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Cronje, Carel Pieter Roche

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THE EPIDEMIOLOGY, OCCURRENCE AND EFFECT OF BROME MOSAIC VIRUS (BMV) ON WHEAT (*Triticum aestivum*) IN THE SUMMER RAINFALL AREA.

Carel Pieter Roché Cronjé

A Thesis Submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the Degree of Doctor of Philosophy.

Johannesburg, 1990

ABSTRACT

THE EPIDEMIOLOGY, OCCURRENCE AND EFFECT OF BMV (Brome Mosaic Virus) ON WHEAT (*Triticum aestivum*) IN THE SUMMER RAINFALL AREA.

CRONJÉ, Carel Pieter Roché, Ph.D. University of the Witwatersrand, Johannesburg, 1990.

The effect of Brome mosaic virus (BMV) on the development and yield of wheat in the summer rainfall area was studied. The methods of transmission of the virus under field conditions included transmission by the Russian wheat aphid *Diuraphis noxia* (Mordwilko). Owing to the severity of this pest on wheat in the eastern Orange Free State, any factors that might enhance the negative effect on yield caused by this aphid had to be studied.

Methods used during the course of the study included ELISA, radial immunodiffusion and several centrifugation techniques. Contrary to statements made by previous researchers it was found that BMV did not constitute a major threat to small grains in the eastern OFS, and that seed-transmission of BMV did not play an important role in the dissemination of the virus under natural conditions. It was also found that some resistance to the virus could be indicated in wheat cultivars. The role of alternate hosts in the epidemiology of the virus, as well as the role of *D. noxia*

as vector for the virus under natural conditions was investigated.

A computer model to simulate BMV epidemics in the summer rainfall area was developed, which represents a useful practical method for advising wheat growers on appropriate chemical control of *D. noxia*.

Simulation runs with the model, confirmed research results, in that even under extreme conditions BMV remained a relatively mild pathogen compared to the detrimental effect of *D. noxia*.

DECLARATION

I declare that this dissertation is my own unaided work and was conducted at the Small Grain Centre, Bethlehem, while in the employ of the Department of Agriculture and Water Supply. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

C.F.R. Cronjé

23rd day of January, 1991.

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Special mention must also be made of Mrs J. Smit for editing the manuscript and Mrs A. Appelgryn for her assistance with the final preparation of the manuscript.

DEDICATION

With gratitude to my wife, Lolla and son, Roché, for their support during the course of this study.

"For everything comes from God alone. Everything lives by His power, and everything is for His glory. To Him be glory evermore." Romans 12:36 The Living Bible

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CHAPTER 1

INTRODUCTION

1.1 THE WHEAT INDUSTRY IN SOUTH AFRICA

Briggle (1980) summarized the role that wheat has played in the development of man as follows:- "Wheat provides more nourishment for people of the world than any other food source, and enters into international trade more than any other food. Its influence has been felt in world and national politics. Wheat has many natural advantages as food and feed. The grain is nutritious, in spite of the fact that wheat protein is lacking in some essential amino acids; it has a concentrated form, so takes little space; it can be readily stored and transported if reasonable caution is exercised; it is easily processed to produce highly refined foods."

Recently the world production and consumption of wheat averaged 500 million tons per year (Fig. 1), which illustrates the importance of this foodstuff to a growing world population. The enormous demand for a cheap and abundant foodstuff has resulted in the selection of high yielding crop cultivars, including wheat cultivars, very often with little consideration for insect and disease resistance. Coupled to this, a swing to monoculture agricultural systems which create ideal conditions for insects and pathogens, can

result in severe crop losses. Although the Republic of South Africa is one of the minor wheat producing countries, it has in recent times usually been able to meet its own demands and in some cases even export a limited quantity.

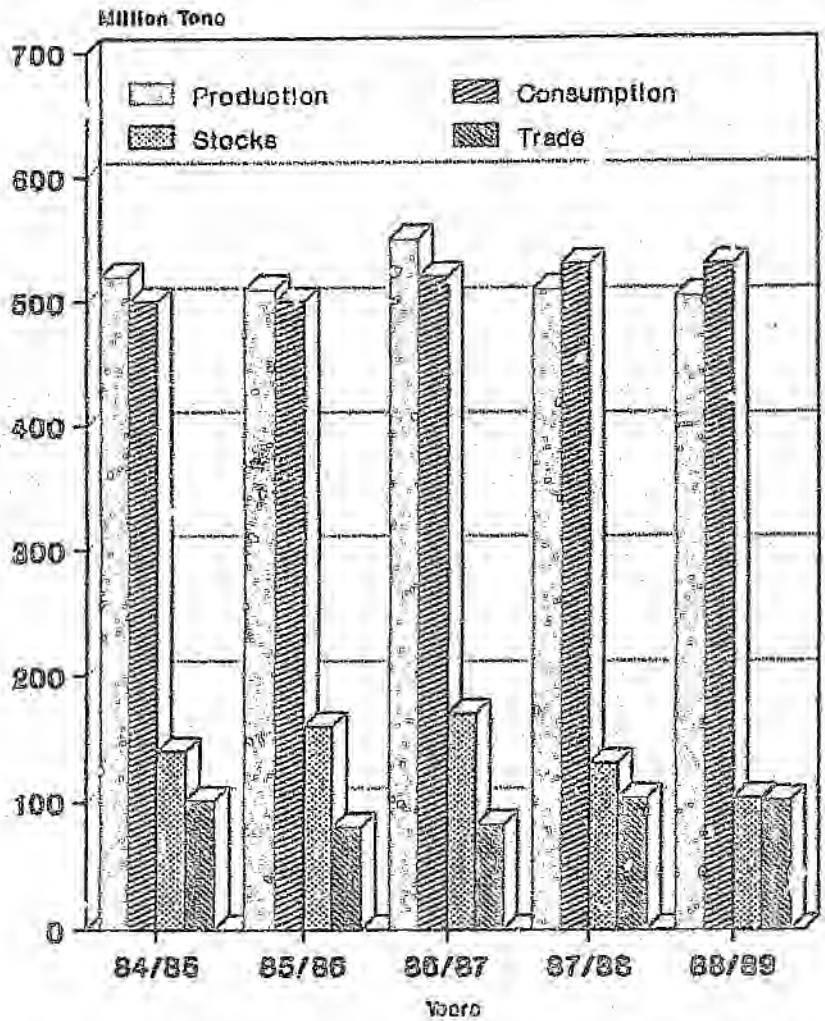


Figure 1. Production and consumption of wheat during the period 1984-89 (Anon, 1989).

The main wheat producing areas in South Africa are located in dry regions of the Republic, and production is therefore lower than some other wheat producing countries in the world.

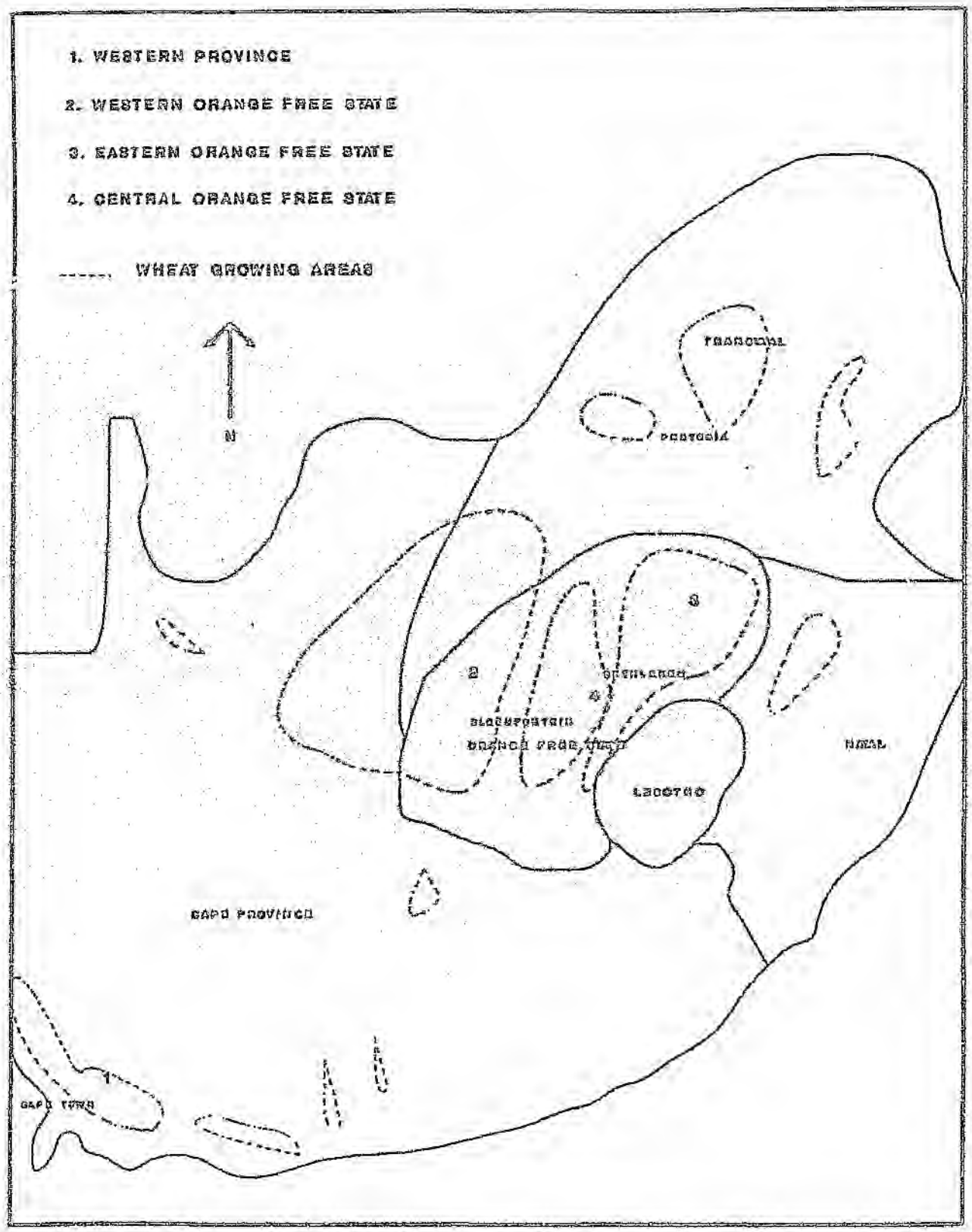


Figure 2. A map of the Republic of South Africa showing the principal wheat producing areas.

Three main areas, namely the western Cape, western Orange Free State (OFS) and eastern OFS, contribute most of the total wheat production in the Republic (Fig. 2).

This study was conducted mainly in the eastern OFS, one of the major dry-land wheat producing areas in the Republic (Fig.3). The highly variable rainfall has undoubtedly a greater effect on yield than all other factors combined.

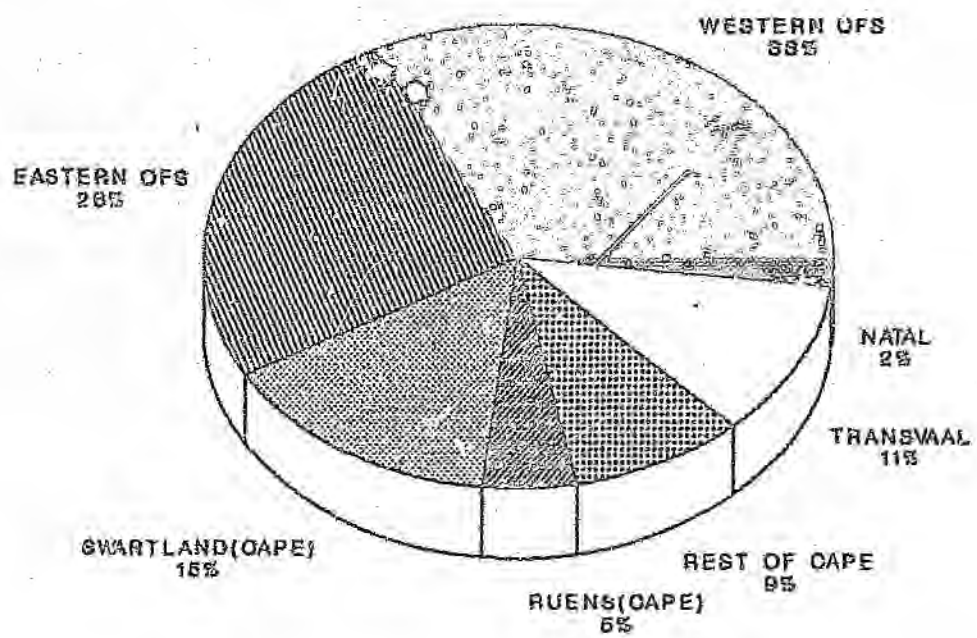


Figure 3. The proportion of total wheat production in South Africa originating from different production areas. Total production during the 1988/1989 season was 3.484 million Tons (Anon,1989).

However insects and diseases can reduce the yield significantly and every effort should be made to minimise losses. A thorough knowledge of all aspects of the ecology of a pest species, or the epidemiology of a pathogen, is essential for the development of successful management programmes (Kriel, 1984).

1.2 THE ADVENT OF THE RUSSIAN WHEAT APHID

The Russian wheat aphid *Diuraphis noxia* (Mordwilko ex Kurdjumov), was first described in the early 1900s as a pest of wheat in the Crimea (Grossheim, 1914), and has since been recorded as a pest of small grains in various countries of the Middle East and the Mediterranean coast of North Africa and Europe (Walters, 1984), and recently, the United States of America (Webster *et al.*, 1987).

During 1978 this pest was found to cause severe damage to wheat near Bethlehem in the OFS (Fig. 2). Infestation was initially confined to part of the Bethlehem district but by September 1979 it had spread over the greater part of the OFS and Lesotho and isolated infestations were also found in the Transvaal and in Natal (Walters, 1984).

