. pots, and placed in an unheated glasshouse. Leaves from these plants were tested by direct ELISA to determine infection by BYDV and the strains of the virus present. Where a single strain of BYDV was detected, it was transferred using the standard aphid transmission procedure (see 2.6) to oat cv. Dula, or perennial ryegrass cv. Talbot.

All oats infected with BYDV were subsequently kept free from contamination by stray aphids by maintaining the oats in perspex cages (36.5 cm x 16 cm x 60 cm high). Each cage housed two 12.5 cm diam. pots per virus strain, the plants from which were used as known-infected material in ELISA tests. In the bottom of each cage was a 2 cm deep layer of sand on which the pots were placed. A removable lid covered in fine netting surrounded a small glass funnel with rubber tubing leading down to the sand. This

enabled watering to take place without removing the covers, thus preventing possible viruliferous stray aphids from entering the cages. The lid was only removed to obtain leaves from the known-infected plants, or when replacing old plants with new ones.

Oats infected with known strains of BYDV were maintained in perspex cages for 1 month before the leaves became too senescent, or the plants too stunted to provide suitable material for use in ELISA or aphid transmission tests. The known strains of BYDV were transferred to fresh oat cv. Dula plants each month.

Two 12.5 cm diam. pots containing BYDV-infected perennial ryegrass cv. Talbot were kept free from infestation by stray aphids in cages which had a wooden frame (40.5 cm x 39.5 cm x 35 cm high) covered in insect-proof netting. A fitted glass cover which could slide out permitted watering of plants from above. The grass plants were fertilized after 1 month with 1 g of SAI2 fertilizer (22:11:11 NPK) to ensure steady growth. The plants were replaced approximately every 4 months.

All BYDV-infected plants were kept in a heated glasshouse under natural daylight supplemented to a 16 h photoperiod by 400 W sodium lamps (Sylvania), with a photosynthetically active radiation (PAR) of 9.8 x 10 µmol $m^{-2}s^{-1}$. During the winter months, supplementary lighting was provided between 06.00 h and 22.00 h. No supplementary lighting was supplied from March to October.

Plants in the glasshouse were kept free from aphid infestations by fumigation with nicotene (Nicotene 40% shreds, Dow chemical company) every 2 weeks.

2.4 PRODUCTION OF PLANTS

All plants, both cereal and grass, were sown in Levington M3 potting compost (Fisons Horticulture, PLC), and maintained in a heated, insect-free glasshouse.

Winter barely cv. Igri plants used to rear virus-free aphid colonies were sown in 12.5 cm diam. pots at a rate of 10-15 seeds per pot. Five seeds of oat cv. Dula, and 50-75 seeds of perennial ryegrass cv. Talbot were sown per 12.5 cm diam. pot for plants to be used as a source of known-infected material.

Cereal test plants were sown at a rate of two seeds per 7.5 cm diam. pot, while grass test plants (perennial ryegrass cv. Talbot) were sown at a rate of 10-15 seeds per 7.5 cm diam. pot. The cereals were thinned to one plant per pot after emergence.

The plants were grown in a heated glasshouse under natural daylight supplemented to a 16 h photoperiod with 400 W mercury vapour lamps (PAR = 1.29 x 10 μmol $m^{-2}s^{-1}$). In winter, supplementary lighting was provided between 06.00 h and 22.00 h. No supplementary lighting was supplied from March to October.

2.5 FOLIAR SYMPTOMS OF BYDV

Leaves from plants grown in the glasshouse which were infected with BYDV were collected for use in ELISA and aphid transmission tests as known-infected material when showing the following symptoms:-

Oats: leaf margins beginning to turn yellow followed by yellow-green blotches near the leaf tip. These progressed down the blade, and soon coalesced leaving the leaf tip yellow (Plate 6). Leaf tips often turned reddish-purple, especially when infected with the PAV strain. Severely infected plants, usually as a result of infection by the RPV or PAV strains of BYDV, often bore deep serrations on the margins of newly emerging leaves (Plate 6). Plants infected with MAV were not as severely stunted as those infected with RPV, or especially PAV, but often bore serrations on the leaf margins.

Winter barley: leaves beginning to turn yellow at the tips, eventually becoming covered in bright yellow blotches along the leaf blade (Plate 7). Again, newly emerging leaves of severely infected plants were deeply serrated at leaf margins (Plate 8). The PAV strain caused the greatest reduction in plant height, and MAV the least (Plate 9).

Winter Wheat: leaves becoming mottled yellow. No plants became severely stunted, and no serration of leaf margins was noted.

Perennial ryegrass: no symptoms were observed.

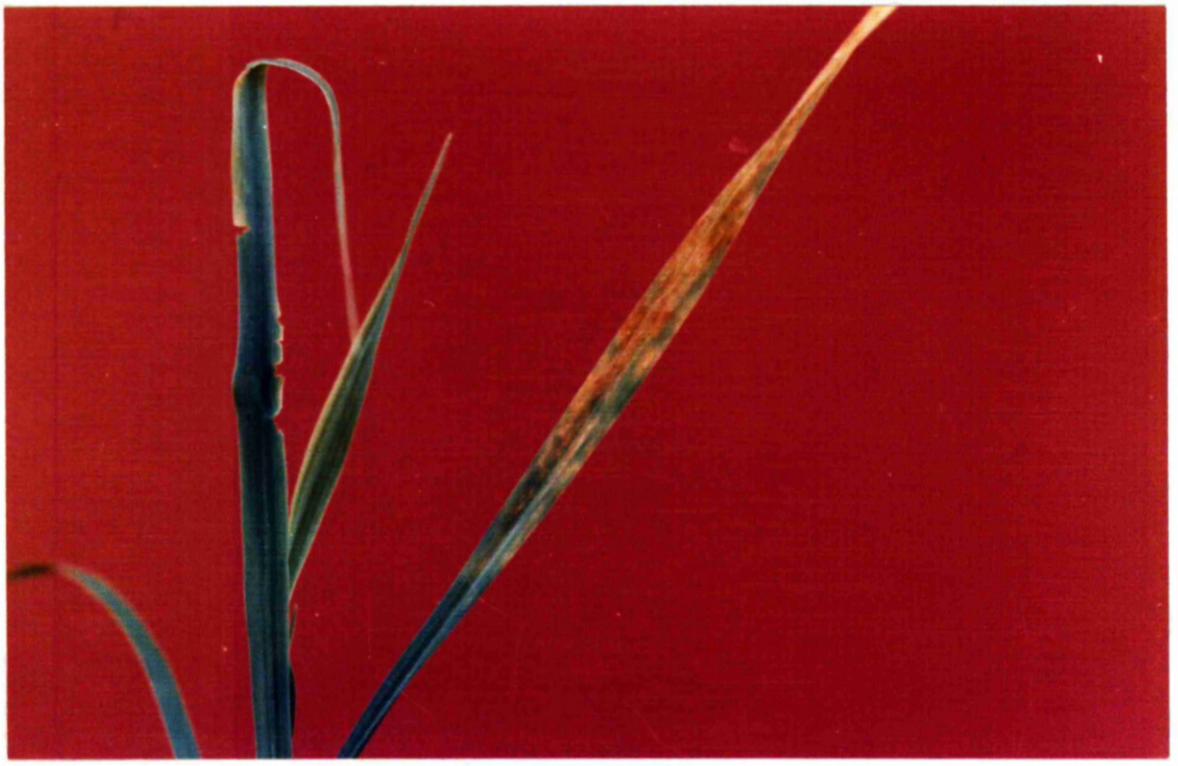


PLATE 6. Symptoms of BYDV on oat cv. Dula.

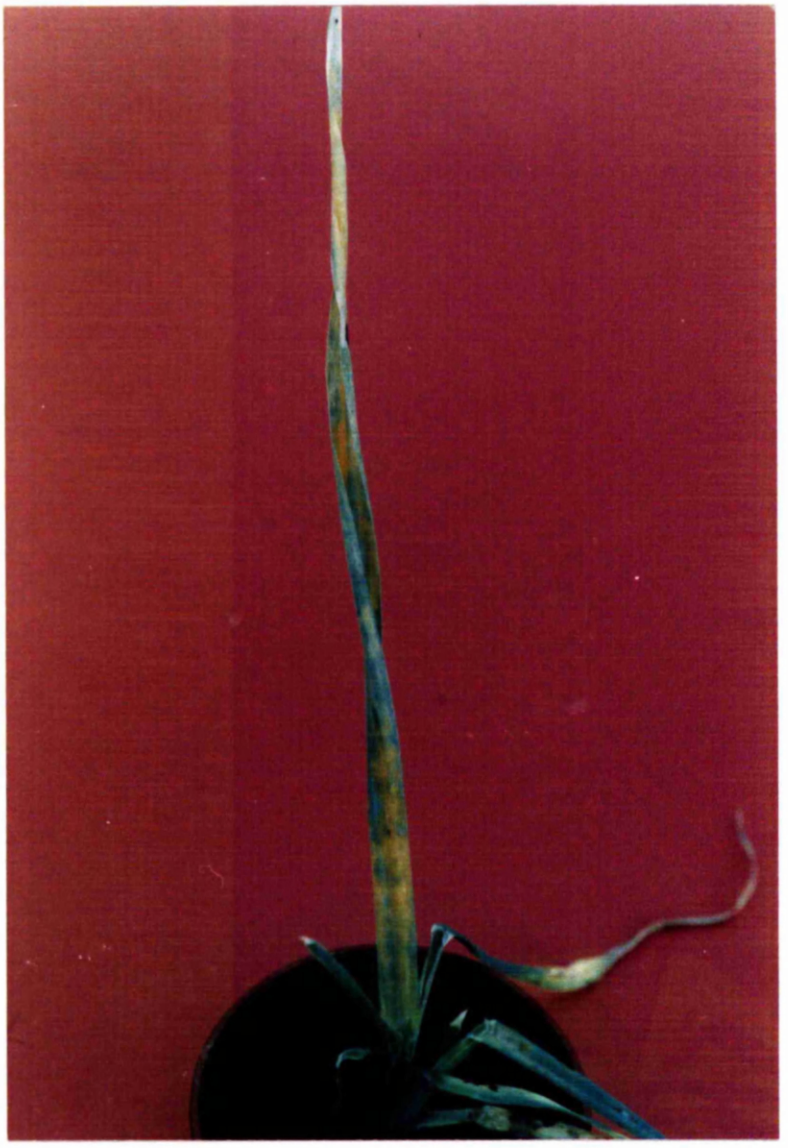
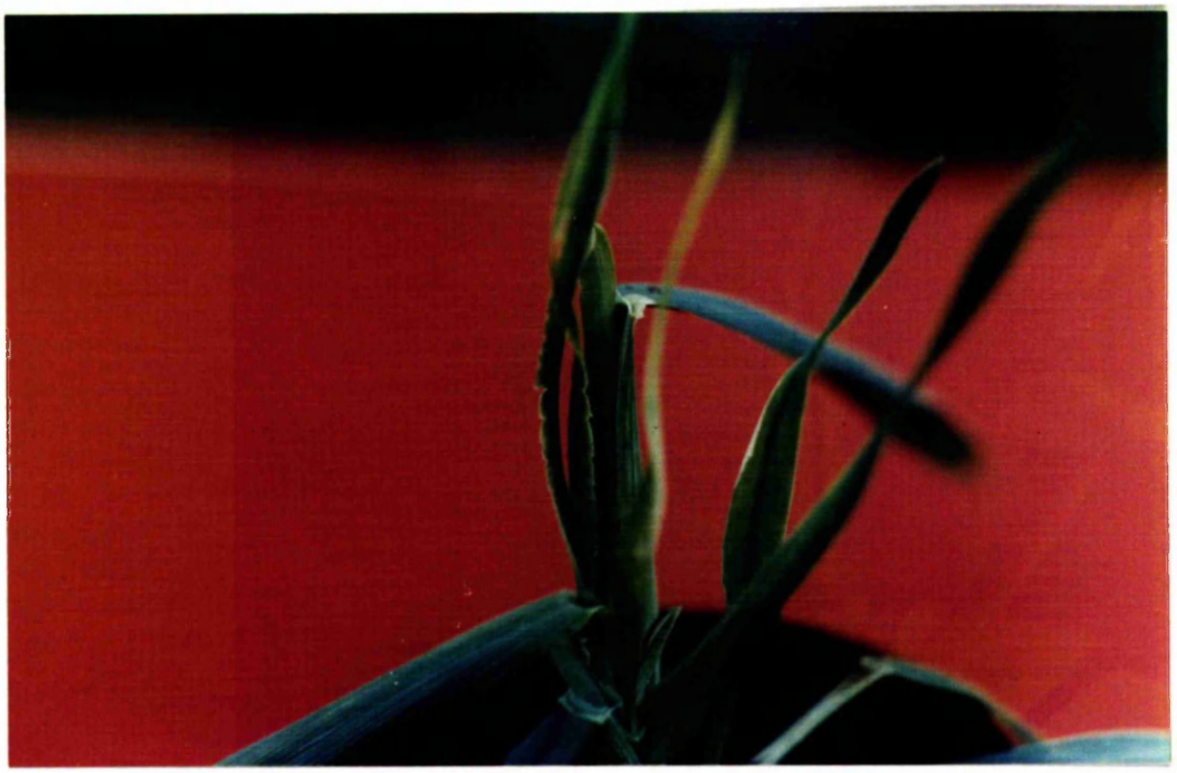


PLATE 7. Symptoms of BYDV on winter barley cv. Igri.

PLATE 8. Symptoms of BYDV on winter barley cv. Igri.

PLATE 9. Effect of the RPV (left), PAV (centre) and MAV (right) strains of BYDV on winter barley cv. Igri.




RPV-INFECTED IGRI
PAV-INFECTED IGRI
MAV-INFECTED IGRI

2.6 APHID TRANSMISSION TESTS

One of the objectives of this research was to determine the transmission rates of the three strains of BYDV, both singly, and in various combinations from differing plant sources to various test plants by two aphid species. This was done using aphid transmission tests as described by Rochow (1969a) and Stoner (1976).

Five to six cereal leaves exhibiting symptoms of BYDV, or 10 ryegrass leaves, were cut from plants known to contain the required BYDV strains, and placed on moist filter paper (4.25 cm diam., Whatman) in a screw-top vial (diam. 4.25 cm, height 10 cm). Virus-free aphids were transferred to the vials with a camel-hair paint brush. Routinely, 70 aphids were placed in each vial. Usually this provided 50 live aphids at the end of the 2-day acquisition feed. Care was taken to pick the aphids up by their rear abdomen in order not to damage the stylet.

The aphids were then transferred similarly to healthy seedlings, and confined there by means of small, clear, plastic propagator covers (diam.7 cm, height 14 cm) sellotaped to the pot thus ensuring that no aphids escaped.

To test transmission rates, there were 50 numbered 7.5 cm diam. pots for each aphid species with a single (12-day-old) cereal seedling, or 15-20 (3-week-old) grass seedlings in each pot. One apterous, adult aphid was placed on each cereal seedling, or on the 15-20 grass

seedlings (per pot). The survival of aphids was monitored over the 2-day inoculation feed. The plants were then sprayed with the aphicide Pirimor (50% pirimicarb, ICI).

Acquisition and inoculation feeding took place in a growth room maintained at 17°C under cool white fluorescent light (Dual tubes, 85 W and 125 W) with a 16 h photoperiod in every 24 h cycle.

The aphicide was applied with a hand-held sprayer in a ventilated, heated glasshouse where the plants were subsequently maintained under natural daylight supplemented to a 16 h photoperiod with 400 W sodium lamps (PAR = 9.80 x 10 µmol $m^{-2}s^{-1}$).

To produce the known-infected source plants, a total of 25-30 aphids were placed on 12-day-old cereal, or on 3-week-old grass seedlings growing in each pot. These aphids were allowed to feed for 5 days.

All plants were watered daily. The incidence of symptoms was noted 4 weeks after inoculation for both cereal and grass test plants. The plants were harvested, placed in labelled polythene bags (20.5 x 27.5 cm, QB Packing) and frozen at -18°C to await analysis. When only one strain was present, approximately five cereal test plants exhibiting symptoms, five with none, and any plants with unclear symptoms were harvested and tested individually. Where a combination of strains was present, all plants with symptoms were harvested to determine in

what proportions the different strains were present. All grass test plants were harvested as ryegrass did not exhibit symptoms of BYDV.

2.7 ELISA TESTS

All test samples and known-infected leaves used in the acquisition feeds were analysed by ELISA.

In the following experiments, two ELISA techniques were used, the direct double antibody sandwich ELISA, and the indirect ELISA. Initially the former was used, but the bulk of analysis was carried out using the indirect ELISA.

Both techniques basically followed the same procedure. The direct ELISA used polyclonal antibodies, while the indirect ELISA used both polyclonal and monoclonal.

2.7.1 Reagents

The same buffers were used in both techniques using chemicals from BDH (Analar grade):-

COATING BUFFER

1.59 g sodium carbonate (Na_2CO_3)

2.93 g sodium hydrogen carbonate ($NaHCO_3$)

1000 ml distilled water

PHOSPHATE-BUFFERED SALINE (PBS)

8.0 g sodium chloride (NaCl)

0.2 g potassium dihydrogen orthophosphate (KH_2PO_4)

2.9 g di-sodium hydrogen orthophosphate dodecahydrate ($Na_2HPO_4\ 12H_2O$)

0.2 g potassium chloride (KCl)

0.5 ml Tween 20 (Polyoxyethylene (20)-Sorbitan monolaurate)

1000 ml distilled water

EXTRACTION BUFFER

2.0 g polyvinyl pyrrolidone (PVP)

0.02 g egg albumen

100 ml PBSTween

SUBSTRATE BUFFER

97 ml diethanolamine ($[CH_2(OH).CH_2]_2NH$)

800 ml distilled water

Adjust to pH 9.8 with concentrated hydrochloric acid (HCl), and then make up to 1000 ml with distilled water.

The enzyme was alkaline phosphatase (Sigma type VII), and the substrate was 2-nitrophenyl phosphate (Sigma) in tablet form.

Coating buffer, substrate buffer and enzyme were stored at 4^oC while the substrate tablets were stored at -18^oC. Extraction buffer was freshly made for every step while PBS-Tween was made in bulk and stored at room temperature, 20^oC.

2.7.2 Equipment

The Nunc-Immuno maxisorp microtitre plates used were supplied by NUNC Intermed (Gibco UK Ltd.). These consisted of 96 flat-bottomed wells arranged in a 12 x 8 format. The capacity of each well was 400 µl. Aliquots of 200 µl of any reagent were deposited in each well.

All containers used to hold antibodies were made of polypropelene as proteins do not stick to this kind of plastic. Clingfilm was used to cover the plates when incubating.

2.7.3 The direct form of ELISA

This procedure was basically that of Clark & Adams (1977). The specific polyclonal antibody, either RPV, PAV, or MAV was diluted 1:1000 with coating buffer (10 µl antibody:10 ml buffer), and 200 µl were placed in each well of a microtitre plate (which was labelled according to the antibody) and incubated for 3 h at 37^{o}C.

Test plant material (1 g) was chopped into small pieces and ground first in liquid nitrogen using a mortar and pestle to ensure even maceration of the material and maximum extraction of virus from the phloem. Automatic sap extraction equipment generally did not yield adequate amounts of virus (Holmes, 1991). Each sample was then ground in 5 ml of extraction buffer for 55 sec and strained through individual cheesecloth squares (R.J. Fullwood & Bland Ltd.) to remove larger pieces of plant

debris. The sap extracts were strained into 30 ml round-bottomed centrifuge tubes (Sarstedt) which were numbered according to the sample. The sap was then poured into 4 ml auto-analyser cups (Dynatech). It was necessary to initially pour the plant sap from the mortar into the 30 ml tubes as the samples could not be easily strained directly into a 4 ml vial.

Following incubation, coated plates were washed three times with PBS-Tween using an automatic plate washer (Dynatech). The buffer was allowed to soak in the wells of the plate for 90 sec between each wash. This removed traces of soluble reactants that could cause non-specific reactions (Clark & Adams, 1977). The Tween 20 prevented post-coating absorption of protein to the well surface (Clark & Adams, 1977).

Plant sap (200 µl) was then added to each well; 39 test samples, one healthy control (repeated three times), and one known-infected sample (repeated twice) per plate. All known-infected samples used as controls in ELISA tests contained the RPV, PAV and MAV strains. To reduce experimental variation, healthy controls were, whenever possible, the same cultivar and age as the test samples. Every sample was added to two adjacent wells.

Any virus present in the test plant samples (and known-infected samples) would stick to the specific antibody coating the plate. The albumen in the extraction buffer filled empty reaction sites on the plate (Clark &

Adams, 1977). Plates containing sap were incubated overnight at 4°C.

Sap components were removed by washing the plates three times as described above. An enzyme-labelled (alkaline phosphatase) specific antibody was diluted 1:1000 in extraction buffer (10 µl antibody:10 ml buffer), and added to its corresponding plate before incubation at 37°C for 5 h.

Plates were washed a further three times with PBS-Tween following incubation. Enzyme substrate, i.e. nitrophenyl phosphate in tablet form, was crushed between two filter papers using a spatula. This was to ensure that it was not touched by hands as it is hygroscopic. The powder was dissolved in substrate buffer at a dilution of 1:13, e.g. six tablets:45 ml buffer, and aliquots of 200 µl added to each well. In the presence of enzyme, the substrate is changed from a colourless to a yellow liquid.

Plates were incubated at room temperature (20°C) for 60 min. At the end of this time, trapped virus was indicated by the colour change described above. This reaction should be very obvious in the wells containing known-infected samples, while the healthy controls should remain virtually colourless. The duplicate wells per sample should show a similar colour change. Any discrepancy between wells would throw doubt on the result, and the sample concerned would be retested.

No antibodies, or plant sap were ever placed in the first column of a plate. In this column, only the appropriate buffer without antisera or plant sap was added at each stage.

The plates were then placed in a spectrophotometer (Titertek multiskan MCC, Flow Laboratories) and the absorbance values were determined at a wavelength of 405 nm. The first column was used by the reader to "blank" the machine, i.e. the machine calibrated itself by asigning the first column an absorbance value of A_{405} = 0.000. The A_{405} values of all other wells on the microtitre plate were determined relative to those in the first column. If this column remained colourless following the addition of substrate it indicated that none of the buffers had deteriorated. Any colour change in test samples would, therefore, be due to trapped virus. The absorbance values increased during the 1 h incubation period.

A sample was considered to have given a positive result for BYDV when the A_{405} value exceeded the mean value of the healthy control wells plus three times their standard deviation, $\bar{x}$ + 3s (Chebychev's equivalent, Sutula *et al.*, 1986).

2.7.4 The indirect form of ELISA

Monoclonal antibodies, MAC 91 (PAV specific), MAC 92 (RPV), and MAFF 2 (MAV) were obtained from ADAS Central

Science Laboratories (Harpenden). The indirect method used for this work followed the procedure of L. Torrance (personal communication). The basic method is described by Koenig (1981) and Torrance *et al.* (1986a).

Plates were coated with polyclonal antibody (diluted 1:1000 in coating buffer) specific to the virus under test. The test and control samples of plant sap were then added and incubated overnight at 4°C. The procedure up to this point was identical to that for direct ELISA. The method differed once the plates were washed three times with PBSTween following the overnight incubation.

Specific monoclonal antibodies, MAC 91, MAC 92 and MAFF 2 were diluted in PBS-Tween at 1:750 (15 µl antibody:12 ml PBSTween), added to their corresponding microtitre plate and incubated at 20°C for 2 h. Plates were then rinsed twice with PBSTween before adding alkaline phosphatase conjugated rabbit anti-rat Ig (Sigma) diluted 1:1000 in PBSTween. Following a further 2 h incubation at 20°C, the plates were washed twice, substrate added (as for the direct method), and the absorbance values determined at 405 nm after 60 min at room temperature (20°C).

The previously described aphid transmission tests were used in all experiments to transfer virus, and the indirect ELISA used to identify/verify the virus present in all plant samples tested, unless otherwise stated. Details of materials and methods specific for individual

experiments are given where appropriate.

2.8 STATISTICAL ANALYSIS

Differences between means of ELISA absorbance values (Sections 3-7) were calculated for significance using the Student's t-test. The following formula was used to obtain the t value:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

where t has $(n_1 + n_2 - 2)$ degrees of freedom (Parker, 1979).

$\bar{x}_1$ = mean of sample 1

$\bar{x}_2$ = mean of sample 2

s_1^2 = variance of sample 1

s_2^2 = variance of sample 2

n_1 = number of observations in sample 1

n_2 = number of observations in sample 2

However, when data were not measurements, but proportions, e.g. analysis of transmission rates (Sections 4-6), s_i (i = 1 or 2) was calculated as follows:

$$s_i = \sqrt{\frac{p_i \, q_i}{n}}$$

which is the binomial sampling-variation (Mead & Curnow, 1983), where p_i = proportion and $q_i = 1 - p_i$, and t has $(n_1 + n_2 - 2)$ degrees of freedom.

The t-value was obtained using the following formula:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{s_1^2 + s_2^2}}$$

Using the general result from Mead & Curnow (1983) that the distribution of the sample mean of p is approximately normal if the sample size is sufficiently large (n > 30), the difference between the two proportions was assumed to follow the normal distribution.

The probability values (P) were obtained using Student's t-tables.

The degree of association between the two variables x and y was analysed using the sample correlation coefficient (Mead & Curnow, 1983):

$$r = \frac{\sum (x_j - x)\ (y_j - y)}{\sum (x_j - x)^2 \sum (y_j - y)^2}$$

which was calculated using MINITAB.

j = 1 to 50 observations

r = correlation coefficient

$\sum$ = the sum of

x = sample 1

y = sample 2

One-way analysis of variance was carried out on the data in Section 7. However, where two factors were influencing the variation, the data were analysed using two-way analysis of variance. The analysis of variance was calculated using MINITAB.

CHAPTER 3

DEVELOPMENT OF EXPERIMENTAL TECHNIQUES

3.1 EXPERIMENT 1: COMPARISON OF POLYCLONAL AND MONOCLONAL ANTIBODIES FOR THE DETECTION OF BYDV IN PERENNIAL RYEGRASS AND OAT CV. M TABARD

3.1.1 Materials and Methods

An experiment was carried out in June 1988 to determine whether polyclonal or monoclonal antibodies gave clearer results for the detection of BYDV in grass and cereal plants. The former employed the direct form of ELISA, the latter, the indirect form as described previously (2.7).

Ten samples (2 g per sample) of perennial ryegrass leaves were collected from a field in Ayrshire, which was known to be infected with BYDV. In addition, ten samples (2 g per sample) of oat cv. M. Tabard grown in a glasshouse were harvested, the leaves of which exhibited typical symptoms of BYDV infection, i.e. yellow or red leaves and stunting. Each sample was finely chopped, and divided into two equal portions, 1 g of plant material being used for each technique, i.e. direct or indirect ELISA.

All samples were tested (in duplicate) for the RPV, PAV and MAV strains of BYDV by both techniques simultaneously, using six microtitre plates, three for each technique. Each of the three strains of BYDV was assayed for on a separate plate.

In the above and all other experiments throughout Chapter 3, the absorbance values (A_{405}) for infected and

uninfected test samples were the mean of a varying number of wells (on the microtitre plates), which are indicated in the tables for each experiment.

3.1.2 Results

The polyclonal antisera gave higher background absorbance values (as indicated by the mean A_{405} values of the healthy control sample) compared to monoclonal antisera ($P<0.001$, Table 1). These absorbance values in turn produced higher minimum positive threshold levels ($\bar{x}$ + 3s).

Similarly, the mean absorbance values for test samples infected with BYDV were higher using polyclonal than monoclonal antibodies, with the exception of RPV in oats. Absorbance values of the oat samples infected with PAV ($P<0.05$) and grass samples infected with MAV ($P<0.001$) were significantly higher using polyclonal than monoclonal antibodies. Otherwise, the differences were not significant.

3.2 EXPERIMENT 2: COMPARISON OF NUNC AND DYNATECH MICROTITRE PLATES

3.2.1 Materials and Methods

A comparison between Nunc-Immuno maxisorp (Gibco UK Ltd.) and Dynatech Hi adsorb (Dynatech Lab. Ltd.) microtitre plates was made in June 1988.

TABLE 1. Comparison of mean absorbance values (A_{405}) of perennial ryegrass and oat cv. M. Tabard samples tested for BYDV by ELISA using polyclonal and monoclonal antibodies.

Plant sample	Strain of BYDV	Mean A_{405} values of BYDV detected using		SE	d.f.
		Polyclonal	Monoclonal		
Healthy control ($\bar{x}$ + 3s)	RPV	0.09[a] (0.15)	0.01[a] (0.02)	0.008	10
Infected oats (range)		0.91[c] (0.25 - 1.61)	1.08[c] (0.33 - 1.83)	0.281	30
Infected grass (range)		0.68[b] (0.57 - 0.70)	0.52[b] (0.25 - 0.78)	0.093	14
Healthy control ($\bar{x}$ + 3s)	PAV	0.10[a] (0.18)	0.01[a] (0.03)	0.011	10
Infected oats (range)		1.71[d] (0.36 - 2.58)	1.04[d] (0.32 - 1.81)	0.319	38
Infected grass (range)		0.52[c] (0.82 - 0.79)	0.42[c] (0.26 - 0.63)	0.103	30
Healthy control ($\bar{x}$ + 3s)	MAV	0.09[a] (0.15)	0.04[a] (0.06)	0.009	10
Infected oats (range)		0.46[c] (0.17 - 1.29)	0.29[c] (0.17 - 0.55)	0.130	30
Infected grass (range)		0.16[b] (0.15 - 0.18)	0.08[b] (0.08 - 0.09)	0.006	14

[a] mean of six wells; [b] mean of eight wells; [c] mean of 16 wells; [d] mean of 20 wells.

$\bar{x}$ + 3s is the minimum positive threshold for BYDV.

One sample (2 g) of perennial ryegrass cv. Talbot leaves and of oat cv. M. Tabard leaves was taken from each of 28 and seven plants, respectively. All plants had been maintained in the glasshouse and were known to contain BYDV. Twenty-two samples of perennial ryegrass were known to contain the RPV strain only, 14 of which were analysed for this strain alone. All other grass and oat samples were analysed for all three strains of BYDV.

Leaf material (2 g per sample) was finely chopped, then divided into two, providing 1 g of plant material, the sap from which would be tested in Nunc microtitre plates, while sap from the remaining 1 g of plant material would be tested in the Dynatech plates.

All microtitre plates were tested simultaneously by indirect ELISA. Three plates of each type were used, one for each strain of BYDV.

3.2.2 Results

The healthy control sample indicated that lower background absorbance values (A_{405}) and variability between wells (indicated by the minimum positive threshold) were obtained with sap analysed in Nunc microtitre plates compared to Dynatech plates (Table 2). The difference was not significant for the RPV strain , but was for the PAV and MAV strains ($P<0.05$).

By contrast, samples containing virus generally gave higher positive absorbance values for the RPV, PAV and MAV

TABLE 2. Comparison of mean absorbance values (A_{405}) of perennial ryegrass cv. Talbot and oat cv. M. Tabard samples tested for BYDV by ELISA using Nunc and Dynatech microtitre plates.

Plant sample	Strain of BYDV	Mean A_{405} values of BYDV detected in Nunc	Mean A_{405} values of BYDV detected in Dynatech	SE	d.f.
Healthy control ($\bar{x}$ +3s)	RPV	0.01[b] (0.04)	0.02[b] (0.08)	0.009	10
Positive grass (range)		0.48[f] (0.10 - 1.51)	0.15[e] (0.09 - 0.23)	0.049	94
Positive oats (range)		1.08[d] (0.13 - 1.60)	1.00[d] (0.09 - 1.20)	0.237	26
Healthy control ($\bar{x}$ + 3s)	PAV	0.00[b] (0.02)	0.03[b] (0.10)	0.010	10
Positive grass (range)		0.31[a] (0.30 - 0.32)	0.12[a] (0.10 - 0.16)	0.012	6
Positive oats (range)		1.24[c] (0.65 - 1.92)	0.60[c] (0.10 - 1.28)	0.248	14
Healthy control ($\bar{x}$ + 3s)	MAV	0.01[b] (0.07)	0.04[b] (0.12)	0.011	10
Positive grass (range)		0.17[c] (0.01 - 0.29)	0.15[a] (0.12 - 0.16)	0.059	10
Positive oats (range)		0.65[c] (0.52 - 0.83)	0.22[c] (0.13 - 0.38)	0.056	14

[a] mean of four wells; [b] mean of six wells; [c] mean of eight wells; [d] mean of 14 wells; [e] mean of 40 wells; [f] mean of 56 wells.

$\bar{x}$ + 3s is the minimum positive threshold for BYDV.

strains present when analysed in Nunc plates compared to in Dynatech plates. This was significant for the RPV and PAV strains detected in grass samples ($P<0.001$), and for the PAV and MAV strains detected in oat samples ($P<0.05$ and $P<0.001$ respectively). In fact, the sap of 8 grass samples known to contain RPV gave positive absorbance values for this strain (mean A_{405} = 0.213) when analysed in Nunc microtitre plates, but gave negative values (mean A_{405} = 0.075) when analysed in Dynatech plates. The sap of infected oats gave positive A_{405} values for all samples on both makes of microtitre plate.

3.3 EXPERIMENT 3: COMPARISON OF EFFICIENCY OF RINSING PLATES MANUALLY AND WITH AN AUTOMATIC PLATEWASHER

3.3.1 Materials and Methods

The objective was to determine which method was most efficient at removing plant sap and reagents containing unbound components from microtitre plates.

Handwashing involved squirting PBS-Tween under pressure from a 500 ml plastic wash bottle (Sterilin) into every well of the plate. After the wells had soaked in buffer for 90 sec, the contents were emptied out and the process was repeated twice more. Finally, the microtitre plates were emptied and banged upside down on paper towels to remove as much liquid and bubbles as possible.

The platewasher (Dynatech Laboratories Ltd.) was programmed to dispense 350 µl of PBS-Tween automatically

into each well of the plate, soak for 90 sec, aspirate the contents, and repeat twice more.

Four samples of healthy oat cv. M. Tabard and one of known-infected cv. M. Tabard were tested against all three strains of BYDV. The sap of each 2 g leaf sample was arranged in six sets of duplicate wells across every microtitre plate, and three plates (one per virus strain) were analysed for each rinsing method, simultaneously, by direct ELISA on 26 July 1988.

3.3.2 Results

After incubating for 1 h in the substrate buffer, bubbles could still be observed in some wells of the microtitre plates rinsed manually. No bubbles were present in plates rinsed automatically.

Comparing the two methods, generally, plates rinsed automatically gave lower background absorbance values (indicated by mean A_{405} values of healthy samples), minimum positive thresholds ($\bar{x}$ + 3s), and variability between wells (range of A_{405} values) than those rinsed by hand (Table 3).

The differences were significant for the mean absorbance values of healthy samples tested for RPV ($P<0.05$), PAV ($P<0.05$) and MAV ($P<0.001$). However, the mean A_{405} values of known-infected samples were not significantly lower in plates rinsed automatically, compared to those rinsed manually.

TABLE 3. Comparison of mean absorbance values (A_{405}) of oat cv. M. Tabard samples tested for BYDV by ELISA in microtitre plates rinsed manually and by an automatic platewasher.

Plant sample	Strain of BYDV	Mean A_{405} values obtained from plates washed		SE	d.f.
		Manually	Automatically		
Healthy ($\bar{x}$ + 3s)	RPV	0.06[b] (0.15)	0.04[b] (0.08)	0.008	94
Known-infected (range)		0.19[a] (0.15 - 0.20)	0.15[a] (0.12 - 0.17)	0.032	22
Healthy ($\bar{x}$ + 3s)	PAV	0.05[b] (0.13)	0.04[b] (0.07)	0.004	94
Known-infected (range)		0.28[a] (0.18 - 0.36)	0.25[a] (0.16 - 0.27)	0.027	22
Healthy ($\bar{x}$ + 3s)	MAV	0.13[b] (0.19)	0.10[b] (0.14)	0.004	94
Known-infected (range)		0.20[a] (0.19 - 0.25)	0.17[a] (0.15 - 0.18)	0.026	22

[a] mean of 12 wells; [b] mean of 48 wells.

$\bar{x}$ + 3s is the minimum positive threshold for BYDV.

Generally, the absorbance values of MAV for healthy samples were higher than expected, for either method, however, this problem was quite common when using polyclonal antibodies raised against MAV. By contrast, the ELISA values obtained with sap from the known-infected sample were lower than expected for all three strains of BYDV.

3.4 EXPERIMENT 4: COMPARISON OF SAP EXTRACTION METHODS

3.4.1 Materials and Methods

Extracting sap with a mortar and pestle is a lengthy, although effective process. It involves the cutting up of leaves, macerating with liquid nitrogen, and finally grinding for 55 sec in buffer. Therefore, a study was done on the efficiency of extracting sap using a plastic bag and homogeniser (Bioreba Ltd.) or seam roller (Colorol). The method would be faster allowing more samples to be tested. It would also save time as no washing of mortars, pestles and test tubes is required, and as the bags are disposable, would cut down possible cross-contamination.

Two methods of sap extraction were investigated, one (1) in June 1990, the other (2) in September 1990:

(1) One cereal leaf, approximately 15 cm long, was placed in a polythene bag (20.5 x 27.5 cm, QB Packing) with 1.75 ml of extraction buffer. A seam roller (5 cm wide) with a handle was pushed back and forth several times

over the bag, crushing the contents. The sap was then poured out of the bag, being careful not to include larger pieces of plant debris, into individually labelled 4 ml auto-analyser cups (Dynatech). The bags were then discarded. The cereal samples analysed were of winter wheat cv. Avalon (16). Six samples of perennial ryegrass cv. Talbot were also analysed by this method. In the case of the latter, three leaves per sample, approximately 10 cm long, were placed in each polythene bag.

(2) This method was similar except that a hand-held homogeniser (Bioreba Ltd.) was used, which resembled a passport stamper with moving metal ball-bearings on the base. As metal was rubbing on polythene, thicker bags were used (13 x 20 cm, Transatlantic Plastics). The homogeniser was very effective in macerating leaves and was more comfortable to hold than the seam roller. Again the sap was poured into individually labelled 4 ml auto-analyser cups. The samples analysed by this method were 15 cm long leaves of winter barley cv. Igri (16) and oat cv. Dula (five).

By either method it took approximately 10 sec to process a leaf prior to pipetting on to microtitre plates. Extraction using a mortar and pestle took 4 min per 1 g sample.

The ELISA method followed was as described for monoclonal antibodies (2.7.4). However, aliquots of only 150 µl instead of 200 µl were placed in each well of the

microtitre plate.

Each method was compared with mortar and pestle extraction of the same plant samples. All samples were tested for the RPV, PAV and MAV strains of BYDV, one plate per strain, per extraction method. Sap from wheat cv. Avalon and ryegrass cv. Talbot were analysed together on the same microtitre plate, while sap from barley cv. Igri and oat cv. Dula were analysed together. Some cereal samples exhibited symptoms of BYDV infection.

3.4.2 Results

Extracting virus from plant material using a polythene bag and crusher was less efficient than by mortar and pestle. ELISA analysis of the sap extracted from wheat cv. Avalon and perennial ryegrass cv. Talbot by a mortar and pestle determined that 50.0% of the wheat and 83.3% of the grass samples were infected with BYDV (Table 4). Analysis of sap extracted from the same plants using a polythene bag and roller indicated that 31.3% of the wheat and 33.3% of the ryegrass contained BYDV.

ELISA analysis of sap extracted by mortar and pestle from winter barley cv. Igri and oat cv. Dula leaves showed that 75.0% of the barley samples and 100.0% of the oat samples were infected with BYDV (Table 4). The sap extracted from the same plants using a polythene bag and hand-held homogeniser did not indicate such a high incidence of BYDV in the barley (56.3%) or oat (80.0%)

TABLE 4. Percentage test samples shown to contain BYDV by ELISA in sap extracted by polythene bag and roller/homogeniser or mortar and pestle.

Plant sample	% Samples positive for BYDV	
	Polythene bag	Mortar and pestle
Winter wheat cv. Avalon	31.3	50.0
Perennial ryegrass cv. Talbot	33.3	83.3
Winter barley cv. Igri	56.3	75.0
Oat cv. Dula	80.0	100.0
Total all samples	50.2	77.1

samples when analysed by ELISA.

The mean absorbance values (A_{405}) for sap from both infected and uninfected test plants were generally higher when extracted using a mortar and pestle than the other methods (Tables 5-8). This applied to wheat, barley, oats and ryegrass with the following exceptions: virus-free ryegrass test sap analysed for PAV extracted by the bag and roller method (Table 6, $P>0.05$); sap from RPV-infected oats extracted by the bag and homogeniser method (Table 8, $P>0.05$). In these two cases, the absorbance values obtained were lower for sap extracted by mortar and pestle compared to bag and crusher.

The MAV strain was not detected in grass test sap extracted by the bag and roller method, but was in sap extracted by mortar and pestle (Table 6). Although these positive absorbance values were relatively low, they were significantly higher than the A_{405} values of uninfected samples ($P<0.001$).

Generally, the A_{405} values obtained were significantly different between the techniques ($P<0.05$ - $P<0.001$). Where they were not, usually the range of A_{405} values was wide, or the number of samples involved was small, both giving high standard errors.

It should be noted that absorbance values obtained for healthy control samples were higher with sap extracted by mortar and pestle than by bag and crusher ($P>0.05$ - $P<0.001$). The exception was the A_{405} values of control

TABLE 5. Comparison of mean absorbance values (A_{405}) of BYDV detected by ELISA in sap extracted from winter wheat cv. Avalon using a polythene bag and roller and a mortar and pestle.

Plant sample	Strain of BYDV	Mean A_{405} values of sap extracted using a		SE	d.f.
		Bag and roller	Mortar and pestle		
Healthy control ($\bar{x}$ + 3s)	RPV	0.01[b] (0.04)	0.01[b] (0.01)	0.003	10
Positive wheat (range)		-	-	-	-
Negative wheat (range)		0.02[g] (0.00 - 0.03)	0.03[g] (0.00 - 0.05)	0.002	62
Healthy control ($\bar{x}$ + 3s)	PAV	0.01[b] (0.02)	0.00[b] (0.03)	0.003	10
Positive wheat (range)		0.49[b] (0.38 - 0.67)	1.37[c] (0.48 - 2.32)	0.221	16
Negative wheat (range)		0.02[e] (0.00 - 0.03)	0.03[d] (0.01 - 0.07)	0.003	44
Healthy control ($\bar{x}$ + 3s)	MAV	0.02[b] (0.04)	0.04[b] (0.08)	0.006	10
Positive wheat (range)		0.41[a] (0.15 - 0.66)	2.19[a] (2.09 - 2.28)	0.149	6
Negative wheat (range)		0.02[f] (0.01 - 0.04)	0.05[f] (0.02 - 0.07)	0.005	54

[a] mean of four wells; [b] mean of six wells; [c] mean of 12 wells; [d] mean of 20 wells; [e] mean of 26 wells; [f] mean of 28 wells; [g] mean of 32 wells.

$\bar{x}$ + 3s is the minimum positive threshold for BYDV.

TABLE 6. Comparison of mean absorbance values (A_{405}) of BYDV detected by ELISA in sap extracted from perennial ryegrass cv. Talbot using a polythene bag and roller and a mortar and pestle.

Plant sample	Strain of BYDV	Mean A_{405} values of sap extracted using a		SE	d.f.
		Bag and roller	Mortar and pestle		
Healthy control ($\bar{x}$ + 3s)	RPV	0.01[b] (0.04)	0.01[b] (0.01)	0.003	10
Positive grass (range)		0.56[a] (0.56 - 0.56)	1.75[a] (1.66 - 1.84)	0.105	2
Negative grass (range)		0.01[c] (0.01 - 0.02)	0.03[c] (0.01 - 0.06)	0.008	18
Healthy control ($\bar{x}$ + 3s)	PAV	0.01[b] (0.02)	0.00[b] (0.03)	0.005	10
Positive grass (range)		0.21[a] (0.20 - 0.21)	0.56[b] (0.11 - 0.93)	0.148	6
Negative grass (range)		0.03[c] (0.01 - 0.07)	0.01[b] (0.01 - 0.03)	0.013	14
Healthy control ($\bar{x}$ + 3s)	MAV	0.02[b] (0.04)	0.04[b] (0.08)	0.006	10
Positive grass (range)		-	0.13[b] (0.11 - 0.14)	0.017	4
Negative grass (range)		0.03[d] (0.02 - 0.06)	0.05[c] (0.02 - 0.07)	0.008	20

[a] mean of two wells; [b] mean of six wells; [c] mean of 10 wells; [d] mean of 12 wells.

$\bar{x}$ + 3s is the minimum positive threshold for BYDV.

TABLE 7. Comparison of mean absorbance values (A_{405}) of BYDV detected by ELISA in sap extracted from winter barley cv. Igri using a polythene bag and hand-held homogeniser and a mortar and pestle.

Plant sample	Strain of BYDV	Mean A_{405} values of sap extracted using a		SE	d.f.
		Bag and homogeniser	Mortar and pestle		
Healthy control ($\bar{x}$ + 3s)	RPV	0.02[c] (0.04)	0.02[c] (0.03)	0.003	10
Positive barley (range)		0.63[b] (0.32 - 0.93)	1.47[d] (0.15 - 2.81)	0.546	10
Negative barley (range)		0.03[i] (0.02 - 0.05)	0.06[h] (0.03 - 0.07)	0.003	50
Healthy control ($\bar{x}$ + 3s)	PAV	0.02[c] (0.04)	0.02[c] (0.05)	0.004	10
Positive barley (range)		0.99[f] (0.27 - 1.49)	2.22[g] (0.12 - 2.83)	0.186	36
Negative barley (range)		0.03[f] (0.00 - 0.06)	0.06[e] (0.04 - 0.07)	0.006	24
Healthy control ($\bar{x}$ + 3s)	MAV	0.02[c] (0.03)	0.05[c] (0.09)	0.005	10
Positive barley (range)		0.13[a] (0.12 - 0.13)	0.18[h] (0.12 - 0.28)	0.014	24
Negative barley (range)		0.04[j] (0.01 - 0.07)	0.09[d] (0.09 - 0.09)	0.009	36

[a] mean of two wells; [b] mean of four wells; [c] mean of six wells; [d] mean of eight wells; [e] mean of 10 wells; [f] mean of 16 wells; [g] mean of 22 wells; [h] mean of 24 wells; [i] mean of 28 wells; [j] mean of 30 wells.