The Department of Agriculture recognized the gravity of the situation after it had been found that the insecticides registered against other wheat aphids were ineffective against

D. noxia. The typical stunting of plant growth and streaking and rolling of leaves caused by the pest led to the assumption that a toxin was involved (Hewitt *et al.*, 1984; Kriel *et al.*, 1984). To date a toxin has not been isolated, but a mechanism for the action of a "Russian aphid extract" on chloroplasts of wheat has been postulated by Krüger and Hewitt (1984).

The extent and severity of *D. noxia* infestation varies from year to year but the reasons for this are not known. Wheat and barley seem to be the preferred hosts of *D. noxia* and alternate hosts for this aphid at present appear to be limited to volunteer cereals or *Bromus* spp. (Hewitt *et al.*, 1984). Evidence from the western OFS suggests that volunteer wheat acts as a source of aphids for the infestation of newly sown crops (Kriel *et al.*, 1984). In the eastern OFS volunteers within crops are rare, but patches of infestation consistent with aphids flying into the crop are seen (Aalbersberg, 1987). The fact that this aphid damages crops is in no doubt (Du Toit and Walters, 1984; Fouché *et al.*, 1984). The large population levels that this species reaches, even if it did not also produce a toxin, would constitute a threat to yield (Plumb, 1984).

1.3 THE RUSSIAN APHID TASK TEAM

During October 1979 a research team called the "Task team for the control of *Diuraphis noxia*" was called into being by

the Department of Agriculture. Members of the team were drawn from the various institutes in the Department, from the University of the Orange Free State (UOFS) and from the University of Cape Town (UCT). The research programme devised by the task team encompassed a wide spectrum of activities involving disciplines such as entomology, virology and plant breeding. All these activities had a common goal namely, to find and utilize all possible avenues of control of the pest and to reduce the damage caused by it (Walters, 1984).

1.4 POSSIBLE VIRUS INVOLVEMENT IN THE RUSSIAN APHID COMPLEX

As a result of the activities of this research group a number of viruses were found in South African small grains. The characteristic symptoms caused by *D. noxia* feeding on wheat are a horizontal flattening of the tillers of young winter wheat plants and whitish yellow streaks or purple and maroon streaks on the leaves and stems. These symptoms are especially severe in the cold season (April to October), and are usually temporary in cases where *D. noxia* are removed from the plants at an early stage (Rybicki, 1984; Bremer and Raatikainen, 1975). In older plants the stems return to the vertical position and appear normal apart from white streaking along the midribs of the younger leaves and occasional bleaching of the leaf sheaths. Leaves colonized by *D. noxia* are often tightly rolled (Stshegolev, 1937;

Borner *et al.*, 1957). According to Von Wechmar (1980) the Russian wheat aphid *per se* is not the only causal agent for the typical streak symptoms caused by this species on wheat plants, but that this symptoms might also be caused by Brome Mosaic virus (BMV) infections. According to Von Wechmar (1984) wheat plants collected from fields in the eastern OFS, which showed symptoms of *D. noxia* infestation, were all infected with BMV. She further stated that BMV could cause yield losses of up to 50 % in wheat (Du Toit, 1986(b)).

Additional symptoms not typical of *D. noxia* infestation were also noted. Marked yellowing of leaves infested early in the season led to the description of the "Free State Streak" disease* of small grains (Von Wechmar and Rybicki, 1981). These symptoms, in addition to the frequent occurrence of plants with intensely yellow tips and bold yellow streaks on the leaf blade, together with evidence of premature death of these leaves, led to the assumption that a virus could be responsible for at least part of the disease syndrome (Rybicki, 1984). Rybicki (1984) also reported that samples collected from the field in 1979 contained a high incidence of BMV at the end of the wheat season (November).

The apparent involvement of aphids in diseases in the field prompted aphid vector studies as well. When *D. noxia* and

* The use of the term "Free State Streak" disease could cause confusion between aphid and virus damage and symptomatology of the pathogens involved and is not used any longer (Du Toit, 1987).

Rhopalosiphum padi (L.) fed on BMV-containing plants were transferred to laboratory grown plants, yellowing symptoms were transmitted by both aphids through several cycles of propagations.

Three types of spherical virus-like particles could be isolated from such plants. These were identified as BMV, barley yellow dwarf virus (BYDV) and *Rhopalosiphum padi* virus (RhPV) (Rybicki, 1984; Von Wechmar and Rybicki, 1981).

Von Wechmar *et al.* (1984) also found that some of the commercially available small grain seed were contaminated with BMV. These findings held far-reaching implications for the future of the small grain industry of South Africa. It was suggested by them that a seed certifying scheme would have to be implemented, because the virus was the major cause of economically important crop losses.

1.5 SCOPE AND OBJECTIVES

The movement of an arthropod-transmitted plant-pathogenic virus from one plant to another requires three basic components: the host plant of the virus, the arthropod vector, and the plant pathogenic virus itself. Without all three, spread cannot occur, unless there are alternative transmission possibilities, for example through mechanical means or seed. This triad of basic components suggests that the phe-

nomenon of spread might be fairly straight-forward and predictable. However, closer scrutiny reveals that the complexities within this triad, and between components of the triad and the environment are sufficient to have perplexed scientists of the world for generations (Irwin and Ruesink, 1986).

The relationships between pathogenic viruses and their plant hosts have been investigated for decades. The general conclusion emerging from these studies to date is that interrelationships are specific and complex (Horsfall and Cowling, 1980; Matthews, 1981). The processes involved in rystemic infection and resistance to infection are evolutionary circuitous and as yet incompletely understood (Irwin and Ruesink, 1986).

Relationships between plant-pathogenic viruses and their arthropod vectors have also been studied for many years, although perhaps not as intensively as those of viruses and their host plants. What has emerged from these investigations suggest interrelationships that are often obligatory and specific, resulting from complex and intricate evolutionary interactions (Pirone and Megahed, 1966; Pirone and Harris, 1977; Pirone, 1981).

In South Africa there is a unique combination of viruses and vectors affecting Gramineae. The interest that has been generated in this area is almost entirely due to the advent

of the Russian wheat aphid and it is not surprising that research into this previously neglected area has revealed new facts and raised more problems (Plumb, 1984). Not only was the presence of BMV and cucumber mosaic virus (CMV) in South African wheat crops discovered for the first time, but also the transmission of BMV through wheat seeds and numerous vectors.

1.5.1 The effect of BMV infection on wheat

Bromoviruses sometimes induce typical mosaic symptoms in their hosts, but a great variety of symptoms can be found under differing conditions and host plants (Lane, 1977). The viruses multiply rapidly in their hosts, producing symptoms within a few days under favorable conditions. The viruses reach high levels of up to two milligrams per gram of tissue within three weeks after inoculation (Proll, 1965; 1967(a); (b); (c)). The viruses remain at high levels in older plants although the rate of synthesis decreases markedly and the symptoms become less obvious. The effects of Bromovirus infection on host metabolism are poorly understood. Brome mosaic virus infection changes respiration and nitrogen accumulation (Proll, 1967(c)) in barley and reduces chlorophyll and increases peroxidase in wheat.

1.5.2 Natural occurrence and distribution of BMV in the wheat producing areas

Von Wechmar and Rybicki (1981) reported that field surveys conducted in the eastern OFS and in the northern and eastern Cape Province showed that a number of grasses were severely affected by BMV and that this virus seemed endemic in the natural vegetation in these areas. They stated that the presence of this virus in perennial grasses contributed to its occurrence in cultivated crops, but suggested no method for the transmission of BMV from the grasses to wheat.

1.5.3 Simultaneous virus infections of wheat under field conditions

Rybicki (1984) reported that when BMV isolates from field grown wheat in the eastern OFS were inoculated into wheat seedlings, the first mechanical inoculation gave rise to a yellowing syndrome unlike the normal BMV mosaic symptoms on most wheat cultivars. Subsequent transfers from the first generation plants gave less and less yellowing until, after the third or fourth transfer, no yellowing symptoms were noticed.

He reported that extraction of diseased field material yielded large amounts of BMV, and that it is probable that due to the high concentration of the BMV any possible con-

taminants were not detectable in the presence of the BMV virions.

Rybicki (1984) reported that the inoculum he used could have contained another, less easily mechanically-transmissible component, as judged by the "dilution" of the yellowing syndrome upon successive mechanical transfers after the original inoculation. It is possible that the BMV was in fact a mixture of strains - a "yellowing" and "mosaic" strain - and that one replicated more slowly, and consequently was diluted out with successive transfers. However, it is also possible that a virus other than BMV was present at very low concentrations in the original extract, and that this was either diluted out during transfers, or was not truly mechanically transmissible, and was only inefficiently transmitted. This evidence, together with the proven occurrence of BYDV in diseased field plants, and the suspected involvement of several species of aphids with the transmission of the disease (Von Wechmar and Rybicki, 1981), led to the hypotheses that a two-virus complex -in all probability BMV and BYDV- was involved (Rybicki, 1984). Rybicki and Von Wechmar (1981) also reported that the OFS isolate of BMV is serologically distinct from other isolates of BMV, although closely related, which could partially explain the apparently aberrant behavior of this virus in the eastern OFS.

Von Wechmar (1985) reported that even under optimal conditions only very small BYDV yields could be isolated from

field diseased wheat in the eastern OFS. Although BYDV is present, climatic conditions are not conducive to the spreading of BYDV. She postulated that the contaminant virus with BMV, was CMV, rather than BYDV (Von Wechmar, 1985).

The samples tested for BMV during the course of this study also incorporated tests for CMV. No synergistic action between BMV and CMV has been reported to date, but synergistic reactions were noted between BMV and barley stripe mosaic (BSMV), and BMV and wheat streak mosaic (WSMV) (Mckinney 1956(a); (b)). Simultaneous infections produced severe symptoms and stunted plants more severely than single infections. The interaction between BMV and BSMV was especially severe (Lal and Sill, 1959). Samples were not tested for BYDV, since no BYDV was found in the eastern OFS during initial surveys.

1.5.4 Alternate hosts of BMV

Von Wechmar and Rybicki (1981) report that BMV infected *Chenopodium hybridum* (L) and *C. quinoa* (Willd) were found in old maize and wheat stubble fields and may serve as overseasoning hosts. However, Kriel (1984) reports that after extensive surveys of *Chenopodium* spp. in the western OFS, no *D. noxia* or signs of aphid colonisation could be found on these plants.

1.5.5 Seed-transmission of BMV

According to Lane (1981) the bromovirusses are not transmitted through seed. There is a single report of seed transmission of broad bean mottle mosaic (BBMMV), a member of the bromovirus group (Lane, 1981). No other reports outside the Republic of South Africa (Von Wechmar *et al.*, 1984) indicate seed-transmission of BMV. This could be because BMV in the eastern OFS is a different strain or distinct biotype (Rybicki, 1984).

The exact mechanism by which seed-transmission of BMV occurs in wheat is not known, but Rybicki (1984) reported that seed surfaces may be contaminated and that contaminated seed need not produce an infected plant. Rybicki (1984) found BMV in non-germinating embryos and postulated that infection of embryos could cause non-viability, but also that only heavily infected embryos died, and those that survived gave rise to infected coleoptiles and infected leaves.

The timing of BMV infection during barley development had an influence on the presence of viable virions in embryos, although BMV seed-transmission in Atlas barley could not be demonstrated (Ednie, 1970). According to Rybicki (1984) the situation in wheat appears to be different, in that seed-transmission of BMV at relatively high incidences could be obtained.

It appears that BMV is naturally aphid- and seed-transmitted in wheat, which could explain its apparent random occurrence at high concentration in wheat in the eastern OFS, where these mechanisms appear to operate (Rybicki, 1984). He stated that seed transmitted virus could be the primary virus source and that aphids play an active role in secondary transmission of these viruses amongst crops (Rybicki, 1984). In conclusion he stated that "- to ensure higher average wheat yields, certified virus-free seed should be available to the farmer-".

1.5.6 Vectors reported to be implicated in the dissemination of BMV

The disease-distribution potential of the aerial migrant aphid is formidable. Aphids combine seemingly simple search behavior with enough reproductive capacity to saturate large areas of terrain by their wind-borne host-finding flights. Some insect species are more likely than others to produce flying migrants and there is no sharp entomological distinction between flying and walking vectors - the same individual may be both, and both may lead to the distribution of populations between plants. However, the scale of redistribution by flight and on foot differs greatly and is of special concern. In general, walking vector movement is within crops and may decide the secondary crop infestation, once it is initiated. It is more easily observed and modelled than aerial movement. The flying migrants are the primary immi-

grant carriers however, and are more erratic and less predictable than local vectors because a small winged insect can cover considerable distances and are difficult to find after arrival (Taylor, 1986).

The role of *D. noxia* as a vector of BMV is uncertain, as tests on seed, produced before this aphid was known to occur in South Africa, showed that most cultivars tested were infected with BMV, some to a large extent (Von Wechmar *et al.*, 1984). Brome mosaic virus and barley stripe mosaic virus (BSMV) have also been shown to be aphid transmitted by *D. noxia* and other cereal aphids such as *Schizaphis graminum* (Rondani) and *R. padi* (Von Wechmar and Rybicki, 1981). The relative efficiency of these vectors in relation to each other has not been determined, whilst BMV has been shown to be transmitted by stem and leaf rust (Erasmus, 1982). Virus infection appears to be widespread, but there is little data on the proportion of individual crops that is infected and this is likely to be the principle factor determining yield loss in crops.

1.5.7 The Russian aphid as vector of BMV

For the purposes of this study only *D. noxia* was used as vector, because it is the most abundant under field conditions, although other aphids, mites and rusts were reported to be able to transmit the virus (Rybicki, 1984).

The Russian aphid is far more numerous than the other aphids occurring in the summer rainfall area. This fact is supported by both Rothamstead suction trap catches (for flying migrants) (Fig. 4), Moericke (yellow pan) trap catches (for aphid migration within a crop) (Fig. 5) and the presence of aphids on tillers in a field (Fig. 6) (G.J. Prinsloo, Unpublished report).