$\bar{x}$ + 3s is the minimum positive threshold for BYDV.

TABLE 8. Comparison of mean absorbance values (A_{405}) of BYDV detected by ELISA in sap extracted from oat cv. Dula using a polythene bag and hand-held homogeniser and a mortar and pestle.

Plant sample	Strain of BYDV	Mean A_{405} values of sap extracted using a		SE	d.f.
		Bag and homogeniser	Mortar and pestle		
Healthy control ($\bar{x}$ + 3s)	RPV	0.02[c] (0.04)	0.02[c] (0.03)	0.003	10
Positive oats (range)		1.17[b] (0.42 - 1.93)	0.98[c] (0.74 - 1.18)	0.415	8
Negative oats (range)		0.03[c] (0.02 - 0.03)	0.06[b] (0.05 - 0.06)	0.008	8
Healthy control ($\bar{x}$ + 3s)	PAV	0.02[c] (0.04)	0.02[c] (0.05)	0.004	10
Positive oats (range)		1.18[c] (1.11 - 1.30)	2.61[c] (2.45 - 2.81)	0.069	10
Negative oats (range)		0.01[b] (0.00 - 0.03)	0.05[b] (0.04 - 0.06)	0.008	6
Healthy control ($\bar{x}$ + 3s)	MAV	0.02[c] (0.03)	0.05[c] (0.09)	0.005	10
Positive oats (range)		0.11[a] (0.09 - 0.12)	0.23[b] (0.13 - 0.33)	0.054	4
Negative oats (range)		0.04[d] (0.01 - 0.07)	0.09[c] (0.08 - 0.09)	0.014	12

[a] mean of two wells; [b] mean of four wells; [c] mean of six wells; [d] mean of eight wells.

$\bar{x}$ + 3s is the minimum positive threshold for BYDV.

samples tested for the RPV strain which were higher for sap extracted by bag and either the roller or the homogeniser ($P>0.05$).

Generally, BYDV was only detected using a polythene bag and crusher (roller or homogeniser) if the plant samples contained much virus. However, the variability of A_{405} values (as indicated by the range), and the minimum positive thresholds were usually greater for sap extracted by mortar and pestle compared to by bag and crusher.

The hand-held homogeniser macerated the leaf blades more successfully than did the roller. However, tiny fragments of plant debris remained in the sap blocking the tips of the Finnpippettes. Bioreba Ltd. manufacture polythene bags lined with cheesecloth which prevent this from occurring by partially filtering the sap. However, none was used in this study. Indeed, they may not be suitable in this case as so little buffer is used, and the cheesecloth may absorb too much liquid not leaving enough to pipette into the microtite plates.

3.5 EXPERIMENT 5: THE USE OF DOT-ELISA TO DETECT BYDV

3.5.1 Materials and Methods

Dot-ELISA has been used successfully to detect viruses such as potato virus Y (Berger *et al.*, 1984), potato leafroll virus (Davis Smith & Bantarri, 1984), tobacco mosaic virus, tobacco ringspot virus and tomato ringspot virus (Powell, 1984).

The method only takes 2-3 h to complete compared to the indirect ELISA method currently used in this laboratory which take 30 h. If Dot-ELISA could be used to detect BYDV, many more samples could be tested than by plate-ELISA. In addition, Dot-ELISA is more economical as the buffers containing the antibodies or conjugate can be retained and re-used. Moreover, less equipment is required compared to plate-ELISA.

3.5.1.1 METHODS OF SAP EXTRACTION

As extraction of the virus is the critical step in the detection of BYDV, several methods were examined:

(1) Leaves exhibiting symptoms plus some healthy control samples were prepared as for plate-ELISA (2.7.3). Leaf material (1 g) was ground in a mortar and pestle with liquid nitrogen. The resulting fine powder was diluted in PBS-Tween as follows:

(i) 1 g powder : 5 ml extraction buffer
(ii) 1 g powder : 2.5 ml extraction buffer
(iii) 1 g powder : 2 ml extraction buffer
(iv) 1 g powder : 1 ml extraction buffer

The fine powder was ground for 55 sec in buffer before it was strained through cheesecloth into 30 ml centrifuge tubes to remove larger pieces of plant material. The extracts were then decanted into 4 ml auto-analyser cups.

(2) One leaf was placed in a polythene bag (13 x 20 cm, Transatlantic Plastics) with 1.75 ml of PBS-Tween buffer and crushed with a hand-held homogeniser (Bioreba Ltd.). Buffer-containing sap was then poured into 4 ml auto-analyser cups.

Leaves of test and control plants used throughout these procedures were of either winter barley cv. Igri, or oat cv. Dula (see below, 3.5.1.4). One known-infected and one healthy control sample (repeated three times) was included in every test.

3.5.1.2 EQUIPMENT AND REAGENTS

The sap was spotted on to a sheet of nitrocellulose paper (Anderman & Co Ltd.) using a 0-20 µl Pipetman (P20, Gilson). The paper could be cut to size according to the number of samples to be analysed, the maximum being approximately 8 x 9.5 cm. A separate sheet of nitrocellulose paper was used for each virus strain, and placed in individual petri dishes (diam. 9 cm or 14 cm, Sterilin), large enough for the sheet to lie flat. Each dish was marked permanently with the strain of virus under test.

As indirect Dot-ELISA was used, the same monoclonal antibodies (MAC 91, MAC 92 and MAFF 2) and anti-rat alkaline phosphatase conjugate were used as for plate-ELISA, the former used at 1:750 and 1:500, the latter at 1:1000. Monoclonals diluted 1:500 were used only when 5

ml of PBS-Tween was used to dilute sap (see 3.5.1.4). Apart from PBS-Tween for sap extraction, the Dot-ELISA required different reagents to those used in plate-ELISA:

TRIS BUFFERED SALINE (TBS, 0.05 M)

6.0 g Tris-(hydroxymethyl)-aminomethane ($(HOCH_2)_3CNH_2$, Aldrich)

11.7 g sodium chloride (NaCl, BDH)

1000 ml distilled water

Adjust to pH 7.4 with concentrated hydrochloric acid (HCl)

NAPTHOL AS-BI STOCK SOLUTION

50 mg Napthol AS-BI phosphate (Sigma)

20 ml N:N dimethyleformamide (Sigma)

20 ml distilled water

Adjust to pH 8.0 with 0.1 M sodium carbonate (Na_2CO_3, BDH) added dropwise, then add 600 ml distilled water

360 ml 0.2 M TBS

FAST-RED SOLUTION

10 mg Fast-red TR salt (Sigma)

10 ml Napthol AS-BI stock solution

Shake well and filter before use.

The Napthol AS-BI stock solution was stored in the dark at 4°C (i.e. refrigerator). The Fast-red solution was freshly made, in a fume cupboard, for every step, while TBS was made in bulk (10 l) and stored at room temperature, 20°C.

3.5.1.3 PROCEDURE FOR INDIRECT DOT-ELISA

This method is basically that of D.H. Mitchell, Department of Agriculture and Fisheries for Scotland (DAFS), East Craigs, Edinburgh (personal communication).

Once the sap had been extracted, 2 µl of each sample was spotted, in duplicate, on to the membranes and allowed to dry. Each sheet was washed briefly in TBS to remove any surface debris and placed in the petri dishes. Unbound sites were blocked with dried milk (Safeways) plus 1% bovine serum albumen (BSA, BDH) in TBS. Normally, 10 g dried milk with 1 g albumen in 100 ml TBS was required for three dishes (diam. 14 cm). It was imperative that the milk in the dishes was shaken constantly or only some sites on the nitrocellulose would be blocked. Dishes were placed in an orbital agitator (Gallenkamp) and shaken for 1 h at 20^{o}C.

Following incubation, all sheets were washed with TBS until all traces of milk were removed. Each membrane was then floated in TBS, agitated for 2 min, and repeated three times in fresh TBS. Inadequate washing after the blocking stage would result in uneven patches of colour on the developed sheet.

Monoclonals diluted in TBS were then added to the relevant dishes and again incubated for 1 h at 20^{o}C with constant shaking. At the end of this stage, each monoclonal antibody, MAC 91, MAC 92 and MAFF 2, could be poured back into its labelled beaker, refrigerated and re-

used for a further 2-3 tests. All papers were rinsed three times for 3 min with TBS before rabbit anti-rat conjugate in TBS was added to all dishes.

Following a further 1 h incubation at 20°C with constant shaking, conjugate was retained as for monoclonals before the membranes were rinsed three times for 3 min in TBS. Poor washing after conjugation would result in a high background reaction and rapid deterioration of the substrate solution.

Presence of virus was then visualised using freshly-made Fast-red solution. The nitocellulose papers were left at room temperature (20°C) without shaking for up to 3 h. Positive identification of virus took place when a red ring appeared round the dotted samples. This reaction should be very obvious round the known-infected extracts, and absent round the healthy control samples.

Once the paper itself began to turn pink, the reaction was stopped by lifting the papers out of the Fast-red TR solution and rinsing with TBS. The papers were then dried and kept as a permanent record.

3.5.1.4 DETAILS OF TEST PLANT SAMPLES USED

The number and cultivar of samples used in the tests described in 3.5.1.1 were as follows:

(1) (i) 37 samples : 16 winter barley cv. Igri (1:750)
(7.8.90) 21 oat cv. Dula (1:750)
eight samples : four cv. Igri (1:500)
(13.8.90) four cv. Dula (1:500)
(ii) six samples : three cv. Igri
(19.9.90) three cv. Dula
(iii) four samples : four cv. Igri
(19.9.90)
(iv) two samples : two cv. Dula
(19.9.90)

(2) 26 samples : 15 winter barley cv. Igri
(5.9.90) 11 oat cv. Dula

Each sample analysed by Dot-ELISA was compared to analyses of the same plants by indirect plate-ELISA.

3.5.2 Results

No virus was detected on the nitrocellulose papers spotted with plant sap extracted by either grinding 1 g of leaf material with 5 ml buffer in a mortar and pestle, or by crushing a leaf in a polythene bag with 1.75 ml buffer. After 30 min in Fast-red solution, all papers for each virus strain began to turn pink and the reaction was stopped. This occured faster for monoclonals diluted at 1:500 than at 1:750. Eventually, the Fast-red solution itself in all dishes turned pale pink after 30 min.

Surrounding each spotted sample was a white circle irrespective of virus content; the latter was determined

from the absorbance values (A_{405}) obtained with sap analysed by plate-ELISA.

The only exceptions were a few necrotic samples which had been used as source leaves in aphid transmission tests. These spotted plant samples (prepared by mortar and pestle) were surrounded by red circles, however, the results did not always correlate with the findings of the plate-ELISA method. These samples were spotted on as brown/red dots compared to all others which were green spots. In no cases did the known-infected samples produce a red ring.

Leaf material ground in smaller amounts of buffer, i.e. 2.5 ml, 2 ml and 1 ml buffer, produced very different results. Again the sheets turned pink overall after approximately 30 min, however if left in solution for 2 h, red rings appeared around the spotted samples. These again did not correlate with plate-ELISA results and did not depend on dilution. In addition red circles, although fainter appeared around the healthy controls.

3.6 EXPERIMENT 6: THE USE OF SQUASH BLOT ELISA TO DETECT BYDV

3.6.1 Materials and Methods

Squash blotting, a novel method of sampling and detecting plant viruses, has been developed at the DAFS Laboratory, East Craigs, Edinburgh. The technique involves direct sampling of plant material in the field. This solves the

problems associated with storage plus eliminating the time-consuming task of sample extraction in the laboratory. This method has been used successfully to detect potato viruses such as potato virus Y (D.H. Mitchell, personal communication).

In the field, sampling with Squash blots is faster and more convenient than leaf sampling. Sample details can be written directly on to the body of the device (plastic label, see below, 3.6.1.1), and once the sap has dried on to the Squash blots, any virus which it may contain will remain stable for at least 6 months (D.H. Mitchell, personal communication).

Like the Dot-ELISA (Experiment 5), the Squash blot technique has not been successfull with BYDV (D.H. Mitchell, personal communication). If this method could detect BYDV particles, it could permit analysis of many more samples due to elimination of extraction time combined with the fact that this technique takes only 3 h to complete.

The procedure, including reagents and buffers, is similar to that for the Dot-ELISA described earlier (3.5.1.2, 3.5.1.3). The difference is that flexible plastic strips are used instead of nitrocellulose paper.

Each sample analysed by indirect Squash blot ELISA was compared to analyses of the same plants by indirect plate-ELISA.

3.6.1.1 PRODUCTION OF SQUASH BLOTS

This method is basically that of D.H. Mitchell, P.J. Howell and D.G. Rose (personal communication).

Squash blots were made using plastic-coated plant labels (15 x 2.5 cm, NBS Clansman). The tips were coated with nitrocellulose. This was done by dissolving 1.5 g of nitrocellulose paper in 25 ml of methanol (Sigma) and 25 ml of dimethyl sulfoxide (Sigma). A very thin layer of the solution was painted on to the top 2.5 cm of the labels with a small paintbrush. The labels were allowed to dry overnight when they were glued together in pairs (Pritstick) with the treated tips innermost left free to move apart.

3.6.1.2 SAMPLING PROCEDURE

The sample was taken by folding the chosen leaf two - three times, placing it between the pointed ends of the Squash blot and squeezing several times using a small pair of pliers. Pliers were washed with clean water between crushing each sample to prevent cross-contamination. Upon removal of the leaf debris, a visible green tinge of plant sap was present on the treated surface. Tests were only done on infected oat cv. Dula (eight) and winter barley cv. Igri (eight) as insufficient sap was expressed from grass leaves.

The blots dried rapidly in the air, after which the treated ends were cut from the rest of the strip, bent

slightly, and placed in RPV, PAV, or MAV labelled plastic petri dishes (diam. 9 cm or 14 cm, Sterilin), the size of the dish depending on the number of samples being analysed. The tips were bent to ensure that they did not stick together in the petri dishes, and each tip was labelled with indelible ink.

As only two strips were used per leaf, each sample could only be tested for two strains of BYDV. However, if the initial results proved negative, sample tips could be washed with TBS and reprobed with a different antibody. Sample leaves requiring analysis of all three strains simultaneously were cut in half lengthways and crushed between two sandwiched strips, giving four tips for analysis. One known-infected and a healthy control sample were included in each dish. Monoclonal antibodies and the conjugate were diluted in TBS at 1:750 and 1:1000 respectively.

3.6.1.3 PROCEDURE FOR INDIRECT SQUASH BLOT ELISA

When the treated tips were in the petri dishes, they were soaked for 1 h at 20^{o}C under constant agitation in dried milk solution to block any unused protein-binding sites on the nitrocellulose. Following triple rinsing with TBS, the tips were incubated at 20^{o}C for 1 h with constant shaking together with the relevant monoclonal antibodies, MAC 91, MAC 92, or MAFF 2. Tips were again washed three times with TBS to remove any antibody which had not bound to virus. Subsequently, enzyme-conjugated rabbit anti-rat

antibody was added to all dishes and incubated for 1 h at 20°C with constant agitation. As with Dot-ELISA, the buffers containing the monoclonal antibodies or conjugate could be retained and re-used.

After a further three rinses with TBS, Fast-red TR solution was added which stains the enzyme label a bright red colour, so any virus stuck to the Squash blot would be visible with a colour change from green to red.

Tips were kept for up to 1 h at room temperature (20°C) to observe any colour changes.

3.6.2 Results

At the end of the process, all tips turned mottled red after approximately 30 min, including the healthy controls. By this stage, no green tinge was observed. The solution in each dish turned pink after standing for 20-30 min.

3.7 DISCUSSION

Previous work has shown that PAV and MAV are serologically related, but not identical (Aapola & Rochow, 1971; Rochow *et al.*, 1971), and a heterologous reaction in direct ELISA was noted by Rochow & Carmichael (1979). This was due to a weak heterologous reaction between PAV antigen and MAV-specific globulin, and a reciprocal reaction between MAV antigen and PAV-specific globulin when using polyclonal immunoglobulins. However, in an indirect ELISA,

monoclonal antibodies are strain-specific and do not cross-react (Halk & De Boer, 1985).

Lister & Rochow (1979) obtained low background readings (A_{405}), with an average mean for healthy controls of 0.008 when using polyclonal antibodies. However, the antiserum used to prepare the globulins had previously been adsorbed with an equal volume of a concentrate of healthy oat sap, a step which Rochow felt contributed to low background readings (Rochow & Carmichael, 1979; Rochow, 1982b).

The antiserum used in the present research was not pre-adsorbed with oat sap, perhaps giving higher background absorbance values. However, it has been found that some plant saps can induce strong non-specific background colour reactions which can make it difficult to distinguish positive from negative results (Torrance & Jones, 1981).

None of the polyclonal antisera, buffers, or microtitre plates was contaminated with, for example, bacteria or dirt, as all these reagents or equipment were used in the indirect method where low background absorbance values were obtained. Therefore, high background absorbance values obtained with polyclonal antiserum may be partly attributable to impure conjugation of enzyme to antiserum.

As the mean of the healthy samples plus three times the standard deviation of the mean ($\bar{x}$ + 3s) was used as a

threshold for ascertaining positive samples, a high background absorbance value combined with the variation between duplicate wells, as found with polyclonals, could mask samples containing virus. Lower background A_{405} values obtained with monoclonals, and less variation between duplicate wells, enabled samples with little virus to be detected. This is because monoclonal antibodies eliminate the qualitative and quantitative variability in specific antibody content found in batches of polyclonal serum from several different bleedings (Halk & De Boer, 1985).

It is acknowledged that there are disadvantages in using monoclonal antibodies. They are only as good as the panel of BYDV isolates against which they were selected, i.e. other variants of a particular strain may not be detected.

Nevertheless, Koenig & Paul (1982) concluded that indirect ELISA methods may be preferable to direct methods as BYDV occurs in an unknown number of serologically different strains (Rochow & Carmichael, 1979). Thus it was decided to use monoclonal antibodies for the routine testing of all plant samples.

However, relying exclusively on ELISA tests for the analysis of plant samples could mean that new variants arising through mutation would not be detected (Rochow, 1979a). This highlights the importance of aphid transmission tests.

In both the direct and indirect methods of ELISA, the variability between wells of infected samples was great, giving large standard errors. However, it should be noted that the mean values given in the results (Table 1) are of 10 different test samples. Each sample contained different levels of virus, giving a range of absorbance values. Ideally, a single known-infected sample should have been tested repeatedly, thus giving a low standard error and more significant results (as was achieved for the healthy sample so analysed). This procedure should have been carried out for all test samples analysed throughout Chapter 3. However, a range of test samples was used in each experiment, thus many results have high standard errors producing lower SED values.

The adsorption of antibodies on to the wells of microtitre plates varies with the manufacturor of the plate (Hill, 1984). In the present research, sap containing BYDV produced higher absorbance values when analysed in Nunc microtitre plates than in Dynatech plates. In contrast, lower absorbance values were obtained with virus-free samples analysed in the former microtitre plates compared to in the latter. It would appear that Nunc microtitre plates allow better adsorption of antibodies to the wells than do Dynatech, without producing a high background reading.

In the past, Nunc plates have also been found to be more suitable than Dynatech. Holmes (1985) found Nunc 1 immunoplates gave greater consistency between wells and

between plates within a batch than Dynatech 129B microtitre plates.

Greater variability between wells containing healthy samples in plates rinsed manually compared to those rinsed by the automatic platewasher appeared to be partly due to bubbles present in the substrate buffer. Although banging the plates on paper towels removed most bubbles, some still remained, even after 1 h in the substrate buffer. Any contamination, such as specks of dust, or bubbles, produces a higher absorbance value than normal in the spectrophotometer. Therefore, some wells containing healthy sap may have bubbles on the surface, others not, but the absorbance values show greater variability between wells which in turn produce a higher minimum positive threshold.

The platewasher does not cause bubbles to be formed on the surface of the buffer, therefore, the absorbance value is due to the correct absorbance of the colour change and variation is reduced. A low control value allows samples containing small levels of BYDV to be detected.

Another factor causing variation in the results obtained from microtitre plates rinsed manually would be the individual rinsing the plates. The operator will not be able to maintain a constant pressure of PBS-Tween entering each well in every plate. Some wells will be rinsed more thoroughly than others. As the platewasher is

automatic, it dispenses the same quantity of buffer into each well with a uniform force, thus reducing variation.

The platewasher has the disadvantage that occasionally the dispensing or aspirating jets become blocked. However, a purge programme at the start of every wash determines where this is a block, i.e. the correct quantity of buffer is dispensed into every well of the microtitre plate. Any well not filled to the correct level indicates a blocked dispensing jet. The reverse situation indicates a blocked aspirating jet. Any blocked jets can be cleared and the plates washed properly.

On the basis of the data from these two experiments, it was concluded that Nunc-Immuno maxisorp ELISA plates rinsed by the automatic platewasher gave the most reliable results. Thus this system was adopted for all ELISA tests.

Grinding leaf material with a mortar and pestle was found to be the most satisfactory method of extracting BYDV from infected plants. Methods employing polythene bags and crushers yield insufficient virus, as indicated by lower absorbance values obtained from infected test samples compared to those obtained for sap extracted by mortar and pestle. Although the leaves appeared suitably macerated in the plastic bags, it is possible that the phloem vessels containing the virus particles were not always broken.

Nevertheless, slightly higher absorbance values were also obtained with uninfected sap extracted by mortar and pestle compared to by bag and crusher. The reason for this is unclear. It should not be a background reaction to plant cell structures as monoclonal antibodies are monospecific for a single antigenic determinant (Halk & De Boer, 1985). It would be expected that the low A_{405} values obtained from healthy control sap extracted by the bag and crusher method, would be beneficial in ascertaining low levels of virus in test samples as the minimum positive thresholds would also be low. However, low levels of MAV in perennial ryegrass cv. Talbot, detected in sap extracted by mortar and pestle, were not detected in sap extracted by the bag and roller method.

As plant samples analysed in all investigations throughout this research were of variable virus content, polythene bags and crushers were unreliable methods for detecting BYDV. Therefore, mortars and pestles were used to extract sap from all plant material tested.

The successful employment of either the Dot-ELISA, or the Squash blot ELISA would have been a tremendous asset in allowing more samples to be analysed; both tests producing results in a matter of hours. Amplified plate-ELISA can also detect BYDV in oat sap in less than 4 h (Torrance, 1987). However, Squash blot ELISA would reduce preparation time, and make it unnecessary to store plant samples.

Neither technique worked satisfactorily for the detection of BYDV. It is acknowledged, nevertheless, that only a limited number of samples were tested by Dot-ELISA (83), and especially by Squash blot ELISA (16).

Results from Dot-ELISA were unreliable; often indicating the presence of virus in uninfected plant sap, or lack of virus in infected sap. The red rings observed around the spots of sap from the necrotic leaves (diluted 1 g : 5 ml) appeared genuine except that they did not correlate fully with plate-ELISA results for the same plant samples. In addition, other green spots on these sheets did not exhibit such red rings despite having equally high or higher virus contents. Therefore, the red rings described above may have been caused by the brown/red colour of the sap seeping through the nitrocellulose paper.

The detection of virus did not differ with the cultivar of the plant, or whether the sap had been extracted using a mortar and pestle, or a hand-held homogeniser.

Although Dot-ELISA generally did not show the presence of virus (i.e. red rings), including known-infected samples, the exception was with low dilutions of plant material. Here red rings appeared around all samples including uninfected extracts, proved to be virus-free by plate-ELISA. In this case, perhaps the ring is a reaction to the plant material as it was more concentrated

than normal. The response is unlikely to be a genuine indication of the presence of BYDV as red rings were also observed around the known-healthy controls.

However, a proper comparison to other nitrocellulose papers in this study cannot be made as those described above were left to incubate in the Fast-red solution for 2 h. At 30 min, all papers in the present research turned pink, at which point the papers spotted with sap diluted in 5 ml TBS were taken out, stopping the reaction with no formation of rings. Sheets spotted with sap diluted in 1-2.5 ml buffer also turned pink at 30 min with no evidence of rings. These were left in solution where the colour of the papers remained static, but red rings developed around the samples. Therefore, it is not known whether this reaction would have occurred to samples on other papers had they been left for 2 h, or whether it is indeed a reaction to the plant material diluted in less buffer.

Failure of Squash blots to detect BYDV successfully are likely to have been due to the extraction method. It is doubtful that enough pressure can be applied with pliers to rupture the phloem cells to release sufficient virus. This method is particularly suited to the detection of viruses that occur in high concentrations, such as PVY (D.H. Mitchell, personal communication) but not, it would appear, to phloem-restricted viruses.

The overall reddening of all papers and plastic tips at the end of the process may be attributable to

inadequate washing of the papers/tips following incubation with the conjugated antiserum. This practice has been reported to produce a high background reaction and rapid deterioration of the substrate solution (D.H. Mitchell, personal communication) This may result in the solution turning pink thus staining the papers or tips. Clark *et al.* (1986) also commented that problems of conjugate penetration and of inadequate washing can cause difficulties in discriminating specific and nonspecific reactions.

In addition, the monoclonal antibodies may have been used too dilute for the methods. However, as the titres of BYDV are often low in test samples, it may be inefficient to use higher dilutions.

This problem combined with the minute quantity of sap (2 µl) employed with Dot-ELISA may mean that these methods of detecting virus are not suitable for BYDV. Nevertheless, further studies investigating other experimental parameters, e.g. dilutions, or reagents, may show one and/or both techniques to be an alternative and efficient method for the detection of BYDV.

CHAPTER 4

THE TRANSMISSION OF THREE STRAINS OF BYDV BY *Rhopalosiphum padi* AND *Sitobion avenae* FROM OAT CV. MARRIS TABARD TO A RANGE OF OAT CULTIVARS

4.1 INTRODUCTION

As 35,647 ha of oats were grown in Scotland in 1988 (Anon., 1988), it was of interest to determine the incidence of BYDV, and whether oats could be a potential reservoir of the virus.

The aim of this study was to establish the transmission rates of the RPV, PAV and MAV strains, both individually and in various combinations by *R. padi* and *S. avenae*. BYDV was transmitted from oat cv. M. Tabard to oat cultivars M. Tabard, Blenda and Pennalt. These cultivars were used as they exhibit clear symptoms of infection by BYDV.

4.2 MATERIALS AND METHODS: EXPERIMENTS 1 - 12

In each experiment, one adult apterous *R. padi*, or *S. avenae* was placed on each of 50 seedlings (12-day-old) of the oat cultivar being tested. The aphids used had been allowed to feed on oat cv. M. Tabard infected with the RPV, PAV and MAV strains of BYDV singly or in combination. The exception was in Experiment 10 in which aphids were allowed to feed on oat cv. Dula infected with PAV + MAV. The acquisition and transmission procedures were as described previously (2.6). After inoculation the test plants were placed in a heated glasshouse.

Approximately 4 weeks after inoculation, foliar symptoms were noted. In Experiments 1-6 and 11-12, on average the leaves of 5-10 oat plants showing symptoms

of infection of BYDV, 5-10 symptomless plants, and leaves of any plants with foliar discoloration which was not typical of BYDV infection were collected, placed in labelled polythene bags, and kept in a deep-freeze at -18^{o}C until tested by ELISA. In Experiments 7-10 all oat plants were harvested as described above, except for Experiment 10 where only half of the plants inoculated by *S. avenae* were harvested.

The average survival rate of aphids during the 48 h inoculation feed was 90.6% for *R. padi*, and 85.3% for *S. avenae* (minimum (min.) 79.3% for *R. padi* in Experiment 7, and 71.0% for *S. avenae* in Experiment 9).

Soon after all plants were placed in the glasshouse, the source leaves used for the acquisition feed (48 h) were analysed by ELISA to confirm the strains of BYDV present.

The transmission rates of *R. padi* and *S. avenae* were investigated simultaneously for each strain, or mixture of strains. Therefore, in every ELISA test, samples of sap from plants fed upon by each aphid species were included in each microtitre plate. Thus, the transmission efficiency of the two species could be compared.

The dates the plants were sown, inoculated and assessed for foliar symptoms, together with the mean, minimum and maximum (max.) glasshouse temperatures are recorded for each experiment in Table 1, Appendix I.

Experiments 11-12 were carried out to investigate whether the species of aphid used to inoculate a plant affected the rate of transmission by a subsequent aphid species from that plant.

4.2.1 Experiment 11

Four RPV-infected oat cv. M. Tabard plants were used for the 48 h acquisition feed. Two had been inoculated by *S. avenae*, and two by *R. padi*. One vial per plant was set up as follows:-

Vials 1 and 2 each contained 70 *S. avenae* feeding on leaves from the two plants previously inoculated by *R. padi*.

Vial 3 contained *S. avenae* and leaves from one of the two plants originally infested by *S. avenae*.

Vial 4 contained a colony of *R. padi* and leaves from the other plant previously exposed to *S. avenae*.

Following the acquisition feed, 50 aphids from each vial were placed singly on seedlings of oat cv. M. Tabard.

4.2.2 Experiment 12

Two PAV-infected oat cv. M. Tabard plants were used for the acquisition feed (48 h). One had been inoculated by *S. avenae*, the other by *R. padi*. The vials were set up as follows:-

Vial 1 contained 70 *S. avenae* feeding on source leaves previously exposed to *R. padi*.

Vial 2 contained a colony of *S. avenae* and some leaves from the plant originally infested by *S. avenae*.

Vial 3 contained *R. padi* and the remaining leaves from the plant previously inoculated by *S. avenae*.

Following the acquisition feed, 50 aphids from each vial were placed singly on to seedlings of oat cv. Blenda.

4.2.3 Calculation of overall percentage transmission

As mentioned previously, not all plants grown in each experiment were analysed by ELISA. Nevertheless, in Experiments 1-12, the presence or absence of virus as determined by foliar symptoms (typical or none) was closely confirmed by ELISA tests (Table 9).

This was especially important in the investigations where only a proportion of the symptomless plants and those with typical symptoms were analysed by ELISA (Experiments 1-6 and 11-12). As foliar symptoms and ELISA results matched exactly in such cases, it was assumed that the remaining plants with and without symptoms also did, or did not contain virus. This allowed the overall percentage transmission to all plants grown in each experiment to be calculated. All plants with atypical symptoms were analysed giving an exact percentage which was included in the overall percentage transmission.

TABLE 9. ELISA analysis of inoculated oat cultivars with and without symptoms of infection (Experiments 1-12).

Symptom expression	No. of plants positive in ELISA test/no. of plants analysed	
None	3/238	(1.3)*
Yellow or red leaves/ stunting (typical)	303/306	(99.0)
Leaf chlorosis (atypical)	51/86	(59.3)

* Figures in parentheses are the percentage infection.

4.3 RESULTS

4.3.1 Experiment 1: The transmission of the RPV strain to oat cv. M. Tabard

Typical symptoms of BYDV infection were observed on the majority of the oat cv. M. Tabard plants fed upon by *R. padi* (45, Table 10). In addition, three plants developed atypical symptoms (yellow leaves). In contrast, the majority of plants fed upon by *S. avenae* remained symptomless (47). However, three plants did develop typical symptoms of infection.

In ELISA tests, all plants analysed with typical or atypical symptoms of BYDV were found to contain RPV. The symptomless plants infested by *S. avenae* were shown to be free of virus, however, RPV was detected in one of the two symptomless plants exposed to *R. padi*.

Overall, *R. padi* transmitted RPV to oat cv. M. Tabard at a much higher frequency (98.0%) than did *S. avenae* (6.0%, $P<0.001$).

4.3.2 Experiment 2: The transmission of the PAV strain to oat cv. M. Tabard

All plants exposed to *R. padi* became severely stunted with yellow or red-tipped leaves (Table 11). Similar symptoms of BYDV also developed on 14 oat plants inoculated by *S. avenae*.

TABLE 10. Symptom expression and detection by ELISA of the RPV strain of BYDV transmitted by *R. padi* and *S. avenae* from oat cv. M. Tabard to oat cv. M. Tabard.

Symptom expression	Number of plants		Virus incidence*	
	R. padi	*S. avenae*	*R. padi*	*S. avenae*
None	2	47	1/2	0/10
Yellow or red leaves/stunting (typical)	45	3	10/10	3/3
Leaf chlorosis (atypical)	3	0	3/3	-
Total	50	50		
% Transmission			98.0	6.0
Standard error			± 1.98	± 3.36

* Expressed as number of plants positive/number analysed in ELISA test.

TABLE 11. Symptom expression and detection by ELISA of the PAV strain of BYDV transmitted by *R. padi* and *S. avenae* from oat cv. M. Tabard to oat cv. M. Tabard.

Symptom expression	Number of plants		Virus incidence*	
	R. padi	*S. avenae*	*R. padi*	*S. avenae*
None	0	36	-	0/5
Yellow or red leaves/stunting (typical)	50	14	10/10	5/5
Leaf chlorosis (atypical)	0	0	-	-
Total	50	50		
% Transmission			100.0	28.0
Standard error			± 0.00	± 6.35

* Expressed as number of plants positive/number analysed in ELISA test.

Analysis of the leaves by ELISA revealed that PAV was present in all plants with typical symptoms of infection. No virus was found in the symptomless plants.

The results indicated that *R. padi* transmitted PAV more successfully (100.0%) to oat cv. M. Tabard than did *S. avenae* (28.0%, $P<0.001$).

4.3.3 Experiment 3: The transmission of the MAV strain to oat cv. M. Tabard

All oat cv. M. tabard plants fed upon by *R. padi* remained symptomless (Table 12). Of those exposed to *S. avenae*, 14 plants developed typical symptoms of BYDV, while atypical symptoms were observed on six plants.

BYDV was not detected in the symptomless leaves analysed by ELISA. However, MAV was detected in all plants analysed with typical symptoms, and in four plants exhibiting atypical foliar discoloration.

From these results it was concluded that *R. padi* was unable to transmit MAV, whereas *S. avenae* did at a significant level (36.0%, $P<0.001$).

4.3.4 Experiment 4: The transmission of a mixture of the RPV and PAV strains to oat cv. M. Tabard

Eighteen and four plants infested by *S. avenae* developed typical and atypical symptoms, respectively (Table 13). Whereas, the majority of the plants fed upon by *R. padi*

TABLE 12. Symptom expression and detection by ELISA of the MAV strain of BYDV transmitted by *R. padi* and *S. avenae* from oat cv. M. Tabard to oat cv. M. Tabard.

Symptom expression	Number of plants		Virus incidence*	
	R. padi	*S. avenae*	*R. padi*	*S. avenae*
None	50	30	0/10	0/5
Yellow leaves/ stunting (typical)	0	14	-	7/7
Leaf chlorosis (atypical)	0	6	-	4/6
Total	50	50		
% Transmission			0.0	36.0
Standard error			± 0.00	± 6.79

* Expressed as number of plants positive/number analysed in ELISA test.

TABLE 13. Symptom expression and detection by ELISA of a mixture of the RPV and PAV strains of BYDV transmitted by *R. padi* and *S. avenae* from oat cv. M. Tabard to oat cv. M. Tabard.

Symptom expression	Number of plants		Virus incidence*	
	R. padi	*S. avenae*	*R. padi*	*S. avenae*
None	6	28	0/5	0/5
Yellow or red leaves/stunting (typical)	38	18	5/5	5/5
Leaf chlorosis (atypical)	6	4	6/6	0/4
Total	50	50		
% Transmission			88.0	36.0
Standard error			± 4.60	± 6.79

* Expressed as number of plants positive/number analysed in ELISA test.

developed typical symptoms of infection (38), with six also developing atypical foliar discoloration.

In ELISA tests, BYDV was detected in all leaves analysed with typical symptoms, and also in all plants exhibiting atypical symptoms which had been fed upon by *R. padi*. No virus was detected in the symptomless leaves analysed.

Overall, the percentage transmission was greater for *R. padi* (88.0%) than for *S. avenae* (36.0%, $P<0.001$).

The incidence of virus in the leaves analysed indicated that *R. padi* transmitted the PAV strain (50.0%) at a higher frequency than RPV + PAV (6.3%, $P<0.01$, Table 14). It was interesting to note that no RPV alone was detected. The transmission of PAV by *S. avenae* (21.4%) was not significantly lower (statistically) than that of *R. padi* due to the small number of plants analysed.

4.3.5 Experiment 5: The transmission of the PAV strain to oat cv. Blenda

The majority of plants exposed to either *R. padi* (37) or *S. avenae* (33) developed typical symptoms of infection (Table 15). A number of plants also exhibited atypical foliar discoloration (six and 16, respectively).

The PAV strain was detected in all plants analysed with typical symptoms, and in 36.4% of the plants exhibiting atypical symptoms.

TABLE 14. The transmission of a mixture of the RPV and PAV strains of BYDV by *R. padi* and *S. avenae* from oat cv. M. Tabard to oat cv. M. Tabard. Results are expressed as the percentage transmission ± the standard error of the mean.

	% Plants tested inoculated by	
Strain of BYDV	*R. padi*	*S. avenae*
RPV	0.0 ± 0.00	0.0 ± 0.00
PAV	50.0 ± 12.50	21.4 ± 10.96
RPV + PAV	6.3 ± 6.07	0.0 ± 0.00

TABLE 15. Symptom expression and detection by ELISA of the PAV strain of BYDV transmitted by *R. padi* and *S. avenae* from oat cv. M. Tabard to oat cv. Blenda.

Symptom expression	Number of plants		Virus incidence*	
	R. padi	*S. avenae*	*R. padi*	*S. avenae*
None	7	1	0/5	0/1
Yellow or red leaves/stunting (typical)	37	33	5/5	5/5
Leaf chlorosis (atypical)	6	16	4/6	4/16
Total	50	50		
% Transmission			78.0	70.0
Standard error			± 5.86	± 6.48

* Expressed as number of plants positive/number analysed in ELISA test.

The results obtained from this experiment indicated that *R. padi* and *S. avenae* transmitted PAV at similar rates from oat cv. M. Tabard to oat cv. Blenda (78.0% and 70.0%, respectively).

4.3.6 Experiment 6: The transmission of the RPV strain to oat cv. Blenda

All plants fed upon by *S. avenae* remained symptomless, whereas the majority of plants infested by *R. padi* developed typical symptoms of BYDV infection (41, Table 16).

Analysis of the leaves by ELISA showed that RPV was present in all plants exhibiting typical symptoms of BYDV, but in none of those plants remaining symptomless.

On the basis of these results, it was concluded that *S. avenae* was unable to transmit RPV from oat cv. M. Tabard to oat cv. Blenda, while *R. padi* was able to at a high frequency (82.0%, $P<0.001$).

4.3.7 Experiment 7: The transmission of a mixture of the RPV, PAV and MAV strains to oat cv. Pennalt

The majority of oat cv. Pennalt exposed to *R. padi* developed typical symptoms of BYDV infection (46, Table 17), with a further two plants developing atypical foliar discoloration. In contrast, the majority of plants fed upon by *S. avenae* remained symptomless (34) with 12 and four of the remaining plants developing typical and

TABLE 16. Symptom expression and detection by ELISA of the RPV strain of BYDV transmitted by *R. padi* and *S. avenae* from oat cv. M. Tabard to oat cv. Blenda.

Symptom expression	Number of plants		Virus incidence*	
	R. padi	*S. avenae*	*R. padi*	*S. avenae*
None	8	50	0/5	0/10
Yellow or red leaves/stunting (typical)	41	0	10/10	-
Leaf chlorosis (atypical)	1	0	0/1	-
Total	50	50		
% Transmission			82.0	0.0
Standard error			± 5.43	± 0.00

* Expressed as number of plants positive/number analysed in ELISA test.

TABLE 17. Symptom expression and detection by ELISA of a mixture of the RPV, PAV and MAV strains of BYDV transmitted by *R. padi* and *S. avenae* from oat cv. M. Tabard to oat cv. Pennalt.

Symptom expression	Number of plants		Virus incidence*	
	R. padi	*S. avenae*	*R. padi*	*S. avenae*
None	2	34	0/2	2/34
Yellow or red leaves/stunting (typical)	46	12	45/46	12/12
Leaf chlorosis (atypical)	2	4	1/2	2/4
Total	50	50		
% Transmission			92.0	32.0
Standard error			± 3.84	± 6.60

* Expressed as number of plants positive/number analysed in ELISA test.

atypical symptoms, respectively.

In ELISA tests, BYDV was detected in all plants bearing typical symptoms of infection, with the exception of one plant infested by *R. padi*. In addition, virus was detected in half of all plants exhibiting atypical foliar discoloration. All symptomless plants were shown to be free of virus except for two which had been infested by *S. avenae*. These were found to be positive for the MAV strain.

The incidence of virus in the leaves analysed indicated that *R. padi* transmitted RPV and PAV together (74.0%) rather than either strain singly ($P<0.001$, Table 18). By contrast, *S. avenae* preferentially transmitted PAV (18.0%) and MAV (8.0%) singly rather than together ($P<0.05$ and $P>0.05$, respectively).

Overall, *R. padi* was a more efficient vector of BYDV (92.0%) than *S. avenae* (32.0%, $P<0.001$).

4.3.8 Experiment 8: The transmission of a mixture of the RPV and MAV strains to oat cv. Pennalt

Typical symptoms of BYDV infection were observed on the majority of plants fed upon by *R. padi* (45) and *S. avenae* (34, Table 19). Moreover, atypical foliar discoloration was seen on the foliage of four plants in each treatment.

All plants bearing typical symptoms were shown to contain BYDV in ELISA tests, with the exception of two

TABLE 18. The transmission of a mixture of the RPV, PAV and MAV strains of BYDV by *R. padi* and *S. avenae* from oat cv. M. Tabard to oat cv. Pennalt. Results are expressed as the percentage transmission ± the standard error of the mean.

	% Transmission by	
Strain of BYDV	*R. padi*	*S. avenae*
RPV	6.0 ± 3.36	0.0 ± 0.00
PAV	12.0 ± 4.60	18.0 ± 5.43
MAV	0.0 ± 0.00	8.0 ± 3.84
RPV + PAV	74.0 ± 6.20	0.0 ± 0.00
RPV + MAV	0.0 ± 0.00	0.0 ± 0.00
PAV + MAV	0.0 ± 0.00	6.0 ± 3.36
RPV + PAV + MAV	0.0 ± 0.00	0.0 ± 0.00

TABLE 19. Symptom expression and detection by ELISA of a mixture of the RPV and MAV strains transmitted by *R. padi* and *S. avenae* from oat cv. M. tabard to oat cv. Pennalt.

Symptom expression	Number of plants		Virus incidence*	
	R. padi	*S. avenae*	*R. padi*	*S. avenae*
None	1	12	0/1	0/12
Yellow or red leaves/stunting (typical)	45	34	45/45	32/34
Leaf chlorosis (atypical)	4	4	3/4	0/4
Total	50	50		
% Transmission			96.0	64.0
Standard error			± 2.77	± 6.79

* Expressed as number of plants positive/number analysed in ELISA test.

plants exposed to *S. avenae*. Virus was not detected in the symptomless oat plants, but was in 37.5% of plants exhibiting atypical symptoms.

ELISA analysis of the leaves showed that oats inoculated by *R. padi* were infected with a combination of RPV and MAV (68.0%) rather than by either strain singly ($P<0.001$, Table 20). *S. avenae* transmitted MAV at a high frequency (60.0%, $P<0.001$), and interestingly 4.0% of *S. avenae* transmitted RPV + MAV.

Overall, *R. padi* inoculated more oat cv. Pennalt with BYDV (96.0%) than did *S. avenae* (64.0%, $P<0.001$).

4.3.9 Experiment 9: The transmission of a mixture of the RPV and PAV strains to oat cv. Pennalt

All plants infested by *R. padi* developed foliar discoloration; 45 showed typical symptoms of infection, and five atypical (Table 21). In contrast, the majority of oat cv. Pennalt fed upon by *S. avenae* remained symptomless (37), with only six and seven plants developing typical and atypical symptoms, respectively.

Analysis of the leaves in ELISA tests revealed that all plants bearing typical symptoms of infection contained BYDV, as did one of the 12 plants exhibiting atypical symptoms. All symptomless plants were shown to be free of virus.

TABLE 20. The transmission of a mixture of the RPV and MAV strains of BYDV by *R. padi* and *S. avenae* from oat cv. M. Tabard to oat cv. Pennalt. Results are expressed as the percentage transmission ± the standard error of the mean.

	% Transmission by	
Strain of BYDV	*R. padi*	*S. avenae*
RPV	28.0 ± 6.35	0.0 ± 0.00
MAV	0.0 ± 0.00	60.0 ± 6.93
RPV + MAV	68.0 ± 6.60	4.0 ± 2.77

TABLE 21. Symptom expression and detection by ELISA of a mixture of the RPV and PAV strains of BYDV transmitted by *R. padi* and *S. avenae* from oat cv. M. Tabard to oat cv. Pennalt.

Symptom expression	Number of plants		Virus incidence*	
	R. padi	*S. avenae*	*R. padi*	*S. avenae*
None	0	37	-	0/37
Yellow or red leaves/stunting (typical)	45	6	45/45	6/6
Leaf chlorosis (atypical)	5	7	1/5	0/7
Total	50	50		
% Transmission			92.0	12.0
Standard error			± 3.84	± 4.60

* Expressed as number of plants positive/number analysed in ELISA test.

R. padi transmitted RPV + PAV to oat cv. Pennalt at a higher frequency (80.0%) than RPV alone (12.0%, $P<0.001$, Table 22). Interestingly, *R. padi* did not transmit the PAV strain from RPV + PAV-infected oat cv. M. Tabard leaves. Overall, *S. avenae* transmitted BYDV to fewer plants ($P<0.001$) than *R. padi* with PAV at 12.0% the only strain detected.

4.3.10 Experiment 10: The transmission of a mixture of the PAV and MAV strains to oat cv. Pennalt

Several plants fed upon by *R. padi* developed typical symptoms of infection (23), while nine were observed with atypical foliar discoloration (Table 23). In contrast, all plants exposed to *S. avenae* remained symptomless.

In ELISA tests, no virus was detected in the symptomless plants. However, all plants with typical symptoms were shown to contain BYDV, as were five of the nine oat plants exhibiting atypical symptoms.

Out of a combination of PAV and MAV, *R. padi* transmitted PAV + MAV (30.0%) and PAV alone (26.0%) at similar levels (Table 24). No MAV alone was transmitted by *R. padi*, and interestingly, no plants were infected by *S. avenae* (despite 98.0% of the seedlings remaining infested during the inoculation access period). It should be noted that the leaves used in the acquisition feed were old.