Damsteegt and Hewings (1967) tested the vector status of a *D. noxia* clone with a Nebraska isolate of BMV and could not show any nonpersistent transmission of the virus. Persistent transmission occurred in 20% of the cases tested.

In South Africa the relative contributions of direct feeding damage caused by *D. noxia* and the damage attributable to virus carried by the aphid are still to be determined (Du Toit, 1986 (b)).

2.5.8 The epidemiology of BMV in the summer rainfall area

The success of a plant virus as a pathogen is determined not only by the properties of the virus but by its source, vectors, host range and the influence of environment on all or these. The interaction of all these factors determines the seriousness of a disease outbreak. A corollary of this is that if any one of these factors becomes unfavorable for virus survival or spread, a plant virus epidemic may decline or fail to develop (Randles, 1986).

2.2.2 Wheat aphid - *Schizaphis graminum*

This cosmopolitan aphid, which may have originated in Asia, has an enormous potential for multiplying and consequent damage to wheat, especially in the eastern OFS (Annecke and Moran, 1982). It is especially in the dry winter and early spring that populations of the wheat aphid can increase virtually to plague proportions, although only one outbreak, in 1958, occurred in the past 50 years (Annecke and Moran, 1982). It is doubtful however whether this aphid has played any significant role since the arrival of *D. noxia* because of the enormous competition for limited resources that the wheat aphid has to face.

During feeding the discharge from this aphid's salivary glands produces discoloured, chlorotic feeding marks, and it is in this way, as numbers increase, that young plants may be severely set back or killed (Annecke and Moran, 1982).

2.2.3 Oat aphid - *Rhopalosiphum padi*

This aphid occurs on all continents. The biology of this species has not been studied in South Africa. The aphid usually occurs in small colonies amongst large numbers of *D. noxia*, and it is only after the emigration of *D. noxia* at the end of the wheat growing season that *R. padi* reaches significant numbers. The oat aphid is usually not regarded as a serious pest (Annecke and Moran, 1982).

(McKinney, 1967). They infect nearly all plant cells, and are normally sap transmissible. Some, however, are restricted to phloem cells where they interfere with nutrient transport. Phloem-limited viruses induce general chlorosis and are transmitted principally by insects (Noordam, 1973).

Until 1950 reports of viruses in wheat were rare even though viruses in other plants were well known. Today, approximately 30 different viruses have been associated with the crop and it is possible that more exist since viral diseases in wheat tend to be overlooked or dismissed as nutritional or other nonparasitic disorders which they resemble (Slykhuis, 1976(a)).

The viruses most commonly found world-wide in wheat are BYDV, BSMV, WSMV, and wheat soil borne mosaic (WSBMV) (Wiese, 1977). Viruses reported to infect wheat in Africa are maize streak (MSV), African cereal streak (ACSV) (Wiese, 1977), BMV, BYDV (Von Wechmar and Wasserman, 1965; Von Wechmar, 1967), and CMV (Von Wechmar, 1985). The viruses mentioned fall into six taxonomic groups i.e., the luteo-, hordei-, bromo-, cucumo-, tobamo- and geminivirus groups (Matthews, 1982), with WSMV and ACSV as yet unclassified. The methods of transmission include leafhopper-, aphid-, mite-, mechanical- and fungal-transmission, with some overlap between some of the viruses (Rybicki, 1984).

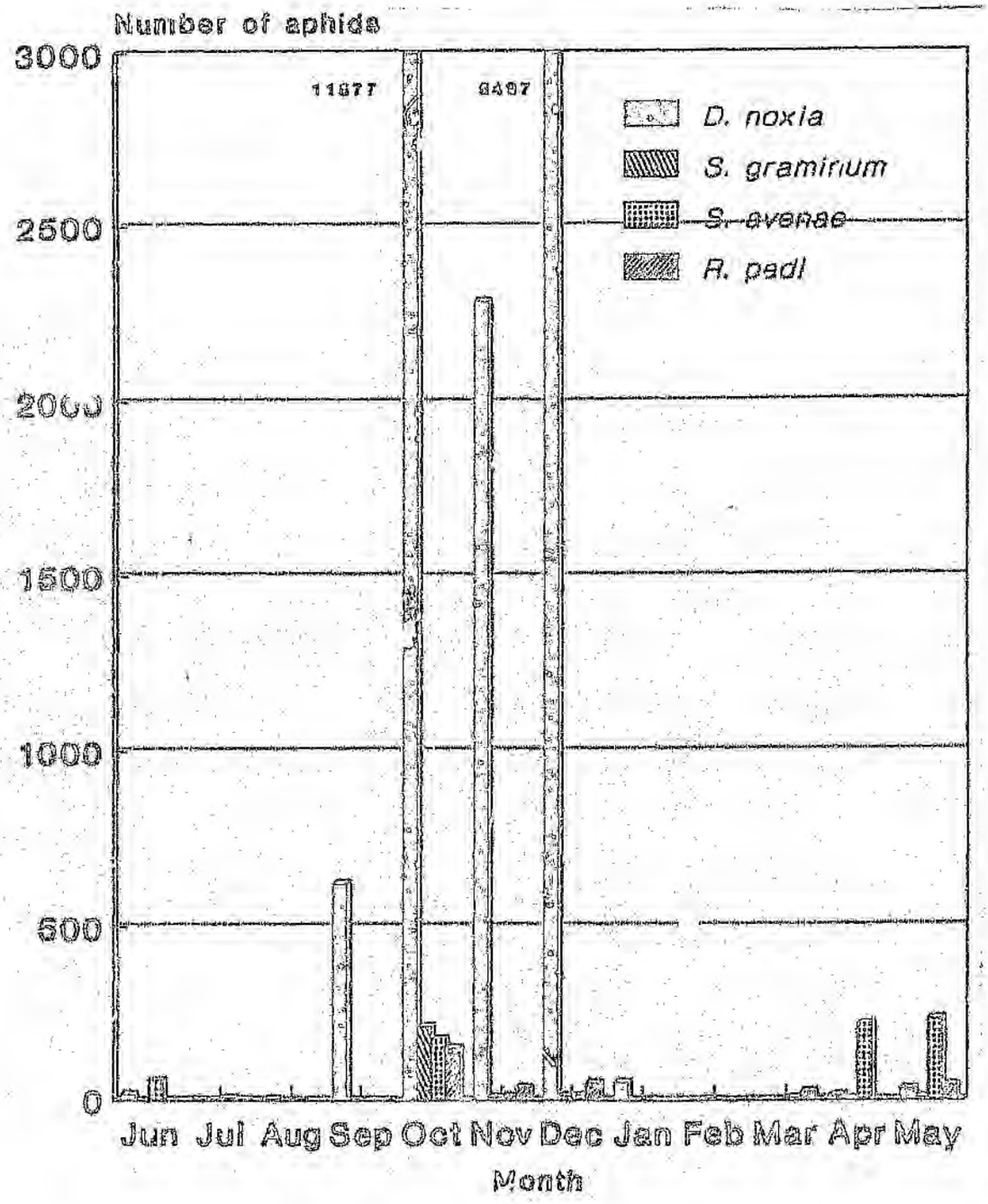


Figure 4. Different aphid species alates caught in a Rothamstead suction trap at Small Grain Centre during the 1989-1990 season. Numbers on the figure indicate aphid numbers in excess of 3000.

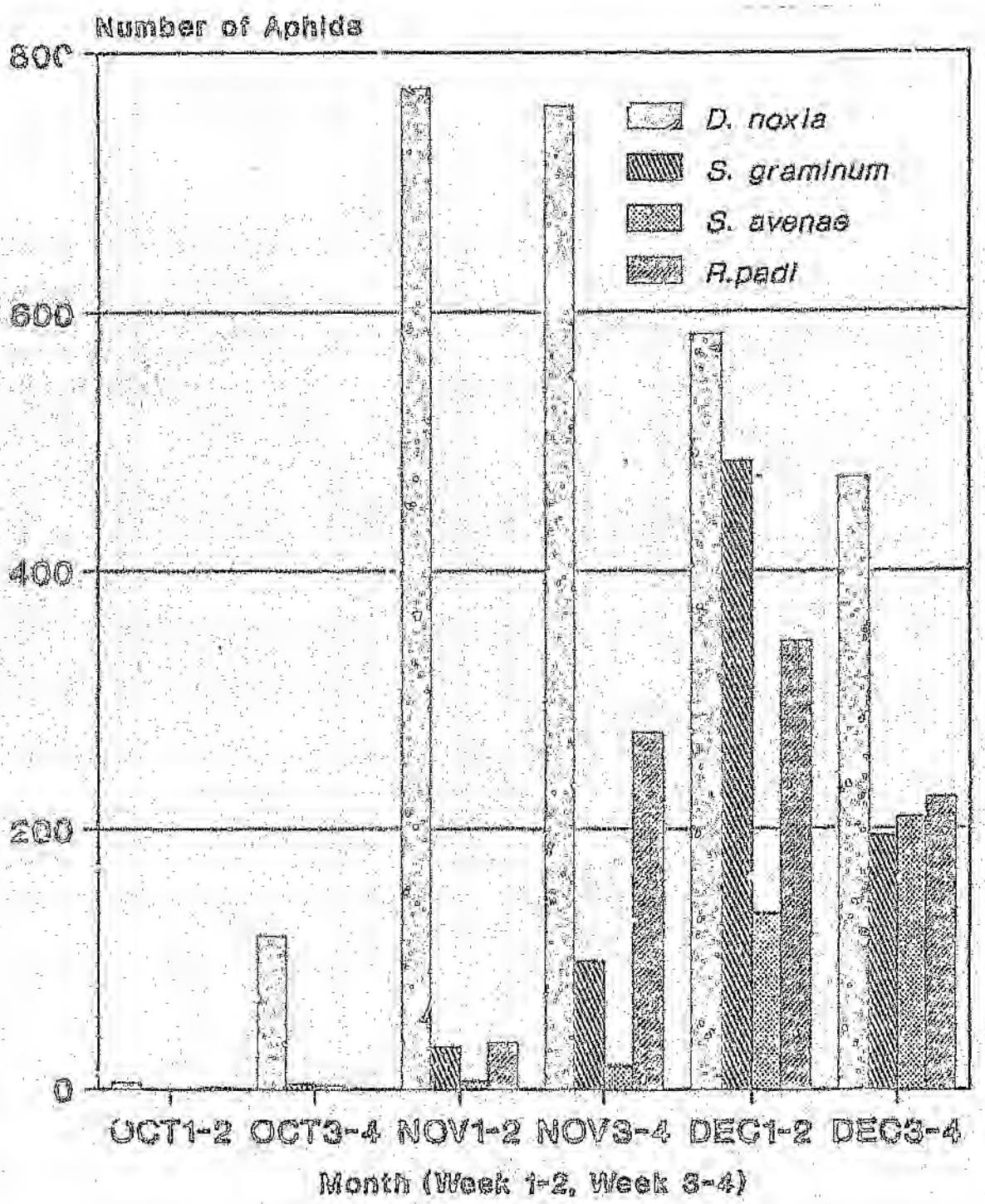


Figure 5. Different aphid species caught in Moericke (yellow pan) traps placed in a wheat field at Small Grain Centre during the 1989 season.

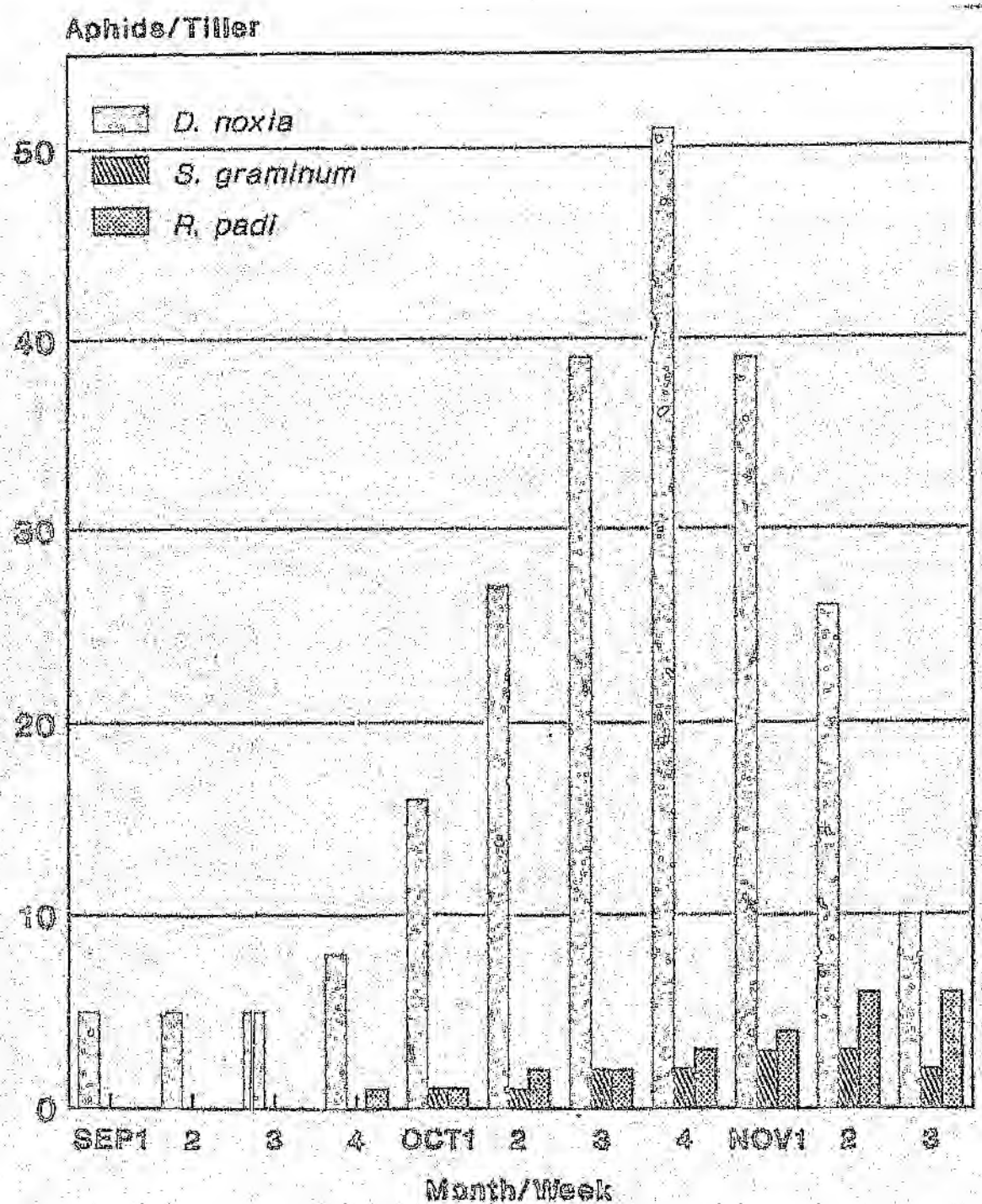


Figure 6. The relative numbers of aphid species colonising wheat tillers in a wheat field during the 1988 season.

The complexity of any virus epidemiological system can be illustrated by enumerating some of the aspects of each of the virus-vector-host-environment components that make up the system. For the virus, its particle stability and biological properties will be the main determinant of its ability to survive, spread, infect and induce disease. Vectors influence spread by their mode of interaction with the virus, as well as by their activity and behavior. Source and crop plants influence virus spread by their genotype, life cycle, size, age, density and colour, and they influence both virus and vectors. Environmental factors such as weather, soil, nutrients and topography are highly variable and their interaction with the other components are complex (Randles. 1986).

The influence of the various factors mentioned will be determined for the epidemiology of BMV. The main purpose of this will be to determine if BMV on its own constitutes a major wheat pathogen in the summer rainfall area, or if this virus is only a secondary side effect of the yield-limiting role that *D. noxia* plays in wheat cultivation in this area.

1.6 Computer simulation of the epidemiology of BMV

Plant virus epidemiology can be simulated using computer models. The quality of the predictions is entirely dependent upon the quality of the data from which the model is

prepared and the validity of the assumptions on which it is based (Frazer, 1977). Computer-based simulation modelling is a recent activity in science, even though modelling is not. There have been many attempts in recent phytopathological literature to define "model" and, although the term is widely used, it still does not have a single clear meaning (Teng, 1985). A model is any representation of an object, system, or idea in some form other than that of the entity itself (Shannon, 1975). Models are by necessity limited sections of reality (Zadoks, 1971), having some but not all the properties of real-life situations. Models are seldom complete, and modelling is an iterative process in which the model provides a closer and closer approximation of reality with each successive iteration (Teng, 1981). Thus, a model is never an end-point in itself (Krams and Doyle, 1978).

Since the inception of research on *D. noxia* in South Africa in 1978, a vast amount of work has been done on this aphid and problems related to it. It was especially the work of Adlbersberg (1987) that paved the way for a model to be constructed for this aphid. The complexity of the interaction between this aphid, its environment and the possible involvement with BMV has necessitated the development of a conceptual model at this stage.

The model represented in this thesis is a first attempt to provide a prediction system for BMV and *D. noxia* epidemiology. As such it only represents the first step on the

"testing-adapting-and-retesting" road. The data used to compile the model was obtained from various sources and the author remains in debt to these persons. The data on aphid behavior in the western OFS was obtained from Kriel (1984), and for the eastern OFS from Aalbersberg (1987), Du Toit (1986(b)) and Prinsloo (1990). The data on BMV was obtained from experiments by the author and by Rybicki (1984).

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION TO WHEAT DISEASES

Wheat plants, in all stages of growth, are subject to numerous injuries and stresses which interfere with their normal development. Any resultant abnormal condition, in a broad sense, is a disease. Each year about 20% of the wheat that otherwise would be available for food and feed is lost to disease. Diseases in the field and in storage claim about equal amounts. Weather, insects, viruses, fungi, nematodes, bacteria and weeds are primary hazards to wheat production. Wheat production hinges on sound management, pest (disease, insect, weed) control, improved cultivars, new technology and mass produced nutrients. Available nutrients and moisture, perhaps, are the most important factors that affect crop prospects (Wiese, 1977).

The actual number of wheat diseases is unknown. About 50 are economically important and nearly 200 have been described. All, whether infectious or non-infectious, are injurious in some areas, in some years and on some plant part. All draw attention because of symptoms or because of detrimental effects on the quality or quantity of plants, straw or grain (Wiese, 1977).

All parts of the wheat plant are subject to disease and one or more diseases occur on virtually every p.ant and in every field. The magnitude of disease damage is environment- and cultivar- dependent. For infectious diseases, the type, population and virulence of pathogens and their vectors also are influential (Wiese, 1977).

2.2 MAJOR PESTS OF SMALL GRAINS IN SOUTH AFRICA

2.2.1 Russian wheat aphid - *Diuraphis noxia*.

This aphid is well known in South Africa, and was first recorded in this country in the eastern OFS in 1978. In 1980 the species was reported in the south-western Cape Province. How it came into the country is not known. Early records of the species from Zimbabwe and Kenya may be questionable and require confirmation. Various aspects of the ecology and damage caused by this aphid are described by Hewitt *et al.* (1984); Kriel *et al.* (1984); Fouché *et al.* (1984); Krüger and Hewitt (1984); Du Toit (1986(b)), and several others.

Diuraphis noxia apparently originated in eastern Europe and western Asia where it is a known wheat pest. It was assumed to be the vector ... a virus infection of wheat because of the resultant yellowish streaks in the leaves, but this damage may be more consistent with extreme toxicity of the salivary secretion passed into the plant during the feeding process (Annecke and Moran, 1982).

2.2.4 Brown wheat ear aphid - *Sitobion avenae*

This aphid is common on wheat in Europe and western Asia, and may have o iginated there. This aphid becomes abundant on wheat ears during the winter and occurs almost everywhere wheat is grown in South Africa (Annecke and Moran, 1982).

There is no evide e to show that this aphid is capable of reducing a wheat crop or of reducing the quality of a wheat crop and is therefore regarded to be of little importance.

2.2.5 Other insects occurring on wheat

The grain chinch bug, *Macchiademus diplopterus*, occurs occasionally on wheat in the western Cape, and is regarded to be of minor importance. Other species of the order Hemiptera also occur on wheat from time to time, but the only other arthropod occurring on wheat in significant numbers is the brown wheat mite, *Petrobia latens*. No evidence of involvement of this mite in crop yield reduction has been reported to date (Annecke and Moran, 1982).

2.3 ECONOMICALLY IMPORTANT SMALL GRAIN VIRUSES

Virus infections in wheat range from latent to lethal, and induce stunting, rosetting, mosaics, yellowing and necrosis. The action of two or more viruses in a given plant (multiple infections) can be additive, synergistic or cross-protective

2.3.1 Barley yellow dwarf (BYDV)

This virus is probably the most widely distributed and researched virus disease of cereals (Rochow and Duffus, 1981). Barley yellow dwarf virus causes fairly significant crop losses in wheat and barley in North America, Britain and Europe, despite stringent control efforts over the past thirty years (Gill, 1970; Wiese, 1977; Carrigan et al., 1981). Barley yellow dwarf virus isolates display variations, especially in terms of serology and specificity of transmission by their aphid vectors (Aapola and Rochow, 1971; Paliwal, 1979; 1980). Rochow (1979) classified the more common isolates as:

(1) *Rhopalosiphum padi*-transmissible (RPV),
(2) *Macrosiphum avenae*-transmissible (MAV),
(3) *R.maidis*-transmissible (RMV),
(4) *Schizaphis graminum*-transmissible (SGV), and
(5) *R.padi* and *M.avenae*-transmissible (PAV).

Barley yellow dwarf virus transmission by its vectors is circulative and persistent. The virus does not multiply in the aphids and can be found specifically bound to accessory salivary glands after acquisition feeding (Rochow, 1969; Paliwal and Sinha, 1970; Gildow and Rochow, 1980). *R. padi*- and *R. maidis*-transmitted viruses are reported to be serologically related, but unrelated to *R. padi* and *R. maidis* non-transmissible isolates (eg. MAV and SGV) (Rochow and Carmichael, 1979).

Symptoms caused by BYDV are ambiguous and often overlooked or associated with nutritional or nonparasitic disorders. Barley yellow dwarf virus is diagnosed by the presence of aphid vectors and the occurrence of yellowed, stunted plants, singly or in small groups among normal plants. Recovery and transmission of the virus by aphids may be required for definitive diagnosis. This may not be a satisfactory means of diagnosis as was shown by Gill (1970), who found that relatively high yields of BYDV could be obtained from symptomless plants from diseased fields.

Leaf discolouration occurs [illegible] llow, red or purple and is found especially from [illegible] and from margin to midrib of the leaves. Seedling infections, if not lethal, slow plant growth and cause prominent or brilliant yellowing of older leaves. Yellowed or reddened flag leaves on otherwise normal plants are reported to be indicative of post seedling infections (Bruehl, 1961).

Most BYDV isolates cause stunting of their hosts, which are limited to the *Poaceae* but include most commercial grains such as oats, barley, wheat, maize, rice and rye, as well as many grasses. Tillering of wheat and oats may be reduced, but symptoms in wheat are less distinctive (Bruehl, 1961). The symptoms in leaves appear to be caused by blockage of phloem vessels due to the accumulation of virions in them (Jensen, 1969, Rochow and Duffus, 1981).

aphids, or mechanically, but there are many reports of seed transmission (Neergaard, 1977).

Von Wechmar (1985) reports that CMV appears to be the dominant virus responsible for diseased wheat in the Chrissiesmeer district, south eastern Transvaal. Alternate hosts for CMV are Japanese Radish in winter and possibly maize in summer.

2.3.4 Maize streak virus (MSV)

Maize streak virus is the type member of the geminivirus group (Matthews, 1982). The virus was first reported from South Africa in 1901, and its insect transmission was discovered in the 1930s (Fuller, 1901; Storey, 1939). It was also isolated and characterized in east Africa (Bock *et al.*, 1974), and has again recently been investigated in South Africa (Von Wechmar, 1967; Von Wechmar and Milne, 1983).

The virus has been reported from western, eastern and southern Africa and from India (Goodman, 1981). Several strains of MSV have been isolated from different Gramineae, and found to differ both serologically and in host range (Bock *et al.*, 1974). Symptoms in maize are characteristic interrupted parallel chlorotic leaf streaks or striations and usually severe stunting of the whole plant. Maize streak virus-infected wheat displays fine chlorotic streaks, and plants are often stunted, tiller excessively, and can be sterile (Von Wechmar, 1967; Wiese, 1977; Goodman, 1981;

2.3.6 Barley stripe mosaic virus (BSMV)

Barley stripe mosaic virus is perhaps the best known seed transmitted virus of small grains (Carroll, 1980). Although it is also seed transmitted in wheat and causes severe laboratory infections, no serious yield losses have been described in this crop (Wiese, 1977; Jackson and Lane, 1981). Owing to international exchange of germplasm and also to commercial seed shipments, the virus has a world-wide distribution in small grains (Timian, 1975).

The virus is the type member of the hordeivirus group and has a fairly wide host range in the Gramineae, infecting barley, wheat, oats, maize, ryegrass, millet and *Bromus* spp. amongst others. The virus also infects some dicotyledons such as some *Chenopodiaceae* (Atabekov and Novikov, 1971; Jackson and Lane, 1981).

Strain variations in BSMV are well documented (McKinney and Greely, 1965; Timian, 1974; Jackson and Lane, 1981), and all the strains described to date are apparently serologically related to varying degrees, although symptom expression and host ranges differ, and severity of infection differs between strains. Infections in wheat may be latent, or expressed as severe mosaic, chlorotic blotches, dwarfing, necrotic streaking, leaf tip necrosis, or a mixture of these (Wiese, 1977). Symptom expression and severity of infection in barley was found to be temperature- as well as strain-de-

the Gramineae and it infects rye, barley and various *Bromus* spp., but has been reported on certain *Chenopodium* spp. as well (Brakke, 1971).

Wheat (soil-borne) mosaic virus has a cosmopolitan distribution, occurring throughout the east and central USA, Japan, Egypt, Italy, Argentina and Brazil. It is claimed that the virus disease rivals the damage caused by BYDV in wheat in the USA and that economic damage to the crop can be severe, although crop loss depends on virus strain, wheat cultivar, environment and time of infection, with autumn-sown wheat in the USA being the most susceptible (Wiese, 1977). Disease symptoms are characteristically mosaics and mild to severe stunting and occasionally rosetting. Disease symptoms are suppressed as temperature increases, as is the severity of the effect on yield (Brakke, 1971; Wiese, 1977).

The virus is transmitted by the soil fungus *Polymyxa graminis* (Led.), an obligate parasite of the roots of many families of higher plants (Brakke, 1971). The virus is borne both internally and externally on motile zoospores, which enter the plants via the root hairs. The fungus flourishes best in cool wet periods when soil moisture content is high, while temperatures above 20° C are reported to inhibit fungus replication (Wiese, 1977).

2.4.1 Virus-Host related factors

2.4.1.1 Virus sources

If there is no source of virus, there would be no problem with virus diseases. This elementary statement is the key to understanding the epidemiology of virus diseases. Elimination or reduction of the amount of primary inoculum will determine to a large extent how readily aphids will spread a particular disease. The virus sources include a) infected seed, b) perpetuating sources (volunteer plants), c) similar or dissimilar crops and d) weed hosts.

Seed-borne virus diseases which are additionally transmitted by aphids occupy a special place in the spread of virus diseases. The subject of seed transmission of plant viruses was reviewed by Bennet (1969). No semipersistent or persistent viruses were mentioned, but several nonpersistent viruses were.