TABLE 22. The transmission of a mixture of the RPV and PAV strains of BYDV by *R. padi* and *S. avenae* from oat cv. M. Tabard to oat cv. Pennalt. Results are expressed as the percentage transmission ± the standard error of the mean.

	% Transmission by	
Strain of BYDV	*R. padi*	*S. avenae*
RPV	12.0 ± 4.60	0.0 ± 0.00
PAV	0.0 ± 0.00	12.0 ± 4.60
RPV + PAV	80.0 ± 5.66	0.0 ± 0.00

TABLE 23. Symptom expression and detection by ELISA of a mixture of the PAV and MAV strains of BYDV transmitted by *R. padi* and *S. avenae* from oat cv. Dula to oat cv. Pennalt.

Symptom expression	Number of plants		Virus incidence*	
	R. padi	*S. avenae*	*R. padi*	*S. avenae*
None	18	50	0/18	0/25
Yellow or red leaves/stunting (typical)	23	0	23/23	-
Leaf chlorosis (atypical)	9	0	5/9	-
Total	50	50		
% Transmission			56.0	0.0
Standard error			± 7.02	± 0.00

* Expressed as number of plants positive/number analysed in ELISA test.

TABLE 24. The transmission of a mixture of the PAV and MAV strains of BYDV by *R. padi* and *S. avenae* from oat cv. Dula to oat cv. Pennalt. Results are expressed as the percentage transmission ± the standard error of the mean.

	% Plants tested inoculated by	
Strain of BYDV	*R. padi*	*S. avenae*
PAV	26.0 ± 6.20	0.0 ± 0.00
MAV	0.0 ± 0.00	0.0 ± 0.00
PAV + MAV	30.0 ± 6.48	0.0 ± 0.00

It appeared that *R. padi* was a better vector (56.0%) of BYDV from leaves containing a mixture of PAV and MAV than was *S. avenae* (0%, P<0.001).

4.3.11 Experiment 11: An investigation on whether the transmission of RPV by one aphid species can alter the subsequent transmission of RPV by another species to oat cv. M. Tabard

Typical symptoms of BYDV were observed on the majority (38) of oat cv. M. Tabard infested by *R. padi* (Table 25). In addition, atypical foliar discolouration was seen on nine plants. All plants exposed to *S. avenae* remained symptomless. The exception was two plants observed with typical symptoms of BYDV which had been exposed to *S. avenae* fed upon source leaves previously infested by *R. padi*.

Virus was detected by ELISA in all plants analysed exhibiting typical symptoms of infection and in none of the symptomless plants. The RPV strain was also detected in four of nine oat plants with atypical symptoms.

R. padi transmitted RPV more frequently (84.0%) than *S. avenae,* irrespective of which species of aphid had introduced virus into the source leaves (P<0.001). It was notable that in one of the two investigations where *S. avenae* had fed on leaves previously infested by *R. padi*, 4.0% of the *S. avenae* transmitted the RPV strain to oat cv. M. Tabard.

TABLE 25. Symptom expression and detection by ELISA of the RPV strain of BYDV transmitted by *R. padi* and *S. avenae* to oat cv. M. Tabard from oat cv. M. Tabard source leaves inoculated by either *R. padi* or *S. avenae*.

Symptom expression	Number of plants				Virus incidence*			
	R. padi (*S. avenae*)	*S. avenae* (*R. padi*)	*S. avenae* (*R. padi*)	*S. avenae* (*S. avenae*)	*R. padi* (*S. avenae*)	*S. avenae* (*R. padi*)	*S. avenae* (*R. padi*)	*S. avenae* (*S. avenae*)
None	3	48	50	50	0/3	0/10	0/10	0/10
Yellow leaves/ stunting (typical)	38	2	0	0	8/8	2/2	-	-
Leaf chlorosis (atypical)	9	0	0	0	4/9	-	-	-
Total	50	50	50	50				
% Transmission					84.0	4.0	0.0	0.0
Standard error					± 5.19	± 2.77	± 0.00	± 0.00

* Expressed as number of plants positive/number analysed in ELISA test.

Aphid species in parentheses indicate the species used to infect the source plant.

4.3.12 Experiment 12: An investigation on whether the transmission of PAV by one aphid species can alter the subsequent transmission of PAV by another species to oat cv. Blenda

Plants with typical symptoms of BYDV were severely stunted with serrated leaf margins. Similar symptoms were seen on the foliage of 47 plants exposed to *R. padi* (Table 26). The majority of oats exposed to *S. avenae* remained symptomless. However, 15 and nine plants developed typical symptoms of BYDV in investigations where the *S. avenae* had fed on source leaves previously infested by *R. padi* and *S. avenae*, respectively.

In ELISA tests, PAV was detected in all plants analysed with typical symptoms of infection. No virus was found in the symptomless plants.

R. padi transmitted PAV more frequently (94.0%) to oat cv. Blenda than did *S. avenae* ($P<0.001$), irrespective of which aphid species had previously infested the source leaves. *S. avenae*, fed upon source leaves previously infested by *R. padi*, transmitted PAV at a higher frequency (30.0%) than did those fed upon leaves previously infested by *S. avenae* (18.0%, $P>0.05$).

4.4 SUMMARY OF RESULTS

R. padi transmitted the RPV (98.0%) and PAV (100.0%) strains of BYDV equally readily to oat cv. M. Tabard (Table 27). Similarly, *R. padi* transmitted both strains

TABLE 26. Symptom expression and detection by ELISA of the PAV strain of BYDV transmitted by *R. padi* and *S. avenae* to oat cv. Blenda from oat cv. M. Tabard source leaves inoculated by *R. padi* or *S. avenae*

Symptom expression	Number of plants			Virus incidence*		
	R. padi (*S. avenae*)	*S. avenae* (*R. padi*)	*S. avenae* (*S. avenae*)	*R. padi* (*S. avenae*)	*S. avenae* (*R. padi*)	*S. avenae* (*S. avenae*)
None	3	35	41	0/3	0/5	0/5
Yellow or red leaves/stunting (typical)	47	15	9	10/10	5/5	5/5
Leaf chlorosis (atypical)	0	0	0	-	-	-
Total	50	50	50			
% Transmission				94.0	30.0	18.0
Standard error				± 3.36	± 6.48	± 5.43

* Expressed as number of plants positive/number tested in ELISA test.

Aphid species in parentheses indicate the species used to inoculate the source plant.

TABLE 27. The transmission of the RPV, PAV and MAV strains of BYDV by *R. padi* and *S. avenae* from oat cv. M. Tabard to oat cvs. M. Tabard and Blenda. Results are taken from Tables 10-12 and 15-16, and are expressed as the percentage transmission ± the standard error of the mean.

Cultivar of test plants	Strain of BYDV	% Transmission by	
		R. padi	*S. avenae*
M. Tabard	RPV	98.0 ± 1.98	6.0 ± 3.36
	PAV	100.0 ± 0.00	28.0 ± 6.35
	MAV	0.0 ± 0.00	36.0 ± 6.79
Blenda	RPV	82.0 ± 5.43	0.0 ± 0.00
	PAV	78.0 ± 5.86	70.0 ± 6.48

(82.0% and 78.0%, respectively) with similar efficiency to oat cv. Blenda. However, transmissions were fewer than to cv. M. Tabard (RPV, $P<0.01$ and PAV, $P<0.001$).

RPV was transmitted by *S. avenae* to oat cv. M. Tabard (6.0%) but not to oat cv. Blenda (0%, $P>0.05$), although, PAV was less readily transmitted to cv. M. Tabard (28.0%) than to Blenda (70.0%, $P<0.001$).

The incidence of MAV in cv. Blenda was not studied, however, *S. avenae* transmitted MAV and PAV similarly to oat cv. M. Tabard (36.0% and 28.0%, respectively, $P>0.05$), but more readily than RPV (6.0%, $P<0.001$).

It was notable that *R. padi* did not transmit the RPV strain to oat cv. M. Tabard from source leaves containing a mixture of the RPV and PAV strains, but did transmit RPV (12.0%, $P<0.05$) from RPV + PAV-infected source leaves to cv. Pennalt (Table 28). In contrast, *R. padi* did not transmit PAV to the latter cultivar but did to the former (50.0%, $P<0.001$). Both strains were transmitted together by *R. padi* more readily to cv. Pennalt (80.0%) than to cv. M. Tabard (6.3%, $P<0.001$). The percentage transmission of PAV by *S. avenae* to the two cultivars was not significantly different.

The RPV strain was transmitted at a higher frequency (28.0%) by *R. padi* to cv. Pennalt from RPV + MAV-infected source leaves than from RPV + PAV-infected leaves (12.0%, $P<0.05$). *R. padi* transmitted PAV (26.0%) to cv. Pennalt from oat cv. Dula sources leaves doubly-infected

TABLE 28. The transmission of strains of BYDV by *R. padi* and *S. avenae* from double-infected oat cv. M. Tabard plants to oat cv. Pennalt. Results are taken from Tables 14, 20, 22 and 24, and are expressed as the percentage transmission ± the standard error of the mean.

Aphid vector	Source leaves infected with	Percentage transmission of BYDV strains to test plants					
		RPV	PAV	MAV	RPV + MAV	RPV + PAV	PAV + MAV
R. padi	RPV + MAV	28.0 ± 6.35	-	0.0 ± 0.00	68.0 ± 6.60	-	-
S. avenae		0.0 ± 0.00	-	60.0 ± 6.93	4.0 ± 2.77	-	-
R. padi	PAV + MAV*	-	26.0 ± 6.20	0.0 ± 0.00	-	-	30.0 ± 6.48
S. avenae		-	0.0 ± 0.00	0.0 ± 0.00	-	-	0.0 ± 0.00
R. padi	RPV + PAV	12.0 ± 4.60	0.0 ± 0.00	-	-	80.0 ± 5.66	-
S. avenae		0.0 ± 0.00	12.0 ± 4.60	-	-	0.0 ± 0.00	-
R. padi	RPV + PAV+	0.0 ± 0.00	50.0 ± 12.50	-	-	6.3 ± 6.07	-
S. avenae		0.0 ± 0.00	21.4 ± 10.96	-	-	0.0 ± 0.00	-

* Source leaves are of oat cv. Dula.

+ Aphid vectors transmitted BYDV to oat cv. M. Tabard test plants.

with PAV and MAV, but not from RPV + PAV-infected cv. M. Tabard leaves. In contrast, *S. avenae* transmitted PAV from the latter (12.0%, $P<0.05$), but not from the former. Moreover, *S. avenae* also failed to transmit the MAV strain from PAV + MAV-infected source leaves, but did so from RPV + MAV-infected leaves (60.0%).

Comparing the parallel transmission of BYDV strains from double-infected leaves, *R. padi* transmitted RPV + PAV (80.0%) to cv. Pennalt more readily (although not significantly) than RPV + MAV (68.0%, $P>0.05$), which in turn was at a higher frequency than PAV + MAV (30.0%, $P<0.001$). The parallel transmission of RPV + PAV by *R. padi* (80.0%) from double-infected leaves was at a similar level to that transmitted from triple-infected leaves to cv. Pennalt (74.0%, $P>0.05$, Table 18).

Combining the transmission rates of RPV obtained in Experiment 11 with those in Experiment 1 (Table 29), it is apparent that *R. padi* transmitted RPV more readily to cv. M. Tabard from known-infected leaves where the virus had been introduced by *R. padi* (98.0%) than when introduced by *S. avenae* (84.0%, $P<0.05$). *S. avenae* succeeded in transmitting RPV from oat leaves inoculated by *R. padi* in two out of the three experiments (6.0% and 4.0%).

Comparing the percentage transmission of PAV obtained from Experiment 12 with those from Experiment 5 (Table 30), *R. padi* transmitted PAV to cv. Blenda more frequently from known-infected leaves previously infested by *S.*

TABLE 29. The transmission of the RPV strain of BYDV by *R. padi* and *S. avenae* to oat cv. M. Tabard from oat cv. M. Tabard source leaves inoculated by either *R. padi* or *S. avenae*. Results are taken from Tables 10 and 25, and are expressed as the percentage transmission ± the standard error of the mean.

RPV introduced into source leaves by:-	% Transmission from source leaves to test plants	
	R. padi	*S. avenae*
R. padi	98.0 ± 1.98	6.0 ± 3.36
		4.0 ± 2.77
		0.0 ± 0.00
S. avenae	84.0 ± 5.19	0.0 ± 0.00

TABLE 30. The transmission of the PAV strain of BYDV by *R. padi* and *S. avenae* to oat cv. Blenda from oat cv M. Tabard source leaves inoculated by either *R. padi* or *S. avenae*. Results are taken from Tables 15 and 26, and are expressed as the percentage transmission ± the standard error of the mean.

PAV introduced into source leaves by:-	% Transmission from source leaves to test plants	
	R. padi	*S. avenae*
R. padi	78.0 ± 5.9	70.0 ± 6.48
		30.0 ± 6.48
S. avenae	94.0 ± 3.36	18.0 ± 5.43

avenae (94.0%) than from those inoculated by *R. padi* (78.0%, $P<0.05$). In two separate experiments, *S. avenae* transmitted the virus from leaves previously infested by *R. padi* with significantly different probabilities (70.0% and 30.0%, $P<0.001$). *S. avenae* transmitted PAV less efficiently from plants infected by *S. avenae* (18.0%) than from those inoculated by *R. padi* ($P<0.001$ and $P>0.05$, respectively).

Statistical analysis of the mean absorbance values (A_{405}) of the source leaves used in the acquisition feeds and the percentage transmission from these leaves (Table 1, Apendix II) showed that there was little correlation between the two factors ($r = 0.015$, $P>0.05$).

4.5 DISCUSSION

R. padi transmitted RPV (88.0%) and PAV (90.7%) from plants infected with either one or the other strain to both oat cvs. M. Tabard and Blenda at comparably high rates similar to those found by Rochow (1969a) and Foxe & Rochow (1975) to oat cv. Coast Black (RPV at 96.4% and PAV at 98.7%). *S. avenae* transmitted MAV to oat cv. *M. Tabard* at a lower rate (36.0%) than was found by the above mentioned authors to oat cv. Coast Black (68.5%). However, the *S. avenae* in the above cited work were allowed a 5-day inoculation feed at 21°C compared to 2 days at 17°C in the present research. In addition, 10 aphids were placed per seedling in the cited work, which, combined with the longer inoculation feed, may increase

the rate of transmission.

The PAV strain was transmitted by *S. avenae* with different efficiencies, not only to the three oat cultivars studied, but also to the same oat cultivar (cv. Blenda) in two separate experiments. These differences were significantly greater than the variations observed with the transmission of PAV or RPV by *R. padi*. This may be attributed to the age of the source leaves as *S. avenae* is more likely to transmit PAV from young oat leaves (86.2%) than from old ones (26.8%, Foxe & Rochow, 1975).

In Experiment 5, predominantly young leaves may have been used for the acquisition feed, perhaps because older leaves were too badly diseased, or senesced. Thus the rate of transmission by *S. avenae* was high (70.0%). Whereas, older leaves were used in Experiment 12, as at the time, they had clearer symptoms than the young ones. As a result, *S. avenae* transmitted PAV at lower frequencies (30.0% and 18.0%). The age of the leaves used as a source of PAV in these experiments was not noted, therefore, this interpretation is speculative.

The ability of a given aphid species to transmit a strain of BYDV was not obviously influenced by the aphid species which introduced the virus into the source leaves. Similar results have been observed by others (Watson & Mulligan, 1960; Timian & Jensen, 1964).

A degree of variability in the above investigations was expected as the different rates of transmission for

each strain were from separate experiments. The transmission of RPV and PAV by *R. padi* from source leaves previously infested by either *R. padi* or *S. avenae* were significantly different. However, the transmission rates obtained were all high, and differences obtained may be as a result of inconsistent aphid feeding behaviour.

S. avenae transmitted PAV at significantly different rates from two sets of source leaves where virus had been introduced by *R. padi*. Therefore, any differences in PAV transmission between experiments were not due to differences in aphid species used, but most likely due to the age of the source leaves, as discussed earlier. It is acknowledged, nevertheless, that only a limited number of experiments were carried out in this investigation.

Rochow (1969a) found that *R. padi* was a more efficient vector of PAV than *S. avenae*, regardless of which aphid species had been used to maintain the virus culture. Similar findings were obtained in the present research both to oat cv. M. Tabard and cv. Blenda.

S. avenae transmitted RPV at a very low frequency in two of four experiments. This was likely to be due to genuine transmission by *S. avenae* and not contamination as no stray aphids were observed on the test plants during their growing period in the glasshouse. Foxe & Rochow (1975) comment that occasional transmissions of RPV by *S. avenae* do occur, but are rare and inconsistent. Similar findings were reported by Rochow (1969a, 1982a) and Rochow

& Gill (1978). In addition, Creamer & Falk (1989) described a Californian isolate of RPV that was transmitted nonspecifically by single *S. avenae* from New York, although the New York RPV isolate was not. RPV isolates found in the west of France were transmitted to barley and grasses by both *R. padi* and *S. avenae* (Henry, 1988).

The ability of *R. padi* or *S. avenae* to transmit RPV or PAV was generally more successful to oat cv. M. Tabard compared to cv. Blenda. However, further studies would be necessary to determine whether this was due to varietal differences in susceptibility.

In experiments investigating transmission from source leaves infected with two strains of BYDV, *R. padi* appeared to selectively transmit both RPV and PAV together to oat cv. Pennalt, rather than either RPV, or PAV alone. This is to be expected as *R. padi* is an efficient vector of both strains (Rochow, 1969a).

As RPV and PAV are serologically distinct strains, they could be recognised by separate receptors within *R. padi* as they do not share significant protein coat similarities (Gildow & Rochow, 1980a). However, *R. padi* preferentially transmitted the PAV strain alone from RPV + PAV-infected leaves to oat cv. M. Tabard. This may have been because four times as much PAV was present in the source leaves compared to RPV (as indicated by ELISA). However, statistical analysis indicated that the

absorbance value of BYDV in the source leaves did not correlate with the percentage transmission to the test plants. Nevertheless, it is unusual that RPV was not transmitted more readily to oat cv. M. Tabard as the PAV strain should not interfere with the transmission of RPV (as they have separate receptors in the aphid). In addition, *R. padi* transmitted RPV to cv. M. Tabard with a high frequency (98.0%) from leaves infected with this strain alone.

R. padi is thought to have receptors for RPV and PAV which do not recognise MAV (Gildow & Rochow, 1980a). Therefore, in the present research, transmission of PAV + MAV, and RPV + MAV by *R. padi* from double-infected plants is likely to result from transcapsidation (Rochow, 1970a, 1972, 1973, 1982a).

R. padi transmitted MAV together with PAV at the same rate as PAV alone from plants doubly-infected with PAV and MAV. Rochow (1982a) found that PAV was effective in enabling *R. padi* to transmit MAV. However, the rates of mixed transmission reported by Rochow were higher (89%) than those described in this chapter (30.0%).

The same vector species in the present study also successfully transmitted the MAV strain together with RPV from leaves doubly-infected with both strains. The transmission of RPV + MAV (68.0%) was at a higher frequency than that of PAV + MAV. This indicates that the transcapsidation of MAV is more successful in the presence

of RPV than PAV. This is not consistent with the previous findings that PAV was as effective a helper as RPV (100%) in enabling *R. padi* to transmit MAV to oat cv. Coast Black (Rochow, 1982a). However, again the experimental conditions were different from those in the present research (Rochow placed 10 aphids per seedling and allowed a 5-day inoculation feed).

A small percentage of RPV + MAV was transmitted by *S. avenae* (4.0%). This is likely to be a genuine result as *S. avenae* occasionally transmits both strains together (1-8%) from a mixture (Rochow & Gill, 1978). It may alternatively be contamination, although no stray aphids were ever noted on the plants.

No definite explanation can be found for the lack of transmission of PAV and MAV by *S. avenae* from double-infected oat leaves. Both *R. padi* and *S. avenae* were fed on source leaves from the same plant, which were proven to contain PAV and MAV, both by ELISA, and by transmission by *R. padi*. Therefore, *S. avenae* was definitely exposed to both virus strains. The lack of transmission is unusual as the aphid species is a vector of both PAV and MAV (Rochow, 1969a), and transmitted each strain singly from RPV + MAV-, and RPV + PAV-infected leaves, respectively. In addition, *S. avenae* did transmit PAV + MAV from a mixture also containing RPV. Therefore, lack of transmission was not a function of the recipient host plant.

The cultivar used for the acquisition feed, oat cv. Dula, may have had an effect on transmission. *S. avenae* transmitted PAV + MAV from triple-infected cv. Dula leaves at a very low frequency in other studies (see Chapter 5). In addition, the leaves used for the acquisition feed in the PAV + MAV experiment were old and so the ability of *S. avenae* to transmit PAV could have been reduced (Foxe & Rochow, 1975). Although the cited authors used oats infected with a single strain of BYDV, perhaps the same principle applies to leaves containing more than one strain of BYDV. The age of the leaf is not thought to affect transmission of MAV by *S. avenae* (Foxe & Rochow, 1975), therefore lack of transmission may be due to other reasons.

It is unlikely that high glasshouse temperatures masked the development of symptoms of BYDV (Rochow, 1969a; Yount & Carroll, 1983) on plants exposed to *S. avenae* in the experiment described above, as the accompanying set of plants inoculated by *R. padi* exhibited clear symptoms of BYDV infection. Although only half of the plants infested by *S. avenae* were analysed, it is doubtful that analysis of all 50 test plants would have detected the presence of PAV or MAV. However, two symptomless plants analysed from the RPV + PAV + MAV experiment were found to contain the MAV strain in ELISA tests. Therefore, one or two of the symptomless plants that were not analysed from the PAV + MAV experiment may have also been infected with MAV. The latter experiment should be repeated using younger leaves

for the acquisition feed in order to draw proper conclusions on the transmission efficiency of either *R. padi* or *S. avenae*.

Little information could be found on plants triple-infected with RPV, PAV and MAV. Jedlinski & Brown (1965) reported that when oats were simultaneously inoculated with these three strains, the plants showed only mild symptoms, recovered completely, and no virus could subsequently be transmitted from these plants. This was the opposite to what was found in the present research. Triple-infected plants became severely stunted, and BYDV could be transmitted easily from such plants.

It was notable that combinations involving MAV were not transmitted by *R. padi* to oat cv. Pennalt from plants containing three strains of BYDV, although they did occur from plants doubly-infected with RPV + MAV or PAV + MAV. The MAV strain was present in source leaves used for the acquisition feed, as proved by ELISA, and by transmission of MAV by *S. avenae*. It may be that the presence of RPV and PAV together somehow affects, or stops the transcapsidation of the MAV strain.

S. avenae transmitted PAV and MAV, both singly and together from triple-infected oat leaves to oat cv. Pennalt. This was of interest as no such transmission took place from double-infected source leaves containing PAV and MAV. However, the lack of transmission of BYDV in the latter study has been partially explained, as

discussed earlier.

Interestingly, the simultaneous transmission of all three strains of BYDV from triple-infected oats was not observed, despite *R. padi* being capable of transmitting MAV through transcapsidation with either RPV, or PAV. Perhaps the vector-virus-plant relationship is more complicated when all three strains co-exist.

It is acknowledged, however, that all experiments investigating the transmission of a mixture of BYDV strains should be repeated to confirm the results obtained.

CHAPTER 5

THE TRANSMISSION OF THREE STRAINS OF BYDV BY *Rhopalosiphum padi* AND *Sitobion avenae* FROM PERENNIAL RYEGRASS CV. TALBOT AND OAT CV. DULA TO A RANGE OF WINTER BARLEY CULTIVARS

5.1 INTRODUCTION

The research in this chapter was concerned with the transmission of BYDV from grass to winter cereals. This was of importance as a large acreage of both are grown throughout Scotland; 69,240 ha of winter barley was grown in 1988 (Anon., 1988), while 61% of the total agricultural land in 1988 was temporary, or permanent grass (Anon., 1988).

The problem of BYDV in autumn-sown cereals in U.K. has been increased by the introduction of earlier sowing dates. Crops are now commonly sown from early to mid-September (Holmes, 1984b; Plumb, 1989). This allows longer exposure of the emerging crop to autumn flights of viruliferous aphids and for subsequent BYDV infection (A'Brook & Dewar, 1980; Holmes, 1984b).

Grass is considered to be a source of viruliferous aphids and a large reservoir of strains of BYDV capable of causing severe damage to cereal crops (Doodson, 1967; Plumb, 1977; Holmes 1984a; Smith *et al.*, 1984).

A series of experiments was conducted, therefore, to determine the transmission of BYDV by *R. padi* and *S. avenae* from perennial ryegrass and oats to a range of winter barley cultivars. The objectives of these experiments were to determine:

(1) the relative efficiency of BYDV-transmission from perennial ryegrass and oats by the two aphid species;

(2) the influence of the recipient cultivar of winter barley upon the transmission rate; and

(3) the influence of strain mixtures in the source leaves on the rate of transmission of individual strains from the mixture.

5.2 MATERIALS AND METHODS

5.2.1 Experiments 1 - 7: The transmission of strains of BYDV by *R. padi* from perennial ryegrass cv. Talbot to a range of winter barley cultivars

In each experiment, one adult apterous *R. padi* was placed on each of 50 seedlings (12-day-old) of the winter barley cultivar being tested, and 25 seedlings of oat cv. Dula (12-day-old). The aphids used had been allowed to feed on perennial ryegrass cv. Talbot infected with either RPV, PAV, or a mixture of the two. The acquisition and transmission procedures were as described previously (2.6). After inoculation the test plants were placed in a heated glasshouse.

Approximately 4 weeks after inoculation, foliar symptoms were noted. Also, on average six barley plants showing symptoms of BYDV, six with no symptoms and any with foliar discoloration which was not typical of BYDV infection were cut at soil level, placed in individual polythene bags, and kept in a deep-freeze at -18^{o}C until tested by ELISA. The procedure was repeated with the oat plants, however, only the leaves were collected. In

Experiment 1, all the barley and oat plants were harvested as described above.

Oat cv. Dula, which is susceptible to BYDV, was included in each experiment as a control to check that the aphids had acquired BYDV from the ryegrass or oat source plants.

The average survival rate of aphids during the inoculation feed (48 h) was 90.3% on winter barley (min. 75.3% in Experiment 1), and 93.0% on oat cv. Dula (min. 88.8% in Experiment 3).

Shortly after all plants were placed in the glasshouse, the source leaves used for the acquisition feed (48 h) were assayed by ELISA. This was to confirm the strains of virus present. When analysing the barley and oat test samples, sap from both cultivars was included in each microtitre plate. The contents of each plate were tested for a separate strain of virus.

The heights of a proportion of the winter barley cv. Igri (49.0%) and oat cv. Dula (43.1%) plants were recorded prior to aphid inoculation, and again 4 weeks later (see Materials and Methods, 5.4).

The dates the plants were sown, inoculated and assessed for foliar symptoms, together with the mean, minimum and maximum glasshouse temperatures are recorded for each experiment in Table 2, Appendix I.

5.2.2 Experimments 8 - 13: The transmission of strains of BYDV by *R. padi* and *S. avenae* from perennial ryegrass cv. Talbot, oat cv. Dula and winter barley cv. Igri to winter barley cv. Igri

The procedure followed for Experiments 8-13 was similar to that described for Experiments 1-7 (5.2.1), with the following exceptions:-

(1) Only winter barley cv. Igri (12-day-old) was investigated (of which 50 seedlings were tested in each experiment).
(2) Fifty seedlings of oat cv. Dula (12-day-old) were used as controls.
(3) The transmission rates of *S. avenae* (as well as *R. padi*) were investigated.
(4) The aphids used had previously been allowed to feed on perennial ryegrass cv. Talbot or oat cv. Dula infected with either the RPV, PAV, or a mixture of the RPV, PAV and MAV strains, or winter barley cv. Igri infected with the MAV strain.
(5) All the barley and oat plants were harvested in Experiments 9 and 12.

The average survival rate of *R. padi* and *S. avenae* during the 48 h inoculation feed was 98.4% and 94.9%, respectively, on cv. Igri (min. 96.0% for *R. padi* in Experiment 12 and 85.0% for *S. avenae* in Experiment 9), and 97.5% and 92.8%, respectively, on cv. Dula (min. 93.3% and 76.0%, respectively, in Experiment 9).

5.2.3 Calculation of overall percentage transmission

As mentioned previously, the foliar symptoms on all plants were noted 4 weeks after inoculation. Typical symptoms of infection were clear on oat cv. Dula. However, the winter barley cultivars were less suited to growing in glasshouse conditions, the leaves tending to turn yellow 2 weeks after inoculation. This sometimes made diagnosis difficult, and also produced many plants with atypical foliar discoloration.

In Experiments 1-13, the presence or absence of virus as determined by foliar symptoms (typical or none) was very similar to that determined by ELISA tests (Table 31). However, barley plants considered to have typical symptoms of BYDV were sometimes found to be uninfected when tested by ELISA. Therefore, the overall percentage transmission to all plants grown in each experiment was based on proportional extrapolation of the results of those plants analysed by ELISA. This was applied to all studies where only a proportion of the symptomless plants and those with typical symptoms were analysed by ELISA (Experiments 2-8, 10-11 and 13). All plants with atypical symptoms were analysed giving an exact percentage which was included in the overall percentage transmission.

For example, in Experiment 3 (Table 34), only five of 25 symptomless winter barley cv. Gerbel were analysed by ELISA. No virus was detected in those five plants, therefore , it was assumed that the remaining 20 plants

TABLE 31. ELISA analysis of inoculated winter barley and oat cultivars with and without symptoms of infection (Experiments 1-13).

	No. of plants positive in ELISA test/no. of plants analysed	
Symptom expression	Barley	Oats
None	2/176 (1.1)*	3/168 (1.8)
Yellow leaves/ stunting (typical)	104/136 (76.5)	116/116 (100.0)
Leaf chlorosis (atypical)	42/538 (7.8)	36/119 (30.3)

* Figures in parentheses are the percentage infection.

were also virus-free. Ten of 13 plants with typical symptoms were analysed by ELISA, of which nine were found to contain BYDV (90.0%). The percentage of plants containing virus was estimated for all 13 cv. Gerbel observed with typical symptoms. This was done by extrapolation of the results of those analysed, i.e. 0.90 x 13 = 11.7 (23.4%). All plants exhibiting atypical symptoms were analysed, of which only four contained BYDV (8.0%). Therefore, the percentage transmission of BYDV by *R. padi* to all 50 oat cv. Gerbel plants grown in the experiment was 0 + 23.4 + 8.0 = 31.4%

5.3 RESULTS

5.3.1 Experiment 1: The transmission of a mixture of the RPV and PAV strains by *R. padi* from perennial ryegrass to winter barley cv. Halcyon

Typical symptoms of BYDV developed on 46 of the winter barley cv. Halcyon plants, and on 50 oat controls (Table 32).

In ELISA tests, BYDV was detected in 39 barley plants with typical symptoms of BYDV and in all of the oat plants.

Analysis of the incidence of strains of BYDV detected, indicated that *R. padi* transmitted PAV alone to winter barley cv. Halcyon at a higher frequency (44.0%) than either RPV alone (12.0%, $P<0.001$), or the two strains together (22.0%, $P<0.05$, Figure 1). Whereas, *R. padi*

TABLE 32. Symptom expression and detection by ELISA of a mixture of the RPV and PAV strains of BYDV transmitted by *R. padi* from perennial ryegrass cv. Talbot to winter barley cv. Halcyon and oat cv. Dula.

Symptom expression	Number of plants		Virus incidence*	
	Halcyon	Dula	Halcyon	Dula
None	4	0	0/4	-
Yellow leaves/ stunting (typical)	46	25	39/46	25/25
Leaf chlorosis (atypical)	0	0	-	-
Total	50	25		
% Transmission			78.0	100.0
Standard error			± 5.86	± 0.00

* Expressed as number of plants positive/number tested in ELISA test.

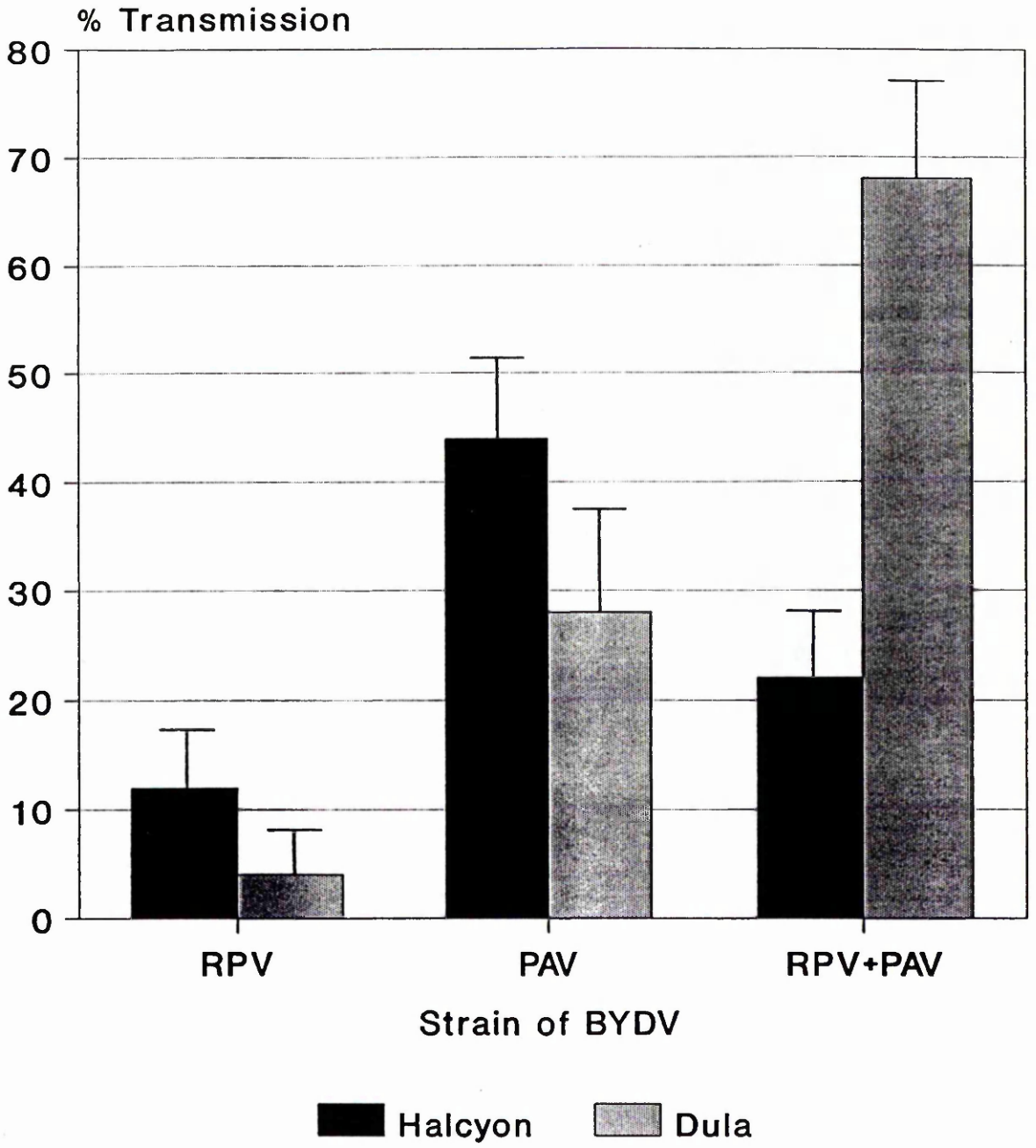


FIGURE 1. The percentage transmission of a mixture of the RPV and PAV strains of BYDV by *R. padi* from perennial ryegrass cv. Talbot to winter barely cv. Halcyon and oat cv. Dula. Vertical bars represent the standard error of the mean.

appeared to selectively transmit both strains together (68.0%) over PAV (28.0%, $P<0.01$), or RPV alone (4.0%, $P<0.001$) to the oat control. Overall, virus transmission was higher to oat cv. Dula (100.0%) than to winter barley cv. Halcyon (78.0%, $P<0.001$).

5.3.2 Experiment 2: The transmisssion of the RPV strain by *R.padi* from perennial ryegrass to winter barley cv. Magie

Neither winter barley nor oats developed typical symptoms of BYDV (Table 33). However, 14 plants of winter barley cv. Magie and six of oat cv. Dula developed atypical symptoms.

In ELISA tests, no virus was detected in winter barley cv. Magie which showed atypical symptoms, or was symptomless. However, all cv. Dula plants exhibiting atypical foliar discolouration contained RPV, giving an overall percentage transmission of 24.0%.

5.3.3 Experiment 3: The transmission of a mixture of the RPV and PAV strains by *R. padi* from perennial ryegrass to winter barley cv. Gerbel

Atypical symptoms were observed on a similar number of winter barley cv. Gerbel plants (12) as were typical symptoms of infection (13, Table 34). Whereas, only four oat cv. Dula plants developed atypical symptoms, the majority developing typical symptoms of BYDV (16).

TABLE 33. Symptom expression and detection by ELISA of the RPV strain of BYDV transmitted by *R. padi* from perennial ryegrass cv. Talbot to winter barley cv. Magie and oat cv. Dula.

Symptom expression	Number of plants		Virus incidence*	
	Magie	Dula	Magie	Dula
None	36	19	0/6	0/3
Yellow leaves/ stunting (typical)	0	0	-	-
Leaf chlorosis (atypical)	14	6	0/14	6/6
Total	50	25		
% Transmission			0.0	24.0
Standard error			± 0.00	± 8.54

* Expressed as number of plants positive/number tested in ELISA test.

TABLE 34. Symptom expression and detection by ELISA of a mixture of the RPV and PAV strains of BYDV transmitted by *R. padi* from perennial ryegrass cv. Talbot to winter barley cv. Gerbel and oat cv. Dula.

Symptom expression	Number of plants		Virus incidence*	
	Gerbel	Dula	Gerbel	Dula
None	25	5	0/5	0/5
Yellow leaves/ stunting (typical)	13	16	9/10	6/6
Leaf chlorosis (atypical)	12	4	4/12	4/4
Total	50	25		
% Transmission			31.4	80.0
Standard error			± 6.56	± 8.00

* Expressed as number of plants positive/number tested in ELISA test.

Analysis by ELISA of barley leaves with typical symptoms showed that nine of 10 contained BYDV, as did four out of 12 with atypical symptoms. All oat plants exhibiting typical and atypical symptoms contained virus, while all symptomless barley and oat plants did not.

R. padi transmitted BYDV more readily to oat cv. Dula (80.0%) than to winter barley cv. Gerbel (31.4%, $P<0.001$). The incidence of BYDV in the leaves analysed indicated that the PAV strain (33.3%) was transmitted to cv. Gerbel at a significantly higher level than the strain combination RPV + PAV (3.7%, $P<0.01$), but not significantly higher than RPV alone (14.8%, $P>0.05$, Figure 2). Although PAV alone was detected at a higher frequency (40.0%) than RPV (13.3%), or RPV + PAV (13.3%) in cv. Dula, it was not significant due to the small number of plants analysed.

5.3.4 Experiment 4: The transmission of the RPV strain by *R.padi* from perennial ryegrass to winter barley cv. Marinka

All winter barley cv. Marinka plants developed atypical foliar discoloration (Table 35). In contrast, few of the oat control plants developed foliar discoloration, with only four and five plants exhibiting typical and atypical symptoms, respectively.

ELISA analysis of the barley leaves showed that 14.0% of the plants contained RPV. This was similar to the

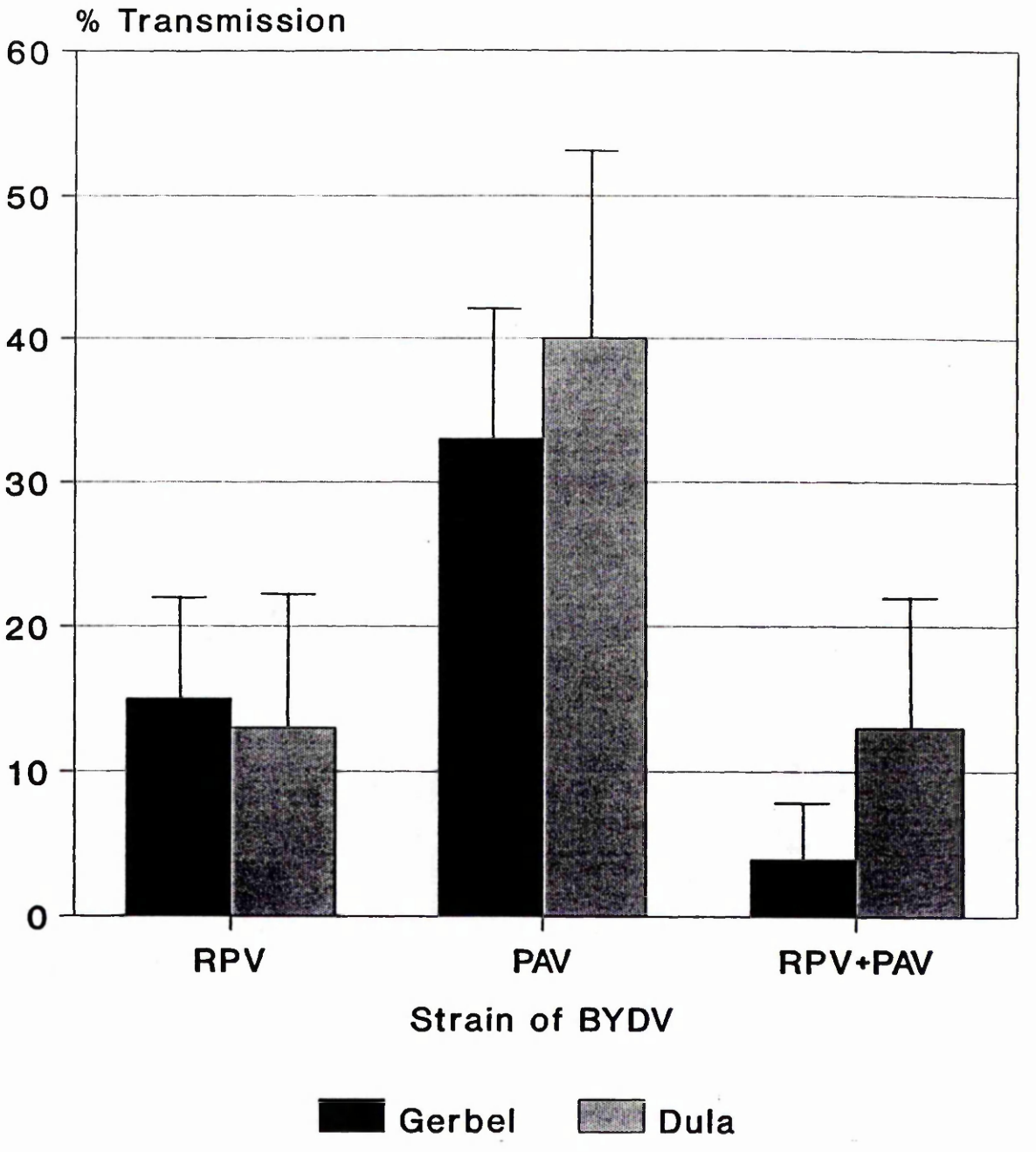


FIGURE 2. The percentage transmission of a mixture of the RPV and PAV strains of BYDV by *R. padi* from perennial ryegrass cv. Talbot to the winter barley cv. Gerbel and oat cv. Dula plants analysed. Vertical bars represent the standard error of the mean.

TABLE 35. Symptom expression and detection by ELISA of the RPV strain of BYDV transmitted by *R. padi* from perennial ryegrass cv. Talbot to winter barley cv. Marinka and oat cv. Dula.

Symptom expression	Number of plants		Virus incidence*	
	Marinka	Dula	Marinka	Dula
None	0	16	-	0/5
Yellow leaves/ stunting (typical)	0	4	-	4/4
Leaf chlorosis (atypical)	50	5	7/50	0/5
Total	50	25		
% Transmission			14.0	16.0
Standard error			± 4.91	± 7.33

* Expressed as number of plants positive/number analysed in ELISA test.

percentage of oat plants found to be infected (16.0%, P>0.05).

5.3.5 Experiment 5: The transmission of the RPV strain by *R.padi* from perennial ryegrass to winter barley cvs. Igri and Magie

The majority of winter barley cv. Igri (46), cv. Magie (49) and oat cv. Dula (21) remained symptomless (Table 36). However, atypical symptoms of BYDV did develop on four cv. Igri and on one cv. Magie plant, while typical symptoms were observed on four of the oat control.

When analysed by ELISA, RPV was not detected in winter barley cv. Magie, but it was in the cv. Igri and oat cv. Dula plants bearing atypical and typical symptoms, respectively. Virus was also detected in one of five symptomless oat plants analysed by ELISA. As only 1.8% of all symptomless oat plants analysed by ELISA were shown to contain BYDV (Table 31), it was assumed that the remaining 16 oat plants which were not analysed in Experiment 5 were free of virus. Consequently, the rate of transmission of RPV by *R. padi* to cv. Dula (20.0%) was not significantly greater than to barley cv. Igri (8.0%).

TABLE 36. Symptom expression and detection by ELISA of the RPV strain of BYDV transmitted by *R. padi* from perennial ryegrass cv. Talbot to winter barley cvs. Igri and Magie, and oat cv. Dula.

Symptom expression	Number of plants			Virus incidence*		
	Igri	Magie	Dula	Igri	Magie	Dula
None	46	49	21	0/10	0/10	1/5
Yellow leaves/ stunting (typical)	0	0	4	-	-	4/4
Leaf chlorosis (atypical)	4	1	0	4/4	0/1	-
Total	50	50	25			
% Transmission				8.0	0.0	20.0
Standard error				± 3.84	± 0.00	± 8.00

* Expressed as number of plants positive/number analysed in ELISA test.

5.3.6 Experiment 6: The transmission of the RPV strain by *R. padi* from perennial ryegrass to winter barley cvs. Igri, Marinka, Panda and Plaisant

Foliar discoloration, either typical or atypical of BYDV infection was observed on plants of a number of the winter barley cvs. Igri, Marinka and Panda (Table 37). However, all winter barley cv. Plaisant and oat cv. Dula remained symptomless.

The presence of RPV was detected by ELISA in all of the cv. Marinka plants, but in only one out of six cv. Igri plants analysed with typical symptoms of infection. No virus was detected in any symptomless plants or ones bearing atypical foliar discoloration.