Where seed acts as the principal source of virus in nature, plants grown from infected seed often serve as the primary source of inoculum for aphids. This appears to be the case for bean common mosaic virus (Bos, 1971), blackeye cowpea mosaic virus (Gay and Winstead, 1970; Zettler and Evans, 1972), peanut mottle virus (Paguio and Kuhn, 1974; Demski and Kuhn, 1975), and lettuce mosaic virus (Grogan *et al.*, 1952; Zitter and Guzman, 1974; Bruckhart and Lorbeer, 1976).

In the western Cape, BYDV occurs in small grains along the west coast, but is not considered a serious problem. Von Wechmar (1988) reports that in the south western coastal region the situation is confused by the presence of other viruses such as CMV and BMV, which cause yellowing symptoms in small grains and are probably responsible for more economically significant crop losses.

Von Wechmar (1988) also stated that in the eastern OFS, wheat has a particularly high incidence of BMV infection, often occurring together with CMV. Barley yellow dwarf virus infection was found to occur as well, but is not considered to be a serious problem in the eastern OFS.

2.3.2 Brome mosaic virus (BMV)

Brome mosaic virus is the type of member the bromovirus group, which includes the serologically related broad bean mottle virus (BBMV) and cowpea chlorotic mottle viruses (CCMV) (Hollings and Horvath, 1978; Lane, 1981; Matthews, 1982).

Brome mosaic virus has been isolated from wheat and pasture grass in the USA (Wiese, 1977), in the UK (Gibson and Kenten, 1978), in Europe (Proll and Richter, 1965), and in South Africa (Von Wechmar and Van Regenmortel, 1966; Von Wechmar, 1967). Brome mosaic virus is found in wheat, barley, maize, oats and pasture grasses under natural infections world-wide, but it is not considered to be of any eco-

economic importance outside South Africa (Wiese, 1977; Lane, 1981). Brome mosaic virus also infects a few dicotyledons such as *Datura* spp. and some of the Chenopodiaceae (Chiu and Sill, 1959; Rochow, 1959; Lane, 1974; 1977; 1981).

The virus sometimes causes mosaic symptoms on Gramineae but some wheat cultivars are apparently symptomless carriers of BMV, whilst others may display chlorotic or white streaking, yellow-green mosaic, yellow mottling, head stunting or other deformities (Von Wechmar, 1967; Wiese, 1977; Lane, 1981). During the course of this study it was often found with both mechanical and natural infections under field conditions that streaking of the leaf laminae only occurred in the distal half of the leaf. Proll (1967(b)) found that the highest concentrations of the virus also occurred in this region of the leaf and that symptom severity and virus concentration could be directly linked.

Until very recently, BMV had no known vectors under field conditions, although Schmidt *et al.* (1963) and Fritsche (1975) succeeded in the laboratory transmission of the virus by *Xiphinema* spp. The cereal leaf beetle, *Oulema melanopus* (L.) has been reported as a natural transmitter under field conditions (Lane, 1981; Gaborjanyi and Szabolcs, 1987). Both stem- and leaf-rust fungi of wheat, *Puccinia graminis* f. sp. *tritici* and *P. recondita* (Von Wechmar, 1980; Erasmus, 1982; Erasmus and Von Wechmar, 1983) have been reported as vectors of BMV. In addition it was also reported that BMV may be transmitted by grain aphids (*R. padi* and *D. noxia*)

both in the laboratory and under natural conditions (Von Wechmar and Rybicki, 1981), although this has not been observed outside South Africa (Lane, 1981). Von Wechmar and Rybicki (1981) suggest that the numerous natural vectors could account for "- the severity of natural BMV infections in small grains in the eastern OFS and elsewhere in South Africa, where the virus appears to be the major cause of economically important crop disease -".

2.3.3 Cucumber mosaic virus (CMV)

Cucumoviruses have a very wide host range which includes herbaceous and woody plant species. By far the most widespread and most studied is the type member, CMV. Cucumoviruses are isometric particles about 30 nm in diameter. They are transmitted mechanically and by aphids (Kaper and Waterworth, 1981).

The most common symptom caused by CMV is mosaic (Kahn and Scott, 1964; Gracia and Feldman, 1977), and severity may range from no obvious symptoms in some crops to death of the host in others (Mckinney, 1953).

Perhaps the most notable characteristic of the natural spread of cucumoviruses is that the viruses are transmitted by numerous species of aphids (Fritzche et *al.*, 1972). Cucumoviruses are transmitted in a nonpersistent or stylet-borne manner (Zitter, 1977). They are not known to be transmitted by fungi, nematodes, or insects other than

Scott *et al.*, 1985). Maize streak virus usually occurs in irrigated wheatlands that are close to maize growing areas, with autumn-sown winter wheat being especially susceptible owing to migration of leafhoppers from ripening maize to young wheat (Scott *et al.*, 1985).

2.3.5 African cereal streak virus (ACSV)

This virus was first described in east Africa in 1973, after causing severe crop damage on oats in Kenya from 1969-1972. ACSV is phloem-limited and is obligatory transmitted by the planthopper *Toya catalina* (Fennah) (Harder and Backer, 1973; Harder, 1975), which could account for its low-land occurrence in Kenya, as the planthoppers' geographical distribution excludes high-lands (Harder and Backer, 1973; Wiese, 1977).

The virus infects barley, oats, rye, triticale, rice and several grasses. Symptoms are faint discontinuous chlorotic leaf streaking and can spread across the entire leaf which may eventually become completely yellowed. Infected seedlings are often stunted and severely chlorotic and usually die (Harder and Backer, 1973).

Toya catalina has not been reported in the OFS wheatlands, which are at a fairly high altitude (average 1500m), and may have a more temperate climate than the Kenyan low-lands. These factors could prevent the spread of the virus in this country (Rybicki, 1984).

pendent. An increase in both symptoms and severity was directly linked to a temperature increase (Jackson and Lane, 1981).

Efficiency of seed transmission of BSMV depends on the virus strain, the host plant and the ambient temperature. Some barley cultivars are not affected by either sap inoculation or seed transmission. No wheat cultivars appear to be resistant although the incidence of seed transmission is apparently lower in wheat than in barley (Hagborg, 1954; Jackson and Lane, 1981). The virus also appears to be pollen transmitted (Carroll, 1974).

Barley stripe mosaic virus has no known natural vectors and it is reported that the virus spreads from seed-infected plants, either by mechanical transmission due to inter-plant contact or damage by farm implements and by pollen transmission. No aphids, beetles, leafhoppers, rust or soil fungi are known to be implicated in natural transmission of this virus (Wiese, 1977; Jackson and Lane, 1981).

2.3.7 Wheat (soil-borne) mosaic virus (WSBMV)

This virus was identified in 1919 and is one of the first described wheat viruses, and also the first reported to be soil-borne (Wiese, 1977). Wheat (soil-borne) mosaic virus is a furovirus, serologically related to TMV and potato mop-top virus (PMTV) (Cooper and Harrison, 1972; Powell, 1976). Its host range appears to be essentially limited to

2.3.8 Maize dwarf mosaic virus (MDMV)

Maize dwarf mosaic virus is a strain of sugar cane mosaic (SCMV) and has been found in wheat in the USA (Wiese, 1977). Maize dwarf mosaic virus only rarely causes wheat disease and occurs in wheat grown near maize or infected Johnson grass, and is presumably spread by *Rhopalosiphum, Schizaphis, Macrosiphum* and *Myzus* spp. (Wiese, 1977). In wheat it produces mild leaf mottling, but is not regarded as being economically important.

2.3.9 Wheat streak mosaic and other filamentous wheat viruses

Wheat streak mosaic virus (WSMV), Hordeum, Agropyron- and ryegrass mosaic viruses are similar in appearance to the potyviruses in that they are elongated and filamentous, their dimensions, however, are regarded as being too short to be true potyviruses (Hollings and Brunt, 1981). All the viruses are, in addition, transmitted by eriophyid mites rather than by aphids, and are not serologically related to any of the recognized potyviruses (Hollings and Brunt, 1981), and are sap-transmissible (Slykhuis, 1973).

Wheat streak mosaic virus causes disease in wheat throughout the USA and in areas of eastern Europe. In the central great plains of north America it can cause significant crop losses (Wiese, 1977). Wheat streak mosaic virus is also found in spring wheat, barley, maize and other grain crops,

as well as in several perennial grasses, indicating a fairly broad host range (Slykhuis, 1973; Wiese, 1977). The other three viruses mentioned all infect Gramineae but only Agropyron mosaic appears to cause serious damage in wheat (Slykhuis, 1976(b)). Symptoms of WSMV in wheat are variable, but are most often manifested as green-yellow parallel streaks (Wiese, 1977).

Wheat streak mosaic virus is reported to be transmitted by *Aceria tulipae* (Keif.) and by *Eriophyes tosichella* (Keif.). Agropyron mosaic is transmitted by *Abacarus hystrix* (Nal.). The vector of Hordeum mosaic is unknown, but is presumed to be a mite (Wiese, 1977). These viruses have not been reported to occur in South Africa, and the mite vectors of WSMV have not yet been found in the wheatfields of the OFS (Rybicki, 1984).

2.4 EPIDEMIOLOGY OF APHID-BORNE VIRUSES

The epidemiology of aphid-borne virus diseases can best be reviewed as the interaction of the virus, host, vector, environment, actions and cultural practices of the grower, and the effects of time (Zitter, 1977).

Lettuce mosaic is a good example of a virus which, although transmitted at a relatively low level in seed, can be spread extensively by aphids. Workers in California (Zink *et al.*, 1956) and England (Broadbent *et al.*, 1951) established that if seed transmission exceeded 0.1%, control would not be satisfactory. The number of infected lettuce seedlings emerging per acre at the 0.1% rate would be 200, or 20000 infected seedlings/100 acres (Greathead, 1966).

Among the important perpetuating virus sources are infected propagative plants, tubers and volunteer plants carried over from the previous season. Historically, perpetuating sources have been the chief means of wide spread dissemination of several virus diseases. For example, the occurrence of sugarcane mosaic virus in most areas can be traced directly or indirectly to infected seed cane from Java (Summers *et al.*, 1948).

Although volunteer plants may not appear to be a particularly important virus source, here again aphids can greatly enhance the role of these sources Examples of aphid-borne virus diseases associated with volunteer plants are onion yellow dwarf (Zitter 1977), beet mosaic and beet western yellows (Wallis, 1967(b); Howell and Mink, 1971), beet yellows (Bennet, 1960), turnip mosaic (Laird and Dickson, 1963), and peanut mottle (Demski and Kuhn, 1975).

Similar or closely related crops can often serve as virus sources. Studies in the United States have indicated that

sugar beet itself is an important source of beet mosaic and yellows viruses for introduction into subsequent beet crops (Shepherd and Hills, 1970; Duffus, 1973).

Weeds are usually intricately linked to the distribution and spread of virus diseases. In terms of the total number of aphid-transmitted virus diseases which depend on weeds as their primary source of inoculum, weed hosts must be considered the single most important virus source (Duffus, 1971).

Aphid-borne virus diseases belonging to virus groups which are nonpersistently transmitted seem to depend heavily upon weeds as virus sources. Chief among these viruses would be those with wide host ranges exemplified by alfalfa mosaic and cucumber mosaic viruses (Litter, 1977).

Two semipersistent viruses in which weed hosts can act as virus reservoirs are beet yellows virus and beet yellow stunt virus (Duffus, 1973). He stated that there was little direct evidence that weeds serve as a major source of infection of beet yellows, but may simply perpetuate the disease.

The occurrence of several diseases caused by persistent viruses is closely linked with weed hosts. This is particularly true with beet western yellows virus which is more widely distributed than any of the other beet yellowing viruses (Duffus, 1964(a); Wallis, 1967(b)). The other major virus which has been widely studied is BYDV. This virus

overwinters in perennial grasses as well as in autumn-sown small grains (Bruehl, 1961).

2.4.1.2 Host response and behavior of virus in the source plant

Factors which influence susceptibility of plants to infection by aphid-borne viruses were reviewed by Swenson (1969). Among the host factors mentioned were inherent genetic differences, plant age and age of leaves at the time of inoculation.

Generally, the older the plant, the more difficult it is to be inoculated by aphids. The age of leaves can also influence susceptibility as was shown in the infection of potato with potato virus Y (Bagnall and Bradley, 1958) and bean with cucumber mosaic virus (Stimmann and Swenson, 1967). An important consideration in evaluating plant susceptibility to virus infection is distinguishing between results obtained by mechanical rubbing and those achieved by aphid inoculation (Nault *et al.*, 1971; Aapola *et al.*, 1974).

Older plants are less susceptible to inoculation by aphids and aphids are less likely to recover virus from older infected plants. This is probably the result of lower virus titers in plants infected for a long time (Zitter, 1977).

Two patterns of host-virus relationships among nonpersistent viruses were discussed by Swenson (1967). In the first re-

lationship, a relatively high concentration of infective virus is maintained in successive new leaves of systemically infected plants, as in the case of bean yellow mosaic virus (Swenson, 1962), potato virus Y (Simons, 1958), cellery mosaic virus (Zitter, 1970), and pepper mottle virus (Zitter, 1975). In the second relationship, a peak concentration of infective virus is reached in certain leaves but is not maintained in these or succeeding leaves, as in the case of cucumber mosaic virus (Simons, 1958; Stimmann and Swenson, 1967; Zitter, 1970) ar an common mosaic virus (Zettler, 1969).

2.4.2 Aphid-Virus Relationships

Viruses exhibiting a nonpersistent (stylet-borne) relationship are those which can be acquired in brief probes and can be just as quickly transmitted to another host. The longer the virus acquisition period is, the less likely aphids are to transmit these viruses, and they are not retained longer than an hour by feeding aphids and usually not more than several hours by non-feeding aphids. Migrant aphids are ideally suited for the spreading of nonpersistent viruses under these conditions, because they alight and probe on many hosts but do not feed or breed. Without further access to another virus source plant, however, the aphid soon loses the virus. The nonpersistent relationship has a great influence on the distance of virus spread. Limited or marginal spread has been noted in many instances (Wellman, 1937; Watson *et al.*, 1951; Simons, 1957; Adlerz, 1972;

1974(b); Demski, 1975), although the distance of spread may be greater depending on circumstances (Shepherd and Hills, 1970).

Viruses with a persistent relationship require more time for virus acquisition, and the longer the acquisition period the greater the chance that the aphid will be viruliferous. A latent period of varying duration is required before the aphids are able to transmit, but once the virus has been acquired, it can be retained by the aphids for many days, if not for life. This long retention factor serves to compensate somewhat for the prolonged acquisition period and vector specificity which seems to be typical for persistently transmitted viruses. By being less dependent on nearby virus sources, virus spread may be more long ranged as in the case of beet western yellows virus (Duffus, 1973), BYDV (Gill, 1970; 1975), and subterranean clover stunt virus (Gutierrez *et al.*, 1971).

Viruses with a semipersistent relationship have characteristics which are intermediate between the other two methods of transmission. Acquisition and inoculation are not accomplished in brief probes, and the probability of acquisition increases with increased acquisition periods of up to 12-24 hours (Swenson, 1968). Preliminary fasting does not enhance acquisition and there is no definite latent period. Aphids can retain these viruses for 1-2 days which allow for a greater distance of spread than with the stylet-borne viruses. This appears to be the case for the spread of beet

yellows virus (Shepherd and Hills, 1970) and beet yellow stunt virus (Duffus, 1973).

2.4.2.1 Aphid populations and resultant virus spread

Of all the factors which can influence the amount of virus spread in a season, probably the single most important factor is the number of aphid vectors available (Zitter, 1977). Usually good correlations have been made between trapping information and the amount of spread in such crops as potato (Broadbent, 1950), sugar beet (Gonzalez and Rawlins, 1969), pepper (Zitter and Ozaki, 1973), and watermelon (Adlerz, 1974(b)).

Aphid activity rather than aphid number was also considered important in the rate of dissemination of strawberry viruses (Shanks, 1965). The timing of aphid flights and its relationship to the age of the susceptible crop can be just as important as aphid numbers (Heathcote, 1966; Gill, 1970; 1975; Ishii, 1972). The distribution and abundance of virus sources can also restrict or delay virus spread (Adlerz, 1974(a); Knocke et al., 1974). Finally, differences in aphid transmission mechanisms mentioned earlier and aphid-virus specificities, can greatly influence the amount of spread (Zitter, 1977).

2.5 SIMULATION OF EPIDEMIOLOGY

Frazer (1977) suggested that simulation modelling could be a useful technique in the study of virus epidemiology but it appears that few have taken up his idea.

Simulation models can either be deterministic, in which case the mean values of parameters and variables are used and as such the model need only be run once with any particular set of parameter values, or stochastic, where values are dependent on the probability functions and hence there is a range of outcomes for each particular set (Carter, 1986). The former type is more convenient to use but may in certain cases lack biological realism (Fransz and Smith, 1974).

2.5.1 Definition of objectives

It would seem obvious that this step is the first and crucial one in a modelling exercise, but it has often been undervalued (Caswell *et al.*, 1972; Overton, 1977). The objectives determine the type of model to be constructed and, consequently, the amount and precision of data required (Berryman and Pienaar, 1974) and the criteria used to evaluate the model (Ruesink, 1976). Conversely, the complexity of the model developed might depend on the data already available and the effort required to collect more, that is, a model might have to be simplified if only limited information can be obtained with the available resources (Carter, 1986). Ideally a model should have generality, realism and

precision but it is difficult to reconcile all three (Levins, 1966). It is likely that the virologist would be concerned with the amount and rate of virus build-up, and how it is influenced by the size of the vector population, its transmission efficiency, host plant resistance and the consequences for yield (Carter, 1986).

2.5.2 Model structure

The first stage is to list the components, interactions and mechanisms thought likely to be important (Hall and Day, 1977) given the objectives of the modelling exercise. This stage can be initially crude and is then continually refined during model development as more information becomes available (Berryman and Pienaar, 1974). Components that effect others within the system, but are not influenced themselves, are placed outside it and in this way the system boundary is positioned.

The components that might influence aphid population development include: temperature, host plant quality and aphid density (Carter, 1986). The next stage is to represent the system diagrammatically, using relational diagrams of which there are several conventions. A convenient one is described by De Wit and Goudriaan (1978). Variables are classified into five categories, each represented by a symbol: a) State variables characterize and quantify the state of the system at any time, for example, numbers of insects, proportion of virus-infected plants, b) driving variables

are those which influence the system from outside but are not affected by processes inside, for example, temperature, c) rate variables quantify the rate of change of state variables, and their values are determined by state and driving variables, d) auxiliary variables are used as intermediate variables in complicated processes to aid understanding, for example, the use of aphid units to equate different instars for the calculation of rates of predation, and e) output variables are those variables which the model produces for the user - they may be state, rate or auxiliary variables and are chosen to fulfil the objectives of the exercise.

2.5.3 Model formulation

This step follows 2.5.2 and is used to quantify the relationships described qualitatively. For aphids and other species the variable-life-table method has been widely used (Hughes and Gilbert, 1968; Gilbert and Hughes, 1971), while for species such as leafhoppers, with several distinct generations per year (Sasaba et al., 1973), or moths with one generation per year (Dempster and Lakhani, 1979; Varley et al., 1973), a series of regression equations have frequently been employed. The variable-life-table method includes three basic population processes which vary with time, development, survival and reproduction (Fraser, 1977). Groups of insects are moved from one instar to the next (development), with the loss of some individuals at each stage (mortality/survival).

Temperature is often considered to be the main driving variable. Extrapolation of the linear relationship to give the temperature corresponding to the zero developmental rate gives the developmental threshold temperature (Campbell *et al.* 1974; Scopes and Biggerstaff, 1977). This threshold is 0.54 °C for *D. noxia* (Aalbersberg, 1987).

The time step of the model can either be real-time or a fixed unit of physiological time. For aphid models one quarter of the physiological units (a quip) of any of the first three instars (an instar period) is a convenient length (Gilbert *et al.*, 1976). The advantage of a real-time step, however, is that the model predictions are immediately comparable to field observations (Carter, 1986). However, if temperatures increase during the season, the amount of daily development also increases and should be taken into consideration.

2.5.4 Model verification and validation

Before a model can be used to give advice on control measures, it has to be verified and validated. These two terms for model testing are often used interchangeably but it is useful to differentiate between them. Verification comprises the comparison of the structure and general behavior of a model with the real system (Jeffers, 1978) and ensures that the model operates on the data in the intended way, that is, it is a kind of debugging (Loomis *et al.*, 1979). Validation is the quantitative comparison of output from the

model with observed results not used to develop the model (Jeffers, 1978; Carter *et al.*, 1982). A model which fails either verification or validation may have errors in its structure or its formulation or both.

One undesirable aspect of modelling is calibration (or tuning) which involves changing parameter values to improve the fit of the model to observations. This may result in the lowering of the model's explanatory role and degenerate into sophisticated curve fitting (Loomis *et al.*, 1979). In certain circumstances it may be impossible to estimate the value of a parameter under field conditions and calibration may be the only way of determining it (Carter, 1986).

The most common way of validating a simulation model is by direct comparison of the model output with independent sets of data (Carter, 1986).

2.5.5 Model manipulation and implementation

Sensitivity analysis is the name given to the process of experimenting with models and it involves the changing of model structure and parameter values. It is carried out after model formulation and should be used continually during formulation to determine the importance of processes (Carter, 1986).

Two forms of sensitivity analyses are recognized; a coarse form where processes are omitted from the model to test

their overall effect, and a fine form where small positive and negative changes are made to parameter values to study the consequences for model output (Carter *et al.*, 1982).

Often only one parameter is changed at a time to test its effect on model output, especially where a model has many parameters. Changing more than one parameter at a time quickly leads to excessive numbers of simulations. A two parameter model requires eight simulations to test all combinations in both directions, and in general 3^{k-1} simulations are needed for a ^{k} parameter model (Carter, 1986).

It would have been advantageous to have been able to give a single procedure for the development of a simulation model and to follow this with descriptions of validated models which are already fully operational. As Frazer (1977) stated, the number of approaches to modelling is roughly equal to the number of modellers.

Some researchers feel that models remove the need to collect data. Nothing could be further from the truth as models help to ensure that the right data at the right precision level are collected (Carter, 1986).

CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

3.1.1 Cultivar choice

In all experiments reported on in this thesis use was made of the cultivar Betta, unless otherwise stated. The reason for this being that this cultivar is one of the most frequently planted under dry-land conditions in the eastern OFS. This cultivar has been one of the top five cultivars ranked according to popularity with wheat producers since 1985 (Anon 1985, 1986, 1987, 1988, 1989).

3.1.2 Plant yield components

After maturity the plants were harvested with a plot harvester, excluding the side rows of every plot. The seeds were then dried, cleaned and weighed. In most cases the yields per plot were transformed to tonne per hectare for statistical analyses. Where yields of single plants were determined, such plants were harvested by hand and the seeds removed from the ears with a single ear threshing apparatus.

Various yield components were measured during the course of experiments. This included the thousand kernel weight (TKW), which is a measure of the kernel weight or "filling" of

individual kernels. The flag leaf and the leaf directly beneath the flag leaf are mainly responsible for the provision of carbohydrates to the developing kernels (Thorne, 1965, Lupton, 1968). Damage to these leaves could therefore lead to the shortage of basic nutrients to the developing kernels. Damage of these leaves could also lead to a lowering of photosynthetic surface which also leads to undernourishment of the kernels. This usually provides a sensitive measurement of yield loss and was frequently used during the course of experiments.

Other measurements used were the number of seeds per plant and the mass of such seeds. These measurements were used to determine TKW according to the following formula : TKW = mass of seeds/number of seeds x 1000.

Although the number of tillers per plant and plant length are not directly involved in eventual yield, the differences in number of tillers and plant length do give an indication of the effect of infection on plant development. Where experimental procedure dictated such measurements, they were done as well. Other measurements included the number of plants per metre, ears per metre and virus concentrations per plant.

3.1.3 Growth stage determination

The growth stages (GS) of wheat were determined according to the scale of Zadocks *et al.* (1974), Tottman and Makepeace

(1979) and Tottman (1987). This scale was used because of the decimal nature of the scale, as well as the provision of numerous sub-classifications within a class. This ensured that accurate determinations of GS were possible. The nature of the scale is also such that rapid determination of GS was possible under field conditions, without first having to laboriously dissect plants to obtain meristems to determine physiological development. A short synopsis of the Zadoks scale is given in Table 1 to ensure clarity of procedures used in the experiments.

Table 1. A synopsis of the Zadoks scale for growth stage determination in cereals, with an approximation of the duration of every stage (Zadoks *et al.*, 1974)

GS	Description	Approximate duration*
0	Germination	36 days
10	Seedling growth	21 days
20	Tillering	27 days
30	Stem elongation	27 days
40	Booting	4 days
45-46	Flag leaf emergence	4 days
50	Ear emergence	14 days
60	Flowering	7 days
70	Milk development	6 days
80	Dough development	7 days
90	Ripening	30 days

* The duration of a GS is extremely variable and is influenced by cultivar and environment.

3.1.4 Chemical aphid control

Where experimental procedures demanded the control of aphid populations during the course of a season, demeton-s-methyl (25% emulsifiable concentrate (e.c.)) and parathion (50% e.c.)

was used in a ratio of 5[illegible] ml:650 ml respectively per hectare. This concentrate was either sprayed with a back-pack spraying apparatus or a tractor drawn spraying apparatus, determined by the size of the experimental plots to be treated.

3.1.5 Field experiments

Annual field experiments were done during the period 1985-1990 at the Small Grain Centre of the Research Institute for Grain Crops at Bethlehem. Wheat was planted in soil of the Avlon type with a depth of 600 to 700 mm.

Seeding densities used, unless otherwise stated, were 25 kg/ha, and the recommended fertilizer applications for the eastern OFS were used for all experiments. The planting dates were usually during the first two weeks of June.

Plot sizes were 10m x 5 or 6 rows of wheat, with inter-row spacing of 350 to 400mm, unless otherwise stated. The plots within an experiment were usually separated on all sides by 2m spaces to limit aphid movement between plots. The side rows of a plot were not used for any measurements and served as additional buffers between plots.

3.1 6 Statistical procedures

The experimental designs used were according to the methods described by Van Ark (1981). Experimental designs varied during the course of the study, but use was mainly made of 6 x 6

Latin square designs, or of random block designs with 5 or 6 repetitions. Where data covering more than one season were needed, care was taken to use the same experimental designs and treatments during the following seasons.

In some cases factorial experiments were used, and in others correlation and regression analyses. Differences between means were tested at the P = 0.05 level of significance, and smallest significant differences between means were determined according to the Tukey test (Steel and Torrie, 1960).

3.2 VIRUS ISOLATION FROM WHEAT IN THE SUMMER RAINFALL AREA

Samples of field-grown winter wheat (cv. Betta) which showed symptoms typical of a virus infection were collected from various localities in the eastern OFS for virus isolation and identification. The normal pH 4.0 acetate extraction method used for BMV was followed (Von Wechmar and Van Regenmortel, 1968; Rybicki and Von Wechmar, 1981).

Plant samples (100 g) were mixed with 200 ml of buffer containing 20mM sodium acetate and 80 mM NaCl, (Miller and Golder, 1950; Rybicki and Von Wechmar, 1981). The samples were homogenized thoroughly, adjusted back to the correct pH with 10 % (v/v) glacial acetic acid and left for 30 min at 22 °C to allow precipitation of plant components. After clarification by centrifugation at 15000 *g* for 10 minutes, the supernatant was adjusted to a final concentration of polyethylene glycol 6000 (PEG 6000) of

7.5 % (w/v) and NaCl of 2.5 % (w/v), stirred for 2 hours at room temperature and again centrifuged at 15000 *g* for 10 min to collect the precipitated virus.