The results indicated that *R. padi* transmitted RPV to winter barley cvs. Igri (4.7%) and Marinka (10.0%) only. The percentage transmission was not significantly different between the two cultivars.

5.3.7 Experiment 7: The transmission of the PAV strain by *R. padi* from perennial ryegrass to winter barley cvs. Igri, Halcyon, Marinka and Plaisant

All the oat control plants remained symptomless, while many of the four winter barley cultivars developed foliar discoloration, either typical or atypical of BYDV infection (Table 38). Typical symptoms were most prevalent on cv. Plaisant (31).

TABLE 37. Symptom expression and detection by ELISA of the RPV strain of BYDV transmitted by *R. padi* from perennial ryegrass cv. Talbot to winter barley cvs. Igri, Marinka, Panda and Plaisant, and oat cv. Dula.

Symptom expression	Number of plants					Virus incidence*				
	Igri	Marinka	Panda	Plaisant	Dula	Igri	Marinka	Panda	Plaisant	Dula
None	16	22	29	50	25	0/5	0/5	0/5	0/5	0/5
Yellow leaves/ stunting (typical)	14	5	0	0	0	1/6	5/5	-	-	-
Leaf chlorosis (atypical)	20	23	21	0	0	0/20	0/23	0/21	-	-
Total	50	50	50	50	25					
% Transmission						4.7	10.0	0.0	0.0	0.0
Standard error						± 2.99	± 4.24	± 0.00	± 0.00	± 0.00

* Expressed as number of plants positive/number analysed in ELISA test.

TABLE 38. Symptom expression and detection by ELISA of the PAV strain of BYDV transmitted by *R. padi* from perennial ryegrass cv. Talbot to winter barley cvs. Igri, Halcyon, Marinka and Plaisant, and oat cv. Dula.

Symptom expression	Number of plants					Virus incidence*				
	Igri	Halcyon	Marinka	Plaisant	Dula	Igri	Halcyon	Marinka	Plaisant	Dula
None	18	36	19	13	25	0/5	0/6	0/5	0/5	0/5
Yellow leaves/ stunting (typical)	15	8	18	31	0	3/7	5/5	8/8	8/8	-
Leaf chlorosis (atypical)	17	6	13	6	0	0/17	0/6	0/13	0/6	-
Total	50	50	50	50	25					
% Transmission						12.9	16.0	36.0	62.0	0.0
Standard error						± 4.74	± 5.19	± 6.79	± 6.86	± 0.00

* Expressed as number of plants positive/number tested in ELISA test.

The PAV strain was not detected in any symptomless plants, or any bearing atypical foliar discolouration when analysed by ELISA, but it was detected in all plants analysed which had typical symptoms of BYDV. The exception to this was cv. Igri where only three out of seven plants with typical symptoms contained virus. Those that did were severely stunted, while the others were not.

R. padi transmitted PAV more successfully to winter barley cv. Plaisant (62.0%) than to cvs. Marinka (36.0%, $P<0.01$), Halcyon (16.0%, $P<0.001$), or Igri (12.9%, $P<0.001$, Figure 3). Winter barley cv. Marinka in turn was more readily infected than cvs. Halcyon ($P<0.05$) and Igri ($P<0.01$). Surprisingly, oat cv. Dula was not infected with the PAV strain.

5.3.8 Experiment 8: The transmission of the PAV strain by *S. avenae* from perennial ryegrass and oats to winter barley cv. Igri

The majority of winter barley cv. Igri plants developed atypical symptoms whether they had been exposed to *S. avenae* previously fed on PAV-infected oat leaves (48), or PAV-infected grass leaves (44, Table 39). Conversely, the majority of the oat control plants remained symptomless (33 and 42, respectively). Nevertheless, a small number of barley and oat plants did develop typical symptoms of BYDV.

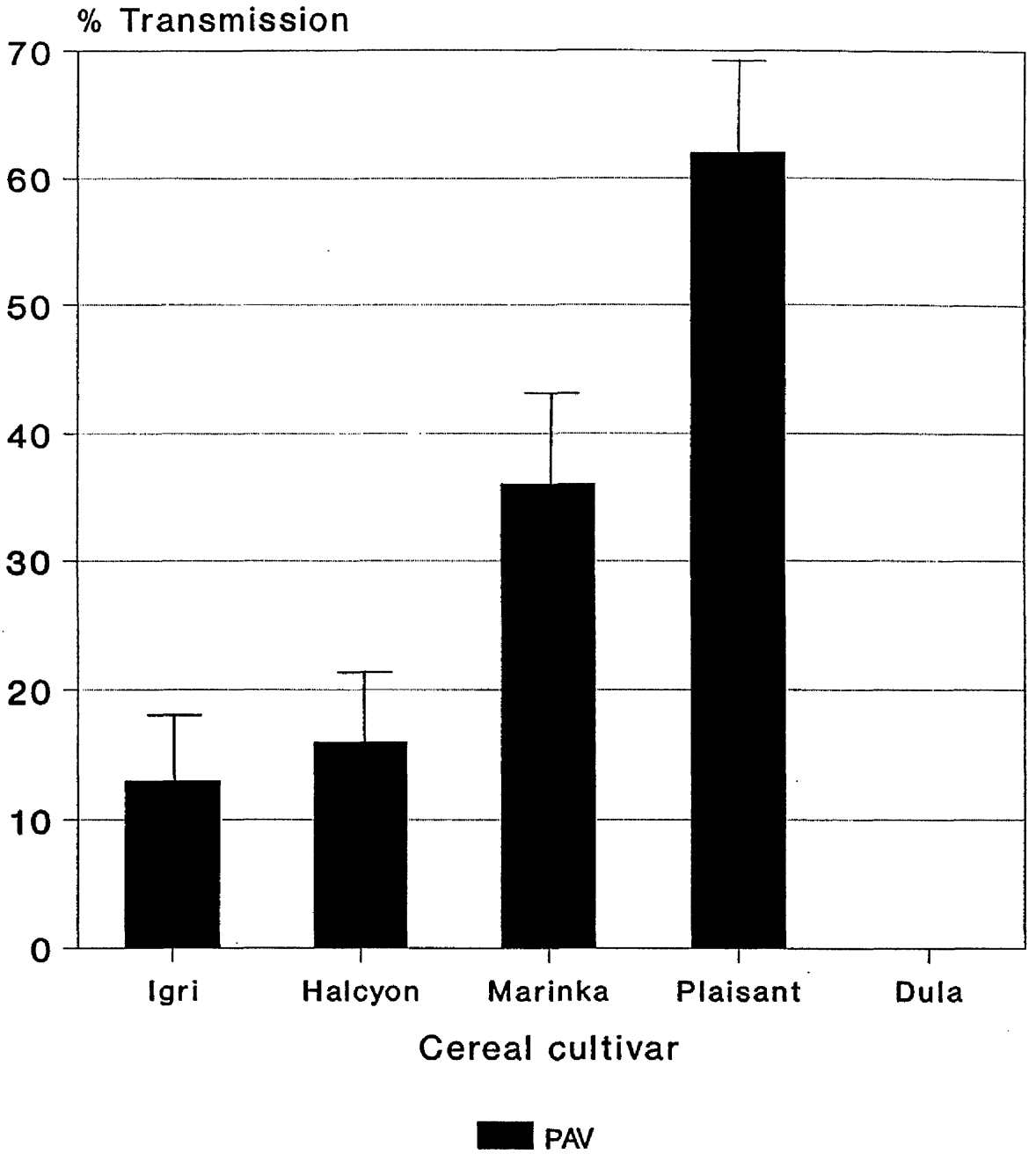


FIGURE 3. The percentage transmission of the PAV strain of BYDV by *R. padi* from perennial ryegrass cv. Talbot to winter barley cvs. Igri, Halcyon, Marinka and Plaisant, and oat cv. Dula. Vertical bars represent the standard error of the mean.

TABLE 39. Symptom expression and detection by ELISA of the PAV strain of BYDV transmitted by *S. avenae* from perennial ryegrass cv. Talbot and oat cv. Dula to winter barley cv. Igri and oat cv. Dula.

	Number of plants				Virus incidence*			
	Oats		Grass		Oats		Grass	
Symptom expression	Igri	Dula	Igri	Dula	Igri	Dula	Igri	Dula
None	1	33	6	42	0/1	0/5	0/6	0/5
Yellow leaves/ stunting (typical)	1	7	0	1	1/1	7/7	-	1/1
Leaf chlorosis (atypical)	48	10	44	7	0/48	1/10	0/44	1/7
Total	50	50	50	50				
% Transmission					2.0	16.0	0.0	4.0
Standard error					± 1.98	± 5.19	± 0.00	± 2.77

* Expressed as number of plants positive/number analysed in ELISA test.

All cereal plants bearing typical symptoms of BYDV infection were shown to contain PAV when analysed by ELISA. Virus was detected in only 11.8% of the oat plants, and in none of the cv. Igri plants exhibiting atypical foliar discoloration.

The transmission of PAV by *S. avenae* to winter barley cv. Igri was low, both from grass (0%), or oat (2.0%) source leaves. *S. avenae* appeared to transmit PAV more efficiently from oats to oats (16.0%) than from grass to oats (4.0%, $P<0.05$), or from oats to barley (2.0%, $P<0.05$).

5.3.9 Experiment 9: The transmission of a mixture of the RPV, PAV and MAV strains by *R. padi* and *S. avenae* from oats to winter barley cv. Igri

Typical symptoms of BYDV infection developed on many of the barley (35) and oat (39) plants infested by *R. padi* (Table 40). In addition, 15 and nine plants, respectively, developed atypical symptoms. No typical symptoms were observed on any of the plants infested by *S. avenae*. All of the barley and 18 of the oat plants developed atypical foliar discoloration.

All the barley and oat plants exhibiting typical symptoms were shown to contain BYDV in ELISA tests. Virus was also detected in 25.0% of plants with atypical symptoms fed upon by *R. padi*, but in only 4.4% of plants exposed to *S. avenae*.

TABLE 40. Symptom expression and detection by ELISA of a mixture of the RPV, PAV and MAV strains of BYDV transmitted by *R. padi* and *S. avenae* from oat cv. Dula to winter barley cv. Igri and oat cv. Dula.

	Number of plants				Virus incidence*			
	R. padi		*S. avenae*		*R. padi*		*S. avenae*	
Symptom expression	Igri	Dula	Igri	Dula	Igri	Dula	Igri	Dula
None	0	2	0	32	-	0/2	-	0/32
Yellow leaves/ stunting (typical)	35	39	0	0	35/35	39/39	-	-
Leaf chlorosis (atypical)	15	9	50	18	4/15	2/9	3/50	0/18
Total	50	50	50	50				
% Transmission					78.0	82.0	6.0	0.0
Standard error					± 5.86	± 5.43	± 3.36	± 0.00

* Expressed as number of plants positive/number tested in ELISA test.

The results indicated that *R. padi* transmitted BYDV from oats to winter barley cv. Igri (78.0%) and oat cv. Dula (82.0%) at similarly high rates ($P>0.05$). Transmission rates to both cultivars were higher than those of *S. avenae* to winter barley cv. Igri (6.0%, $P<0.001$), or oat cv. Dula (0%, $P<0.001$). Comparing levels of infection between these two cultivars, *S. avenae* transmitted BYDV to a greater number of barley than oat plants, but the difference was not significant.

Analysis of the incidence of individual strains of BYDV and combinations of strains detected (Table 41), indicated that *R. padi* transmitted PAV to winter barley cv. Igri at a higher level (48.0%) than all other strain/s detected ($P<0.001$). The PAV strain was also transmitted to oat cv. Dula more readily (36.0%) than all other strains, or mixture of strains detected ($P<0.05$), especially RPV (2.0%, $P<0.001$).

The percentage transmission by *R. padi* of each strain or strain combination was similar for oats and barley.

S. avenae only succeeded in transmitting PAV (6.0%) to winter barley cv. Igri.

No MAV, or RPV + MAV was transmitted by either vector to either cereal cultivar.

TABLE 41. The transmission of a mixture of the RPV, PAV and MAV strains of BYDV by *R. padi* and *S. avenae* from oat cv. Dula to winter barley cv. Igri and oat cv. Dula. Results are expressed as the percentage transmission ± the standard error of the mean.

Strain of BYDV	% Transmission to Igri by		% Transmission to Dula by	
	R. padi	*S. avenae*	*R. padi*	*S. avenae*
RPV	4.0 ± 2.77	0.0 ± 0.00	2.0 ± 1.98	0.0 ± 0.00
PAV	48.0 ± 7.07	6.0 ± 3.36	36.0 ± 6.79	0.0 ± 0.00
MAV	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
RPV + PAV	10.0 ± 4.24	0.0 ± 0.00	16.0 ± 5.19	0.0 ± 0.00
RPV + MAV	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
PAV + MAV	10.0 ± 4.24	0.0 ± 0.00	14.0 ± 4.91	0.0 ± 0.00
RPV + PAV + MAV	6.0 ± 3.6	0.0 ± 0.00	14.0 ± 4.91	0.0 ± 0.00

5.3.10 Experiment 10a - b: The transmission of the RPV and PAV strains by *R. padi* from oats to winter barley cv. Igri

5.3.10.1 EXPERIMENT 10a: TRANSMISSION OF RPV

All winter barley cv. Igri developed atypical symptoms of BYDV (Table 42). In contrast, few of the inoculated oat controls developed foliar discoloration, with typical symptoms observed on 10 plants and atypical on five.

In ELISA tests, the RPV strain was detected in all oat plants analysed with typical symptoms of BYDV, and in 12 of the barley plants bearing atypical symptoms. BYDV was not detected in the symptomless plants analysed.

The percentage transmission of RPV by *R. padi* was similar to both barley (24.0%) and oats (20.0%, $P>0.05$).

5.3.10.2 EXPERIMENT 10b: TRANSMISSION OF PAV

Typical symptoms of BYDV were observed on 31 barley and 30 oat plants. However, 18 barley and five oats plants also developed atypical foliar discoloration (Table 42).

All plants analysed with typical symptoms were found to contain PAV in ELISA tests, while virus was detected in 19.2% of plants exhibiting atypical symptoms. Again, no virus was detected in symptomless plants.

R. padi transmitted PAV similarly to both winter barley cv. Igri (66.0%) and oat cv. Dula (62.0%, $P>0.05$).

TABLE 42. Symptom expression and detection by ELISA of the RPV and PAV strains of BYDV transmitted by *R. padi* from oat cv. Dula to winter barley cv. Igri and oat cv. Dula.

Symptom expression	Number of plants				Virus incidence*			
	RPV		PAV		RPV		PAV	
	Igri	Dula	Igri	Dula	Igri	Dula	Igri	Dula
None	0	35	1	15	-	0/5	0/1	0/5
Yellow leaves/ stunting (typical)	0	10	31	30	-	5/5	5/5	6/6
Leaf chlorosis (atypical)	50	5	18	5	12/50	0/5	2/18	1/5
Total	50	50	50	50				
% Transmission					24.0	20.0	66.0	62.0
Standard error					± 6.04	± 5.66	± 6.70	± 6.86

* Expressed as number of plants positive/number analysed in ELISA test.

Comparing the percentage transmission of RPV with PAV (Figure 4), *R. padi* transmitted the PAV strain to barley and oats more successfully than the RPV strain ($P<0.001$).

5.3.11 Experiment 11: The transmission of the RPV strain by *S. avenae* from perennial ryegrass and oats to winter barley cv. Igri

The majority of winter barley cv. Igri and oat cv. Dula remained symptomless, irrespective of whether the *S. avenae* had fed previously on RPV-infected grass (48 and 49, respectively), or oats (49 and 50, respectively, Table 43).

No virus was detected in any of the plants analysed by ELISA indicating that *S. avenae* did not transmit the RPV strain.

5.3.12 Experiment 12: The transmission of a mixture of the RPV, PAV and MAV strains by *R. padi* and *S. avenae* from perennial ryegrass to winter barley cv. Igri

Foliar discoloration, both typical and especially atypical of BYDV infection were observed on several barley and oat plants fed upon by *R. padi* or *S. avenae* (Table 44).

All winter barley and oat control plants exhibiting typical symptoms were shown to contain BYDV in ELISA tests. In addition, virus was detected in 8.3% of cv. Igri, and 60.5% of cv. Dula bearing symptoms atypical of

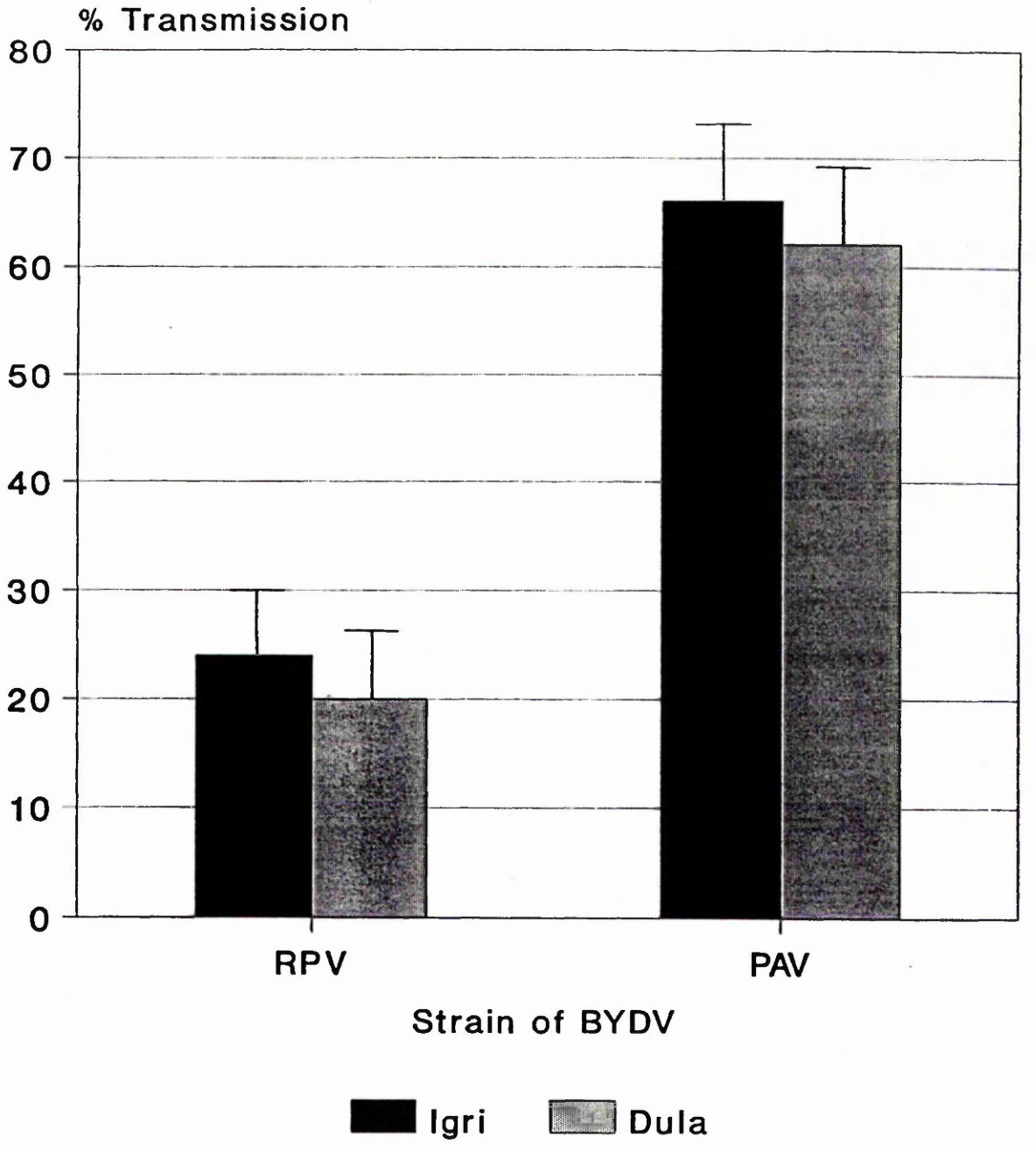


FIGURE 4. The percentage transmission of the RPV and PAV strains of BYDV by *R. padi* from oat cv. Dula to winter barley cv. Igri and oat cv. Dula. Vertical bars represent the standard error of the mean.

TABLE 43. Symptom expression and detection by ELISA of the RPV strain of BYDV transmitted by *S. avenae* from perennial ryegrass cv. Talbot and oat cv. Dula to winter barley cv. Igri and oat cv. Dula.

Symptom expression	Number of plants				Virus incidence*			
	Oats		Grass		Oats		Grass	
	Igri	Dula	Igri	Dula	Igri	Dula	Igri	Dula
None	49	50	48	49	0/10	0/10	0/10	0/10
Yellow leaves/ stunting (typical)	0	0	0	0	-	-	-	-
Leaf chlorosis (atypical)	1	0	2	1	0/1	-	0/2	0/1
Total	50	50	50	50				
% Transmission					0.0	0.0	0.0	0.0
Standard error					± 0.00	± 0.00	± 0.00	± 0.00

* Expressed as number of plants positive/number analysed in ELISA test.

TABLE 44. Symptom expression and detection by ELISA of a mixture of the RPV, PAV and MAV strains of BYDV transmitted by *R. padi* and *S. avenae* from perennial ryegrass cv. Talbot to winter barley cv. Igri and oat cv. Dula.

Symptom expression	Number of plants				Virus incidence*			
	R. padi		*S. avenae*		*R. padi*		*S. avenae*	
	Igri	Dula	Igri	Dula	Igri	Dula	Igri	Dula
None	16	24	32	32	1/16	1/24	0/32	2/32
Yellow leaves/ stunting (typical)	16	3	0	3	16/16	3/3	-	3/3
Leaf chlorosis (atypical)	18	23	18	15	3/18	17/23	0/18	6/15
Total	50	50	50	50				
% Transmission					40.0	42.0	0.0	22.0
Standard error					± 6.93	± 6.98	± 0.00	± 5.86

* Expressed as number of plants positive/number analysed in ELISA test.

BYDV. Interestingly, 3.9% of the symptomless test plants were also shown to contain virus in ELISA tests.

The above results indicated that *R. padi* transmitted BYDV similarly from perennial ryegrass cv. Talbot to winter barley cv. Igri (40.0%) and oat cv. Dula (42.0%, $P>0.05$). Levels of transmission by *R. padi* to the barley and oat cultivars were higher than those of *S. avenae* to cv. Igri (0.0%, $P<0.001$) or cv. Dula (22.0%, $P<0.05$). *S. avenae* appeared to be more successful at transmitting BYDV to oats than to barley ($P<0.001$).

Analysis of the incidence of individual strains and mixtures of strains detected in infected plants (Table 45) indicated that *R. padi* transmitted PAV to winter barley cv. Igri more readily (32.0%) than RPV (2.0%, $P<0.001$), or PAV + MAV (6.0%, $P<0.01$). Similarly, *R. padi* preferentially transmitted PAV to oat cv. Dula at a higher frequency (26.0%) than RPV (6.0%, $P<0.01$), or RPV + PAV (10.0%, $P<0.05$).

S. avenae did not transmit BYDV to cv. Igri, but did transmit PAV (18.0%) and to a lesser extent RPV + PAV (2.0%, $P<0.01$) and PAV + MAV (2.0%, $P<0.01$) to oat cv. Dula.

No MAV, RPV + MAV, or RPV + PAV + MAV was transmitted by either aphid species to either cereal cultivar.

TABLE 45. The transmission of a mixture of the RPV, PAV and MAV strains of BYDV by *R. padi* and *S. avenae* from perennial ryegrass cv. Talbot to winter barley cv. Igri and oat cv. Dula. Results are expressed as the percentage transmission ± the standard error of the mean.

Strain of BYDV	% Transmission to Igri by		% Transmission to Dula by	
	R. padi	*S. avenae*	*R. padi*	*S. avenae*
RPV	2.0 ± 1.98	0.0 ± 0.00	6.0 ± 3.36	0.0 ± 0.00
PAV	32.0 ± 6.60	0.0 ± 0.00	26.0 ± 6.20	18.0 ± 5.43
MAV	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
RPV + PAV	0.0 ± 0.00	0.0 ± 0.00	10.0 ± 4.24	2.0 ± 1.98
RPV + MAV	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
PAV + MAV	6.0 ± 3.36	0.0 ± 0.00	0.0 ± 0.00	2.0 ± 1.98
RPV + PAV + MAV	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

5.3.13 Experiment 13: The transmission of the MAV strain by *R. padi* and *S. avenae* from barley to winter barley cv. Igri

Atypical foliar discoloration was observed on the majority of barley plants infested by *R. padi* (41) and *S. avenae* (39, Table 46). In contrast, many oat cv. Dula plants exposed to *R. padi* remained symptomless (37), but 25 oat plants exposed to *S. avenae* did develop typical symptoms of infection.

No virus was detected by ELISA in plants exposed to *R. padi*. Of the plants exposed to *S. avenae*, those analysed which bore typical symptoms were found to contain MAV, as did 10.0% of those with atypical symptoms.

Based on these results it was concluded that *R. padi* was unable to transmit the MAV strain, while *S. avenae* transmitted MAV more successfully to oats (52.0%) than to barley (8.0%, $P<0.001$).

5.4 MATERIALS AND METHODS

5.4.1 The effect of BYDV on the height of winter barley cv. Igri and oat cv. Dula

Between February 1990 and September 1990, the heights of 413 of 843 winter barley cv. Igri seedlings (12-day-old), and 337 of 782 oat cv. Dula seedlings (12-day-old) were recorded immediately prior to aphid inoculation.

TABLE 46. Symptom expression and detection by ELISA of the MAV strain of BYDV transmitted by *R. padi* and *S. avenae* from winter barley cv. Igri to winter barley cv. Igri and oat cv. Dula.

Symptom expression	Number of plants				Virus incidence*			
	R. padi		*S. avenae*		*R. padi*		*S. avenae*	
	Igri	Dula	Igri	Dula	Igri	Dula	Igri	Dula
None	9	37	10	24	0/5	0/5	0/5	0/5
Yellow leaves/ stunting (typical)	0	0	1	25	-	-	1/1	10/10
Leaf chlorosis (atypical)	41	13	39	1	0/41	0/13	3/39	1/1
Total	50	50	50	50				
% Transmission					0.0	0.0	8.0	52.0
Standard error					± 0.00	± 0.00	± 3.84	± 7.07

* Expressed as number of plants positive/number analysed in ELISA test.

Four weeks later, the heights of the same plants were again recorded, and the growth increment was calculated. The height of the plant was measured from soil level to the tip of the seedling, or plant. The plants measured consisted of a mixture of infected and uninfected plants as shown by ELISA tests at harvest.

Plant measurements were collated for each BYDV strain, or strain combination, and differences in heights between infected and uninfected plants were compared for each strain of BYDV, and also between strains.

5.5 RESULTS

All three strains of BYDV caused a reduction in growth of both winter barley cv. Igri and oat cv. Dula (Figure 5). The reduction caused by RPV was small relative to the uninfected control ($P>0.05$) in both cv. Igri (Plate 10) and cv. Dula (Plate 11), as was that by MAV ($P>0.05$) in cv. Dula (Plate 12). The MAV strain caused marked stunting in cv. Igri plants (Plate 13). However, few plants were measured and the difference was not statistically significant. The PAV strain caused significant stunting in both barley ($P<0.01$, Plate 14) and oats ($P<0.01$, Plate 15).

The stunting caused by PAV in barley was similar to that caused by MAV, but greater than that caused by RPV ($P<0.01$). In contrast, the stunting caused by PAV in oats was not significantly greater than by RPV but was

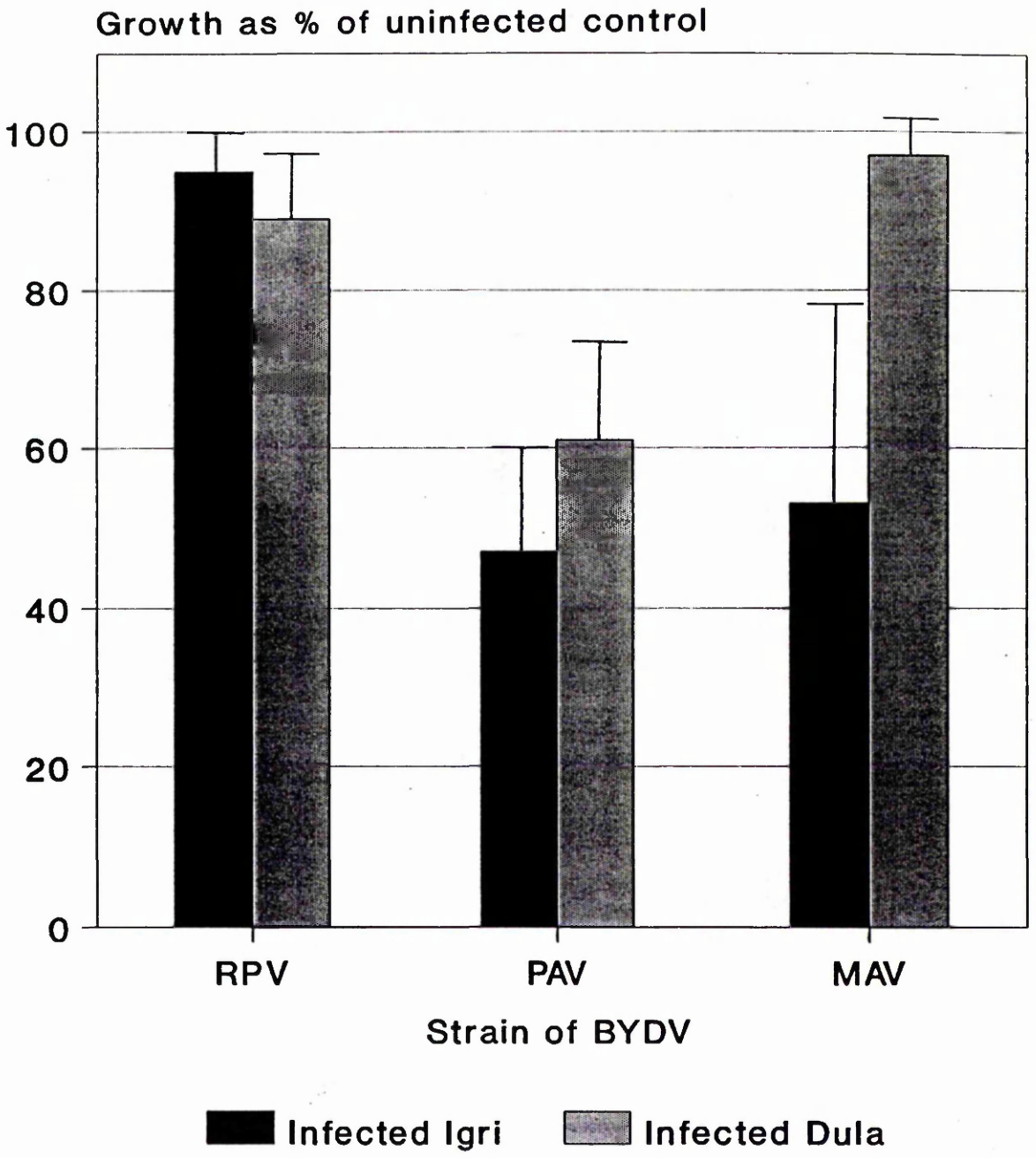


FIGURE 5. The mean growth increment from aphid inoculation to harvesting of winter barley cv. Igri and oat cv. Dula infected with the RPV, PAV, or MAV strains of BYDV. Vertical bars represent the standard error of the mean.

PLATE 10. Uninfected (left) and RPV-infected (right) winter barley cv. Igri plants, 4 weeks after inoculation.

PLATE 11. Uninfected (left) and RPV-infected (right) oat cv. Dula plants, 4 weeks after inoculation.

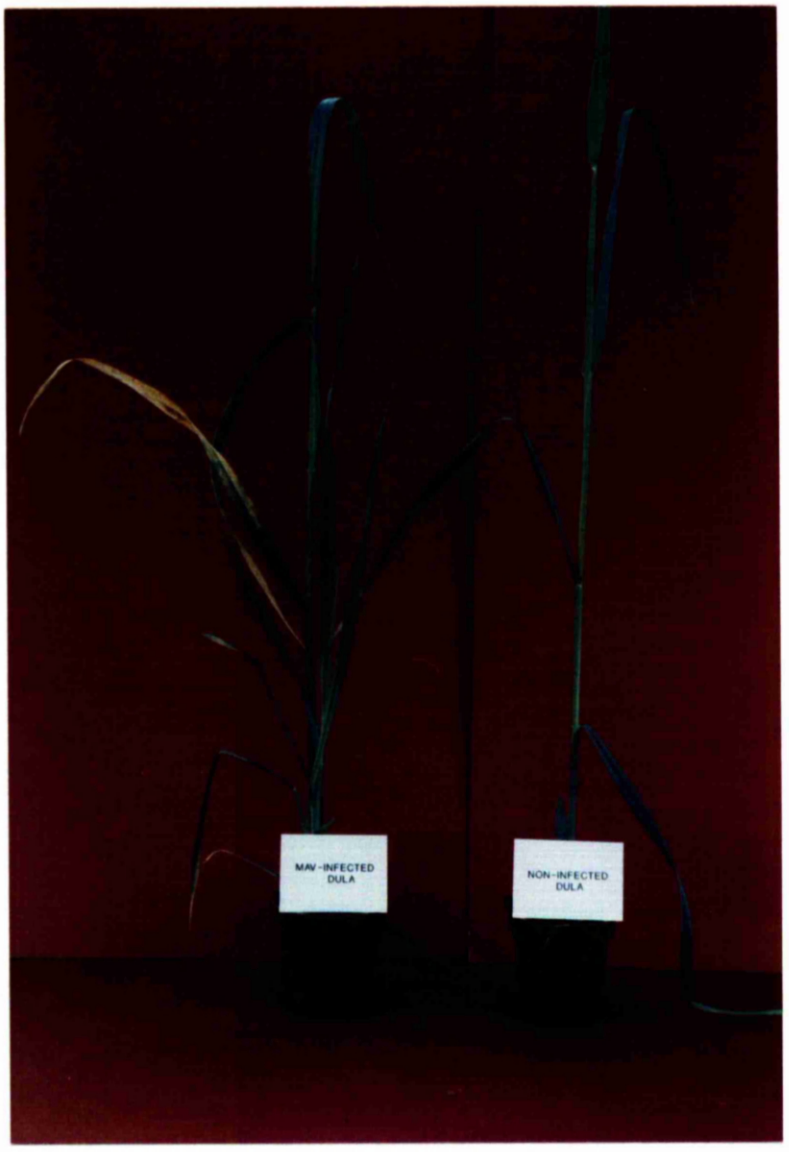


PLATE 12. Uninfected (right) and MAV-infected (left) oat cv. Dula plants, 4 weeks after inoculation.

PLATE 13. Uninfected (left) and MAV-infected (right) winter barley cv. Igri plants, 4 weeks after inoculation.

PLATE 14. Uninfected (left) and PAV-infected (right) winter barley cv. Igri plants, 4 weeks after inoculation.

PLATE 15. Uninfected (left) and PAV-infected (right) oat cv. Dula plants, 4 weeks after inoculation.

significantly greater than by MAV ($P<0.05$).

The mean growth rates of plants were also analysed in experiments with a mixture of the three strains of BYDV (Figure 6). All strains, or mixtures of strains reduced the growth of barley cv. Igri (Plate 16) and oat cv. Dula (Plate 17). The effects of only some strains, or mixtures of strains were statistically significant, however, as certain strains or combinations were detected in few plants. PAV ($P<0.001$) and PAV + MAV ($P<0.05$) caused significant stunting in cv. Igri, while PAV ($P<0.001$) and RPV + PAV ($P<0.05$) caused significant stunting in cv. Dula.

Strains of BYDV when present in mixtures (RPV + PAV, PAV + MAV, and RPV + PAV + MAV) caused more severe stunting in barley than when present alone (RPV and PAV). However, these differences were not significant due to the small number of plants which were infected. The stunting caused by mixtures of strains in cv. Dula was similar to that caused by PAV, but greater than by RPV. Again, differences were not significant due to the few plants found infected.

5.6 SUMMARY OF RESULTS: EXPERIMENTS 1 - 13

Transmission of BYDV to cereals varied greatly between experiments.

The transmission of RPV by *R. padi* to oat cv. Dula varied from 0% to 24.0%, making comparisons of

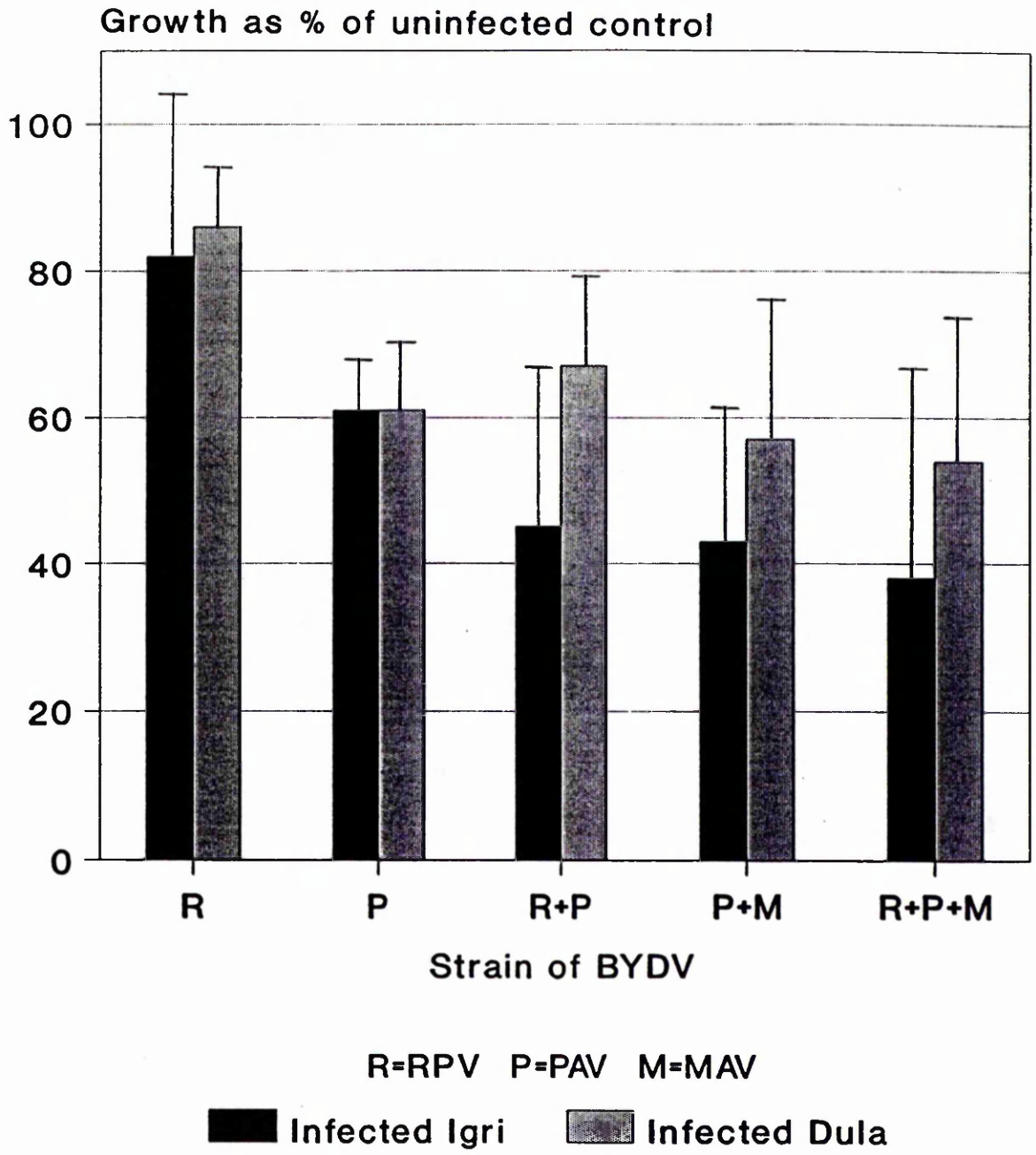


FIGURE 6. The mean growth increment from aphid inoculation to harvesting of winter barley cv. Igri and oat cv. Dula infected with a mixture of the RPV, PAV and MAV strains of BYDV. Vertical bars represent the standard error of the mean.

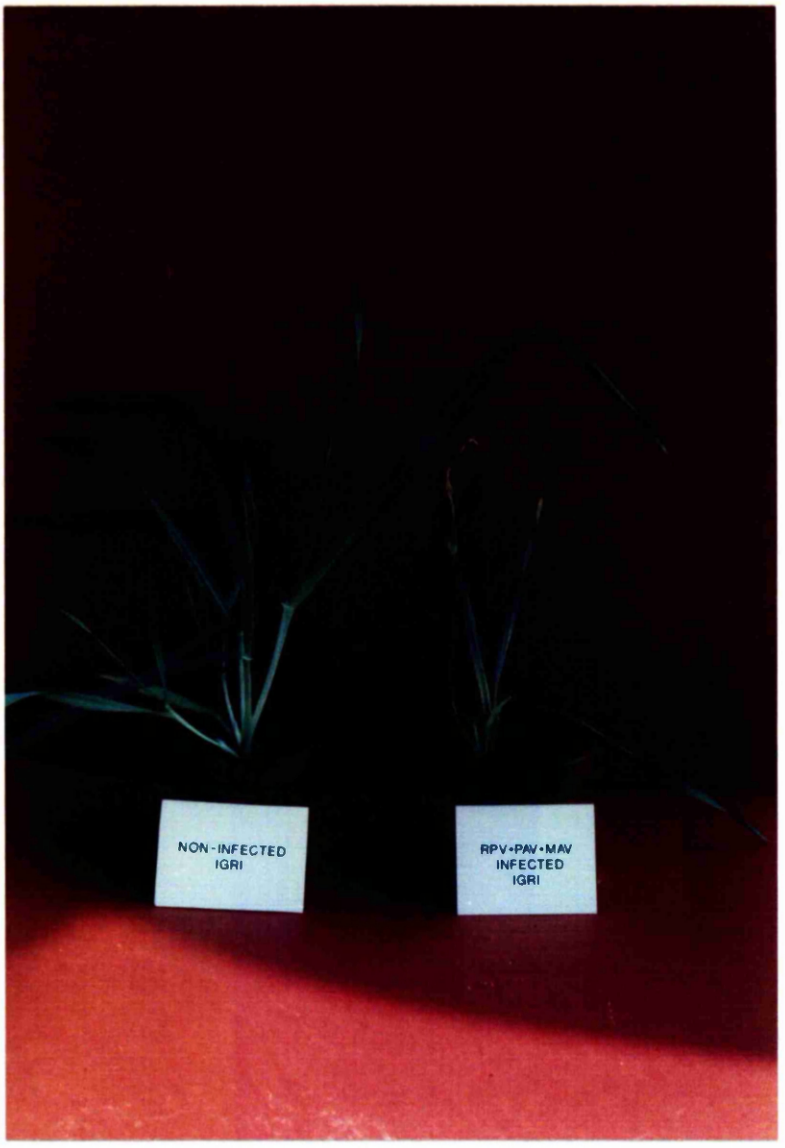


PLATE 16. Uninfected (left) and RPV + PAV + MAV-infected (right) winter barley cv. Igri plants, 4 weeks after inoculation.

PLATE 17. Uninfected (left) and RPV + PAV + MAV-infected (right) oat cv. Dula plants, 4 weeks after inoculation.

transmission rates to the different barley cultivars difficult (Table 47). Nevertheless, the rates of transmission to the oat controls were similar in four of five experiments. Therefore, a level of consistency was observed.

In addition, similar rates of transmission were obtained when repeated for three separate winter barley cultivars. For example, *R. padi* transmitted RPV similarly from ryegrass to cv. Igri in two separate experiments (8.0% and 4.7%, $P>0.05$). RPV was also transmitted similarly to cv. Marinka in two separate studies (14.0% and 10.0%, $P>0.05$). Moreover, it was notable that *R. padi* did not transmit RPV from grass to cv. Magie on two occasions.

The results indicated that *R. padi* transmitted RPV to cv. Igri more successfully from oats (24.0%) than from grass (8.0%, $P<0.05$ and 4.7%, $P<0.01$). Whereas, *S. avenae* did not transmit RPV from either grass or oats to cv. Igri or cv. Dula.

R. padi and *S. avenae* appeared to transmit PAV more readily from oats to both winter barley cv. Igri (66.0% and 2.0%, respectively) and oat cv. Dula (62.0% and 16.0%, respectively) than from grass to either cv. Igri (12.9%, $P<0.001$ and 0%, $P>0.05$, respectively), or cv. Dula (0%, $P<0.001$ and 4.0%, $P<0.05$, respectively, Table 48). As *R. padi* did not transmit PAV from grass to the oat control in this experiment, the comparison between the barley

TABLE 47. The transmission of the RPV strain of BYDV by *R. padi* and *S. avenae* from perennial ryegrass cv. Talbot and oat cv. Dula to a range of winter barley cultivars and oat cv. Dula. Results are taken from Tables 33, 35-37 and 42-43, and are expressed as the percentage transmission ± the standard error of the mean.

Source of inoculum	Barley cultivar	Percentage transmission by *R. padi*		Percentage transmission by *S. avenae*	
		Barley	Oats	Barley	Oats
Grass	Magie	0.0 ± 0.00	24.0 ± 8.54	-	-
	Marinka	14.0 ± 4.91	16.0 ± 7.33	-	-
	Magie	0.0 ± 0.00	20.0 ± 8.00	-	-
	Igri	8.0 ± 3.84		-	
	Igri	4.7 ± 2.99		0.0 ± 0.00	0.0 ± 0.00
	Marinka	10.0 ± 4.24	0.0 ± 0.00	-	
	Panda	0.0 ± 0.00		-	
	Plaisant	0.0 ± 0.00		-	
Oats	Igri	24.0 ± 6.04	20.0 ± 5.66	0.0 ± 0.00	0.0 ± 0.00

TABLE 48. The transmission of the PAV and MAV strains of BYDV by *R. padi* and *S. avenae* from perennial ryegrass cv. Talbot, oat cv. Dula, and winter barley cv. Igri to a range of winter barley cultivars and oat cv. Dula. Results are taken from Tables 38-39, 42 and 46, and are expressed as the percentage transmission ± the standard error of the mean.