Precipitates were then resuspended in 1/10 of the original volume of the acetate buffer, and subjected to 2 cycles of differential centrifugation by alternating a run at 235000 *g* for 80 min with a low speed run at 15000 *g* for 10 min.

3.3 CHARACTERIZATION OF VIRUS ISOLATED FROM WHEAT IN THE SUMMER RAINFALL AREA

Various analytical procedures were used to determine the identity and purity of virus isolates. Criteria used to determine purity were dictated by available apparatus and included ultraviolet spectroscopy, sucrose density gradient banding, electron microscopy and serology.

3.3.1 Ultraviolet Spectroscopy

The absorbance in the range 220 nm to 320 nm of all purified preparations was measured in a spectrophotometer, using an 1 cm path-length quartz cuvette.

The concentration of the isolates was determined according to the method described by Rybicki (1984). An extinction coefficient of 5.2 (Lane, 1974) was used for BMV isolates. The A_{260}/A_{280} absorbance ratio was calculated for every

isolate and the concentration calculated according to the following formula:

$$C_1 = A_{260}/A_{280} \times 2.6$$

where C_1 = Concentration of virus in mg/ml
A_{260}/A_{280} = Absorbance ratio
2.6 = Extinction coefficient/2

To determine the concentration of virus in the original isolate use was made of the following

$$C_2 = (C_1/m) \times b$$

where C_2 = Concentration of virus in original plant (g/kg)
m = Mass of plant material before isolation(g)
b = Volume of buffer used to resuspend isolate(ml)

3.3.2 Rate-zonal Centrifugation

Small aliquots (1.2 ml per tube) of the acetate-prepared samples were layered onto 10-40 % (w/v) sucrose gradients in 0.05 M phosphate pH 6.0 and centrifuged in a SW 28 Beckman rotor at 112000 g at 10° C for 3 hours. The sucrose columns were fractionated into 1 ml samples with a fraction collector, and the absorbance at 254 nm measured.

The method of McEwin (1967) for estimating the sedimentation coefficient of a particle banded at the centre of a tube (approximately 25 % w/w Sucrose) was used. The solute concentration corresponding to extrapolation of a linear gradient distribution to zero radius (Z_0) for the rotor and gradient was calculated from the formula:

$$z_0 = z_1 r_2 - z_2 r_1 \; / \; r_2 - r_1$$

where z_1 = minimum % w/w of sucrose gradient (10 %)
z_2 = maximum % w/w of sucrose gradient (40 %)
r_1 = meniscus of gradient i.e. minimum radial distance from centrifugal axis *
r_2 = bottom of tube, i.e. maximum radial distance from centrifugal axis *

* (These values were found in Table 9, Griffith, 1979.)

By using the tables in appendix 1 (Griffith, 1979) the Time integral ($\int$) values for sucrose at the meniscus of the gradient and at the separated zone for the particle was found.

The sedimentation coefficient (s) was calculated from the formula :

$$s_{20,w} = \int(25\%) - \int(10\%) \; / \; w^2 t$$

where $w^2 = (0.10472 \times RPM)^2$
t = duration of run

3.3.3 Infectivity Assays

Five-day-old wheat plants and *Chenopodium hybridum* were mechanically inoculated with the BMV isolates. A paste consisting of varying concentrations of the purified virus, in acetate buffer (pH 4), and celite (as abrasive) was prepared. This paste was rubbed into the leaves of the wheat plants with a 10 x 10 cm piece of sterile cheesecloth. Twenty minutes after application of the paste, the plants were sprayed with water to remove excess celite and other debris from the leaves. Wheat of the same age was also inoculated with celite and acetate buffer alone to serve as controls.

The wheat plants were then allowed to develop in an aphid-free greenhouse, and examined every second day for the development of macroscopic symptoms on leaves.

3.3.4 Anti-body preparation

Goat antiserum was prepared as described by Walkey, (1985) and Von Wechmar and Van Regenmortel (1968). IgG fractions were prepared from the serum by the method of Clark and Adams (1977). Serum was incubated at 37° C for 20 minutes with 1 ml of host p purified from virus-free plants. The host sap was obtained by grinding healthy plants in acetate buffer (pH 4, 0.03M) and filtering the sap through a single layer of cheesecloth. The serum was then centrifuged at 10000 *g* for 10 min. The supernatant was reincubated with host sap for 20 min at 37° C and centrifuged in the same manner.

The two precipitates obtained in this manner were combined, the pH adjusted to 2.9 with 0.1 N HCl, and centrifuged at 186000 *g* for 30 min. Ten ml of supernatant was decanted into 1 ml of 0.5 M phosphate buffer (pH 7), and after adjusting the pH back to 7 with 0.1 N NaOH, the globulin fraction was precipitated with an equal volume of saturated ammonium sulfate solution. The precipitate was resuspended in a 0.15 M NaCl solution and centrifuged again at 10000 *g* for 10 min to remove any denatured host material.

The globulin was then precipitated with ammonium sulfate solution and centrifuged at 10000 g for 10 min. The globulins were resuspended in 0.25M PBS pH 7.4 and dialysed in 12000 m.w. cutoff tubing overnight against a large volume of the same buffer.

Small aliquots were passed through an anion exchange column (Whatman DE 52, equilibrated with 0.25M PBS buffer). Fractions with absorbance of 1.4 or higher at 280 nm were pooled, and diluted to give an OD_{280} of 1.4 (= 1 mg/ml)

The purified IgG was conjugated with high-activity alkaline phosphatase (2500 U/mg, Seravac Laboratories) by incubation with 0.05 % (v/v) glutaraldehyde (Fluka) for 4 hours at 22°C (Rybicki, 1979). The enzyme:anti-body ratio was 2:1 (w/w). Conjugates were stored at 4°C in pH 7.4 PBS buffer with 1 % (w/v) bovine serum albumin and 0.05 % (w/v) sodium azide as preservative.

3.3.5 Serological tests

The BMV isolated with the acetate extraction procedure was compared to other isolates of BMV using the double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Rybicki and Von Wechmar, 1981). The antisera used were either obtained from the UCT or made as described in 3.3.4. These were conjugated as described by Erasmus (1982).

The RID technique (Slack and Sheperd, 1975; Von Wechmar *et al.*, 1984) was used for testing for seed borne BMV, as this method, although not as sensitive as the ELISA, provided a means for the rapid screening of a large number of samples.

3.3.5.1 Double antibody Sandwich ELISA (DAS-ELISA)

The procedure followed was essentially the same as that described by Clark and Adams (1977). Antibody was diluted in 0.05 M phosphate buffer (pH 7.0) to yield a final concentration of 4-5 µg/ml. Wells were coated with 200 µl of diluted antibody per well and incubated at 37° C for 1-2 hours. Plates were washed 4 times through a 5-minute soaking in PBS/0.05 % (v/v) Tween-20 pH 7.4 (PBS-T), then drained upside down on paper towelling. A post-coating step was usually performed to reduce background. This consisted of incubation with PBS-T/0.2 % (w/v) BSA pH 7.4 (PBS-T-BSA) the wells at 37° C for 30 minutes.

Samples of material to be tested were suspended in PBS-T-BSA pH 7.4. For most assays, antigens were serially diluted 2-, 4- or 5-fold. The samples (200µl/well) were incubated in trays at 37° C for 90-120 minutes. Trays were washed with PBS-T as before.

Conjugate suspensions (1 mg/ml specific antibody) were diluted in PBS-T-BSA, as determined previously by grid titration (Rybicki, 1979), to give an OD (max) value, after 60 minutes substrate incubation, of between 1.0 and 1.5 OD units. The

conjugate suspension dilutions were usually in the region of 1/250 - 1/500 (2-4 µg/ml antibody). The conjugate was incubated for a minimum of 2 hours at 37° C, or 16 hours at 4° C (Rybicki, 1984). The wells were washed as before.

The substrate solution was prepared freshly for each assay by dissolving p-nitrophenyl phosphate hexahydrate (1 mg/ml) in 10 % (v/v) diethanolamine, pH 9.8. The substrate (300µl per well) was added to the plate and then incubated for 30-60 minutes at room temperature.

Plates were usually read several times (at 405 nm) during the latter stages of incubation. A useful feature of the Titertek Multiskan as reported by Rybicki (1984) was the "matrix mode" option, which was used extensively to standardize results in different trays by printing OD values as percentages of a preselected maximum OD. Where quantitative values were needed, at least 10 repetitions per sample were done on the same plate. The intensity of the colour reaction of a standardized control (virus of a known titer) was used as a comparison of the reaction between different plates.

Although some controversy exists on using the ELISA as a quantitative test, use was made of the following:

$A = \Sigma v/a.m/v = \Sigma\ m/a$

where A = absorbance
m = mass of absorbing substance
v = volume of solution in microtitration plate well
a = cross-sectional area of microtitration plate well

(Titertek Multiskan operation manual, Eflaboy co., Finland)

Since Σ/a is a constant, the absorbance was proportional to the mass of the absorbing substance in the plate well. As the sample volume and dilution was known, and procedures standardized as far as possible, it was possible to determine the concentration of the virus present in the samples.

The procedure used to determine the validity of results presented included using all the negative control readings on a particular plate as a blanking control and subtracting these values from the readings of the positive tests. The positions of positive controls were randomized on the plates to eliminate errors due to position on the plate. Extensive use was made of various lattice designs for sample loading on plates as was recommended by Burrows et al. (1984), and a cut-off value was determined that correlated with virus titers obtained during isolation. Borderline cases were repeated and if a normal distribution of results was not obtained, the cut-off value was lowered. The absorbance value for cut-off at 405 nm generally used in tests was 0.15.

3.3.5.2 Radial immunodiffusion assays

The procedure used in these assays was essentially the same as that described by Slack and Shepherd (1975) and Von Wechmar et al. (1984). Plastic Petri dishes (10 cm diameter) were flooded with a thin layer (5 ml) of 0.5 % (w/v) Difco-Noble agar in 0.05 M phosphate/0.15 M NaCl (PBS) buffer pH 6.0, with 0.1 % sodium azide added as preservative.

Approximately 100 seeds were embedded in the cooling agar, and over-layered with 20 ml agar at 45 °C containing 1/50 diluted anti-BMV serum. Plates were incubated in humid boxes at room temperature overnight before examination by direct illumination, and manual scoring of positive results. The formation of precipitates around infected seeds was an indication of the presence of BMV.

3.3.6 Electron microscopy

Purified samples of acetate isolates were examined by electron microscope at the Department of Microbiology, University of the Witwatersrand. Samples were applied to Formvar-coated carboned grids, negatively stained with 2 % neutral phosphotungstate, and examined in a JEM-100S electron microscope.

3.3.7 Isolation and identification of other viruses occurring simultaneously with BMV under field conditions

Field collected material was also subjected to the following purification protocol for the isolation of CMV. All the steps in the procedure were carried out at 4° C.

Leaves were briefly rinsed in deionized water and then homogenized in 0.1 M sodium phosphate buffer, pH 8.0, containing 0.1 % diethyldithiocarbamate (DIECA) and 0.1% thioglycolic acid (TGA), in a leaf (g) to buffer (ml) ratio of 1:2. The homogenate was then centrifuged at 10000 *g* for 10 min.

The precipitate which consisted mostly of plant debris was discarded. Triton X 100 was added to the supernatant fluid to a final concentration of 2 % (v/v) and was stirred at 4°C for 15 min. The supernatant was adjusted to final concentration of 7.5 % (w/v) PEG 6000 and 2.5 % (w/v) NaCl, and stirred for a further 15 min at 4° C, and again centrifuged at 10000 *g* for 10 min. The supernatant was discarded and the pellet resuspended in a small volume of 0.05 M borate buffer, pH 9.0. The isolate was centrifuged again at 10000g for 10 min to remove the denatured host components, and then centrifuged at 235000 *g* for 70 min. The supernatant was discarded and the pellet resuspended in 0.05 M borate buffer. To keep virions intact a 4 % (v/v) formaldehyde solution in 0.05 M borate was added to the resuspension buffer in a 1:1 ratio.

Samples of CMV in dried lupin leaves supplied by Professor M.D. von Wechmar, Department of Microbiology, University of Cape Town, were ground in 0.05 M potassium phosphate buffer, pH 7, containing 0.02 M B-mercaptoethanol and 0.02 M DIECA, and inoculated into 5 day old wheat plants using the same procedure as described in 3.3.3. These plants were harvested 10 days after inoculation and the virus was then isolated with the method previously described. The isolate tested positively against an anti-CMV serum supplied by UCT and was then used to raise an anti-serum in goats.

The plants collected during surveys were also tested for CMV with the ELISA. Isolations of CMV were also done with the technique described previously.

The CMV isolation from field collected plants yielded no detectable virus both spectrophotometrically and when tested against BMV and CMV anti-sera with the ELISA. These samples were not tested any further.

3.3.6 Transmission of BMV from alternate hosts to wheat

Plants collected from the OFS and Natal were tested for BMV. Some of these plants were ground up and the sap used to inoculate wheat (cv. Betta) grown from a virus free seed source. These inoculated plants were tested for BMV six weeks after the inoculation.

Samples of these plants were replanted in pots in the greenhouse and placed singly in aphid-proof cages. Virus-free *D. noxia* were placed on these plants inside the cages (10 aphids/plant). On plants where successful colonisation took place, aphids were allowed to feed for two weeks, and were then transferred to virus-free wheat seedlings grown in pots. Six weeks after colonization the aphids were killed, and the wheat plants tested for BMV with the ELISA.

3.3.9 Natural occurrence of BMV in the summer rainfall area

A survey of BMV in the eastern and western OFS and Natal was made during the period February 1985 to April 1987. The plants tested included *Chenopodium* spp., indigenous perennial grasses (*Bromus* and *Eragrostis* spp.) and volunteer wheat. Any aphids found on such plants were collected and tested for BMV. The stratified random sample design (SRSD) technique described by Delp et al. (1986) was used for sampling the plants.