Source of inoculum	Strain of BYDV	Barley cultivar	Percentage transmission by			
			R. padi		*S. avenae*	
			Barley	Oats	Barley	Oats
Grass	PAV	Halcyon	16.0 ± 5.19	0.0 ± 0.00	-	-
		Marinka	36.0 ± 6.79		-	
		Plaisant	62.0 ± 6.86		-	
		Igri	12.9 ± 4.74		0.0 ± 0.00	4.0 ± 2.77
Oats	PAV	Igri	66.0 ± 6.70	62.0 ± 6.86	2.0 ± 1.98	16.0 ± 5.19
Barley	MAV	Igri	0.0 ± 0.00	0.0 ± 0.00	8.0 ± 3.84	52.0 ± 7.07

cultivars should be interpreted with caution.

In general, *R. padi* transmitted PAV at a higher frequency than RPV both from grass to barley cvs. Marinka ($P<0.05$), Plaisant ($P<0.001$) and Igri ($P>0.05$), and from oats to cv. Igri ($P<0.001$). However, as no PAV was transmitted from grass to oat cv. Dula, *R. padi*, on average, transmitted RPV at a higher level ($P<0.05$). In contrast, PAV was transmitted at a higher frequency than RPV from oats to the oat control ($P<0.001$).

S. avenae transmitted MAV more readily than PAV from cereals to both barley cv. Igri (8.0% and 2.0%, respectively, $P>0.05$) and oat cv. Dula (52.0% and 16.0%, respectively, $P<0.001$).

Although the percentage transmission of BYDV from RPV + PAV-infected grass to oat control plants was different in the two experiments (100.0% and 80.0%, $P<0.05$), both rates were high (Table 49). This permits a valid comparison between the percentage transmissions to winter barley cvs. Halcyon and Gerbel.

R. padi transmitted BYDV from RPV + PAV-infected grass leaves to cv. Halcyon at a higher frequency (78.0%) than to cv. Gerbel (31.4%, $P<0.001$). Again, the PAV strain, either alone or in combination, was transmitted at a higher frequency than RPV to both barley cv. Halcyon ($P<0.001$) and the oat control ($P<0.05$) or cv. Gerbel ($P>0.05$) and the accompanying oats ($P>0.05$).

TABLE 49. The transmission of a mixture of the RPV and PAV strains of BYDV by *R. padi* from perennial ryegrass cv. Talbot to winter barley cvs. Halcyon and Gerbel and oat cv. Dula. Results are taken from Figures 1-2, and are expressed as the percentage transmission ± the standard error of the mean.

		Percentage transmission to	
Barley cultivar	Strain of BYDV	Barley	Oats
Halcyon	RPV	12.0 ± 4.60	4.0 ± 3.92
	PAV	44.0 ± 7.02	28.0 ± 8.98
	RPV + PAV	22.0 ± 5.84	68.0 ± 9.33
	Total	78.0 ± 5.86	100.0 ± 0.00
Gerbel	RPV	14.8 ± 6.83	13.3 ± 8.77
	PAV	33.3 ± 9.07	40.0 ± 12.65
	RPV + PAV	3.7 ± 3.63	13.3 ± 8.77
	Total*	31.4 ± 6.56	80.0 ± 8.00

* Total percentage transmission of all plants grown in the trial based on proportional extrapolation of those plants analysed by ELISA.

The overall percentage transmission of BYDV by *R. padi* and *S. avenae* from triple-infected leaves to winter barley cv. Igri was greater from oats (78.0% and 6.0%, respectively) than from grass (40.0%, $P<0.001$ and 0%, $P>0.05$, respectively, Table 50). Similarly, BYDV was transmitted by *R. padi* to oat cv. Dula at a higher frequency from oats (82.0%) than from grass (42.0%, $P<0.001$). However, the reverse was observed for *S. avenae* (0% and 22.0%, respectively, $P<0.001$).

As before, the PAV strain, either alone or in combination, was transmitted by *R. padi* from both grass and oats more readily than RPV to cv. Igri ($P<0.001$) or oats ($P<0.05$ and $P<0.001$, respectively).

It was notable that *R. padi* did not transmit RPV + PAV from grass to cv. Igri, but did from oats (10.0%, $P<0.05$). In addition, *R. padi* failed to transmit all three strains together from grass to both cv. Igri or the control, but did to both cultivars from oats (6.0%, $P<0.05$ and 14.0%, $P<0.01$, respectively). Furthermore, no MAV or RPV + MAV was transmitted by either aphid species from triple-infected grass or cereal leaves, to either winter barley cv. Igri or oat cv. Dula.

Surprisingly, *S. avenae* seemed unable to transmit any strain of BYDV between oats, or from grass to barley. Consequently, any comparisons between the two experiments should be treated with caution for both *S. avenae* and *R. padi*.

TABLE 50. The transmission of a mixture of the RPV, PAV and MAV strains of BYDV by *R. padi* and *S. avenae* from perennial ryegrass cv. Talbot and oat cv. Dula to winter barley cv. Igri and oat cv. Dula. Results are taken from Tables 40 and 44, and are expressed as the percentage transmission ± the standard error of the mean.

Source of inoculum	Strain of BYDV	Percentage transmission by			
		R. padi		*S. avenae*	
		Barley	Oats	Barley	Oats
Grass	RPV	2.0 ± 1.98	6.0 ± 3.36	0.0 ± 0.00	0.0 ± 0.00
	PAV	32.0 ± 6.60	26.0 ± 6.20	0.0 ± 0.00	18.0 ± 5.43
	RPV + PAV	0.0 ± 0.00	10.0 ± 4.24	0.0 ± 0.00	2.0 ± 1.98
	PAV + MAV	6.0 ± 3.36	0.0 ± 0.00	0.0 ± 0.00	2.0 ± 1.98
	RPV + PAV + MAV	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
	Total	40.0 ± 6.93	42.0 ± 6.98	0.0 ± 0.00	22.0 ± 5.86
Oats	RPV	4.0 ± 2.77	2.0 ± 1.98	0.0 ± 0.00	0.0 ± 0.00
	PAV	48.0 ± 7.07	36.0 ± 6.79	6.0 ± 3.36	0.0 ± 0.00
	RPV + PAV	10.0 ± 4.24	16.0 ± 5.19	0.0 ± 0.00	0.0 ± 0.00
	PAV + MAV	10.0 ± 4.24	14.0 ± 4.91	0.0 ± 0.00	0.0 ± 0.00
	RPV + PAV + MAV	6.0 ± 3.36	14.0 ± 4.91	0.0 ± 0.00	0.0 ± 0.00
	Total	78.0 ± 5.86	82.0 ± 5.43	6.0 ± 3.36	0.0 ± 0.00

Statistical analysis of the mean absorbance values (A_{405}) of the source leaves (Tables 2 and 3, Appendix II) revealed that the positive A_{405} values of RPV and PAV were greater for oat than for grass source leaves (RPV, $P<0.05$ and PAV, $P<0.001$).

In addition, a correlation study was carried out between the absorbance values of the source leaves, and the percentage transmission from these leaves to the winter barley cultivars and their controls. The results indicated that little relationship existed between the A_{405} values and the percentage transmission by *R. padi* and *S. avenae* to barley ($r = 0.489$, $P<0.05$) or oats ($r = 0.691$, $P<0.001$).

5.7 DISCUSSION

One area of concern throughout this chapter is the degree of variability in the percentage transmission of BYDV to the oat control plants. The lack of transmission to oat plants in some experiments was unusual as either a number of winter barley cultivars within the study became infected, or many oat control plants in a separate experiment became infected with the same strain of BYDV.

The variation in transmission to the oat controls makes the comparison of transmission rates between barley cultivars difficult. Nevertheless, three experiments, when repeated, gave similar rates of transmission for RPV for three individual winter barley cultivars. This

indicated some degree of consistency in the transmission to barley. Although other experiments were repeated for the various strains or combinations of strains, these were carried out using different cultivars of test plants and varieties of source leaves.

It was important to assess the cause of the variation in transmission rates to oats from a similar plant source.

It is known that at high glasshouse temperatures, symptoms of infection in oats will not develop (Rochow, 1969a; Jensen, 1973; Rochow, 1979a). The temperature at which plants are grown can also influence BYDV purification (Rochow *et al.*, 1971). Thus preparations made from oats grown at 15-20^{o}C contained approximately 2-5 times more virus than did preparations from plants grown at temperatures above 25^{o}C.

Rochow & Gill (1978) reported variation in rates of transmission to oat cv. Coast Black. They deduced that high glasshouse temperatures reduced the titre of some BYDV strains, especially RPV, and that fluctuating environmental factors during the tests led to variation between experiments.

In the present research, all oat cv. Dula plants observed either with atypical symptoms, or without any foliar discoloration, were grown in the glasshouse at relatively high temperatures (average mean temperature 22.1^{o}C, average max. temperature 31.5^{o}C), compared to those experiments where plants exhibited typical symptoms.

The virus titre may have been reduced by these temperatures, resulting in atypical symptoms. In many cases, the titre in such plants may have been too low for detection by ELISA. This in turn would produce a low percentage transmission to oat cv. Dula. However, temperature cannot be the cause for all the variation in the percentages of RPV or PAV detected in winter barley. Several cultivars were grown simultaneously in some experiments, and thus were subjected to identical temperatures within each study.

Symptoms of infection on winter barley were difficult to diagnose, and BYDV was detected in few of many plants which developed atypical leaf chlorosis. Some of the plants with foliar discoloration which appeared typical of BYDV infection were also found to be free of virus. As many barley plants exhibited foliar discoloration, a large number were analysed by ELISA making it unlikely that any plants containing virus were missed. It is possible, nevertheless, that high glasshouse temperatures would reduce the virus titre in these winter barley cultivars also.

Reasons for the prevalent development of leaf chlorosis in barley can only be speculated upon. Apart from the effects of high glasshouse temperatures, perhaps the potting compost lacked some minerals essential for the healthy development of winter barley plants. Fewer oat cv. Dula plants developed atypical symptoms, but a third of them contained virus. Perhaps, the oat plants bearing

atypical leaf chlorosis at 4 weeks, which were shown to contain BYDV by ELISA, would have developed typical symptoms at 5 weeks.

The absence of BYDV in the oat controls in two experiments, one investigating the transmission of RPV, the other PAV, may have been due to the harvesting method. As neither typical, nor atypical symptoms were observed in these experiments, only 20.0% of the plants were harvested at random and tested by ELISA. This may have resulted in some plants containing BYDV being missed. However, only 1.8% of all symptomless oat plants analysed by ELISA throughout this chapter contained BYDV. Therefore, it is unlikely that BYDV was present in many infected oat plants.

Lack of transmission to either the oat controls or certain winter barley cultivars was not due to poor aphid survival during the inoculation feed (see 5.2.1), or the age of the source leaves used for the acquisition feed. Several cultivars were inoculated simultaneously by aphids previously fed on the same BYDV-infected source leaves. In addition, the age of cereal leaves has no effect on the transmission efficiency of RPV or PAV by *R. padi* (Foxe & Rochow, 1975), provided leaves are not too senesced. This may also be true for grass leaves (none of which was senesced).

The absorbance values (A_{405}) obtained with BYDV-infected source leaves used in acquisition feeds varied.

As all ELISA tests were conducted in a uniform manner, the range of A_{405} values obtained indicated differences in virus content.

Pereira *et al.* (1989) reported no significant relationship between virus transmission efficiency by *S. avenae* or *R. padi* and virus content. This agrees with the statistical analysis of positive absorbance values of source leaves relative to the percentage transmission by *R. padi* and *S. avenae* reported in Chapter 5. Therefore, differences in transmission rates from known-infected grass or oat leaves must be due to other factors.

Experiments where *R. padi*, from a single acquisition feed, were placed on several cereal cultivars simultaneously, were of particular interest as subsequent infection appeared in some cultivars and not in others. This would suggest that *R. padi* were able to acquire the virus, but were unable to transmit it to certain cereal cultivars. As mentioned previously, the inability is unlikely to be a result of environmental conditions as all plants within each experiment were subjected to indentical conditions. Lack of transmission to certain barley cultivars may be a function of the test plant itself, such as leaf surface structure. Tsumuki *et al.* (1989) suggested that surface wax on leaves, which is an inherant trait, was an important component of the resistance of barley to colonizing aphids, expecially *R. padi*. It was found that resistance levels correlated positively with surface wax. However, this may not be relevant with

cereal cultivars used in this research for two reasons:-

(1) Observations of *R. padi* on winter barley and oat seedlings during the inoculation feed indicated that the aphids were settled, and apparently feeding. In addition, several nymphs were produced which also appeared to settle and feed. This suggests that the leaf surface offered no resitance to colonization by *R. padi*.

(2) Winter barley cv. Plaisant failed to become infected with RPV, but became infected with PAV at a significantly high level. *R. padi*, therefore, appeared to have no difficulty in probing the leaf surface.

The reason for lack of transmission of RPV by *R. padi* from grass to winter barley cvs. Magie (twice), Panda and Plaisant is unclear as BYDV has been identified in these cultivars in advisory samples collected from crops in the west of Scotland (S.J.I. Holmes, personal communication). By contrast, the lack of transmission of RPV by *S. avenae* complies with the vector's inability to transmit RPV efficiently (Rochow, 1969a). Similarly, *R. padi* could not transmit MAV to barley cv. Igri as it is a non-vector of this strain when present alone in a plant (Foxe & Rochow, 1975).

Overall, *R. padi* was a more efficient vector of PAV from grass and oats to cereals than *S. avenae*. However, it would be necessary to repeat these experiments in order

to prove this observation. It is notable that Rochow (1969a) also reported that *R. padi* transmitted PAV more often than *S. avenae*, but at higher rates (100% and 75%, respectively) than those obtained in the present research, albeit with different numbers of aphids.

Generally, transmission of BYDV to barley was greater by both aphid species, from infected oats than from infected grass. ELISA analysis of the source leaves showed that absorbance values of the RPV and PAV strains were higher in infected oat leaves than in the grass leaves. Perhaps the aphid vectors dislike the surface structure of grass leaves, and feed more successfully on cereal leaves. Hence the higher rate of transmission from the latter. This may be important to plant geneticists breeding for BYDV resistant cereal cultivars.

R. padi tended to transmit the PAV strain to winter barley more successfully than the RPV strain from grass and oat source leaves infected with one strain of BYDV. This trend was also observed in studies using double-infected grass leaves, and triple-infected grass and oat leaves. As mentioned earlier, the above comparisons should be treated with caution due to the variability in transmission to the oat controls.

Nevertheless, considering the results obtained, the difference in transmission of RPV and PAV by *R. padi* from mixed infections is interesting as Gildow & Rochow (1980a) found no interference between RPV and PAV in *R. padi*.

Therefore, competition for sites would not occur. However, in the present research, very little RPV was transmitted, either alone, or in combination to winter barley cv. Igri. It has been reported that PAV multiplies faster than RPV in cereal plants (Skaria *et al.*, 1985). This may explain the prevalence of PAV compared to RPV in barley and oat test plants inoculated by *R. padi* previously fed on triple-infected oat and grass leaves.

The differences in transmission and subsequent detection of BYDV may have been due to factors such as aphid feeding behaviour, or the effect of glasshouse temperatures on the systemic movement of the virus within plants (Jensen, 1973). The most rapid movement of BYDV occurs at 21^{o}C with a slower movement at higher or lower temperatures. At 10^{o}C, symptoms of BYDV are slow to develop, but eventually become very pronounced.

The transmission of PAV + MAV by *R. padi* to test plants from triple-infected source leaves is attributable to transcapsidation of MAV by PAV (Rochow, 1982a). However, it is unusual that no MAV was transmitted together with RPV as it is a common form of dependent transmission by *R. padi* (Rochow, 1970a, 1972, 1973; Gildow & Rochow, 1980a). Studies on these forms of dependent transmission were all on double-infected plants. It is possible that the situation is altered where all three strains co-exist.

It is unclear as to why *R. padi* transmitted PAV + MAV from triple-infected grass to barley, but not to oats.

The transmission of all three strains simultaneously to barley and oat test plants inoculated by *R. padi* previously fed on triple-infected oat leaves may be as a result of transcapsidation of MAV with either the RPV or PAV virus. Again, the lack of transmission of RPV + PAV + MAV from grass leaves is unclear.

The presence of RPV + PAV in one oat plant fed upon by *S. avenae* is most likely due to a rare transmission of the RPV strain (Foxe & Rochow, 1975; Rochow & Gill, 1978).

Gildow & Rochow (1980a) postulated that the PAV and MAV strains have common receptors in *S. avenae* due to a similarity in coat protein structure. The poor transmission of PAV + MAV and failure to transmit MAV from triple-infected grass leaves to oat cv. Dula may be because all the receptor sites on the salivary gland were saturated with PAV virions, thus inhibiting the transmission of MAV.

However, the lack of transmission of BYDV by *S. avenae* from triple-infected oat and grass leaves to oat and barley test plants, respectively, makes any deductions on the mixtures of strains transmitted by *S. avenae* in these experiments difficult. Repeated tests using triple-infected source leaves may reveal that *S. avenae* is indeed inefficient in transmitting strains of BYDV from a mixture of all three strains. Total lack of transmission

is unusual, nevertheless, as Rochow & Gill (1978) reported *S. avenae* transmitting MAV at 100% (10 aphids per seedling) out of a mixture containing RPV and MAV. (No previously published results could be found for the transmission by *S. avenae* of PAV from RPV + PAV-, or either PAV or MAV from PAV + MAV-infected leaves). In addition, *S. avenae* transmitted PAV, MAV and PAV + MAV at 18.0%, 8.0% and 6.0%, respectively, from triple-infected oat cv. M. Tabard source leaves to oat cv. Pennalt in the present research (see Chapter 4).

Further investigations may provide some insight into the interaction of RPV, PAV and MAV existing simultaneously in a plant, or on their effect on the transmission efficiency of individual aphid species.

Previous studies on the effect of BYDV on cereals (Aapola & Rochow, 1971; Baltenberger *et al.*, 1987) showed that plant heights of both barley and oats were decreased more when cultivars were infected by two strains of the virus than by either strain singly. Similar results were obtained in the present research.

The PAV strain of BYDV had the most damaging effect on cereals, causing more severe stunting of winter barley cv. Igri and oat cv. Dula than the RPV, or MAV strains. A similar phenomenon was reported in oat cv. Coast Black where the PAV, RPV and MAV strains were stated as being strongly, weakly and moderately virulent respectively (Slykhuis, 1976). In the present study, the severity of

stunting caused by mixed infections was similar to that caused by PAV alone in both barley and oats.

Obviously, it is necessary to carry out extensive research on the interactions of virus, plant and vector with triple-infected plants. To date, most work on mixed infections has concentrated on double-infected plants only, and different principles may apply where three strains of BYDV co-exist.

CHAPTER 6

THE TRANSMISSION OF THREE STRAINS OF BYDV BY *Rhopalosiphum padi* AND *Sitobion avenae* FROM OAT CVS. MARRIS TABARD AND DULA AND WINTER BARLEY CV. IGRI TO PERENNIAL RYEGRASS CV. TALBOT

6.1 INTRODUCTION

Several studies have investigated the transmission of BYDV from oats to a variety of grasses, and its effect on the yield and productivity of the ryegrass plants (Catherall, 1966, 1987; Panayotou, 1985). The symptoms and susceptibility of grasses to BYDV have also been documented (Stoner, 1976).

In such studies, it was usual to use oat plants as a known-infected source of BYDV as they gave clear symptoms of infection (Catherall, 1987), and therefore a good indication that virus was present. However, few investigations (Stoner, 1976) have reported using winter barley plants as a source of BYDV. In addition, little information exists on the percentage transmission of BYDV by the different aphid vectors to perennial ryegrass.

The objectives of the studies described in this chapter were to determine the rates of transmission of strains of BYDV by two aphid vector species from oats and winter barley to perennial ryegrass, and also to ascertain whether the rates of transmission differed from those of grass to winter barley (see Chapter 5).

The work carried out in Experiment 1 had additional objectives:

(1) To discover if the likelihood of grass becoming infected with BYDV increased with the number of aphids placed on the leaves.

(2) To determine when to harvest the grass for maximum BYDV content.

Objectives (1) and (2) were important in the production of known-infected grass source plants, whilst the main objective of the work reported in Chapter 6 was to develop a picture of BYDV epidemiology.

6.2 EXPERIMENT 1a - b: Aphid infestations and harvesting dates of grass plants in relation to BYDV incidence in perennial ryegrass cv. Talbot.

6.2.1 Materials and Methods

6.2.1.1 EXPERIMENT 1a: TRANSMISSION OF PAV

Prior to the inoculation feed, 117 pots (7.5 cm diam.) of perennial ryegrass cv. Talbot plants (4-week-old) were cut using scissors to a uniform height of 5 cm. Each pot contained approximately 10-15 seedlings. Any seedlings growing near the edge of the pot were removed.

R. padi were placed on the grass plants at the rate of one, five and 10 aphids per pot, giving 36 pots of grass plants per infestation treatment. The plants in the remaining nine pots were used as healthy controls. Non-viruliferous aphids from the stock-rearing colony were put on these plants using the same infestation rate, three pots of plants per treatment.

The viruliferous aphids used for the 108 test plants had been allowed to feed (48 h) on leaves of oat cv. M.

Tabard infected with the PAV strain of BYDV. The acquisition and transmission procedures were as described in Chapter 2 (2.6).

Following the 48 h inoculation feed, all test plants were placed in a heated glasshouse. Twelve test plants and one control plant per treatment were harvested after 3, 4, or 5 weeks. The leaves from a separate group (12) of plants were harvested each week rather than the same group repeatedly. This was to ascertain the time for BYDV to reach a level detectable by ELISA in grass, and therefore when to sample for the virus. The harvesting date was counted from the end of the inoculation feed.

All plants were cut at soil level, placed in labelled plastic bags (20.5 x 27.5 cm, QB Packing), and kept in a deep-freeze at -18^{o}C until tested by ELISA.

The dates the plants were sown, inoculated and assessed for foliar symptoms, together with the mean, minimum and maximum glasshouse temperatures are recorded for each experiment throughout Chapter 6 in Table 3, Appendix I.

6.2.1.2 EXPERIMENT 1b: THE TRANSMISSION OF RPV

The procedure followed for this experiment was similar to that described for Experiment 1a with the following exceptions:-

(1) Seeds of perennial ryegrass cv. Tablot were sown in 207 pots.

(2) The aphids (*R. padi*) used had previously been fed on oat cv. M. Tabard infected with the RPV strain of BYDV.

(3) Twenty-two test plants and one healthy control plant per infestation rate (one, five and 10) were harvested after 3, 4 and 5 weeks (totalling 198 test plants and nine healthy controls).

Again, all samples were stored and frozen until tested by ELISA.

6.2.2 Results

The highest level of transmission for PAV was recorded in plants harvested at 3 weeks which had been infested with 10 aphids (50.0%, Figure 7). Although this level of transmission was greater than the others recorded (16.7% - 33.3%), it was not statistically significant as too few plants were employed for each treatment (12). However, it was significantly greater than 0% ($P<0.01$), as was the percentage transmission by 10 aphids to plants harvested at 4 and 5 weeks (33.3%, $P<0.05$).

Generally, the percentage transmission of PAV was greater by 10 aphids (38.9%) than by five aphids (11.1%, $P<0.001$), although the latter was not significantly greater than that by one aphid (5.5%).

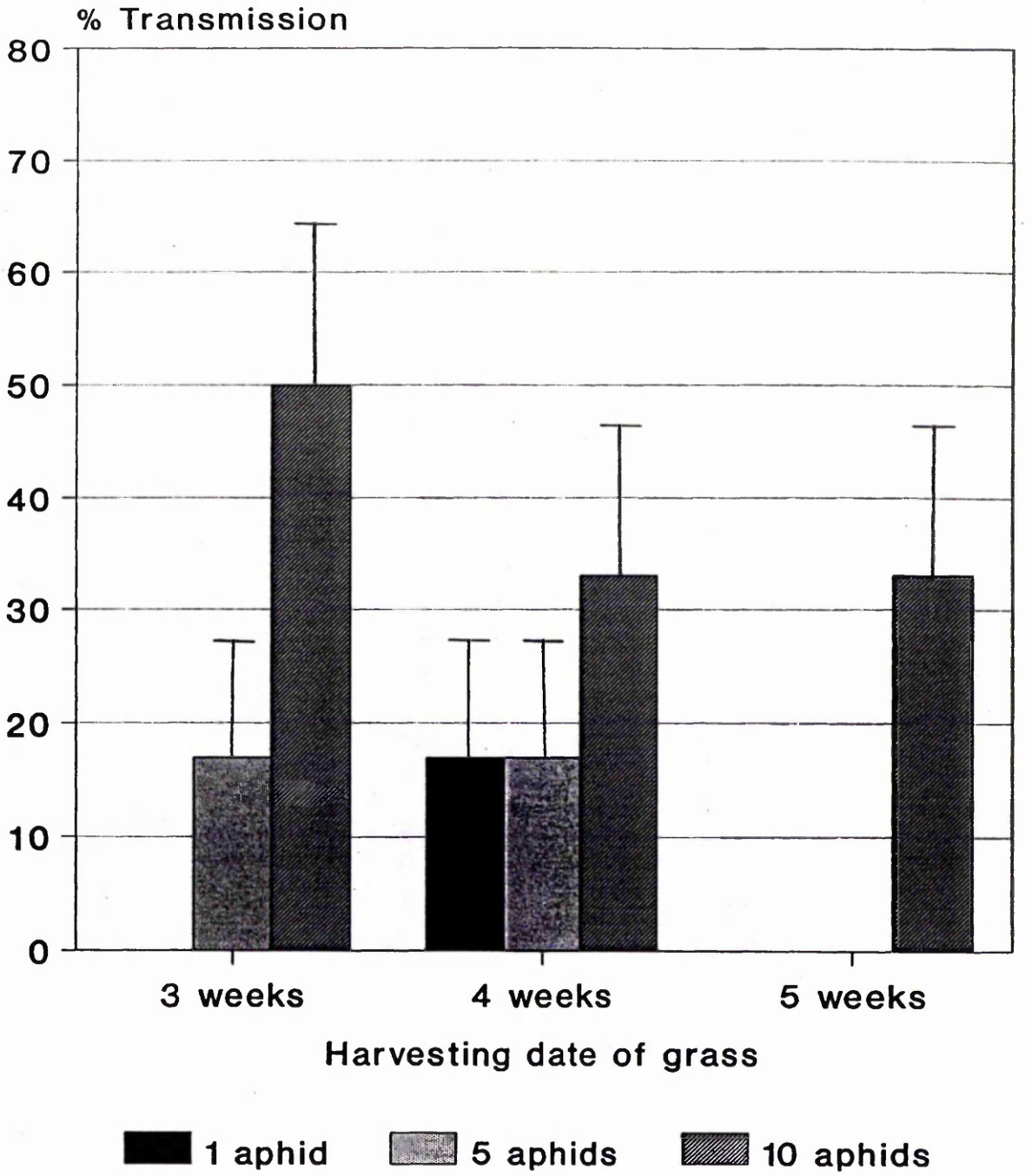


FIGURE 7. The percentage transmission of the PAV strain of BYDV by one, five, or 10 *R. padi* transferred from oat cv. M. Tabard to perennial ryegrass cv. Talbot which was harvested at 3, 4 and 5 weeks after inoculation. Vertical bars represent the standard error of the mean.

The number of plants found to contain PAV at 3 and 4 weeks (22.2%) was greater than at 5 weeks (11.1%, $P<0.01$), irrespective of the infestation rates.

There were significant differences ($P<0.05$ - $P<0.001$), between the percentage of aphids feeding (Table 51) and the percentage transmission, with the exception of 10 aphids loaded on plants harvested at 3 weeks. This indicated that the number of infected plants was fewer than the number of plants remaining infested with aphids during the inoculation period.

The transmission of RPV by *R. padi* was succesful by all three infestation rates (Figure 8). BYDV was detected in a greater number of plants previously infested by 10 aphids per plant than by one or five aphids at all harvesting dates ($P<0.001$), but particularly in plants harvested at 4 weeks (90.9%). Levels of transmission by five aphids per plant were not significantly different between the harvesting dates. However, the number of plants infected with RPV transmitted by one aphid was less in those harvested at 3 weeks (4.6%) than at 4 or 5 weeks (31.8%, $P<0.05$).

As with PAV, significantly more plants remained infested with aphids during the inoculation feed (Table 52) than became infected. This was observed with one aphid and five aphids at all harvesting dates ($P<0.001$) and 10 aphids at the 3 week harvesting date ($P<0.001$).

TABLE 51. Percentage of live *R. padi* remaining on perennial ryegrass cv. Talbot during the 48 h PAV inoculation feed, and the percentage transmission to test plants.

No. of aphids	% Aphids remaining during 48 h inoculation feed on plants harvested at n weeks			% Transmission to plants harvested at n weeks		
	3	4	5	3	4	5
1	83.3 ± 10.77	91.7 ± 7.96	66.7 ± 13.61	0.0 ± 0.00	16.7 ± 10.77	0.0 ± 0.00
5	89.2 ± 8.96	92.5 ± 7.60	59.2 ± 13.13	16.7 ± 10.77	16.7 ± 10.77	0.0 ± 0.00
10	61.3 ± 14.06	79.6 ± 11.63	90.4 ± 8.50	50.0 ± 14.43	33.3 ± 13.61	33.3 ± 13.61

No PAV was transmitted by the non-viruliferous aphids to the virus-free plants (healthy control).

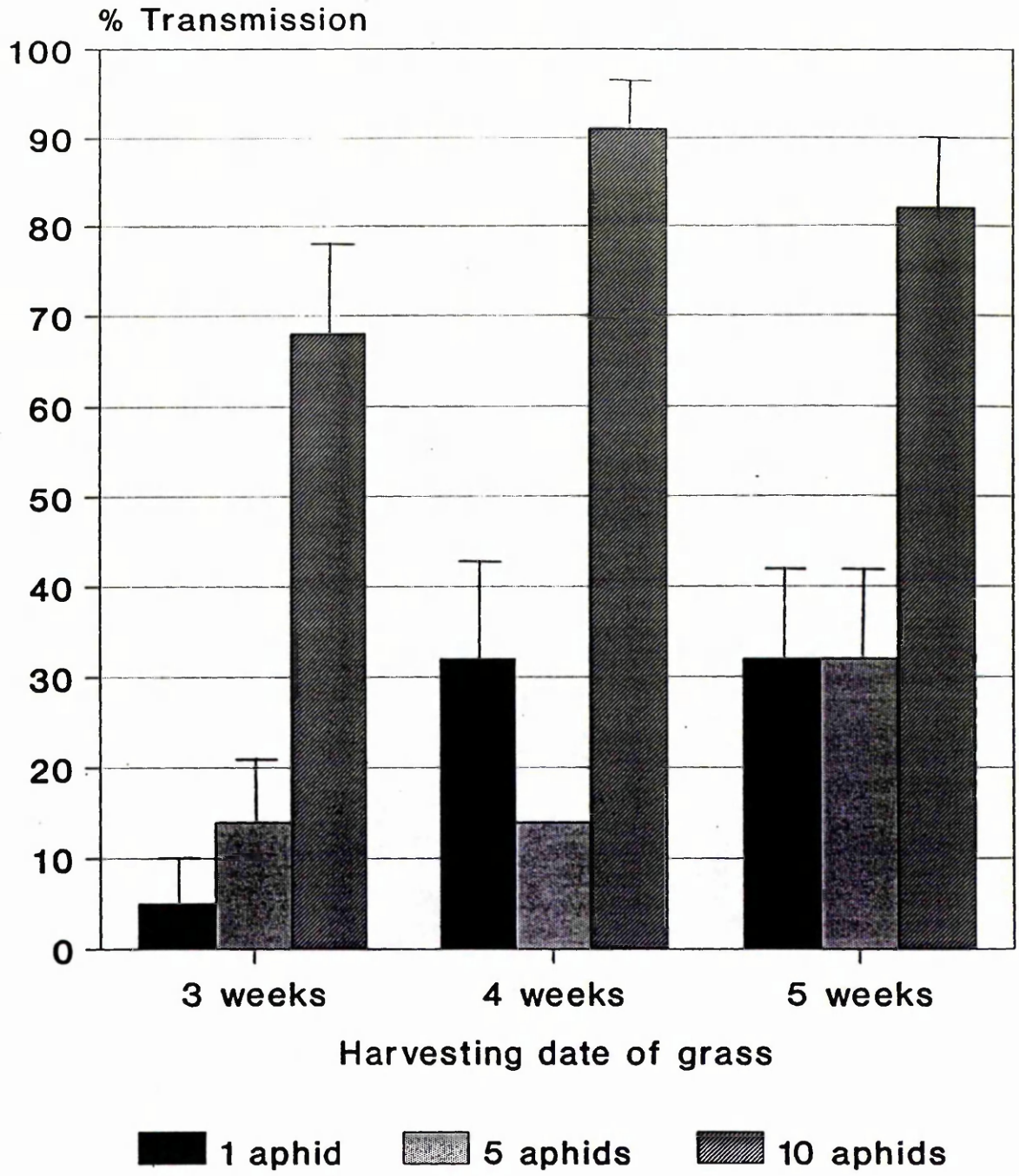


FIGURE 8. The percentage transmission of the RPV strain of BYDV by one, five, or 10 *R. padi* transferred from oat cv. M. Tabard to perennial ryegrass cv. Talbot which was harvested at 3, 4 and 5 weeks after inoculation. Vertical bars represent the standard error of the mean.

TABLE 52. Percentage of live *R. padi* remaining on perennial ryegrass cv. Talbot during the 48 h RPV inoculation feed, and the percentage transmission to test plants.

No. of aphids	% Aphids remaining during 48 h inoculation feed on plants harvested at n weeks			% Transmission to plants harvested at n weeks		
	3	4	5	3	4	5
1	91.7 ± 5.88	100.0 ± 0.00	91.7 ± 5.88	4.6 ± 4.47	31.8 ± 9.93	31.8 ± 9.93
5	96.4 ± 3.97	100.0 ± 0.00	100.0 ± 0.00	13.6 ± 7.31	13.6 ± 7.31	31.8 ± 9.93
10	100.0 ± 0.00	96.4 ± 3.97	96.4 ± 3.97	68.2 ± 9.93	90.9 ± 6.13	81.8 ± 8.23

No RPV was transmitted by the non-viruliferous aphids to the virus-free plants (healthy control).

Comparing the incidence of RPV and PAV in perennial ryegrass, the biggest differences in transmission were observed between experiments where one and five aphids were placed on plants which were subsequently harvested at 5 weeks ($P<0.01$), and 10 aphids on plants subsequently harvested at 4 ($P<0.001$) and 5 weeks ($P<0.01$).

Overall, even considering the difference in plant sample numbers, *R. padi* transmitted RPV from oats to a greater number of perennial ryegrass plants (40.9%) than PAV (18.5%, $P<0.001$). More aphids remained on grass plants during the inoculation feed in the RPV study (97.0%) than in the PAV study (76.0%, $P<0.001$). However, the higher level of transmission by *R. padi* of the RPV strain compared to the PAV strain was not due to a greater mortality of aphids in the PAV experiment. There was little correlation between the number of plants infested with aphids during the inoculation period and the number of plants infected with PAV ($r = 0.324$, $P>0.05$), or RPV ($r = -0.037$, $P>0.05$).

6.3 EXPERIMENT 2: THE TRANSMISSION OF A MIXTURE OF THE RPV, PAV AND MAV STRAINS BY *S. avenae* FROM OAT CV. DULA TO PERENNIAL RYEGRASS CV. TALBOT

6.3.1 Materials and Methods

Three adult, apterous *S. avenae* were placed on the perennial ryegrass cv. Talbot seedlings (18-day-old) growing in each pot (7.5 cm diam., 10-15 seedlings per

pot), 50 pots in total. Three *S. avenae* were also placed on each of 10 seedlings of oat cv. Dula (18-day-old), five seedlings per pot (12.5 cm diam.). The aphids used had previously been fed on oat cv. Dula leaves infected with a mixture of the RPV, PAV and MAV strains of BYDV. The acquisition and transmission procedures were as described in Chapter 2 (2.6). After inoculation the test plants were placed in a heated glasshouse.

Approximately 4 weeks after inoculation, any foliar symptoms was noted. All grass plants were cut at soil level, placed in labelled polythene bags, and kept in a deep-freeze at -18^{o}C until tested by ELISA. The procedure was repeated with the oat plants except that only the leaves were collected. All five seedlings per pot were analysed collectively.

Oat cv. Dula, which is known to be susceptible to BYDV, was included in each experiment as a control to check that the aphids had acquired BYDV from the cereal source plants.

Shortly after all plants were placed in the glasshouse, the source leaves used for the acquisition feed (48h) were analysed by ELISA. This was to ascertain the strains of virus present. When analysing the ryegrass and oat samples, sap from both cultivars were included in each microtitre plate.

The average survival rate of aphids during the inoculation period was 68.0%.

6.3.2 Results

After 4 weeks' growth, no symptoms of BYDV were present on the grass plants. However, all the oat control plants bore typical symptoms of BYDV .

ELISA analysis of the leaves showed that none of the perennial ryegrass plants contained BYDV, but the oat plants from both pots contained PAV and MAV.

6.4 EXPERIMENT 3: THE TRANSMISSION OF THE MAV STRAIN BY *S.avenae* FROM OAT CV. DULA TO PERENNIAL RYEGRASS CV. TALBOT

6.4.1 Materials and Methods

The procedure followed for this experiment was similar to that described for Experiment 2, with the following exceptions:-

(1) The perennial ryegrass used was 4-week-old when aphids were placed on the seedlings, and was cut to a height of 6 cm.
(2) Three pots of oat cv. Dula (containing five 18-day-old seedlings per pot) were used as a control.
(3) The aphids used had been allowed to feed on oat cv. Dula leaves infected with the MAV strain of BYDV.
(4) The average survival rate of aphids during the inoculation period (48h) was 86.5%.

6.4.2 Results

All grass plants were symptomless after 4 weeks' growth, however, typical symptoms of BYDV were observed on all oat control plants.

In ELISA tests, MAV was detected in 38.0% of the perennial ryegrass plants, and in all oat plants with typical symptoms of BYDV. However, the test samples were analysed in two ELISA tests one week apart. The first ELISA test gave positive MAV absorbance values (mean A_{405} = 0.199) for 76.0% of the grass test samples. The second ELISA test analysing sap from the remaining half of the grass test samples did not give positive MAV absorbance values (mean A_{405} = 0.029) for any samples except the known-infected, and one of the oat controls. The mean absorbance value of the latter grass test samples was lower than the negative MAV value (mean A_{405} = 0.068) of the uninfected test samples from the first ELISA test ($P<0.001$).

A number of the positive and negative samples from both tests were re-analysed (25.0%). Samples previously appearing positive gave borderline (positive) absorbance values (mean A_{405} = 0.100). The mean values of the healthy controls in the first and second tests were A_{405} = 0.033 and 0.032 respectively. All ELISA tests used the same reagents and technical procedures.

6.5 EXPERIMENT 4a - c: THE TRANSMISSION OF A MIXTURE OF THE RPV, PAV AND MAV STRAINS BY *R. padi* AND *S. avenae* FROM WINTER BARLEY CV. IGRI TO PERENNIAL RYEGRASS CV. TALBOT

6.5.1 Materials and Methods

The procedure followed for Experiment 4a-c was similar to that described for Experiment 2, with the following exceptions:-

(1) Only one aphid was placed on the perennial ryegrass seedlings growing in each pot. This was to ascertain the absolute percentage transmission.

(2) Fifty seedlings of oat cv. Dula (one seedling per 7.5 cm diam. pot) accompanied every 50 pots of perennial ryegrass, 50 pots per aphid species.

(3) The transmission rates of *R. padi* as well as *S. avenae* were investigated in each study.

(4) The source leaves used for the acquisition feed were of winter barley cv. Igri.

(5) The age of the plants used in each study varied as follows:

Experiment 4a: 3-week-old grass cut to a height of 6.0 cm, and 7-day-old oats.

Experiment 4b: 4-week-old grass cut to a height of 8 cm, and 16-day-old oats.

Experiment 4c: 18-day-old grass cut to a height of 6 cm, and 18-day-old oats.

In each ELISA test, samples of sap from both ryegrass and oats were included in each microtitre plate.

The average survival rate of aphids during the inoculation period (48 h) was 98.4% for *R. padi*, and 99.3% for *S. avenae* on perennial ryegrass plants (min. 97.1% for *R. padi* in Experiment 4a and 98.0% for *S. avenae* in Experiment 4c), and 95.3% and 97.3%, respectively, on oat cv. Dula (min. 92.0% for *R. padi* in Experiment 4c and 96.0% for *S avenae* in Experiment 4a).

6.5.2 Results

6.5.2.1 EXPERIMENT 4a

Unfortunately, a number of the *R. padi* died during the acquisition feed. As a result, only 35 ryegrass plants and 22 oats were infested with *R. padi*.

All grass plants were symptomless, as were the majority (44) of oat plants infested by *S. avenae* (Table 53). However, the remaining plants did develop typical or atypical symptoms of infection (three of each). A number of oat plants fed upon by *R. padi* also developed typical (10) or atypical (four) foliar discoloration.

Analysis by ELISA showed that all oat plants exhibiting typical symptoms contained BYDV, as did three of the plants exhibitng atypical symptoms. No virus was detected in the symptomless oat plants.

TABLE 53. EXPERIMENT 4a. Symptom expression and detection by ELISA of a mixture of the RPV, PAV and MAV strains of BYDV transmitted by *R. padi* and *S. avenae* from winter barley cv. Igri to perennial ryegrass cv. Talbot and oat cv. Dula.

	Number of plants				Virus incidence*			
	R. padi		*S. avenae*		*R. padi*		*S. avenae*	
Symptom expression	Grass	Dula	Grass	Dula	Grass	Dula	Grass	Dula
None	35	8	50	44	9/35	0/8	16/50	0/44
Yellow or red leaves/stunting (typical)	0	10	0	3	-	10/10	-	3/3
Leaf chlorosis (atypical)	0	4	0	3	-	2/4	-	1/3
Total	35	22	50	50				
% Transmission					25.7	54.6	32.0	8.0
Standard error					± 7.39	±10.62	± 6.60	± 3.84

* Expressed as number of plants positive/number tested in ELISA test.

R. padi transmitted BYDV at a higher frequency from winter barley to oat cv. Dula (54.6%) than did *S. avenae* (8.0%, $P<0.001$). On the other hand, *R. padi* and *S. avenae* transmitted BYDV at similar rates from barley to ryegrass (25.7% and 32.0%, respectively, $P>0.05$).

Comparing the percentage transmission by *R. padi* to oats and ryegrass, BYDV was transmitted more readily to the former (54.6%) than to the latter (25.7%, $P<0.05$). In contrast, *S. avenae* transmitted BYDV to a greater number of grass plants (32.0%) than oat plants (8.0%, $P<0.001$).

Analysis of the incidence of strains in the leaves showed that *R. padi* transmitted RPV (2.9%) and RPV + MAV (5.7%) to a small number of ryegrass plants, but, interestingly, transmitted MAV to a larger number (17.1%), but not to a significantly greater extent (Table 54). The percentage transmission of MAV by *R. padi* was less than that transmitted to grass by *S. avenae* (32.0%), but again not significantly. No other strains of BYDV were transmitted by *S. avenae*, except PAV + MAV (8.0%) to the oat control. On the other hand, *R. padi* transmitted PAV (45.5%), and to a lesser extent RPV + PAV (9.1%, $P<0.01$) to oat cv. Dula.

6.5.2.2 EXPERIMENT 4b

The grass plants were approximately 8-week-old at the time of harvesting and foliar assessment (which was 4 weeks after inoculation). As a result, a few of the leaves of

TABLE 54. EXPERIMENT 4a. The transmission of a mixture of the RPV, PAV and MAV strains of BYDV by *R. padi* and *S. avenae* from winter barley cv. Igri to perennial ryegrass cv. Talbot and oat cv. Dula. Results are expressed as the percentage transmission ± the standard error of the mean.

	% Transmission to grass by		% Transmission to oats by	
Strain of BYDV	*R. padi*	*S. avenae*	*R. padi*	*S. avenae*
RPV	2.9 ± 2.84	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
PAV	0.0 ± 0.00	0.0 ± 0.00	45.5 ± 10.62	0.0 ± 0.00
MAV	17.1 ± 6.36	32.0 ± 6.60	0.0 ± 0.00	0.0 ± 0.00
RPV + PAV	0.0 ± 0.00	0.0 ± 0.00	9.1 ± 6.13	0.0 ± 0.00
RPV + MAV	5.7 ± 3.92	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
PAV + MAV	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	8.0 ± 3.84
RPV + PAV + MAV	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

each had senesced. Symptoms of BYDV were not seen on any of the grass plants. Typical symptoms of BYDV were seen, however, on 14 of the oat cv. Dula fed upon by *R. padi* (Table 55). No symptoms of BYDV were observed on those inoculated by *S. avenae*, but atypical foliar discoloration did develop on four of the plants.

All oat plants with typical symptoms that were analysed by ELISA were found to contain BYDV. Virus was not detected in oats with either atypical or no symptoms.

The results showed that *S. avenae* failed to transmit BYDV to oat cv. Dula, whereas *R. padi* did succeed to a significant level (28.0%, $P<0.001$). In contrast, BYDV was detected in every ryegrass plant inoculated by either *R. padi*, or *S. avenae*. Consequently, this level of transmission was higher than the level of transmission to the oat controls ($P<0.001$).

Surprisingly, *S. avenae* transmitted RPV + MAV to every perennial ryegrass plant in the experiment (Table 56), while *R. padi* transmitted RPV + MAV to fewer plants (62.0%, $P<0.001$). Interestingly, MAV alone was transmitted to 24.0% of grass plants by *R. padi*, but not to the oat control. *R. padi* also succeeded in transmitting RPV + PAV + MAV to 14.0% of grass plants. Completely different strains and combinations of strains were detected in the oats fed upon by *R. padi*: PAV (16.0%), and to a lesser extent PAV + MAV (10.0%, $P>0.05$) and RPV (2.0%, $P<0.05$).

Table 55. EXPERIMENT 4b. Symptom expression and detection by ELISA of a mixture of the RPV, PAV and MAV strains of BYDV transmitted by *R. padi* and *S. avenae* from winter barley cv. Igri to perennial ryegrass cv. Talbot and oat cv. Dula.

	Number of plants				Virus incidence*			
	R. padi		*S. avenae*		*R. padi*		*S. avenae*	
Symptom expression	Grass	Dula	Grass	Dula	Grass	Dula	Grass	Dula
None	50	36	50	46	50/50	0/36	50/50	0/46
Yellow or red leaves/stunting (typical)	0	14	0	0	-	14/14	-	-
Leaf chlorosis (atypical)	0	0	0	4	-	-	-	0/4
Total	50	50	50	50				
% Transmission					100.0	28.0	100.0	0.0
Standard error					± 0.00	± 6.35	± 0.00	± 0.00

* Expressed as number of plants positive/number tested in ELISA test.

TABLE 56. EXPERIMENT 4b. The transmission of a mixture of the RPV, PAV and MAV strains of BYDV by *R. padi* and *S. avenae* from winter barley cv. Igri to perennial ryegrass cv. Talbot and oat cv. Dula. Results are expressed as the percentage transmission ± the standard error of the mean.

	% Transmission to grass by		% Transmission to oats by	
Strain of BYDV	*R. padi*	*S. avenae*	*R. padi*	*S. avenae*
RPV	0.0 ± 0.00	0.0 ± 0.00	2.0 ± 1.98	0.0 ± 0.00
PAV	0.0 ± 0.00	0.0 ± 0.00	16.0 ± 5.19	0.0 ± 0.00
MAV	24.0 ± 6.04	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
RPV + PAV	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
RPV + MAV	62.0 ± 6.86	100.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
PAV + MAV	0.0 ± 0.00	0.0 ± 0.00	10.0 ± 4.24	0.0 ± 0.00
RPV + PAV + MAV	14.0 ± 4.91	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.0

As high percentages of MAV and RPV were detected in the ELISA analysis of the grass test samples, the absorbance values of these two strains were investigated. The mean positive absorbance value for MAV obtained with sap from grass test plants was A_{405} = 0.365, while that for RPV was A_{405} = 0.189. The mean MAV and RPV absorbance values for the oats tested simultaneously with the above grass samples were A_{405} = 2.030 and A_{405} = 0.168, respectively. A correlation study on the RPV and MAV absorbance values of the grass samples showed that a strong relationship did exist ($r = 0.816$, $P<0.001$). Furthermore, regression analysis indicated that the absorbance values of RPV rose positively with those of MAV (Figure 1, Appendix III). In contrast, no relationship existed between the absorbance values of MAV and RPV obtained with oats ($r = 0.003$, $P>0.05$, Figure 2, Appendix III).

6.5.2.3 EXPERIMENT 4c

All oat cv. Dula fed upon by *S. avenae* remained symptomless (Table 57). Similarly, the majority of oats infested by *R. padi* remained symptomless. However, foliar discoloration, both typical and atypical of BYDV were observed on a few plants (eight and two, respectively). All perennial ryegrass plants were symptomless.

Following analysis by ELISA, BYDV was detected in all oat plants with typical symptoms of infection, and in none bearing atypical symptoms, or that were symptomless.

TABLE 57. EXPERIMENT 4c. Symptom expression and detection by ELISA of a mixture of the RPV, PAV and MAV strains of BYDV transmitted by *R. padi* and *S. avenae* from winter barley cv. Igri to perennial ryegrass cv. Talbot and oat cv. Dula.

	Number of plants				Virus incidence*			
	R. padi		*S. avenae*		*R. padi*		*S. avenae*	
Symptom expression	Grass	Dula	Grass	Dula	Grass	Dula	Grass	Dula
None	50	40	50	50	49/50	0/40	39/50	0/50
Yellow or red leaves/stunting (typical)	0	8	0	0	-	8/8	-	-
Leaf chlorosis (atypical)	0	2	0	0	-	0/2	-	-
Total	50	50	50	50				
% Transmission					98.0	16.0	68.0	0.0
Standard error					± 1.98	± 5.19	± 6.60	± 0.00

* Expressed as number of plants positive/number tested in ELISA test.

Virus was detected in 68.0% and 98.0% ($P<0.001$) of the ryegrass plants exposed to *S. avenae* and *R. padi* respectively. *R. padi* and *S. avenae* transmitted BYDV to more grass plants ($P<0.001$) than oat plants (16.0% and 0%, respectively).

Out of a mixture, *R. padi* transmitted mainly the RPV and MAV strains together (72.0%) to ryegrass (Table 58). Surprisingly, *S. avenae* also transmitted RPV + MAV, but with a lower frequency than *R. padi* (24.0%, $P<0.001$). Interestingly, *R. padi* transmitted MAV alone (24.0%), as did *S. avenae*, but to a greater number of plants (44.0%, $P<0.05$). *R. padi* transmitted RPV to one grass sample (2.0%). Again, *S. avenae* failed to transmit BYDV to oats while *R. padi* transmitted RPV to 10.0% of plants, and PAV to 6.0%.

All of the positive RPV absorbance values (mean A_{405} = 0.153 were accompanied by positive MAV absorbance values (mean A_{405} = 0.281) in the grass test samples which had been exposed to *R. padi*. However, a few samples which had positive values for MAV (mean A_{405} = 0.152) were not accompanied by positive RPV absorbance values (mean A_{405} = 0.076).

A similar observation was noted for the first ELISA test analysing half of the ryegrass plants exposed to *S. avenae* (mean positive RPV absorbance value, A_{405} = 0.189, mean accompanying MAV value, A_{405} = 0.268). Whereas, in the ELISA test analysing the other half of the

TABLE 58. EXPERIMENT 4c. The transmission of a mixture of the RPV, PAV and MAV strains of BYDV by *R. padi* and *S. avenae* from winter barley cv. Igri to perennial ryegrass and oat cv. Dula. Results are expressed as the percentage transmission ± the standard error of the mean.

	% Transmission to grass by		% Transmission to oats by	
Strain of BYDV	*R. padi*	*S. avenae*	*R. padi*	*S. avenae*
RPV	2.0 ± 1.98	0.0 ± 0.00	10.0 ± 4.24	0.0 ± 0.00
PAV	0.0 ± 0.00	0.0 ± 0.00	6.0 ± 3.36	0.0 ± 0.00
MAV	24.0 ± 6.04	44.0 ± 7.02	0.0 ± 0.00	0.0 ± 0.00
RPV + PAV	0.0 ± 0.00	0.0 ± 0.0	0.0 ± 0.00	0.0 ± 0.00
RPV + MAV	72.0 ± 6.35	24.0 ± 6.20	0.0 ± 0.00	0.0 ± 0.00
PAV + MAV	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
RPV + PAV + MAV	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

grass plants infested by *S. avenae*, the mean positive MAV absorbance value was A_{405} = 0.178, while that for RPV of the same samples was negative, A_{405} = 0.041. (The mean A_{405} value for the RPV known-infected leaves was 0.873). A correlation study on the grass samples in the latter ELISA test showed no relationship between the absorbance values for RPV and MAV ($r = -0.020$, $P>0.05$, Figure 3, Appendix III). Interestingly, a very strong relationship did exist between the RPV and MAV ELISA values obtained in all the other tests on grass samples in this experiment ($r = 0.909$, $P<0.001$). Further analysis by regression indicated that the absorbance values for one strain were rising positively with those for the other strain (Figure 4, Appendix III). Again, no relationship existed between the MAV and RPV A_{405} values obtained with the oat controls ($r = -0.133$, $P>0.05$, Figure 5, Appendix III).

6.6 SUMMARY OF RESULTS: EXPERIMENT 4a - c

Generally, the rate of infection seemed to be affected by the age of the oat cv. Dula plants at the time of inoculation (Table 59). Plants inoculated by *R. padi*, or *S. avenae* when 7-day-old had a greater incidence of infection, (54.6% and 8.0%, respectively), than those inoculated at 16-days (28.0% and 0%, respectively, $P<0.05$), or at 18-days (16.0%, $P<0.01$ and 0.0%, $P<0.05$, respectively).

However, a similar trend was not noted with the ryegrass plants (Table 59). More perennial ryegrass were

TABLE 59. The transmission of BYDV by *R. padi* and *S. avenae* from winter barley cv. Igri to perennial ryegrass cv. Talbot and oat cv. Dula inoculated at three different ages. Results are expressed as the percentage transmission ± the standard error of the mean.

Experiment	Plant cultivar	Age of plants	% Transmission	
			R. padi	*S. avenae*
4a	Ryegrass	21 days	25.7 ± 7.39	32.0 ± 6.60
	Oats	7 days	54.6 ± 10.62	8.0 ± 3.84
4b	Ryegrass	28 days	100.0 ± 0.00	100.0 ± 0.00
	Oats	16 days	28.0 ± 6.35	0.0 ± 0.00
4c	Ryegrass	18 days	98.0 ± 1.98	68.0 ± 6.60
	Oats	18 days	16.0 ± 5.19	0.0 ± 0.00

infected when infested at 18-day-old (83.0%) than at 21-day-old (28.9%, P<0.001), but fewer than at 28-day-old (100.0%, P<0.001).

Overall, *R. padi* transmitted BYDV to a greater number of ryegrass plants in Experiments 4b (100.0%) and 4c (98.0%) than in 4a (25.7%, P<0.001, Figure 9). The number of plants inoculated by *S. avenae* was greater in Experiment 4b (100.0%) than in 4c (68.0%, P<0.0.01), which in turn was greater than in 4a (32.0%, P<0.001)

The transmission of RPV + MAV by *R. padi* to perennial ryegrass in Experiments 4b and 4c was similar (62.0% and 72.0%, respectively, P>0.05), but both were much greater than in 4a (5.7%, P<0.001, Tables 54, 56, and 58). However, transmission of RPV + MAV by *S. avenae* was greater in Experiment 4b (100.0%) than in either 4a (0%, P<0.001), or 4c (24.0%, P<0.001). No RPV + MAV was detected in infected oats from any experiment.

The MAV strain was transmitted at similar rates by *R. padi* to grass in Experiment 4a-c (17.1%, 24.0%, and 24.0%, respectively, P>0.05). On the other hand, *S. avenae* did not inoculate ryegrass plants with the MAV strain alone in Experment 4b, but did at a slightly higher rate in 4c (44.0%) than in 4a (32.0%, P>0.05). Again no MAV was detected in any oat cv. Dula plants.

No PAV alone was detected in any ryegrass plants and was found only in combination with the RPV and MAV strains in Experiment 4b (14.0%) in grass plants fed upon by *R.*

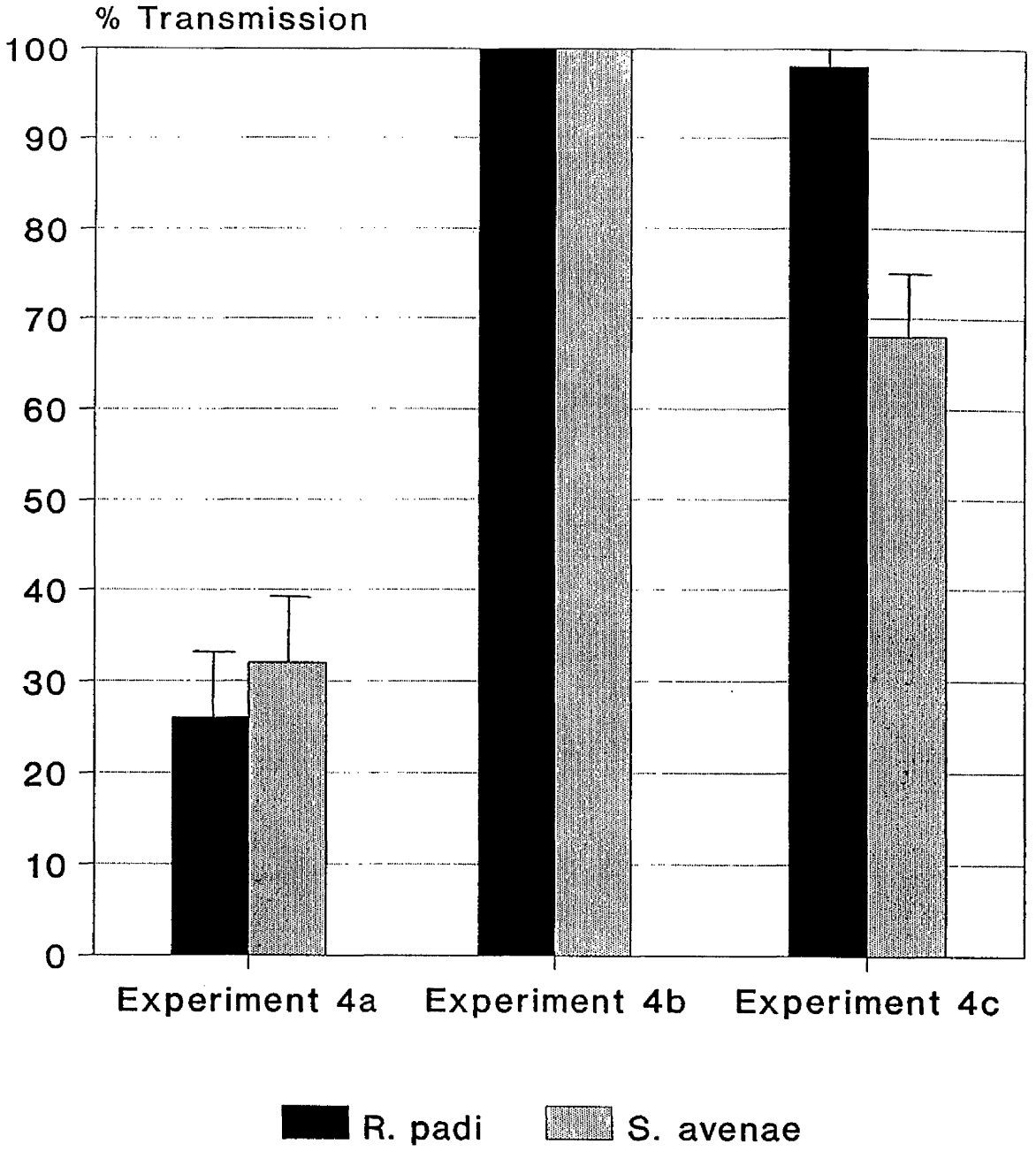


FIGURE 9. Comparison of the percentage transmission of a mixture of the RPV, PAV and MAV strains of BYDV by *R. padi* and *S. avenae* from winter barley cv. Igri to perennial ryegrass cv. Talbot in three separate experiments. Vertical bars represent the standard error of the mean.

padi. However, PAV was transmitted by *R. padi* to the oat controls in Experiment 4a (45.5%) at a higher rate than in Experiments 4b (16.0%, $P<0.05$) or 4c (6.0%, $P<0.001$).

It should be noted that there was little correlation between the mean absorbance values of RPV and MAV of either the grass or oat test samples grown in Experiment 4a ($r = 0.064$, $P>0.05$ and $r = 0.273$, $P>0.05$, respectively, Figures 6-7, Appendix III).

6.7 DISCUSSION

Perennial ryegrass cv. Talbot plants appeared to become more readily infected with the RPV strain of BYDV, than by the PAV strain, irrespective of the number of *R. padi* placed on each plant.

This was previously demonstrated with perennial ryegrass artificially infected with the French isolates of RPV and PAV (Henry, 1988). With three *R. padi* per plant, 57% of the plants became infected with an RPV isolate (R568) compared to 3% by the PAV isolate (PR1 de Rennes). Similarly, only 3% of the perennial ryegrass plants became infected with an MAV isolate (F148) transmitted by *S. avenae* (Henry, 1988).

Although *S. avenae* (three aphids per plant) in the present research transmitted MAV to 38.0% of the perennial ryegrass plants from source leaves infected with MAV alone, this rate of transmission should be treated with caution. Many samples analysed in one ELISA test appeared

positive for MAV, while others from the same experiment all appeared negative in a second ELISA test. Re-testing of samples did not resolve whether or not the samples were definitely positive for MAV.

Nevertheless, these results indicate the difficulty in working with grass which is symptomless, and a virus which is transmitted only by aphids. There may be a degree of variability in the procedure used to detect the virus between tests, however, the technique was always followed in a uniform manner.

In the studies investigating aphid infestation rates, separate plants were harvested at each date, i.e. not repeatedly harvested weekly. Therefore, differences in levels of BYDV transmission could partly be due to aphid feeding behaviour. However, as all plants in each experiment were inoculated simultaneously, and kept under the same conditions, this should not be the determining factor.

Skaria *et al.* (1984) found that wheat, barley and oat plants infected with PAV did not show any differences in symptom expression, tissue weight, or virus content (as assessed by ELISA) whether they had been infested with two, or 10 aphids. This lack of BYDV dosage effect on virus content in cereals may be similar in perennial ryegrass. However, it appears in the present research that the number of grass plants found to contain BYDV increased with the number of aphids placed on each plant.

The number of plants infected with RPV or PAV differed between the harvesting dates, irrespective of aphid infestation rates. A greater number of ryegrass plants were shown to contain PAV when harvested at 3 and 4 weeks than at 5 weeks. Whereas, the mean percentage of plants infected with RPV was greatest at 4 and 5 weeks. These results indicated that the best time to harvest perennial ryegrass cv. Talbot for the optimum levels of RPV, or PAV infection, whether using one, five, or 10 aphids was at 4 weeks.

Henry (1988) reported that levels of PAV and RPV in fescue (*Festuca arundinacea* Schreb) and perennial ryegrass leaves, respectively, were higher at 24 and 30 days than at 18 days. These findings lend support to harvesting perennial ryegrass at 4 weeks for the detection of optimum levels of BYDV.

Generally, more grass plants remained infested with *R. padi* during the inoculation period than the number of plants becoming infected. This may indicate that *R. padi* have difficulty in transmitting the virus. Perhaps they are unable to probe the leaf surface due to the epidermal structure, such as cuticle wax. Their feeding behaviour would have to be closely studied to ascertain whether this was a factor. Alternatively, a number of aphids may not have been carrying virus, i.e. a proportion of the aphids may have failed to acquire BYDV from the oat source leaves during the acquisition feed.

Amplified ELISA tests (Torrance, 1987), or serologically specific electron microscopy (Paliwal, 1982a) could be used to determine what proportion of the aphids were carrying BYDV. If it was shown that most did contain virus, then the lack of transmission may have been conferred by the recipient grass plants themselves. However, if the analysis of aphids revealed that a proportion was not carrying virus, further studies may reveal the cause of the inability of some aphids to acquire virus.

However, both experiments investigating aphid infestation rates, particularly the study involving the PAV strain, should be repeated on a larger scale to determine significant statistical differences.

Strains from a mixture containing the RPV, PAV and MAV strains of BYDV were not transmitted by *S. avenae* from oat cv. Dula to perennial ryegrass cv. Talbot (Experiment 2). The aphids were able to acquire and transmit BYDV from the oats as they were successful in infecting the control, oat cv. Dula, with both PAV and MAV. Again it would appear that the aphids were able to acquire the virus, but were unable to transmit it to the perennial ryegrass. As discussed earlier, the direct analysis of the aphids used in the experiment would indicate whether this was so.

On the other hand, *R. padi* and *S. avenae* transmitted BYDV succesfully from winter barley cv. Igri infected with

the RPV, PAV and MAV strains to perennial ryegrass cv. Talbot. Transmission by *S. avenae* in these studies was by fewer aphids, as only one aphid was placed per test plant compared to three in the experiment discussed above. Only one aphid was used instead of three, five, or 10 as the absolute transmission rate of the aphid was under study.

The marked difference in transmission by *S. avenae* between Experiment 4a-c and Experiment 2 is interesting as both experiments were carried out under similar temperatures, at approximately the same time of year, although one year apart.

The source of virus was from a different plant species (winter barley instead of oats), however, it is doubtful that this would cause such a marked difference in the level of transmission to ryegrass.

Also of interest was the difference in transmission of BYDV by both aphid species to the ryegrass test plants within Experiment 4. All tests were carried out in an identical manner, within days of each other, and maintained under similar conditions. Furthermore, all samples were analysed by indirect ELISA within one month.

Differences in rates of transmission by *R. padi* may have been attributable to the acquisition feed. A number of the *R. padi* died during the acquisition period in Experiment 4a, indicating that either the state of the *R. padi* colony was poor, or that conditions were not suitable for their survival. Therefore, despite 97.1% of the

survivors remaining on the plants during the inoculation feed, many may not have acquired virus. Similarly, although the *S. avenae* did not die during the acquisition period in Experiment 4a, the conditions may not have been suitable for the acquisition of virus resulting in a low rate of transmission compared to in Experiments 4b and 4c.

The aphid feeding behaviour during the inoculation feed would also affect the percentage detection of BYDV. Each grass sample consisted of 10-15 plants (to give 1 g of leaf material used for the ELISA test), and one aphid was placed per sample. Some aphids may move from plant to plant, feeding, while others may stay on the one plant for the 48 h period. With the former, BYDV will be easily detected if the aphid was viruliferous as several plants may be infected, while BYDV may not be detected with the latter as one plant will be infected and the remainder not, thus diluting the quantity of virus in the 1 g sample.

Alternatively, variations in the detection of BYDV in ryegrass plants may be a function of the ryegrass plants themselves. The seeds are produced by cross-breeding and therefore each variety is a heterogenous mixture in which virus may multiply at different rates and so influence detection.

The transmission of RPV and MAV together by *R. padi* may be due to transcapsidation (Rochow, 1982a). However, *S. avenae* rarely transmits both strains together from a

mixture containing RPV and MAV (Rochow & Gill, 1978). The presence of PAV in the source leaves may affect the transmission of RPV by *S. avenae*. However, this is very unlikely as no RPV + MAV was transmitted by *S. avenae* to the accompanying oat controls. Furthermore, this combination has never previously been transmitted by *S. avenae* from triple-infected oat or grass plants to either winter barley or oats (See Chapters 4 and 5).

The high rate of MAV transmission by *R. padi* is also unusual (Rochow, 1969a). Interestingly, of the number of *R. padi* caught in the commode trap at Auchincruive during 1989-1990, 21.4% were viruliferous. Of those, 13.6% and 4.6% contained the MAV and RPV + MAV strains, respectively (G.N. Foster, personal communication).

Foxe & Rochow (1975) commented that *R. padi* was more likely to transmit MAV to oats from young oat cv. Coast Black leaves (14%) than from old leaves (1%). However, levels of transmission to perennial ryegrass cv. Talbot in the present research were still higher than expected, even if in fact, young barley leaves had been used for the acquisition feed.

Alternatively, the prevalent detection of both RPV and MAV in ryegrass plants may be attributed to the contamination of reagents or antibodies used in the ELISA technique. Statistical analysis of the positive absorbance values (A_{405}) for RPV and MAV indicated that there was a positive relationship between them. However,

no such relationship existed between the RPV and MAV A_{405} values obtained for any oat controls, grass samples from Experiment 4a, or in one of the ELISA tests analysing grass samples from Experiment 4c. In the latter case low positive absorbance values of MAV were observed, but were not accompanied by RPV. The negative A_{405} values obtained for RPV were not due to a failure of the RPV monoclonal antibody (MAC 92) in detecting virus as the known-infected control for RPV gave a high absorbance value.

A cross-reaction between RPV and MAV antisera, as occasionally encountered between PAV and MAV when using monoclonal antibodies (Hsu *et al.*, 1984; Torrance *et al.*, 1986a), should not occur as RPV and MAV are serologically unrelated (Aapola & Rochow, 1971).

The unusual association of RPV and MAV in several ryegrass samples would indicate that the ELISA technique had not been carried out efficiently. However, as mentioned earlier, no such relationship existed between the A_{405} values of RPV and MAV obtained with sap from the accompanying infected oat controls analysed on the same microtitre plates. Therefore, the levels of RPV and MAV detected in ryegrass must have risen at a similar rate to each other due to the multiplication of the virus in the plants.

The unusual strains or combinations of strains detected in the ryegrass plants, e.g. RPV + MAV in plants inoculated by *S. avenae*, and MAV alone in those inoculated

by *R. padi*, could be a result of contamination by stray viruliferous aphids in the glasshouse. Two reasons make the latter seem unlikely:-

(1) No aphids were observed on the plants at any time during their 4 week post-inoculation duration in the glasshouse.

(2) Blocks of 50 perennial ryegrass cv. Talbot plants were interspersed with blocks of 50 control oat cv. Dula. No aphids were noted on these either, and no RPV + MAV, or MAV alone was detected by ELISA in any oat plant.

It is unlikely that stray aphids would feed purely on ryegrass plants and not on the neighbouring oat seedlings. Therefore high levels of RPV and MAV in perennial ryegrass were unlikely to be caused by contamination by viruliferous aphids.

Furthermore, the leaves used for the acquisition feed in each test were from one known-infected plant. Each leaf was cut in half, and all were divided between four vials. Aphids were taken at random off these leaves at the end of the acquisition period and placed on the grass and oat seedlings. Any strains of BYDV acquired by the aphids would certainly be dispersed between all seedlings, both grass and oats, unless some factor in the plant cultivar somehow conferred selection of strains.

Most of the infected oat plants were positive for PAV, which was detected in few grass samples. This

indicated either preferential transmission by aphids, or a difference in the ability of the virus to multiply within the plants.

Strains of BYDV detected in oats were generally in accordance with their vector specificity (Rochow, 1969a): PAV alone, or in combination with MAV transmitted by *R. padi*. The latter could be due to transcapsidation (Rochow, 1982a); PAV + MAV transmitted by *S. avenae*; RPV and RPV + PAV transmitted by *R. padi*.

It was unusual, nevertheless, that *S. avenae* failed to transmit BYDV from triple-infected barley to oats in two of the three experiments. However, *S. avenae* also failed to transmit BYDV from triple-infected oats to oats in one of two previous investigations (see Chapters 4 and 5). Furthermore, there was some concern as to the low rates of transmission by *R. padi* to oats, especially in Experiment 4c. However, the poor transmission by both *R. padi* and *S. avenae* may be attributable to the age of the oat plants at inoculation.

Although the subsequent rate of infection by BYDV did not appear to be dependent on the age of the grass at the time of inoculation, it was a factor with oats. It is acknowledged that there was a limited number of experiments with other variables affecting the percentage transmission of BYDV, such as aphid feeding behaviour. Nevertheless, oat plants inoculated at a younger age were infected more readily than did older plants. Eweida

(1985) found that PAV in oats infected at the 1-2 leaf stage (GS 11-12) reached its highest levels in the leaves sooner than those inoculated at the 4-5 leaf stage (GS 14-15). Endo & Brown (1963) reported that oats inoculated at the 3-leaf stage (GS 13) were damaged most; each delay in inoculation causing successively less damage.

Generally it would appear that the RPV and MAV strains enter perennial ryegrass cv. Talbot more readily than the PAV strain, whereas PAV and MAV are transmitted more readily than RPV to oat cv. Dula.

CHAPTER 7

THE INCIDENCE OF STRAINS OF BYDV DETECTED IN PERENNIAL RYEGRASS CROPS SITUATED IN SOUTH-WEST AND CENTRAL SCOTLAND

7.1 INTRODUCTION

BYDV is a prevalent pathogen of ryegrass pastures in the U.K.; 93% of 112 perennial ryegrass crops sampled in England and Wales were infected (Doodson, 1967), as were 70% of 37 sampled in Scotland (Holmes, 1977).

The main objective of this study was to determine the incidence of BYDV in grass leys in Scotland in 1988-1989, and the strains present. A further objective was to determine the fluctuations in strain incidence throughout the year, and also if strain incidence varied with geographical location.

7.2 MATERIALS AND METHODS

Four areas in south-west and central Scotland were sampled: Ayrshire, Dumfriesshire, Stirlingshire (which included south Perthshire) and Wigtownshire. In each area four fields were sampled at the following locations:-

Ayrshire:	Field 1 (A-1)	Minnybae
	Field 2 (A-2)	Kirkoswald
	Field 3 (A-3)	Monkton
	Field 4 (A-4)	Auchincruive
Dumfriesshire:	Field 1 (D-1)	Carswadda
	Field 2 (D-2)	East Preston
	Field 3 (D-3)	Beechbush
	Field 4 (D-4)	Holestane

Stirlingshire	Field 1 (S-1)	Shearerston
	Field 2 (S-2)	East Third
	Field 3 (S-3)	Tod Hill
	Field 4 (S-4)	Kirkintilloch
Wigtownshire:	Field 1 (W-1)	Portencalzie
	Field 2 (W-2)	Kirranrae
	Field 3 (W-3)	Ardwell Mill
	Field 4 (W-4)	Whitecrook

See Figure 10.

Each field was sampled at 12 monthly intervals from March 1988 to February 1989. This would determine if strain incidence followed a seasonal pattern, or was affected by farming methods. The condition of each field at sampling was noted, i.e. long/short grass conservation/grazing, presence of animals, and where possible crop age.

Five points on a diagonal transect in each field were sampled . Thus a total of 20 samples were collected from each area. At each point, sampling was carried out within a 1 m^2 quadrat. Grass was cut with a pair of scissors from 10 places within the quadrat, and placed in a labelled polythene bag (20.5 x 27.5 cm, QB Packing). A note was taken of where the five sampling points were in each field so that the monthly samples would be taken from approximately the same area to better correlate BYDV fluctuation levels over the year.

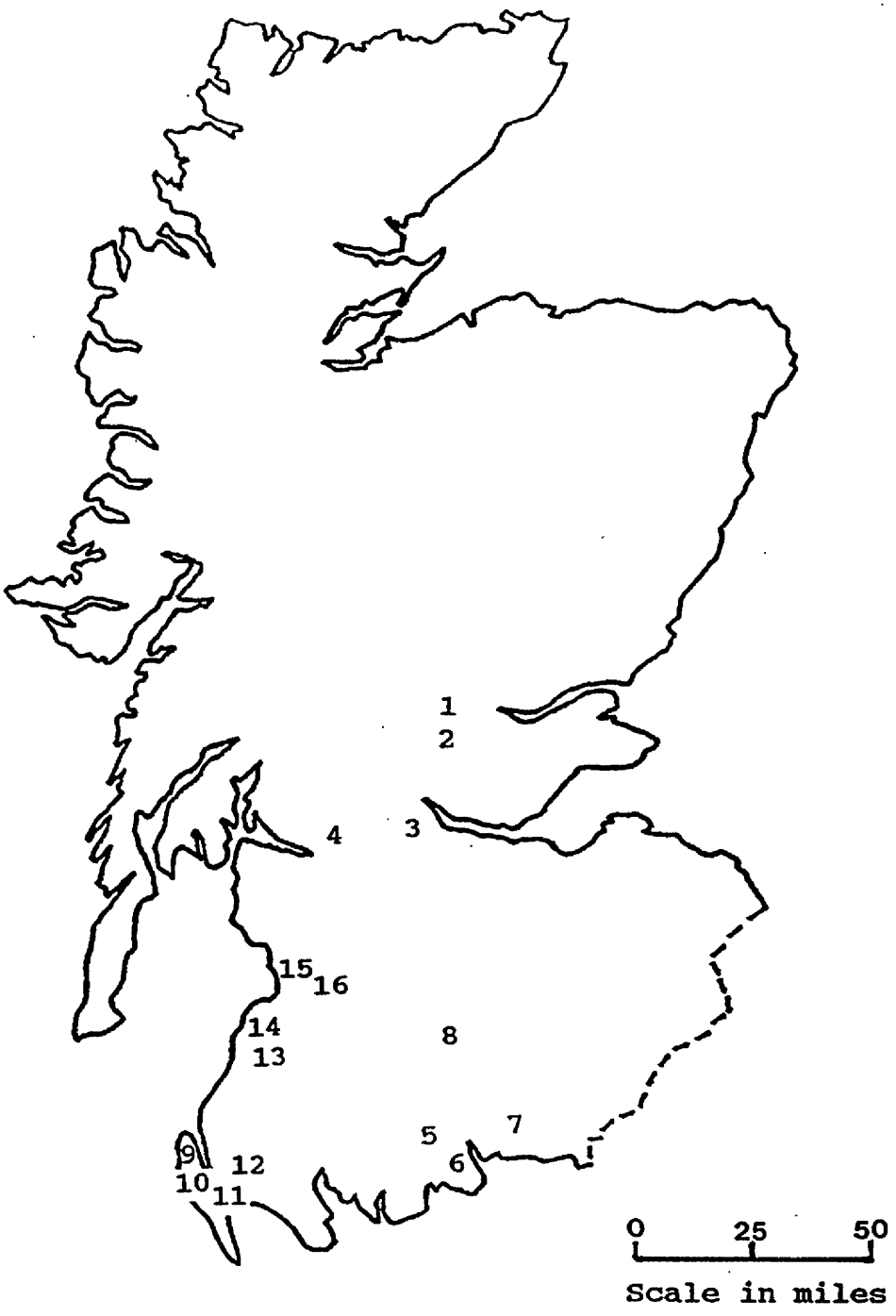


FIGURE 10. Key of ryegrass crops sampled in four areas of Scotland: Stirlingshire (S), Dumfriesshire (D), Wigtownshire (W) and Ayrshire (A):-

1. S-1	5. D-1	9. W-1	13. A-1
2. S-2	6. D-2	10. W-2	14. A-2
3. S-3	7. D-3	11. W-3	15. A-3
4. S-4	8. D-4	12. W-4	16. A-4

The cut grass samples were stored in a deep-freeze at -18°C. Samples were accumulated for 6 months when they were analysed by indirect ELISA. All the samples from one field were analysed together to avoid inter-test variation. The samples collected between March 1988 and August 1988 were tested first, while the second group comprised those samples harvested from September 1988 to February 1989.

7.3 RESULTS

Ryegrass collected from all the regions sampled in Scotland contained the RPV, PAV and MAV strains of BYDV (Table 60).

ELISA analysis of ryegrass samples showed that RPV was most prevalent in Ayrshire (63.3%), and lowest in Dumfriesshire (33.3%, $P<0.05$ - $P<0.01$). Similarly, the incidence of PAV was significantly lower in the latter area (12.5%) compared to in Stirlingshire (29.2%, $P<0.05$), Wigtownshire (32.9%, $P<0.05$), but not Ayrshire (20.4%, $P>0.05$). The number of ryegrass samples shown to contain MAV was greatest in Dumfriesshire (38.7%) and lowest in Ayrshire (24.6%), although the differences were not significant.

The incidence of RPV, PAV and MAV in ryegrass crops between March 1988 and February 1989 is represented for each area in Figures 11-15.

TABLE 60. The percentage detection of BYDV in perennial ryegrass crops sampled in four regions of south-west and central Scotland. Results are expressed as the mean percentage of samples infected with BYDV between March 1988 to February 1989.

	Mean % of BYDV strains detected		
Region (Fields)	RPV	PAV	MAV
Ayrshire (4)	63.3	20.4	24.6
Dumfriesshire (4)	33.3	12.5	38.7
Stirlingshire (4)	48.7	29.2	37.1
Wigtownshire (4)	55.0	32.9	35.8
SED (188 d.f.)	7.7	5.9	7.6

Two-way analysis of variance on the percentage of infected samples indicated that there were significant differences between the incidence of each strain of BYDV detected in Ayrshire ($P<0.01$, $F = 26.26$), Dumfriesshire ($P<0.01$, $F = 13.15$), Stirlingshire ($P<0.05$, $F = 3.33$) and Wigtownshire ($P<0.01$, $F = 5.80$). However, the incidence of BYDV changed significantly over the year in Dumfriesshire ($P<0.01$, $F = 7.25$) and Wigtownshire ($P<0.01$, $F = 4.21$) only.

Generally, RPV was more prevalent than PAV and MAV in Ayrshire ($P<0.001$, Figure 11), Wigtownshire ($P<0.01$, Figure 14), Stirlingshire ($P<0.05$ and $P>0.05$, respectively, Figure 13), but not Dumfriesshire where the incidence of MAV was slightly higher than RPV ($P>0.05$, Figure 12). PAV was significantly lower than RPV and MAV in the latter area ($P<0.001$), but only slightly lower than MAV in the other three areas mentioned above ($P>0.05$).

The overall incidence and seasonal fluctuation of BYDV in fields sampled over the 12 months (Figure 15) showed that the RPV, PAV and MAV strains followed a similar trend throughout the year. The incidence declined between March and July 1988, and began to rise in August before a large increase in the detection of BYDV in September, especially of RPV and MAV. Incidence of PAV continued to increase slightly in October. The percentage detection of all three strains then gradually decreased over the winter months before rising (RPV and PAV) or falling slightly (MAV) in February 1989.

FIGURE 11. Seasonal fluctuation in the detection of strains of BYDV in perennial ryegrass crops sampled in Ayrshire between March 1988 and February 1989. SED ($P>0.05$) for monthly fluctuation in overall strain incidence is 13.06 (108 d.f.). SED ($P<0.01$) for mean strain incidence of RPV, PAV and MAV is 6.53 (108 d.f.).

FIGURE 12. Seasonal fluctuation in the detection of strains of BYDV in perennial ryegrass crops sampled in Dumfriesshire between March 1988 and February 1989. SED ($P<0.01$) for monthly fluctuation in overall strain incidence is 10.81 (108 d.f.). SED ($P<0.01$) for mean strain incidence of RPV, PAV and MAV is 5.40 (108 d.f.).

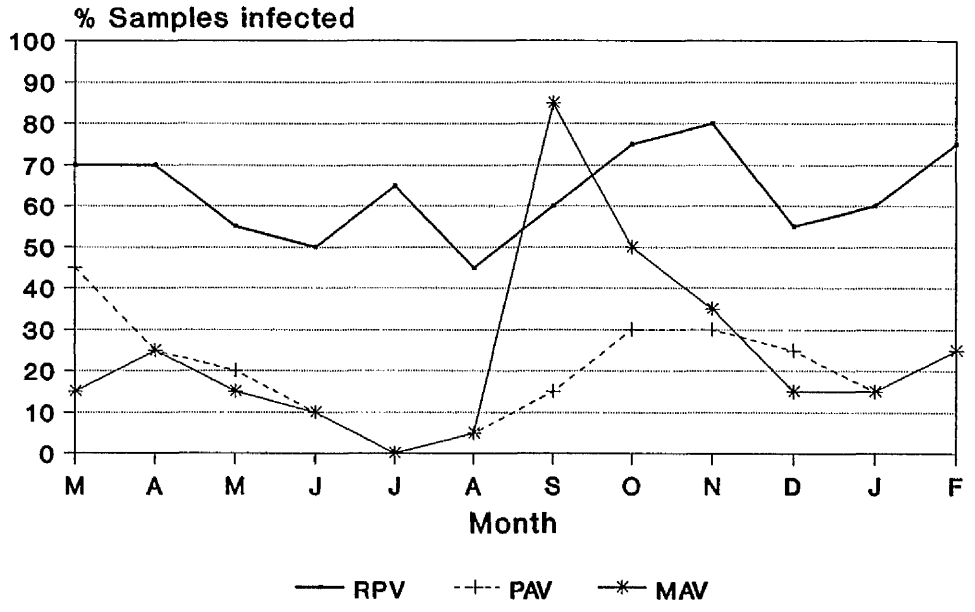


FIGURE 11. Ayrshire.

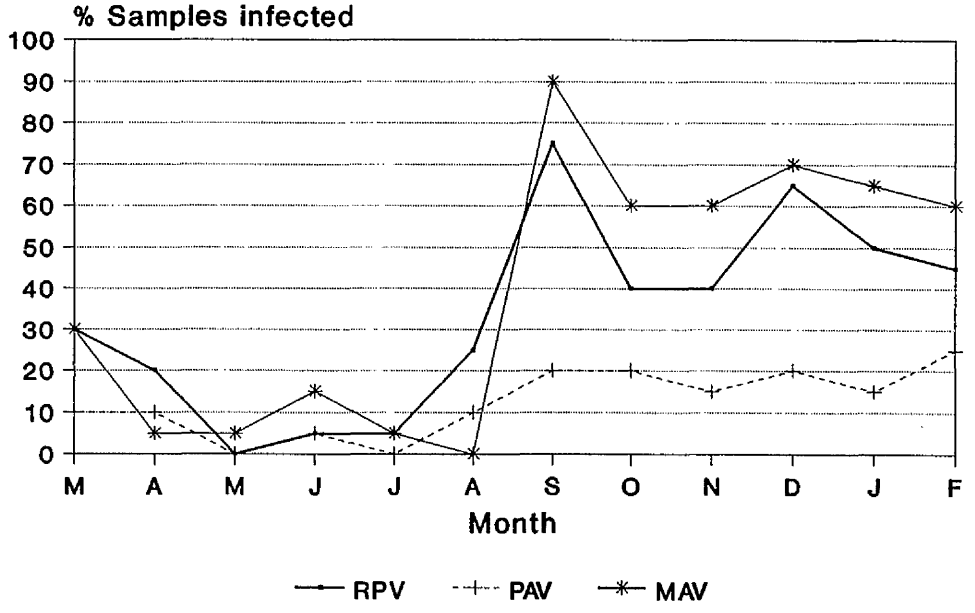


FIGURE 12. Dumfriessshire.

FIGURE 13. Seasonal fluctuation in the detection of strains of BYDV in perennial ryegrass crops sampled in Stirlingshire between March 1988 and February 1989. SED ($P>0.05$) for monthly fluctuation in overall strain incidence is 15.26 (108 d.f.). SED ($P<0.05$) for mean strain incidence of RPV, PAV and MAV is 7.63 (108 d.f.).

FIGURE 14. Seasonal fluctuation in the detection of strains of BYDV in perennial ryegrass crops sampled in Wigtownshire between March 1988 and February 1989. SED ($P<0.01$) for monthly fluctuation in overall strain incidence is 14.09 (108 d.f.). SED ($P<0.01$) for mean strain incidence of RPV, PAV and MAV is 7.05 (108 d.f.).

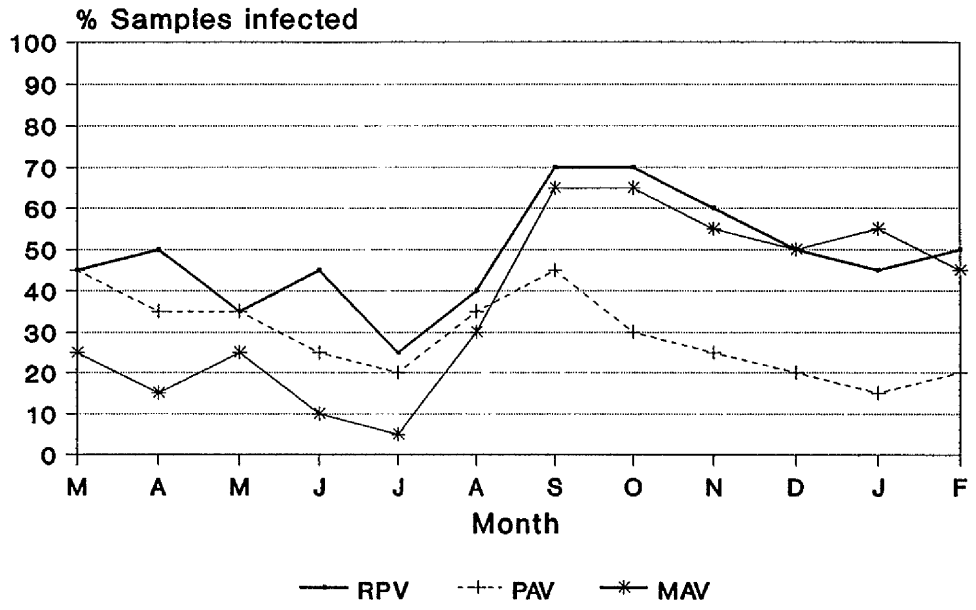


FIGURE 13. Stirlingshire.

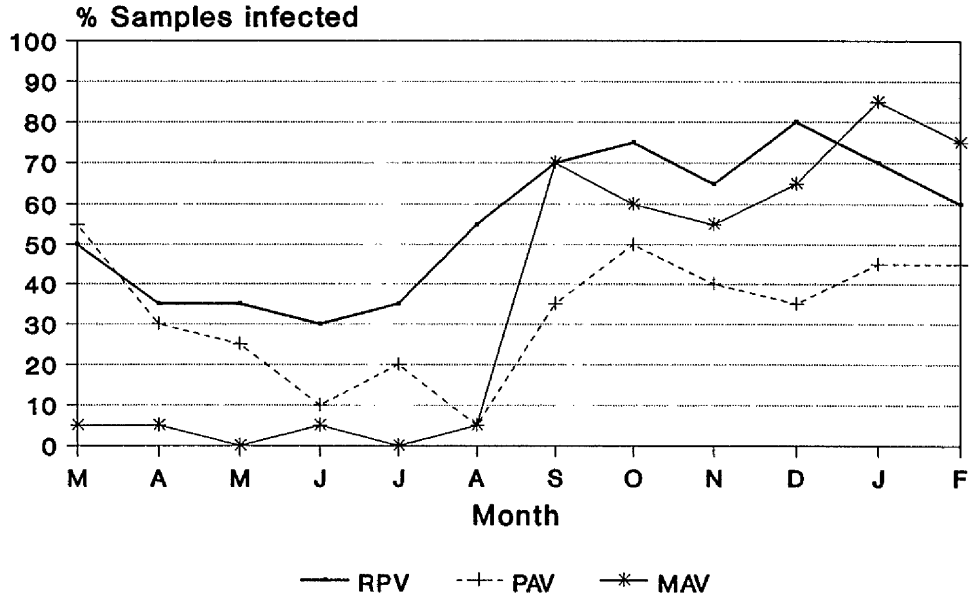


FIGURE 14. Wigtownshire.

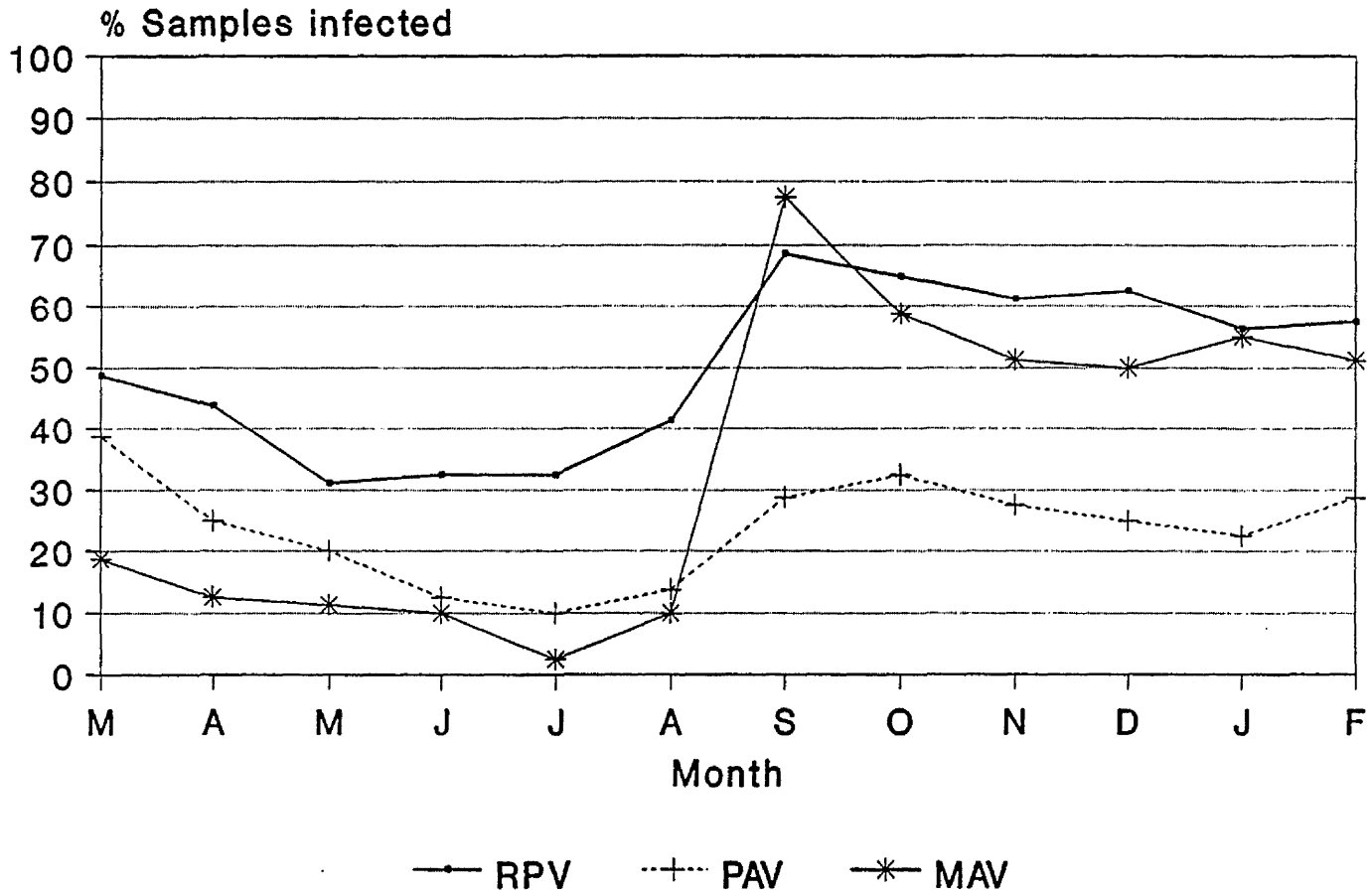


FIGURE 15. Overall seasonal fluctuation in the detection of strains of BYDV in ryegrass crops sampled in south-west and central Scotland between March 1988 and February 1989. SED for monthly fluctuation of RPV ($P<0.05$) is 13.40, of PAV ($P>0.05$) is 10.42 and of MAV ($P<0.01$) is 10.22 (180 d.f.).

Exceptions to the general trend described above were observed in certain areas. The decline in detection of RPV in ryegrass after September was not gradual in Ayrshire, Dumfriesshire, or Wigtownshire, the percentage detection rising again later in the season (November, December and December, respectively). Similarly, the detection of MAV in ryegrass samples increased again in December and January in Dumfriesshire and Wigtownshire, respectively.

As mentioned earlier, the incidence of the three strains differed between the regions. However, although the incidence of the three strains fluctuated in all four areas, the general trend followed by BYDV throughout the year did not appear to alter significantly accross the country, i.e. between south-west (Ayrshire, Dumfriesshire and Wigtownshire) and central (Stirlingshire) Scotland.

The incidence of RPV, PAV and MAV was generally higher in samples analysed from September 1988 to February 1989 than from March to August 1988 in Dumfriesshire ($P<0.001$, $P<0.01$ and $P<0.001$, respectively), Wigtownshire ($P<0.001$, $P<0.01$, and $P<0.001$, respectively), and RPV and MAV only in Stirlingshire ($P<0.01$ and $P<0.001$, respectively), and MAV only in Ayrshire ($P<0.001$). However, by February 1989, the percentages of samples infected were similar to those found one year earlier in March 1988. The exceptions were MAV in Dumfriesshire ($P<0.001$) and Wigtownshire ($P<0.05$) which were higher than in March 1988.

There was no correlation between the absorbance values of RPV and MAV in ryegrass samples ($r = 0.008$, $P>0.05$, Figure 8, Appendix III). This indicated that there was no cross-reaction between the antibodies used to detect MAV (MAFF 2) and those used to detect RPV (MAC 92, see Chapter 6).

Analysis of the incidence of individual strains of BYDV and combinations of strains detected (Table 61) indicated that RPV either alone (22.9%), or in combination with PAV (20.8%) was most prevalent in Ayrshire, compared to the other areas under study, especially Dumfriesshire (4.2%, $P<0.01$ and 6.3%, $P<0.05$, respectively) and Stirlingshire (8.3%, $P<0.05$ and 10.4%, $P>0.05$, respectively). In contrast, the incidence of RPV + MAV was greater in Dumfriesshire (29.2%) than in any other region ($P<0.05$ - $P<0.01$).

A greater proportion of infected samples from each region contained all three strains with the exception of Dumfriesshire (where RPV + MAV was more prevalent). This was most obvious in Stirlingshire where the incidence of RPV + PAV + MAV (45.8%) was higher than the other strain/s detected within that area ($P<0.001$).

Interestingly, PAV alone, or in combination with MAV was detected in Wigtownshire only (6.3% and 4.2%, repectively). As a result, the overall incidence of PAV (1.6%) and PAV + MAV (1.1%) was significantly lower than any other strain, or combination of strains ($P<0.001$),

TABLE 61. The strains and combinations of strains of BYDV detected by ELISA in perennial ryegrass crops sampled in four areas of south-west and central Scotland from March 1988 to February 1989. Results are expressed as the percentage detection ± the standard error of the mean.

Strain of BYDV detected	% Crops infected with strains of BYDV in each area				
	Ayrshire	Dumfriesshire	Stirlingshire	Wigtownshire	Overall
RPV	22.9 ± 6.07	4.2 ± 2.90	8.3 ± 3.98	18.8 ± 5.64	13.6 ± 4.95
PAV	0.0 ± 0.000	0.0 ± 0.00	0.0 ± 0.00	6.3 ± 3.51	1.6 ± 1.81
MAV	8.3 ± 3.98	12.5 ± 4.77	2.1 ± 2.07	0.0 ± 0.00	5.8 ± 3.37
RPV + PAV	20.8 ± 5.86	6.3 ± 3.51	10.4 ± 4.41	12.5 ± 4.77	12.5 ± 4.77
RPV + MAV	8.3 ± 3.98	29.2 ± 6.56	10.4 ± 4.41	10.4 ± 4.41	14.6 ± 5.10
PAV + MAV	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	4.2 ± 2.90	1.1 ± 1.51
RPV + PAV + MAV	29.2 ± 6.56	22.9 ± 6.07	45.8 ± 7.19	39.6 ± 7.06	34.4 ± 6.86

with the exception of MAV (5.8%). RPV alone (13.6%), RPV + PAV (12.5%) and RPV + MAV (14.6%) were detected with a similar frequency, which was lower than RPV + PAV + MAV (34.4%, $P<0.001$).

Tables 62-65 show the percentage detection of BYDV in each individual field from the four areas sampled in south-west and central Scotland. It can be seen from these that little or no BYDV was detected in certain fields, e.g. A-2 (Table 62), S-1 (Table 64), while the percentage of BYDV in others was high, e.g. A-4 (Table 62), D-2 (Table 63), S-2 (Table 64) and W-1 (Table 65).

Generally, as the age of the ryegrass crop increased, the incidence of BYDV also increased (Table 66).

Overall, 93.8% of grass crops sampled in this study contained BYDV (Table 67), i.e. only one field was virtually free of virus (S-1). The RPV and MAV strains were detected in 93.8% of the fields, while PAV was found in 81.2% of the fields sampled throughout the year. The RPV, PAV and MAV strains were detected in 81.3%, 62.5% and 50.0% of the grass crops, respectively, sampled from March 1988 to August 1988. This rose to 93.8%, 75.0% and 93.8%, respectively, from September 1988 to February 1989. The increase in the incidence of RPV, PAV and MAV in ryegrass crops in the second half of the year was significant ($P<0.01$, $P<0.05$ and $P<0.001$, respectively).

TABLE 62. The percentage detection of BYDV in perennial ryegrass samples from four fields in Ayrshire sampled from March 1988 to February 1989.

	Percentage detection of strains of BYDV in each field											
	RPV				PAV				MAV			
Month	A-1	A-2	A-3	A-4	A-1	A-2	A-3	A-4	A-1	A-2	A-3	A-4
March	100	0	80	100	60	0	60	60	40	0	20	0
April	80	0	100	100	20	0	40	40	60	20	20	0
May	80	0	40	100	40	0	0	40	60	0	0	0
June	20	0	80	100	0	0	0	40	20	20	0	0
July	40	20	100	100	0	0	0	0	0	0	0	0
August	0	20	80	80	0	0	0	20	20	0	0	0
September	60	0	80	100	40	0	0	20	80	60	100	100
October	60	40	100	100	60	0	20	40	80	20	0	100
November	80	40	100	100	80	0	0	40	60	0	20	60
December	20	0	100	100	40	0	20	40	60	0	0	0
January	40	0	100	100	40	0	20	0	60	0	0	0
February	60	40	100	100	60	0	20	20	80	0	0	20

20-100 per cent detection represents 1-5 samples positive for BYDV.

TABLE 63. The percentage detection of BYDV in perennial ryegrass samples from four fields in Dumfriesshire sampled from March 1988 to February 1989.

	Percentage detection of strains of BYDV in each field											
	RPV				PAV				MAV			
Month	D-1	D-2	D-3	D-4	D-1	D-2	D-3	D-4	D-1	D-2	D-3	D-4
March	0	80	0	40	0	20	0	20	60	40	0	20
April	0	60	0	20	0	0	0	40	20	0	0	0
May	0	0	0	0	0	0	0	0	0	0	0	20
June	0	20	0	0	0	20	0	0	0	0	0	60
July	0	20	0	0	0	0	0	0	0	0	0	20
August	0	100	0	0	0	40	0	0	0	0	0	0
September	40	100	60	100	20	60	0	0	80	80	100	100
October	20	100	0	40	20	60	0	0	20	60	60	100
November	20	80	20	40	0	60	0	0	20	40	80	100
December	60	100	40	60	0	80	0	0	40	40	100	100
January	40	100	40	20	0	60	0	0	60	60	80	60
February	20	100	40	20	20	80	0	0	60	40	80	60

20-100 per cent detection represents 1-5 samples positive for BYDV.

TABLE 64. The percentage detection of BYDV in perennial ryegrass samples from four fields in Stirlingshire sampled from March 1988 to February 1989.

	Percentage detection of strains of BYDV in each field											
	RPV				PAV				MAV			
Month	S-1	S-2	S-3	S-4	S-1	S-2	S-3	S-4	S-1	S-2	S-3	S-4
March	0	80	40	60	0	100	60	20	0	80	20	0
April	0	80	60	60	0	100	20	20	0	60	0	0
May	0	40	20	80	0	80	0	60	0	80	0	20
June	0	80	20	80	0	80	0	20	0	40	0	0
July	0	40	20	40	0	80	0	0	0	20	0	0
August	0	40	80	40	0	100	20	20	20	80	0	20
September	0	100	100	80	0	100	0	80	0	80	100	80
October	0	100	100	80	0	60	0	60	0	80	100	80
November	0	80	100	60	0	40	20	40	0	60	100	60
December	0	80	100	20	0	40	20	20	0	60	100	40
January	0	80	60	40	0	60	0	0	0	80	80	60
February	0	80	80	40	0	60	0	20	0	80	60	40

20-100 per cent detection represents 1-5 samples positive for BYDV.

TABLE 65. The percentage detection of BYDV in perennial ryegrass samples from four fields in Wigtownshire sampled from March 1988 to February 1989.

	Percentage detection of strains of BYDV in each field											
	RPV				PAV				MAV			
Month	W-1	W-2	W-3	W-4	W-1	W-2	W-3	W-4	W-1	W-2	W-3	W-4
March	100	20	20	60	80	0	80	60	0	0	0	20
April	100	0	0	40	100	0	20	0	20	0	0	0
May	100	0	0	40	60	0	40	0	0	0	0	0
June	100	0	0	20	0	0	20	20	20	0	0	0
July	100	20	0	20	80	0	0	0	0	0	0	0
August	100	80	20	20	20	0	0	0	20	0	0	0
September	100	60	20	100	40	0	80	20	100	40	100	40
October	100	100	20	80	100	0	60	40	100	40	100	0
November	100	80	0	80	60	0	80	20	80	0	100	40
December	100	80	40	100	60	20	60	0	80	40	100	40
January	100	80	20	80	80	0	60	40	80	100	100	60
February	100	60	0	80	20	20	60	80	20	100	100	80

20-100 per cent detection represents 1-5 samples positive for BYDV.

TABLE 66. The percentage detection of BYDV in perennial ryegrass crops of varying ages, sampled from March 1988 to February 1989. Results are expressed as the overall percentage detection for all three strains of BYDV ± the standard error of the mean.

Field	Age of field (years)	% BYDV detected
S-1	2	1.7 ± 1.67
A-2	2	21.7 ± 5.32
D-1	3	31.7 ± 6.01
D-4	4	58.3 ± 6.37
S-3	5	68.3 ± 6.01
A-3	6	90.0 ± 3.87
S-4	8	60.0 ± 6.32
A-4	8	98.3 ± 1.67
W-1	15	100.0 ± 0.00

TABLE 67. Overall percentage detection of BYDV in perennial ryegrass crops sampled in south-west and central Scotland between March 1988 to February 1989.

	Mean % of BYDV strains detected		
Month	RPV	PAV	MAV
March 1988-February 1989	93.8	81.2	93.8
March 1988-August 1988*	81.3	62.5	50.0
September 1988-February 1989+	93.8	75.0	93.8
SED of * and + (96 d.f.)	4.0	6.0	5.0

7.4 DISCUSSION

The high percentage of BYDV-infected grass samples detected in this field survey (93.8%) agrees with what has been reported previously by other researchers elsewhere in Britain. Doodson (1967) in 1966 recovered the virus from 93% of S.24 perennial ryegrass fields in England and Wales. In 1975, 73% of the ryegrass pastures were infected in Northern Ireland rising to 86% in 1976 (Cooper, 1977). In the west of Scotland in 1976, 70% of the ryegrass fields examined were infected (Holmes, 1977). This rose to 100% infection in 13 farm crops surveyed in the same area in 1983 (Holmes, 1985).

As only five samples were taken per field in the present survey, the high incidence of detection indicates that large areas of the fields must be infected.

There appeared to be a definite seasonal fluctuation in the detection of BYDV, with a large increase in September in the incidence of RPV, PAV and MAV detected. The occurrence of BYDV then gradually declined over the winter months, returning to the levels found in the previous spring.

Generally, the percentage detection of the MAV strain was higher in the second group of samples (collected from September 1988 to February 1989), as compared to the first group of samples (collected from March to August 1988). It was originally thought to be attributable to a factor in the ELISA procedure as the two groups of samples were

analysed at different times. However, analysis of the results suggests that the difference in detection of MAV between the groups was genuine for the following reasons:-

(1) The absorbance values (A_{405}) of the samples positive for MAV were significantly higher than those of the healthy control samples.

(2) The numbers of samples infected with MAV had already begun to rise in August in three out of four areas. Samples collected in August were analysed earlier than those collected from September onwards.

(3) In two of four areas sampled, the incidence of MAV in February had dropped to a percentage similar to that found in March 1988.

(4) The incidence of RPV also rose considerably.

(5) The percentage detection in the second half of the year fluctuated as did those in the first half. If a high incidence of virus obtained in September was due to a factor in the ELISA procedure e.g. contamination, it is likely that all samples throughout that test would appear positive. However, this was not so, indicating that fluctuations were seasonal.

Considering the above points, it can be assumed that the detection of MAV, and also of RPV and PAV was correct. The low positive absorbance values (A_{405}) that were usually obtained for MAV indicated this strain, although widespread, was present at a low frequency throughout the ryegrass crops. This is consistent with the fact that a

serious BYDV problem in winter cereals was recognised in spring 1989, of which the majority of the infection was caused by the MAV strain (Holmes *et al.*, 1990). Few aphids were found in autumn 1988, but between October and November 1988, and especially by spring 1989, the predominant aphid species in most of the cereal fields was *S. avenae* (Holmes *et al.*, 1990). Biological testing of aphids collected from these crops revealed that 21.6% of the *S. avenae* carried BYDV, 80.0% of which was the MAV strain. Although *R. padi* was dominant in only 9.3% of the winter cereal fields surveyed, the proportion infective (50.9%) was greater than for *S. avenae*. A mixture of the RPV and PAV strains was detected in 63.0% of infective *R. padi* (Holmes *et al.*, 1990).

Despite the relatively low infectivity of the *S. avenae*, their population was so high (dominant in 67.4% of the autumn-sown winter barley fields surveyed), that the MAV strain could have been widely disseminated. Indeed, winter barley crops growing in the same regions as the grass fields under investigation had a high incidence of MAV, but little or no PAV or RPV, except in Wigtownshire (Holmes *et al.*, 1990).

It could be assumed that the MAV strain was transmitted by *S. avenae* from the grass to the winter barley crops, but if this occurred, then how did the sudden increase in the incidence of MAV in grass originate in autumn 1988?

Work carried out in 1983, 1985 and 1986 identified widespread reservoirs of BYDV in perennial ryegrass fields in the west of Scotland. The PAV strain predominated, with a relatively low incidence of MAV in 1983 (Holmes, 1985). By 1985 an antiserum to the RPV strain had been obtained which enabled RPV-like isolates to be detected for the first time. Further studies in 1985 and 1986 demonstrated that the RPV strain was more widespread than the PAV strain, while little MAV was detected (Holmes, 1991). These findings correspond with the percentage detection of BYDV in the grass crops during the spring and summer of 1988.

The increase in the incidence of all three BYDV strains, especially of RPV and MAV in August to September, could result from infective aphids migrating from cereal fields to grass crops in the interval between harvest and the emergence of autumn-sown cereals in September to November. Aphids migrating from barley to grass during midsummer (A'Brook & Dewar, 1980; Wiktelius, 1987; Hand, 1989) may introduce BYDV which would take approximately one month to be detected by ELISA. This would give a sudden surge in the detection of virus in September.

However, very little BYDV was reported in winter cereal crops in the spring of 1988, while 1988 on the whole was regarded as a low risk year for primary spread of BYDV by *R. padi*, as calculated by the Infectivity Index (II) at Auchincruive (Holmes *et al.*, 1989). Aphids entering grass would have to become infected from another

source. As few aphids were found in grass crops during the summer months, especially where the grass was kept cut and/or grazed (Holmes *et al.*, 1989), it is unlikely that *S. avenae* would introduce BYDV to the grasslands either.

S. avenae and *R. padi* can overwinter anholocyclically on Gramineae (Hand, 1989), especially if winters are mild (Gair, 1953; Hand, 1980). *S. avenae* prefers barley to mature grasses (Orlob, 1961; Kieckhefer & Lunden, 1983), while *R. padi* prefers grass to cereals in autumn (Leather & Dixon, 1982; Wiktelius, 1987; Hand, 1989). This agrees with the findings from grass and cereal crops in Scotland in autumn 1988, where no *S. avenae* were found in grass fields after August, but *R. padi* were numerous.

Field examinations of grass swards between January and April 1989 yielded virtually no aphids. However, both aphid species could still be found in winter cereals by January, probably as a result of the exceptionally mild winter of 1988/89 (Holmes *et al.*, 1989). Therefore, it would appear that the outbreak of BYDV, especially of MAV in cereals in spring 1989 did not come from the grasslands, despite the high incidence of virus existing within.

Few *S. avenae* were found in grass crops after August when the incidence of MAV became high. Therefore, the spring infection of winter cereals is more likely to have come from *S. avenae* overwintering in the cereals. Although some *R. padi* were found in grass crops in late

autumn and also in cereals over winter, very little RPV or PAV was found in cereals in the spring, except in Wigtownshire. It would appear that *R. padi* was transmitting neither the RPV, nor the PAV strains from grass leys to winter cereal crops in at least three of the four areas studied.

The MAV, and especially PAV strains of BYDV were found mainly in combination with RPV. The detection of these strain combinations in grass samples would indicate a predominance in *R. padi* activity rather than *S. avenae*. This also agrees with the fact that more *R. padi* were found in grass swards, particularly in the autumn, compared to *S. avenae* (Holmes *et al.*, 1989).

Although biological testing of *R. padi* collected from winter barley crops in spring 1989 did not detect any MAV in combination with RPV, or PAV (Holmes *et al.*, 1990), these results may not reflect the infectivity of those *R. padi* present in the grasslands. The combination of genomic masking (Rochow, 1970a, 1972, 1973), together with the fact that *R. padi* feeding on MAV-infected leaves for a long time are more likely to transmit MAV (Rochow, 1973), could introduce sufficient MAV into grass crops without much *S. avenae* activity. Although 1988 was considered a low risk year for primary spread of BYDV by *R. padi*, the arbitrary spray threshold value of 50 was exceeded in late October 1988 (Holmes *et al.*, 1989), indicating that infective *R. padi* did exist.

Few *Rhopalosiphum insertum* (Walker) were found in grass crops, while *M. dirhodum* predominated in only one cereal field. However, 25.0% of the latter were found to contain MAV when analysed by amplified ELISA in spring 1989 (Holmes *et al.*, 1990). Therefore, *M. dirhodum* and *S. avenae* together may account for the high incidence of MAV in cereal crops. As so few of these species were detected in grass crops, again it is unlikely that the grass swards acted as a source of infection to the cereals.

Alternatively, the sudden increase in the incidence of BYDV detected in pasture crops in September was not due to the introduction of more virus from infective aphids, but to the increased multiplication of virus within ryegrass plants already infected, thus facilitating increased detection of BYDV. Subsequently, the large incidence of BYDV in the pastures could have been maintained over the winter months as in many fields the grass grew longer than it had during the summer (grass was no longer being cut for silage). This could possibly maintain high concentrations of virus as it multiplied with the growth of the grass, amounts gradually declining as the growth rate slowed over winter. The incidence of virus may have remained low until August due to the ryegrass crops being cut for silage and the activity of animals grazing.

The incidence of BYDV varied between fields within districts and also with the geographical location of pastures. This situation was also noted by Lindsten &

Gerhardson (1969) in Sweden and by Latch (1977) in New Zealand. Strain incidence also varied between the geographical locations indicating a prevalence of particular aphid species in certain areas over the years.

Virus incidence has been found to be higher at sites where grass was grown on heavy soil as opposed to light soil (Guy, 1988). However, the incidence of BYDV at the four geographical locations in south-west and central Scotland altered throughout the year, indicating that a factor other than soil was affecting the occurrence of BYDV, e.g. seasonal factors.

Some of the variation in the detection of BYDV in ryegrass within a field are due to sampling technique. Despite the same area of the field being sampled at each visit, the same individual plants would not be sampled every time. This in itself leads to variation.

Older established grass crops generally had a higher incidence of BYDV than younger ones. Other workers reported a similar phenomenon in other countries (Latch, 1977; Guy *et al.*, 1986; Henry, 1988). The incidence of BYDV infection did vary slightly between pastures of a similar age. The variability could have been incurred by differences in the species, or number of aphids present in each area. Variability in strain incidence could also be due to the management and quality of the grasslands. Holmes (1985) speculated that frequent grazing would tend to favour *R. padi* which prefers the base of plants

(George, 1978), while infrequent removal of top growth would allow colonization of *S. avenae* as they tend to prefer the upper part of plants (Smith *et al.*, 1984).

Latch (1977) found volunteer wheat seedlings in a 1-year-old sward with a high incidence of BYDV (47%). A neighbouring field of the same age with only a 1% level of infection contained no volunteer seedlings. He thought it likely that the wheat seedlings became infected with BYDV during the autumn aphid flight, and in turn became a source of infection for the ryegrass. No volunteer seedlings were observed in the 16 grass crops involved in the present study. They were all established swards where volunteer seedlings if present would have disappeared, even in the 2-year-old leys. However, initially they may have served as a source of infection.

Generally, it would appear that the incidence of BYDV is widespread in south-west and central Scotland, no doubt increasing in area over time as the aphids disseminate the disease. Although perennial ryegrass is not adversely affected by BYDV (Panayotou, 1985), cereals can be. It is therefore important to establish from what source they are becoming infected.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

8.1 GENERAL DISCUSSION

The research presented and discussed in the previous chapters highlights the degree of variability in transmission rates of BYDV by the two aphid species to the various plant cultivars investigated. This underlines the difficulty of working with BYDV. The results are dependent on several factors such as the efficiency of the technique used to detect the virus, the effect of temperature on the development of foliar symptoms of infection (Rochow, 1969a; Yount & Carroll, 1983), the cultivar of test plant, and the cultivar and age of the known-infected source leaves used for the acquisition feed (Foxe & Rochow, 1975). Ultimately, however, the acquisition of the virus is dependent on the ability of the aphid to probe the phloem cells that contain the virus, irrespective of the overall virus content of the leaf (Pereira *et al.*, 1989).

Data collated throughout the present research indicates that *R. padi* is generally a more efficient vector of BYDV than *S. avenae*. This is consistent with findings by other workers studying transmission of BYDV between cereals (Rochow, 1969a; Osler *et al.*, 1990).

Investigations on the transfer of virus to perennial ryegrass from cereals infected with one strain of BYDV, indicates that the RPV strain is introduced to grass plants more readily by *R. padi* than the PAV strain. Similar results have been obtained in other laboratories

(Henry, 1988).

However, no studies could be found on the transmission rates by *R. padi* and *S. avenae* of the RPV, PAV and MAV strains when present in a mixture, from either cereal or grass source leaves to grass test plants. In the present research, both vectors were found to transmit BYDV similarly from mixed infections to grass. As with the single strain experiments, RPV was transmitted more readily than PAV, which was never transmitted alone and rarely in combination with RPV or MAV to ryegrass. By contrast, PAV was transmitted to the oat cv. Dula controls, both alone or with either of the other two strains. Interestingly, both *R. padi* and *S. avenae* often transmitted the RPV strain together with MAV to ryegrass plants (but not to oats), from source leaves containing three strains of BYDV.

It was also interesting to note that RPV + MAV was detected in 14.6% of ryegrass samples from crops in south-west and central Scotland. The incidence of PAV was low, unless in combination with the other two strains. This may indicate that RPV and MAV replicate in grass more readily than PAV.

Henry (1988) studied the multiplication rates of the French RPV (R568), PAV (PR1) and MAV (F148) isolates in grasses, the former in perennial ryegrass. Unfortunately, the ryegrass was not infected by either the PAV or MAV isolates, but both were transmitted to fescue by *R. padi*

and *S. avenae*, respectively. Comparing the multiplication rate of RPV in perennial ryegrass with that of PAV and MAV in fescue, the RPV and MAV isolates multiplied similarly until 18 days, after which MAV declined. RPV continued to multiply at a high rate. The PAV isolate multiplied at a rate slower than both RPV and MAV (Henry, 1988). If similar rates of multiplication applied to PAV and MAV in perennial ryegrass, it may partially explain the extensive detection of RPV, and perhaps MAV, compared to PAV, in the ryegrass test plants in the glasshouse.

The prevalent detection of MAV together with RPV in the grass test samples grown in the glasshouse was interpreted with caution. The transmission of these two strains together by *R. padi* from triple-infected source leaves to ryegrass plants could have been a result of transcapsidation (Rochow, 1970a, 1972, 1973; Rochow & Gill, 1978; Gildow & Rochow, 1980a; Rochow, 1982a). However, the transmission of RPV + MAV to ryegrass by *S. avenae* is unusual as the vector was rarely found to transmit these two strains together to cereals, both in this study, and in other laboratories (Rochow & Gill, 1978). Interestingly, very few *R. padi* (5.7%) transmitted RPV + MAV in one of the three RPV + PAV + MAV grass studies, while the *S. avenae* from this experiment did not transmit the RPV + MAV combination. Therefore, the incidence of this combination in the two remaining experiments, by both aphid species, would appear inconsistent. However, as dicussed earlier in Chapter 6,

the poor transmission in this experiment (Experiment 4a) may be due to the conditions prevailing during the acquisition feed.

Of further interest was the transmission of the MAV strain alone by *R. padi* from cereals to grass. This transmission was unusual as *R. padi* is considered a non-vector of MAV alone (Rochow, 1969a). Furthermore, *R. padi* did not transmit MAV alone from triple-infected grass, or cereal leaves to any of the cereal test plants studied in the present research.

As no research to date has been published on ease of transmission from triple-infected plants, it is not known whether PAV would have any effect on the transmission of RPV and MAV in ryegrass. It is unlikely that PAV would cause RPV to be transmitted together with MAV by *S. avenae*. Indeed, it is unusual that PAV rather than RPV was not transmitted by *S. avenae*, as it is an efficient vector of the former (Rochow, 1969a). However, in the present study, PAV was detected less frequently than RPV and MAV in ryegrass.

No MAV, or RPV + MAV was detected in the oat controls accompanying any of the grass test plants. As the grass and oat test samples were analysed simultaneously by ELISA, the detection of the incidence of strains of BYDV in grass would appear to be genuine. However, the explanation for the anomalous transmission of MAV alone by *R. padi* and RPV + MAV by *S. avenae* remains unclear.

Differences in transmission rates or the incidence of strains could be due to different clones of each aphid species used. However, the stock colonies of non-viruliferous *R. padi* and *S. avenae* were renewed every week or every two weeks, respectively, from a small number of nymphs. Therefore, very few clones of each aphid species were present in the colony.

The correlation between the absorbance values of RPV and MAV obtained with sap from perennial ryegrass test plants was of interest. There should be no cross-reaction between the monoclonal antibodies and the virus strains as RPV and MAV are serologically unrelated (Aapola & Rochow, 1971). Nevertheless, the relationship between the absorbance values of the two strains was positive, either as a result of the incidence of both strains rising together, or due to a cross-reaction between antibody and virus. As expected, very little correlation existed between the absorbance values (A_{405}) of RPV and MAV in the grass field samples, or in any experiments investigating the transmission of RPV and MAV together (often in the presence of PAV) to cereals (Appendix III). This proves that the correlation was not due to a cross-reaction but due to another factor, such as the genuine presence of virus in the samples.

As no work has previously been published on the transmission of BYDV from triple-infected leaves, the results of such studies in the present research are of particular interest.

The strains, or combinations of strains transmitted to cereals by *R. padi* and *S. avenae* from such source leaves, agreed with those reported by other workers from leaves infected with only one or two strains of BYDV, although at lower levels (Rochow, 1969a; Rochow & Gill, 1978; Gildow & Rochow, 1980a; Rochow, 1982a). The exceptions to the above cited works were, obviously, the transmission of RPV + PAV + MAV, and interestingly RPV + MAV, which was never transmitted from triple-infected leaves to cereals. The latter was unexpected as RPV + MAV was transmitted by *R. padi* from source leaves infected with both strains to oat cv. Pennalt in the present study. A possible explanation is that the PAV strain somehow inhibits or influences the genomic masking of MAV with the RPV coat protein. Alternatively, as PAV and MAV are serologically related and RPV is not (Aapola & Rochow, 1971), perhaps, when present in a mixture, preferential transcapsidation between PAV and MAV occurs rather than between RPV and MAV. However, the above points would not explain the prevalent transmission of RPV + MAV by *R. padi* and *S. avenae* to ryegrass test plants.

The strain mixtures in the source leaves did not appear to affect the rate of transmission of individual strains from the mixture by either vector species.

As mentioned previously, glasshouse experiments carried out in the preceeding chapters, indicated that the RPV and PAV strains, were transmitted less readily from grass to cereal, than from cereal to cereal. This was

particularly true for the RPV strain. In contrast, the RPV strain was transmitted more efficiently than the PAV strain from cereal source leaves to ryegrass test plants.

Studies of ryegrass crops throughout west and central Scotland showed that the RPV strain occurred alone more frquently than PAV. However, many grass leys sampled contained a mixture of all three strains of BYDV. Studies investigating transmission of BYDV strains from triple-infected source leaves indicated that predominantly PAV, or PAV + MAV was transmitted from grass or cereal leaves to winter barley and oats. No RPV + MAV was ever transmitted. However, this combination was detected in 44.3% of grass plants inoculated by *R. padi* and *S. avenae* previously fed on triple-infected cereal leaves. No PAV, or PAV + MAV was detected in any grass plant.

If the transmission of RPV and PAV from ryegrass to winter cereals and oats is indeed poor, as in the previously-discussed experiments, then the large reservoir of RPV and PAV in grass leys would not be as great a threat to cereal crops as it appears. The outbreak of MAV reported in cereals in spring 1989 (Holmes *et al.*, 1990) could have come from the ryegrass crops. However, results from glasshouse experiments indicated that no MAV alone was transmitted from triple-infected grass leaves to winter barley, or oats. No glasshouse studies were carried out on the transmission of the MAV strain alone from grass to cereals. Therefore, an analogy to the field situation cannot be made.

The threat of BYDV to cereal crops, whether from grass swards or elsewhere, is ultimately dependent on the aphid population and species present in the area. Nevertheless, it would still appear that the strains, or combinations of strains which were present in the grass pastures were not the principal source of inoculum to the cereal crops. This speculation is based on the results of glasshouse experiments obtained throughout the present research. It is acknowledged, however, that all experiments investigating the transmission of BYDV from triple-infected cereal or grass source leaves to either cultivar, need to be repeated to verify the preferential selection of strains by aphid vectors. Although a number of experiments in the present research were not repeated, those that were for each particular plant cultivar did produce similar results.

The glasshouse experiments were carried out in a controlled environment with cloned aphids transmitting one isolate each of the RPV, PAV and MAV strains. In the field, the situation is very different. Many different isolates exist of each strain of BYDV, some of which have been found in other countries not to be strain-specific with their designated vector. Sward & Lister (1988) found that the MAV-like isolates in grasses and cereals sampled in Australia had *R. padi* as an efficient vector species. The type isolate of RPV, described from New York, is transmitted specifically by *R. padi* (Rochow, 1970a), whereas a variant strain from California was reported

recently to be transmitted by two additional aphid species, *S. avenae* and *S. graminum* (Creamer & Falk, 1989).

The introduction of BYDV to grass test plants in the glasshouse showed that, in addition to perennial ryegrass remaining symptomless, infection by BYDV even at a young growth stage does not adversely affect growth. Indeed, in the field, BYDV-infected plants produce extra tillers (Catherall, 1966; Catherall & Wilkins, 1977). The initial aggressiveness and persistence of diseased ryegrass plants may retard, or inhibit compensatory growth from healthy individuals and, under certain managements, the diseased plants may dominate the sward (Catherall, 1966). Thus producing a permanent and usually symptomless reservoir of BYDV.

Between 1988 and 1989, few aphids were observed in the ryegrass fields sampled compared to the numbers noted in cereal crops throughout Scotland (Holmes *et al.*, 1989). Orlob *et al.* (1961) considered that large populations of cereal aphids would be necessary to maintain BYD as a relatively small segment of the vector population carries the virus. However, Fargette *et al.* (1982) speculated that virus transfer from perennial ryegrass to cereals need not be efficient for it to be significant in the epidemiology of BYDV.

Results from the glasshouse experiments (especially transmission rates of *R. padi*) indicate that only a small number of aphids would be required to cause substantial

infection in grass/cereal crops within several days. However, several factors can affect the feeding behaviour of the vector and the subsequent level of infection in the field.

Each aphid species prefers to feed on, and subsequently reproduce on different host plants at various growth stages (Williams, 1987; Kieckhefer & Gellner, 1988; Johnstone *et al.*, 1990 among others). In addition, the development time, longevity, and reproductive capacity of aphids are altered by feeding on BYDV-infected host plants compared to on uninfected plants (Kieckhefer *et al.*, 1976; Araya & Foster, 1987; Fereres *et al.*, 1989). Often the reproductive capacity is increased on BYDV-infected plants, thus facilitating disease spread. Moreover, Gildow (1980) found that a higher percentage of winged progeny were produced on BYDV-infected oats than on uninfected plants. This would cause the virus to be spread even further afield.

All these parameters together with environmental factors such as temperature and wind would affect the introduction and dissemination of BYDV within a crop to amounts, no doubt, very different from those indicated by the glasshouse experiments.

Therefore, although much work in this thesis suggests that grass swards were not the primary source of infection for winter cereals, or vice versa, they are nevertheless a permanent reservoir of BYDV. Changes in environmental

conditions, virus isolates, or aphid genotypes over the years may change this situation.

There have been many different views as to whether grass is a source of infection to cereal crops. Several authors agree that perennial ryegrass swards are a large reservoir of BYDV which constitute a serious potential hazard to cereal crops (Doodson, 1967; Lindsten & Gerhardson, 1969; Smith *et al.*, 1984; Holmes, 1985; Kendall, 1986). However, several studies have shown that the predominating strain of BYDV in grass species differs from that causing the epidemic in the nearby cereal crops (Gill, 1970; Rochow & Muller, 1976; Plumb, 1977; Rochow, 1979b; Fargette *et al.*, 1982; Paliwal 1982b; Henry, 1988). In such cases, winter cereals are thought to be an important source of BYDV infection to spring grains (Rochow & Muller, 1976). In areas where maize is cultivated, it is thought to be a source of infection to autumn-sown crops (Stoner, 1977; Brown *et al.*, 1984; Henry *et al.*, 1989).

Other sources of infection to cereals are thought to be caused by strong winds carrying viruliferous aphids over long distances from BYDV-infected areas (Gill, 1970; Paliwal, 1982b). A combination of all these factors is most likely to cause infection in both cereal and grass crops as was suggested by Fargette *et al.* (1982).

8.2 CONCLUSIONS AND CONSIDERATIONS FOR FUTURE RESEARCH

The results of the current research indicate that perennial ryegrass swards may not constitute the main source of primary BYDV infection to cereal crops. Nevertheless, they do act as a perennial source of BYDV and aphids will undoubtedly introduce some infection from these swards to cereals. However, the threat to cereals from other cereals appears to be of greater importance, especially in iniating secondary spread.

Developing a forecasting system for BYDV is difficult due to the degree of variation found on the effect and incidence of BYDV in grasses and cereals. The efficiency of transmission between plant cultivars by each vector species can give an indication as to what to expect in the field. However, these would have to be modified according to the different parameters existing in the area concerned, e.g. plant cultivar, age of plants, vector species, aphid numbers, temperature, wind, presence of natural enemies of the aphid species, and seasonal fluctuation of the virus incidence in ryegrass crops.

Although the influence of the above factors may decrease or increase the incidence of BYDV in plants, the occurrence of significant amounts of simultaneous strain transmission from double-, or triple-infected plant sources heightens the threat that vectors can have on cereal crops. The possible introduction of BYDV strains

by non-vectors to crops through transcapsidation further complicates the production of a forecasting system.

The continued use of broad spectrum pesticides to control aphid populations is undesirable (Kendall, 1985). Not only are the environmental hazards potentially very great, but the frequent use of pesticides is likely to increase the risk of vector resistance. In addition, with broad spectrum activity there is the likelihood of undesirable side-effects on non-target organisms (Kendall, 1985).

Any forecasting system adopted should be combined with breeding for host plant resistance. Results from the present research indicate that generally the transmission of BYDV between grasses and cereals is less successful than between cereals (with the exception of the transmission of BYDV from triple-infected barley to ryegrass, which was not satisfactorily explained). This indicates that perhaps the aphid vectors found some factor in grass a barrier to the transmission of the virus. Whatever factor was responsible, such as the leaf surface of the grass, or a gene within the grass that conferred resistance to replication of the virus, incorporation of such a vector/virus-resistant characteristic into an agronomically adapted cereal cultivar would be an economic and environmentally viable solution. Indeed, such studies are already under progress. Germplasm of a line called Zhong 4, produced by crossing a grass, *Thinopyrum intermedium* Host, with two Chinese wheats was found to

inhibit the replication of BYDV (Guang-he *et al.*, 1990).

However, before such a characteristic is available in commercially grown cereal cultivars, many aspects of the dynamics and epidemiology of BYDV remain to be studied and collated. Evidently, it is important to integrate control strategies such as the proper timing of autumn-applied aphicides, crop rotation of BYDV-host plants with non-hosts, resistance or tolerance factors bred into commercially grown cereal cultivars, and sowing crops in relation to vector flight activity. Further studies on the epidemiology of BYDV would undoubtedly improve such strategies.

Future research should concentrate on obtaining a more detailed study of the strains of BYDV present in ryegrass and neighbouring cereal crops throughout Scotland. This may be achieved by sampling the same range of fields for a set number of years. A large number of samples should be taken per field to give a more accurate percentage detection. In addition, sampling error may be reduced by permanently marking the areas in each field. The details gained from such an investigation may indicate whether the same or different strains predominate in each cultivation.

Also worthy of further research, and of paramount importance, is to ascertain the source of primary infection to both cereal and grass crops. Sampling of field margins, weed grasses, volunteer seedlings and

ploughed grass in cereal crops would widen the knowledge of which strains were present and their incidence in each area. Such information combined with aphid flight activity, both locally and from distant sources, wind direction, population dynamics, aphid strain specificities, rates of transmission, and extent of aphid overwintering could lead to an efficient alternative forecasting system for BYDV in cereals.

Detailed glasshouse research on the rates of transmission by different aphid vectors to the varieties of commercially grown cereals and grasses is important in order to complement the field research. Studies combining aphid transmission tests with routine serological analysis of aphids either by electron microscopy or amplified ELISA, would determine what proportion of aphids could acquire the virus, but were unable to transmit it. Reasons (outwith strain specificity) for lack of transmission may then be investigated, and if possible exploited to break the disease cycle of BYDV. Serological testing of aphids may also determine whether the transmission of strains of BYDV by non-vectors is genuine, or simply due to contamination from stray viruliferous aphids, or a cross-reaction between monoclonal antibodies and virus strains.

It is important in future research to study triple-infected plants as a large proportion of ryegrass swards are infected with all three strains in nature. This is an area which has been neglected, and results from such

research may contribute to the understanding of the epidemiology of BYDV.

This thesis describes the extent of BYDV infection in south-west and central Scotland, and the complexity of devising a forecasting system for the disease. The results obtained from this research may help to orientate future studies designed to combat this serious disease of cereals.

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APPENDIX I

DATES TEST PLANTS WERE SOWN, INOCULATED AND ASSESSED, AND THE GLASSHOUSE TEMPERATURES

TABLE 1. The dates test plants were sown, inoculated and assessed for foliar symptoms and the mean, minimum and maximum temperatures recorded in the glasshouse where plants were grown from inoculation to assessment (Chapter 4).

Experiment No.	Date test plants			Temperature °C		
	Sown	Inoculated	Assessed	Mean	Min.	Max.
1	07.04.88	19.04.88	18.05.88	24.2	18.3	30.1
2	17.04.88	29.04.88	30.05.88	24.7	18.5	30.8
3	01.10.88	13.10.88	10.11.88	18.3	13.6	22.9
4	08.05.88	20.05.88	22.05.88	25.7	19.1	32.3
5	17.06.88	29.06.88	26.06.88	25.2	20.4	29.9
6	30.05.88	11.06.88	09.08.88	24.2	18.3	30.1
7	23.01.89	04.02.89	06.03.89	13.6	7.2	20.0
8	10.03.89	22.03.89	20.04.89	15.7	7.7	23.6
9	10.04.89	22.04.89	25.05.89	19.8	10.8	28.8
10	02.08.90	14.08.90	12.09.90	21.5	12.1	30.9
11	06.05.88	18.05.88	16.06.88	24.5	17.0	32.0
12	13.07.88	25.07.88	25.08.88	22.4	15.0	29.7

TABLE 2. The dates test plants were sown, inoculated and assessed for foliar symptoms, and the mean, minimum and maximum temperatures recorded in the glasshouse where plants were grown from inoculation to assessment (Chapter 5).

Experiment No.	Date test plants			Temperature °C		
	Sown	Inoculated	Assessed	Mean	Min.	Max.
1	20.06.89	01.07.89	02.08.89	22.0	14.9	29.0
2	10.07.89	22.07.89	28.08.89	23.2	14.5	31.9
3	13.07.89	25.07.89	28.08.89	22.3	14.1	30.4
4	25.07.89	06.08.89	07.09.89	19.5	14.0	25.0
5	23.01.90	04.02.90	05.03.90	12.1	7.0	17.1
6	01.05.90	13.05.90	14.06.90	21.8	14.1	29.5
7	07.05.90	19.05.90	18.06.90	21.8	12.3	31.3
8	11.05.90	23.05.90	20.06.90	22.5	11.8	33.2
9	18.05.90	30.05.90	26.06.90	21.0	11.6	30.3
10 a & b	25.05.90	06.06.90	06.07.90	22.3	12.0	32.6
11	15.06.90	27.06.90	25.07.90	23.3	12.5	34.0
12	13.07.90	25.07.90	23.08.90	22.9	13.0	32.8
13	03.08.90	15.08.90	17.09.90	21.3	11.2	31.3

TABLE 3. The dates test plants were sown, inoculated and assessed for foliar symptoms, and the mean, minimum and maximum temperatures recorded in the glasshouse where the plants were grown from inoculation to assessment (Chapter 6).

Experiment No.	Date test plants			Temperature °C		
	Sown	Inoculated	Assessed	Mean	Min.	Max.
1a	23.06.88	18.07.88	10.08.88	23.8	16.6	30.9
			17.08.88	22.6	15.5	29.7
			24.08.88	21.4	14.6	28.2
1b	26.01.89	01.03.88	23.03.89	13.8	7.3	20.3
			30.03.89	14.3	7.4	21.2
			06.04.89	14.8	7.7	21.8
2	10.07.89	28.07.89	29.08.89	21.1	14.5	27.6
3	20.12.89	21.01.90	20.02.90	15.7	12.4	18.9
4a	12.07.90	02.08.90	03.09.90	21.7	12.9	30.5
4b	16.07.90	13.08.90	12.09.90	21.5	12.1	30.9
4c	30.07.90	17.08.90	14.09.90	22.8	11.6	34.0

APPENDIX II

MEAN ABSORBANCE VALUES OF SOURCE LEAVES AND PERCENTAGE TRANSMISSION BY APHIDS TO TEST PLANTS

TABLE 1. The mean absorbance values (A_{405}) of oat cv. M. Tabard source leaves and the percentage transmission of the RPV, PAV and MAV strains of BYDV from these leaves by *R. padi* and *S. avenae* to three oat cultivars.

Mean absorbance value (A_{405})			Percentage transmission to oat cultivars					
			M. Tabard		Blenda		Pennalt	
RPV	PAV	MAV	R.p.	S.a.	R.p.	S.a.	R.p.	S.a.
0.475	0.023	0.033	98	6				
0.029	**0.534**	0.086	100	28				
0.006	0.011	**0.146**	0	36				
0.209	**0.817**	0.102	88	36				
0.010	**2.537**	0.052			78	70		
0.311	0.017	0.005			82	0		
2.432	**2.391**	**0.415**					92	32
0.972	0.020	**2.002**					96	64
1.139	**1.186**	0.027					92	12
0.014	**1.921**	**0.165**[a]					56	0
1.730	0.006	0.029[s]	84					
2.499	0.049	0.055[r]		4				
2.535	0.028	0.087[r]		0				
1.866	0.037	0.036[s]		0				
0.023	**2.199**	0.078[s]			94	18		
0.007	**1.701**	0.073[r]				30		
Mean healthy $\bar{x}$ + 3s								
0.041	0.046	0.069						

[a]Source leaves were oat cv. Dula.
Source leaves previously infested by [r]*R. padi* (R.p.) or [s]*S. avenae* (S.a.)
$\bar{x}$ + 3s is the minimum positive threshold for BYDV.
Figures in bold are the strain of BYDV under study.

TABLE 2. The mean absorbance values (A_{405}) of perennial ryegrass cv. Talbot source leaves and the percentage transmission of the RPV, PAV and MAV strains of BYDV from these leaves by *R. padi* and *S. avenae* to a range of winter barley cultivars and oat cv. Dula.

Source of inoculum	Mean absorbance values (A_{405})			Percentage transmission by *R. padi*		Percentage transmission by *S. avenae*	
	RPV	PAV	MAV	Barley	Oats	Barley	Oats
Grass	**0.489**	0.032	0.074	0	24		
	0.599	0.076	0.034	14	16		
	0.944	0.021	0.077	0	33		
				8			
	0.315	0.026	0.070	5			
				10	0		
				0			
				0			
	2.215	0.070	0.086			0	0
	0.011	**0.485**	0.064	16			
				13	0		
				36			
				62			
	0.075	**0.265**	0.083			0	4
	2.341	**0.933**	0.086	78	100		
	2.226	**1.803**	0.124	31	80		
	0.275	**0.467**	**0.415**	40	42	0	22
$\bar{x}$ + 3s	0.067	0.059	0.089				

$\bar{x}$ + 3s is the minimum positive threshold for BYDV.
Figures in bold are the strains of BYDV under study.

TABLE 3. The mean absorbance values (A_{405}) of oat cv. Dula and winter barley cv. Igri source leaves and the percentage transmission of the RPV, PAV and MAV strains of BYDV from these leaves by *R. padi* and *S. avenae* to a range of winter barley cultivars and oat cv. Dula.

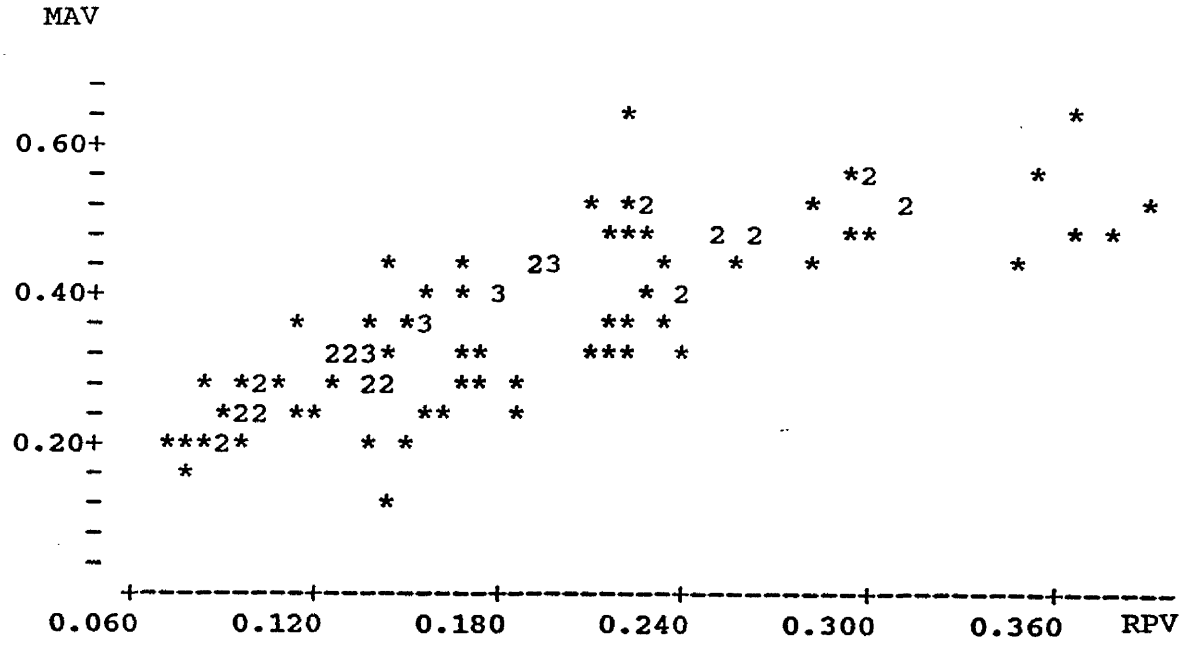
Source of inoculum	Mean absorbance values (A_{405})			Percentage transmission by *R. padi*		Percentage transmission by *S. avenae*	
	RPV	PAV	MAV	Barley	Oats	Barley	Oats
Oats	**2.468**	0.027	0.061	24	20		
	2.383	0.000	0.047			0	0
	0.067	**2.344**	0.161	66	62		
	0.040	**2.211**	0.114			2	16
	1.655	**2.334**	0.209	78	82	6	0
Barley	0.037	0.063	**1.498**	0	0	8	52
$\bar{x}$ + 3s	0.063	0.054	0.092				

$\bar{x}$ + 3s is the minimum positive threshold for BYDV.

Figures in bold are the strains of BYDV under study.

APPENDIX III

CORRELATION AND REGRESSION ANALYSIS OF MEAN ABSORBANCE VALUES



Correlation of MAV and RPV = 0.816

The regression equation is
MAV = 0.129 + 1.25 RPV

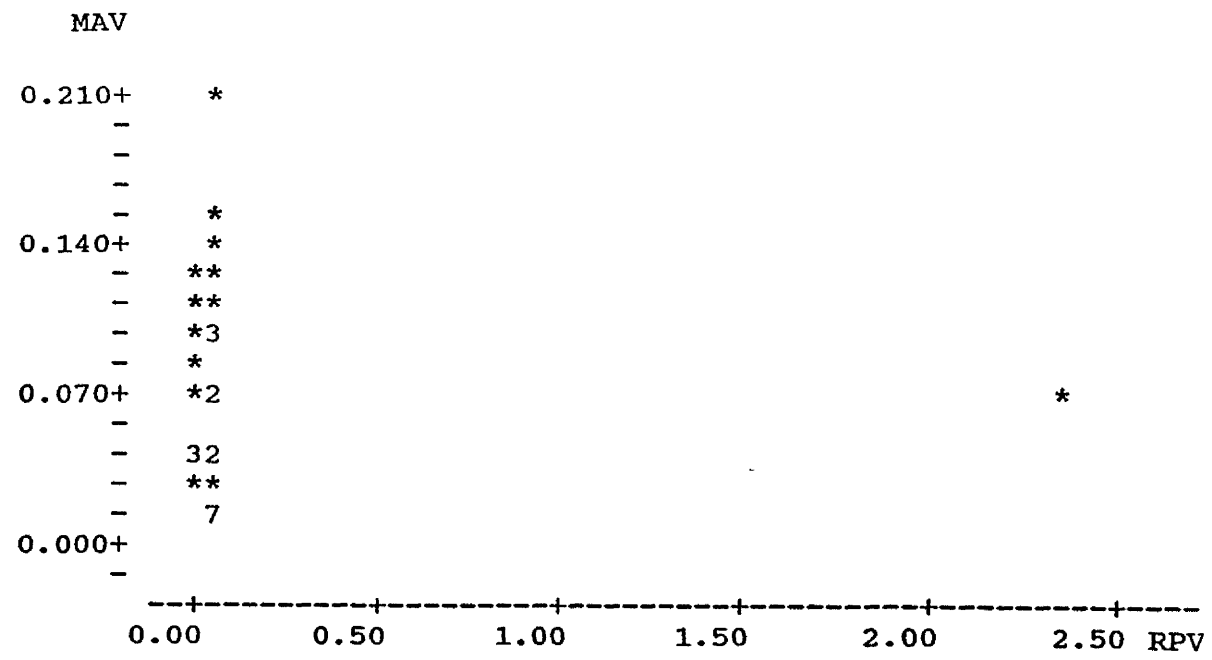
Predictor	Coef	Stdev	t-ratio	p
Constant	0.12937	0.01819	7.11	0.000
RPV	1.25112	0.08951	13.98	0.000

s = 0.06807 R-sq = 66.6% R-sq(adj) = 66.3%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.90521	0.90521	195.38	0.000
Error	98	0.45404	0.00463		
Total	99	1.35925			

FIGURE 1. The correlation and regression analysis of the absorbance values (A_{405}) of RPV and MAV obtained from perennial ryegrass cv. Talbot test plants in Experiment 4b (Chapter 6).



Correlation of MAV and RPV = 0.003

The regression equation is
MAV = 0.0697 + 0.0004 RPV

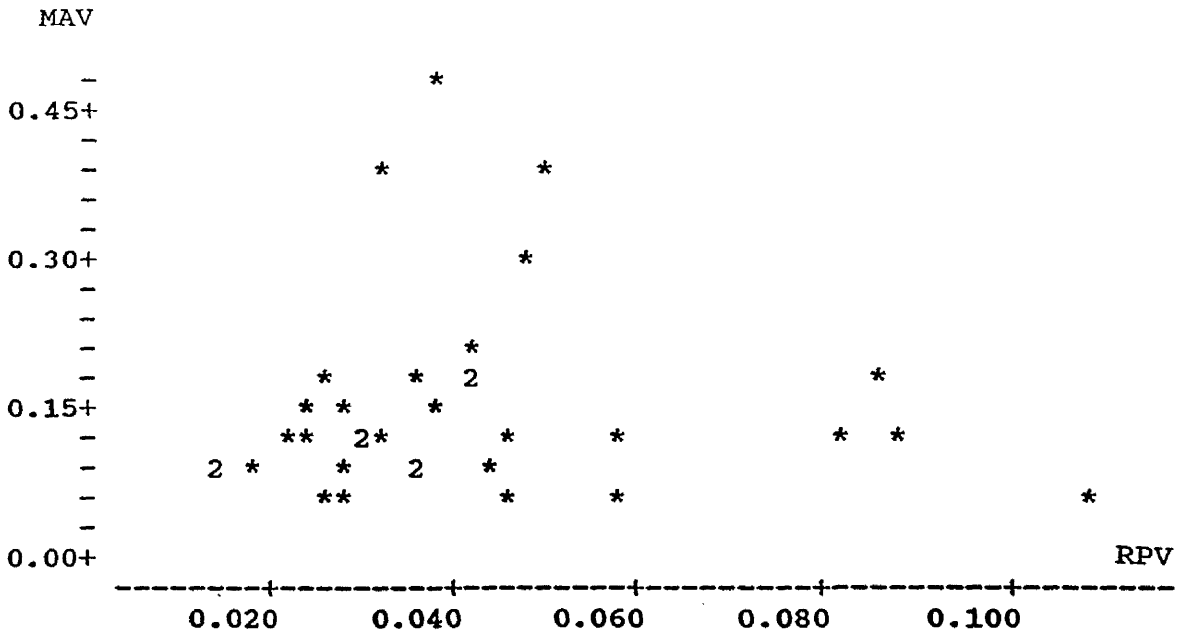
Predictor	Coef	Stdev	t-ratio	p
Constant	0.069728	0.009584	7.28	0.000
RPV	0.00036	0.02245	0.02	0.987

s = 0.05080 R-sq = 0.0% R-sq(adj) = 0.0%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.000001	0.000001	0.00	0.987
Error	28	0.072253	0.002580		
Total	29	0.072253			

FIGURE 2. The correlation and regression analysis of the absorbance values (A_{405}) of RPV and MAV obtained from the oat cv. Dula control in Experiment 4b (Chapter 6).



Correlation of MAV and RPV = -0.020

The regression equation is
MAV = 0.153 - 0.088 RPV

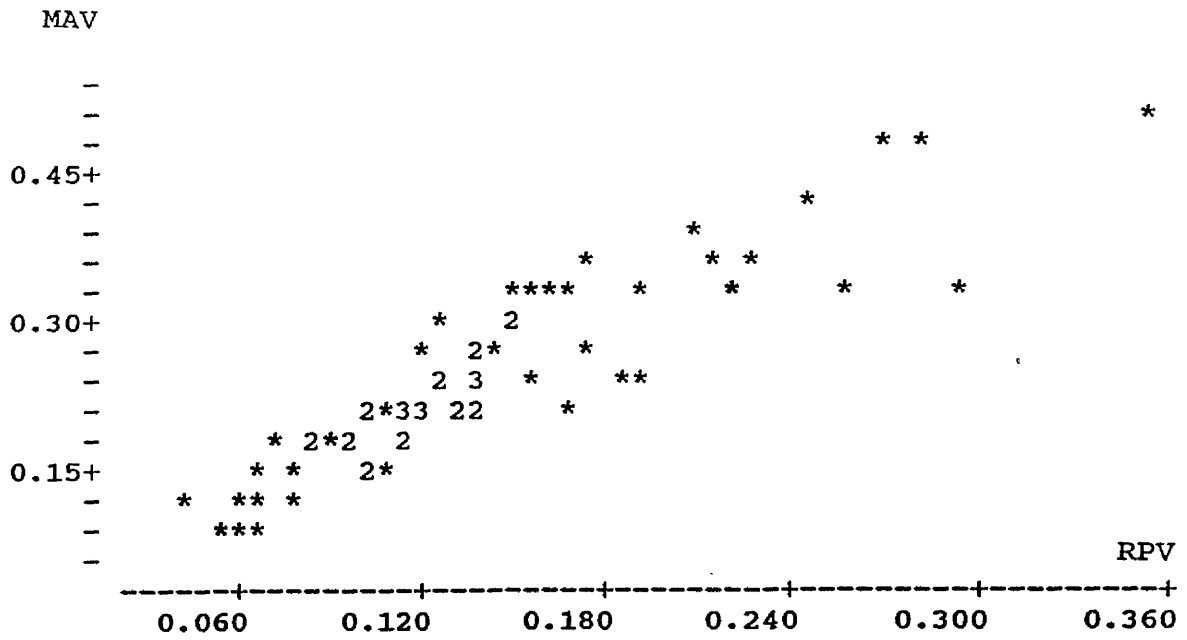
Predictor	Coef	Stdev	t-ratio	p
Constant	0.15318	0.03632	4.22	0.000
RPV	-0.0880	0.7840	-0.11	0.911

s = 0.09753 R-sq = 0.0% R-sq(adj) = 0.0%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.000120	0.000120	0.01	0.911
Error	32	0.304370	0.009512		
Total	33	0.304490			

FIGURE 3. The correlation and regression analysis of the absorbance values (A_{405}) of RPV and MAV obtained from perennial ryegrass cv. Talbot test plants infested with *S. avenae* (Nos. 1-34) in Experiment 4c (Chapter 6).



Correlation of MAV and RPV = 0.909

The regression equation is
MAV = 0.0522 + 1.36 RPV

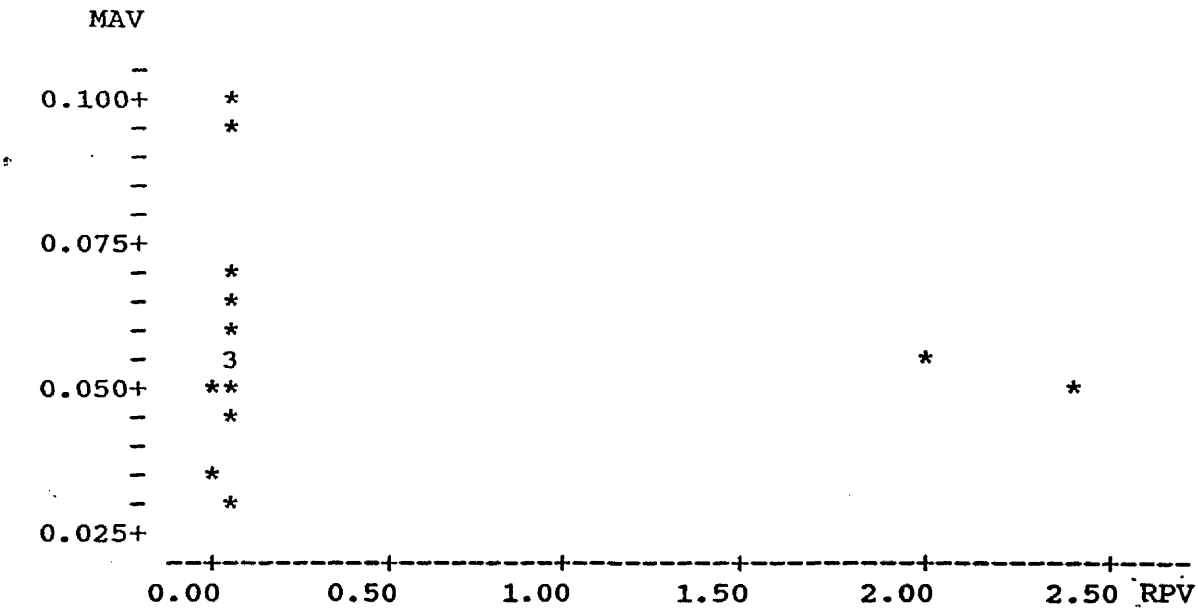
Predictor	Coef	Stdev	t-ratio	p
Constant	0.05217	0.01196	4.36	0.000
RPV	1.35981	0.07804	17.42	0.000

s = 0.03940 R-sq = 82.6% R-sq(adj) = 82.3%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.47142	0.47142	303.61	0.000
Error	64	0.09938	0.00155		
Total	65	0.57080			

FIGURE 4. The correlation and regression analysis of the absorbance values (A_{405}) of RPV and MAV obtained from the remaining perennial ryegrass cv. Talbot test plants in Experiment 4c (Chapter 6).



Correlation of MAV and RPV = -0.133

The regression equation is
MAV = 0.0590 - 0.00318 RPV

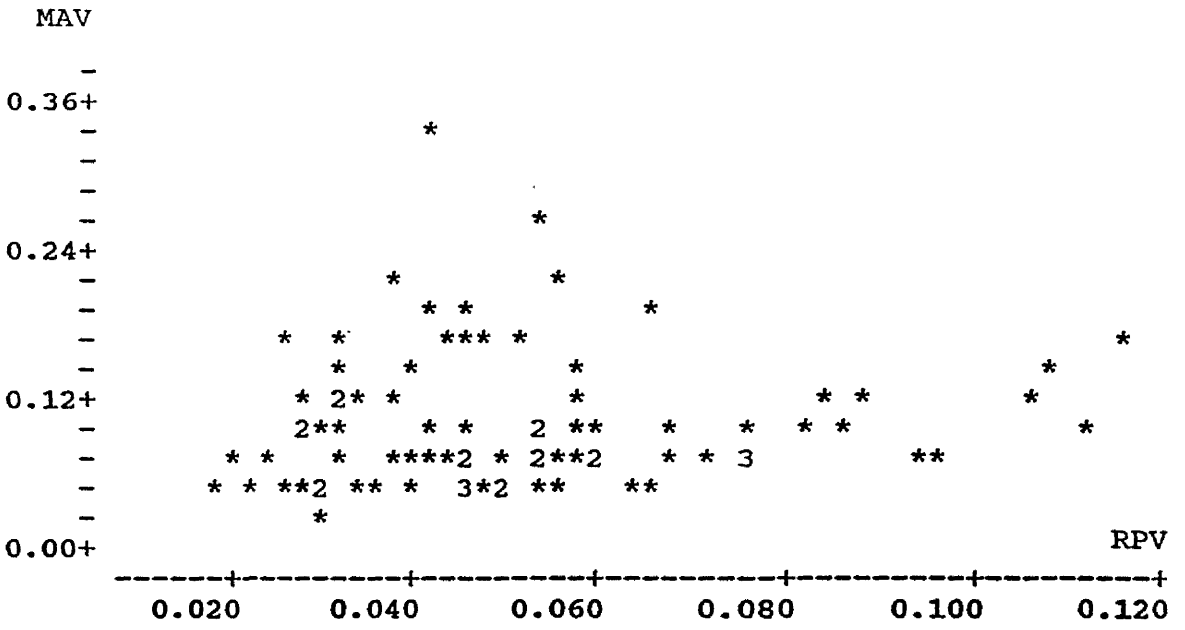
Predictor	Coef	Stdev	t-ratio	p
Constant	0.059024	0.005305	11.13	0.000
RPV	-0.003177	0.006571	-0.48	0.637

s = 0.01884 R-sq = 1.8% R-sq(adj) = 0.0%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.0000830	0.0000830	0.23	0.637
Error	13	0.0046130	0.0003548		
Total	14	0.0046960			

FIGURE 5. The correlation and regression analysis of the absorbance values (A_{405}) of RPV and MAV obtained from the oat cv. Dula control in Experiment 4c (Chapter 6).



Correlation of MAV and RPV = 0.064

The regression equation is
MAV = 0.0911 + 0.163 RPV

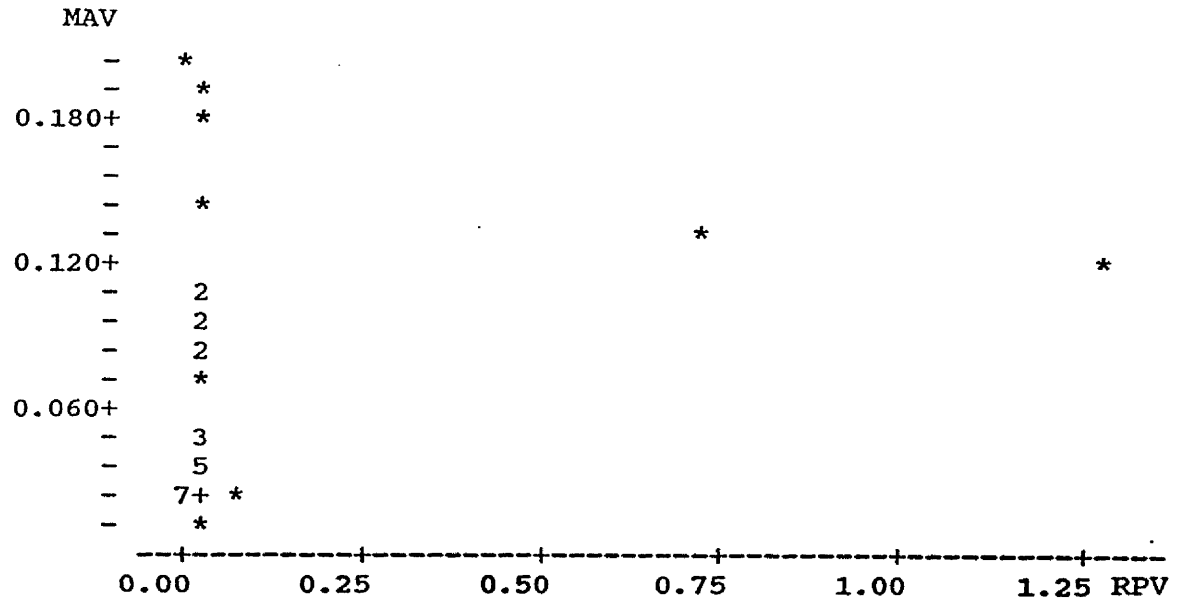
Predictor	Coef	Stdev	t-ratio	p
Constant	0.09114	0.01559	5.85	0.000
RPV	0.1626	0.2771	0.59	0.559

s = 0.05584 R-sq = 0.4% R-sq(adj) = 0.0%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.001073	0.001073	0.34	0.559
Error	83	0.258774	0.003118		
Total	84	0.259847			

FIGURE 6. The correlation and regression analysis of the absorbance values (A_{405}) of RPV and MAV obtained from perennial ryegrass cv. Talbot test plants in Experiment 4a (Chapter 6).



Correlation of MAV and RPV = 0.273

The regression equation is
MAV = 0.0523 + 0.0641 RPV

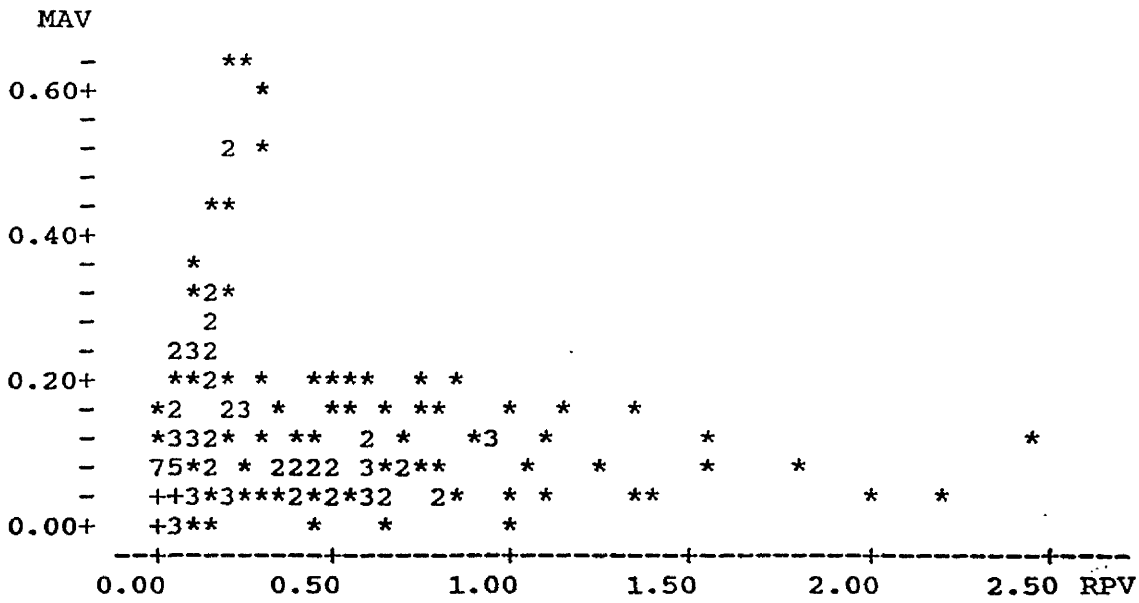
Predictor	Coef	Stdev	t-ratio	p
Constant	0.052338	0.007742	6.76	0.000
RPV	0.06410	0.03481	1.84	0.073

s = 0.04917 R-sq = 7.5% R-sq(adj) = 5.3%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.008199	0.008199	3.39	0.073
Error	42	0.101540	0.002418		
Total	43	0.109739			

FIGURE 7. The correlation and regression analysis of the absorbance values (A_{405}) of RPV and MAV obtained from the oat cv. Dula control in Experiment 4a (Chapter 6).



Correlation of MAV and RPV = 0.008

The regression equation is
MAV = 0.106 + 0.0022 RPV

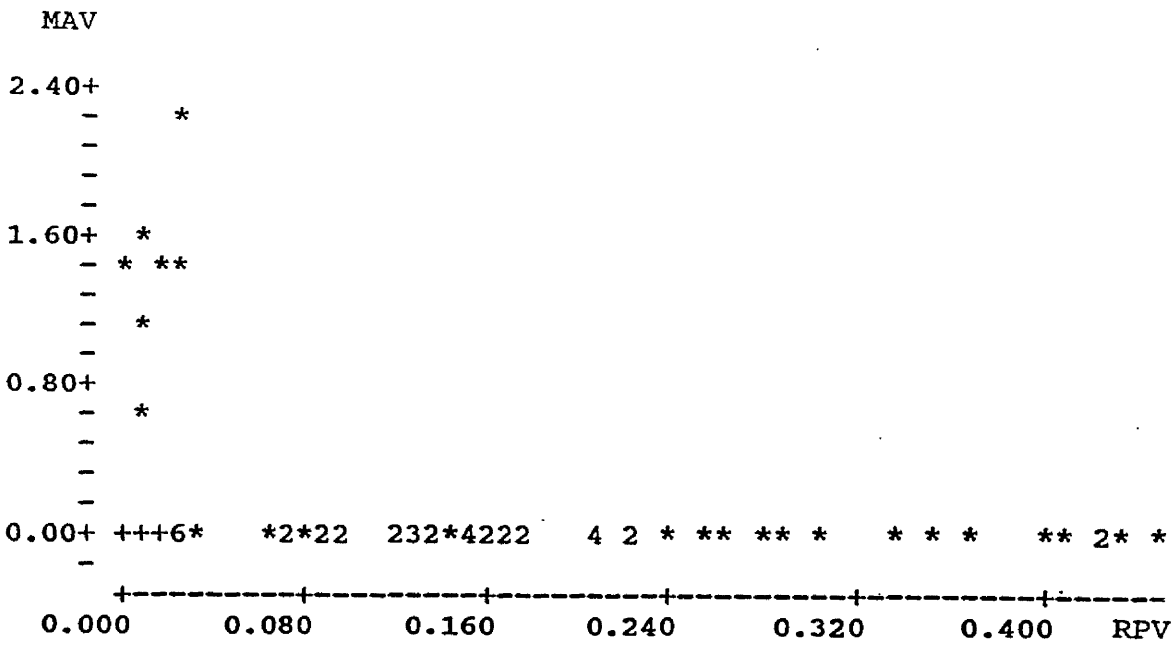
Predictor	Coef	Stdev	t-ratio	p
Constant	0.10614	0.01009	10.52	0.000
RPV	0.00217	0.01863	0.12	0.907

s = 0.1148 R-sq = 0.0% R-sq(adj) = 0.0%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.00018	0.00018	0.01	0.907
Error	203	2.67620	0.01318		
Total	204	2.67638			

FIGURE 8. The correlation and regression analysis of the absorbance values (A_{405}) of RPV and MAV obtained from ryegrass samples from fields throughout Scotland (Chapter 7).



Correlation of MAV and RPV = -0.175

The regression equation is
MAV = 0.171 - 0.516 RPV

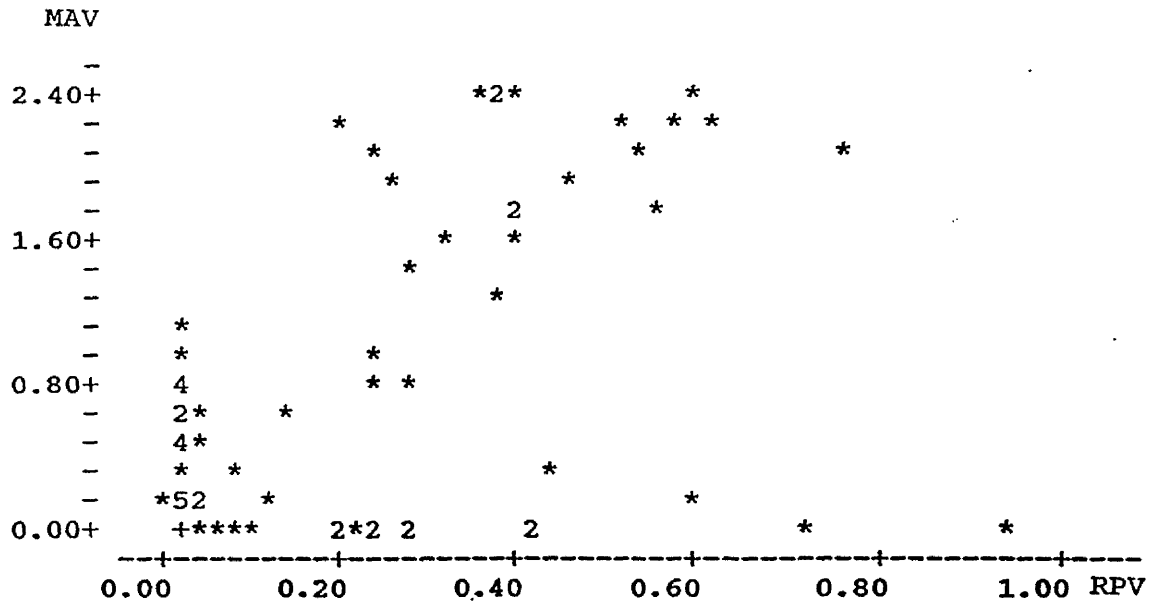
Predictor	Coef	Stdev	t-ratio	p
Constant	0.17145	0.04702	3.65	0.000
RPV	-0.5159	0.2912	-1.77	0.080

s = 0.3637 R-sq = 3.1% R-sq(adj) = 2.1%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.4153	0.4153	3.14	0.080
Error	99	13.0965	0.1323		
Total	100	13.5118			

FIGURE 9. The correlation and regression analysis of the absorbance values (A_{405}) of RPV and MAV obtained from oat cv. Pennalt in Experiment 7 (Chapter 4).



Correlation of MAV and RPV = 0.525

The regression equation is
MAV = 0.338 + 1.96 RPV

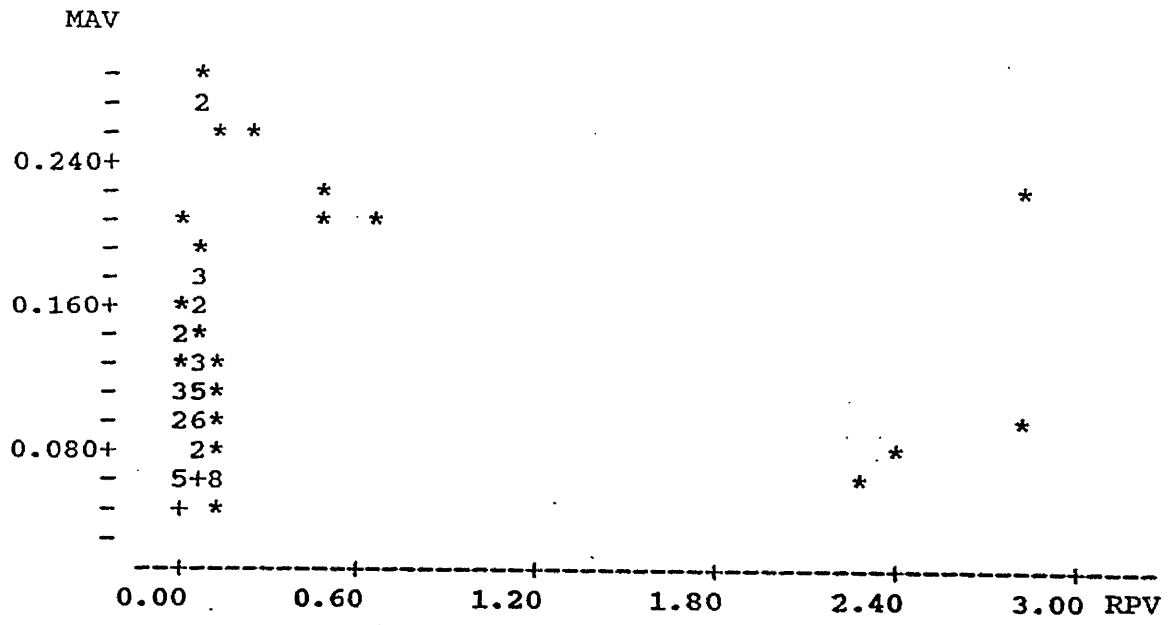
Predictor	Coef	Stdev	t-ratio	p
Constant	0.3385	0.1098	3.08	0.003
RPV	1.9590	0.3618	5.41	0.000

s = 0.7127 R-sq = 27.6% R-sq(adj) = 26.6%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	14.888	14.888	29.31	0.000
Error	77	39.106	0.508		
Total	78	53.994			

FIGURE 10. The correlation and regression analysis of the absorbance values (A_{405}) of RPV and MAV obtained from oat cv. Pennalt in Experiment 8 (Chapter 4).



Correlation of MAV and RPV = 0.119

The regression equation is
MAV = 0.102 + 0.0137 RPV

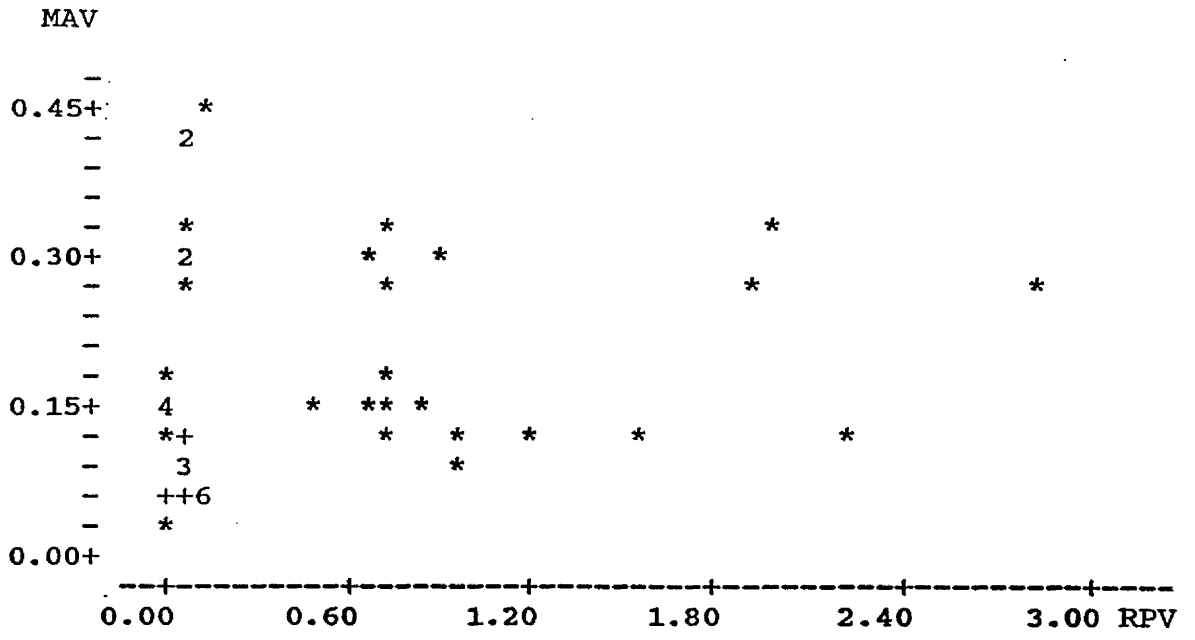
Predictor	Coef	Stdev	t-ratio	p
Constant	0.101519	0.006835	14.85	0.000
RPV	0.01368	0.01219	1.12	0.265

s = 0.06103 R-sq = 1.4% R-sq(adj) = 0.3%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.004694	0.004694	1.26	0.265
Error	88	0.327751	0.003724		
Total	89	0.332445			

FIGURE 11. The correlation and regression analysis of the absorbance values (A_{405}) of RPV and MAV obtained from winter barley cv. Igri in Experiment 9 (Chapter 5).



Correlation of MAV and RPV = 0.325

The regression equation is
MAV = 0.120 + 0.0559 RPV

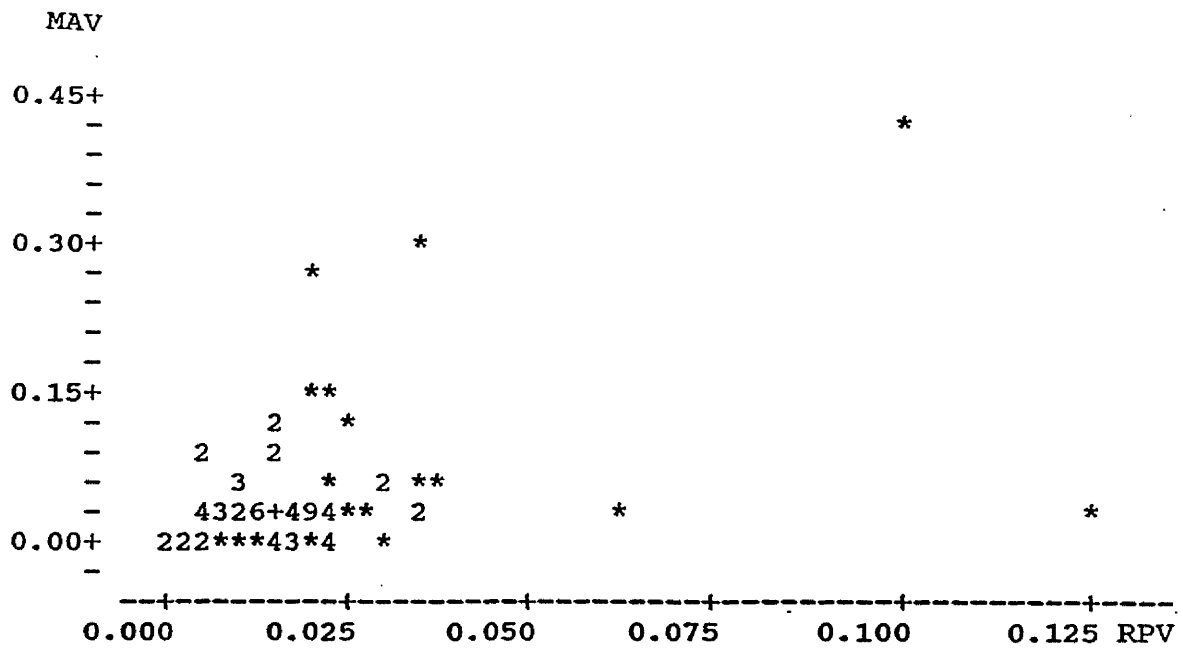
Predictor	Coef	Stdev	t-ratio	p
Constant	0.12018	0.01227	9.79	0.000
RPV	0.05593	0.01902	2.94	0.004

s = 0.09291 R-sq = 10.6% R-sq(adj) = 9.4%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.074663	0.074663	8.65	0.004
Error	73	0.630183	0.008633		
Total	74	0.704846			

FIGURE 12. The correlation and regression analysis of the absorbance values (A_{405}) of RPV and MAV obtained from the oat cv. Dula control in Experiment 9 (Chapter 5).



Correlation of MAV and RPV = 0.399

The regression equation is
MAV = 0.0159 + 1.46 RPV

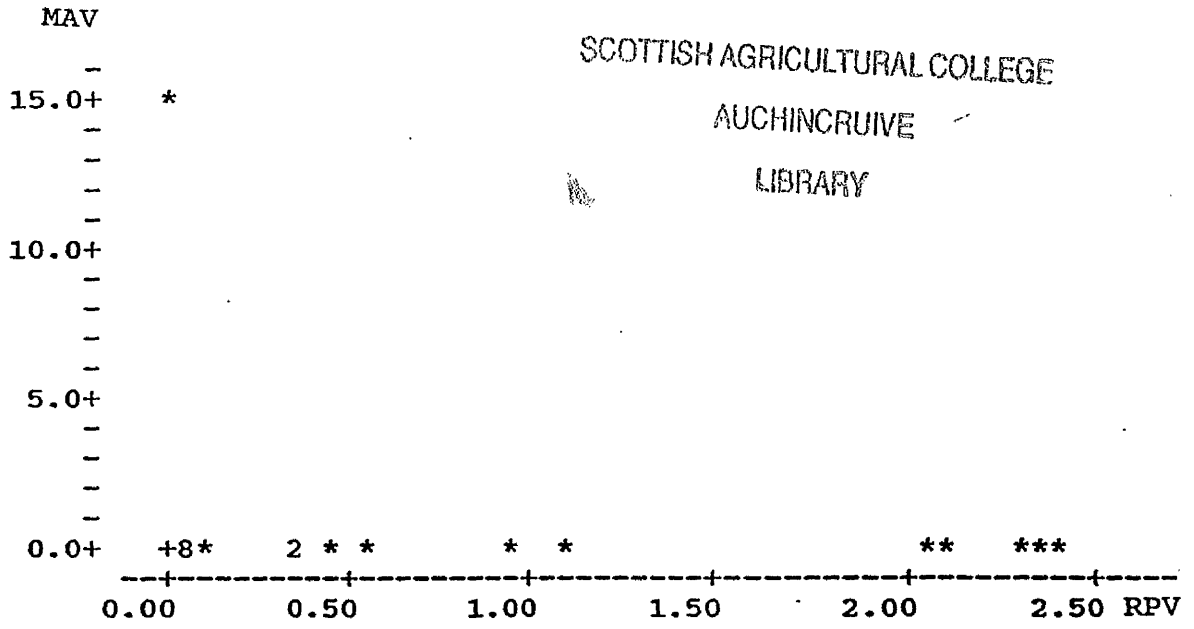
Predictor	Coef	Stdev	t-ratio	p
Constant	0.015932	0.008534	1.87	0.065
RPV	1.4550	0.3435	4.24	0.000

s = 0.05550 R-sq = 15.9% R-sq(adj) = 15.0%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.055278	0.055278	17.95	0.000
Error	95	0.292615	0.003080		
Total	96	0.347893			

FIGURE 13. The correlation and regression analysis of the absorbance values (A_{405}) of RPV and MAV obtained from winter barley cv. Igri in Experiment 12 (Chapter 5).



Correlation of MAV and RPV = -0.059

The regression equation is
MAV = 0.344 - 0.181 RPV

Predictor	Coef	Stdev	t-ratio	p
Constant	0.3439	0.2831	1.21	0.230
RPV	-0.1813	0.4068	-0.45	0.658

s = 1.979 R-sq = 0.4% R-sq(adj) = 0.0%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.778	0.778	0.20	0.658
Error	56	219.351	3.917		
Total	57	220.129			

FIGURE 14. The correlation and regression analysis of the absorbance values (A_{405}) of RPV and MAV obtained from the oat cv. Dula control in Experiment 12 (Chapter 5).