This technique is based on the division of the entire area to be sampled into uniform sectors. These sectors had to be non overlapping, and together they had to comprise the entire area to be sampled. Once the sectors had been defined, a random sample was collected from each sector. A feature of this sampling design was that every plant in the field had an equal chance of being sampled and provided an unbiased estimate of virus incidence. Another advantage of the technique was that the sectors were uniform and independent, and could thus be compared with variance analyses (Cochran, 1953).

At every monitoring point, 5' plants from the sectors laid out on the edges of ploughed fields were randomly selected. These plants were placed separately in marked plastic bags and brought to the laboratory and tested for BM'

3.4 EFFECT OF BMV ON THE WHEAT PLANT

3.4.1 Spread of BMV through infected plants

Sixty pots in the greenhouse were planted with ' plants each, using virus-free seed. The seeds were vernalized for six weeks at 4° C after germination in petri dishes to enhance tiller formation. The plants were divided randomly into three groups of 20 pots each.

The lower two leaves of every plant in the first group were mechanically infected with a purified BMV isolate during GS 15, using the method described in 3.3.3. This procedure was repeated with the next set of 20 pots at GS 30, and the last set of pots were infected at GS 45.

After two weeks the distal 2 cm of the leaves which had been infected initially, as well as parts of any new leaves which had appeared in the interim were removed and tested for BMV with the ELISA. The same procedure was repeated fortnightly until the plants started to ripen. At the end of the season the internodes, as well as any ears that had appeared, were tested for BMV.

3.4.2. Effect of BMV in the greenhouse

A trial was conducted in the greenhouse over two seasons. Wheat from a virus-free source, was grown in pots, after the seed had been vernalized for six weeks after germination at 4°C. In an attempt to simulate natural conditions, the night temperatures in the greenhouse were allowed to vary with ambient temperatures (-4 to 0°C) and the day temperatures were kept at 15° C for the first three months, and at 20° C for the rest of the growing season.

The plants were divided randomly into three groups of 160 each. These groups were mechanically inoculated with BMV at three different growth stages using the method described in 3.3.3. Initial infections were done at GS 15 (seedling), GS 30 (stem elongation) and GS 45 (flag leaf) respectively.

The three main groups were subdivided into four groups of 40 plants each and treated in the following manner: a) A control group which was treated with inoculation buffer and abrasive only, b) A group which was infected with purified BMV at a concentration of 0.001 mg/ml of inoculation buffer (Five ml of buffer per plant), c and d) The same as above but with virus concentrations of 0.01 and 0.1 mg/ml respectively.

These initial infection levels were chosen after isolations of BMV, from virus infected *D. noxia*, indicated virus concentrations in the same range.

The normal yield components were measured at the end of the season. Virus in leaves of five randomly selected plants of each group was isolated and the concentration determined spectrophotometrically. This was done by removing the bottom leaf of each plant two weeks after the first infection and thereafter another leaf every three weeks. In addition these leaves were tested for BMV with the ELISA. The yield measurements of these plants were not used in statistical analyses, as the removal of leaf fragments could have had a detrimental effect on the plants.

3.4.3 Effect of BMV under field conditions

Wheat, from a virus-free source, was planted over two seasons in the field. Plots of 2m x 5m with five rows of plants per plot were planted using a randomized block design with three treatments and five replicates of each. Only the middle three rows of each plot were used for experimental purposes.

The plots were divided randomly into three groups of five each. Eighty wheat plants per row in the middle three rows of every plot in the groups were mechanically inoculated with BMV at three different growth stages using the method described in 3.3.3. Initial infections were done at GS 15, GS 30 and GS 45 respectively.

The three main groups were subdivided into four groups of 20 plants each and treated in the following manner: a) A control

group which was treated with inoculation buffer and abrasive only, b) A group which was infected with purified BMV at a concentration of 0.001 mg/ml of inoculatic buffer, using five ml of buffer per plant, c and d) The same as above but with virus concentrations of 0.01 and 0.1 mg/ml respectively.

Strict chemical control of aphids was applied on a weekly basis throughout the season to ensure that no colonisation of the experimental plots took place.

The number of tillers per plant, seeds per plant, mass of seeds per plant and plant height was recorded at the end of the season.

3.4.4 Yield losses associated with BMV in different cultivars

Five of the cultivars commonly planted in the eastern OFS were planted over two seasons in plots of 10 x 4 meters in a 5 x 5 Latin square design. The planting date which was selected, was a mean of the recommended planting dates for the different cultivars.

The plots had ten rows of plants instead of the five normally planted in the other experiments. Twenty percent of the plants in the middle three rows of five adjacent rows of plants in a plot of each cultivar were r ndomly selected, and then mechanically infected with BMV just prior to the stage that all the cultivars were approaching GS 30. This infection

stage was chosen after it was found that infection at this growth stage had the greatest effect on yield.

Aphid colonisation was controlled chemically fortnightly throughout the season. Observations were made on a weekly basis to ensure that aphid colonisation did not take place, and the number of plants per plot developing BMV infection symptoms was recorded.

The plants were harvested at the end of the season and the yield per plot determined. This data were then correlated with the BMV infection levels of the plot concerned. The normal yield components were also measured.

3.5 SEED TRANSMISSION OF BMV

3.5.1 Localization of the site of BMV infection on seed

Four hundred seeds, from a source tested to be 88 % BMV infected with the ELISA, were individually soaked for 30 minutes in 1 ml of distilled water, removed, washed under running water for two minutes, and then dissected into embryo, seed-coat and endosperm. The three dissected parts per seed were then ground separately in PBS-Tween-BSA (pH 7)(400 µl per seed) and incubated overnight at 4° C. The dissected parts were then tested for the presence of BMV with the ELISA.

3.5.2 Effect of surface-sterilization of BMV infected seeds

Sixty seeds from the same source used in 3.5.1 were soaked in 1 % sodium hypochlorite solution for five minutes to determine the effect of surface-sterilization on BMV infection on seed. The seeds were rinsed five times in 1 ml distilled water per seed, dissected and tested in the same manner as mentioned in 3.5.1. Germination tests on seeds were done in the same manner as described in 3.5.3, both before and after sterilization to determine whether the loss in seed viability caused by BMV infection was influenced.

3.5.3 Effect of BMV seed-infection on germination

Nine groups of seeds with BMV infection rates of 0 %, 5 %, 10 %, 15 %, 20 %, 25 %, 30 %, 35 % and 40 % were identified with the radial immunodiffusion technique (400 seeds per test). These seeds (250 per group) were divided into groups of 50 and were allowed to germinate on moist tissue paper in petri dishes at 22 °C in the dark for 48 hours. After 48 hours the number of germinated seeds was recorded.

3.5.4 Effect of inoculum concentration and time (growth stage) of infection with BMV of the seed parent on seed produced

Twelve groups of seeds were obtained from the groups of plants used in 3.5.2. Five hundred seeds of each group were tested for BMV infection with the RID technique, and a further 120

seeds from each group were tested using the ELISA. Four hundred seeds from each group were used for germination tests (See 3.5.3). A further 100 seeds from each group were used for germination tests in soil in groups of 50 seeds each. These seeds were planted in seed trays in the laboratory and were allowed to germinate at room temperature without any additional lighting. Thereafter the percentage germination was recorded.

3.5.5 Transmission of BMV through seed to progeny plants

The seedlings in 3.5.4 were transferred to a greenhouse and allowed to grow for six weeks. The day/night temperatures were set at 20/10° C and the photoperiod at a 15/9 hour light/dark cycle. At the end of this period the plants, excluding the roots, were tested for BMV infection with the ELISA. These results were correlated with the initial seed infection and germination percentage.

3.5.6 Seed-transmission of BMV in different breeding lines

Thirty six of each of the winter and intermediate elite breeding lines were tested for BMV infection. Two hundred seeds of each line were used, half of which were tested for BMV and the other half used for germination tests (See 3.5.3).

3.5.7 Economic importance of seed transmitted BMV in wheat

A number of seed lots (cv. Betta) was tested with the ELISA until sources with 0 %, 10 %, 20 %, 30 % and 40 % infection levels with BMV were found. These seeds were planted in 10 x 2 m plots in a 5 x 5 Latin square design over two seasons.

After emergence of the plants they were sprayed with insecticide at weekly intervals. The number of plants per m^2 in the middle three rows of every plot was recorded every 25 days up to 150 days after emergence. The number of plants per plot, number of tillers per plant and number of plants per metre were also recorded at the end of the season. The plots were harvested individually at the end of the season and the yield and TKW determined.

3.6 APHID TRANSMISSION OF BMV

3.6.1 Incidence of naturally occurring viruliferous *D. noxia*

A number of aphids were collected at random from volunteer wheat and *Brome* grass. Half of these aphids were tested individually with the ELISA for BMV. The others were transferred to wheat grown from virus free seed, using an average five aphids per plant. Two weeks after initial colonisation these plants were sprayed with insecticide to kill the aphids. The plants were tested for BMV with the ELISA after another six weeks had elapsed.

3.6.2 Transmission method of BMV by *D. noxia*

3.6.2.1 Nonpersistent transmission

A colony of *D. noxia* raised on virus free wheat was used in this experiment. Aphids were removed from the initial plants and transferred to wheat which had been mechanically inoculated with BMV. Before transfers were done, the aphids were subjected to preacquisition starvation periods lasting from 5 - 60 minutes. The aphids were then transferred to wheat grown from virus free seed. Two hundred of the aphids were placed in a funnel covered with a double layer of cheesecloth, and washed for two minutes under running water to remove possible external BMV contamination. They were then homogenized and tested for the presence of BMV with the ELISA.

The rest of the aphids (5 per plant) were left on the new sets of plants for periods of 5 - 25 minutes after probing had started. The plants were then sprayed with insecticide and kept free of aphids until the assays were done. Ten replicates of each feeding time were done and the plants were tested for BMV with the ELISA four weeks after the aphids had been removed.

3.6.2.2 Persistent transmission

Aphids from the same colony of *D. noxia* used in 3.6.2.1 were used in a serial transmission experiment. After a preacqui-

3.6.2.1, five aphids per plant were transferred to wheat grown from virus-free seeds. The aphids were allowed to feed on the plants for 48 hours after initial colonisation and then transferred to a new set of plants. After five transfers the aphids were killed by spraying the plants with insecticide. The plants were kept in aphid-free conditions for a further four weeks and then tested for BMV.

3.6.2.3 The influence of the GS of the target plant and duration of exposure to viruliferous *D. noxia* on BMV transmission

Aphids from the same colony of aphids as in 3.6.2.1 was used for a third experiment. One pot of wheat mechanically infected with BMV and colonized by *D. noxia* was placed among six pots with three virus-free wheat plants each (replicated six times). At the start of the experiment all these plants were at GS 20. Aphids were allowed to colonize the surrounding pots naturally in a variation of the arena test described by Irwin and Ruesink (1986)

The aphids on the first set of six pots were allowed to feed for one week before being killed with insecticide. At this stage all the *D. noxia* on the mechanically infected wheat had colonized the new wheat in the surrounding pots, and the pots with the mechanically infected wheat were removed from the greenhouse. The second set of pots were colonized for two weeks before the aphids were removed. This procedure was continued for four more weeks. All plants previously sprayed

with insecticide were resprayed at weekly intervals to prevent recolonisation by aphids.

After the last set of pots had been treated in this manner a further four weeks were allowed to elapse before the purification of BMV from individual plants in the different treatments was started. The isolates were tested with the ELISA to confirm the identity of the virus. The concentration of BMV was then determined spectrophotometrically.

3.6.2.4 Vertical transmission of BMV in *D. noxia*

Samples of aphids that had become reproductive, determined by the presence of offspring on leaves colonised by the aphids, during the course of the experiment described in 3.6.2.3 were removed from the experimental plants and placed on moist paper in covered petri dishes. Random samples of these aphids were individually tested for BMV with the ELISA. This procedure was repeated with samples of the offspring.

The new-born aphids were transferred to virus-free wheat seedlings and allowed to feed for three weeks. After three weeks these aphids were killed with insecticide. The plants were allowed to develop further for four more weeks before being tested for BMV with the ELISA.

3.6.2.5 Effect of BMV on the longevity of *D. noxia*

Field collected samples of *D. noxia* were placed on pots of virus-free wheat grown in the greenhouse. When these aphids started reproducing, the new-born aphids were removed with a moist Camel hair brush and transferred to pots containing virus-free wheat and pots containing mechanically infected wheat in a growth cabinet. The temperature in the cabinet was controlled at 17 °C day and 11 °C night temperatures (12h:12h). Initially five aphids were placed on the two sets of plants. Ten pots of each treatment (virus-free and virus-infected) were used.

Clip cages were used to confine the aphids to the leaves (Aalbersberg, 1987). In order to ensure that the test aphids were exposed to the same or similar conditions to field aphids, they were transferred with a Camel hair brush to the youngest leaf as soon as it unfolded. The aphids were examined every 12 hours, and after the aphids had begun to reproduce, the nymphs were removed and counted every 12 hours. This was continued until the parent aphid died.

All the transfers were done simultaneously, using aphids as near to the same age as possible. The time from initial colonisation up to death was recorded. Instars of the aphids were determined according to the key of Aalbersberg (1987).

ii) Driving variables:

Temperature, Wind, Rainfall.

iii) Rate variables:

Change in growth stage, Change in virus concentration, Change in aphid population, Change in number of tillers per plant, Change in number of aphids per tiller.

iv) Auxiliary variables:

New plants infected, New plants infested, *D. noxia* population growth factor, BMV concentration change factor, Chemical control of *D. noxia* effectivity factor.

v) Output variables:

Days post emergence, Total number of *D. noxia* infested plants, Percentage of *D. noxia* infested plants, Total number of BMV infected plants, Percentage of BMV infected plants, Summary of environmental factors, Growth Stage of plants.

3.9.2 Model formulation

Model formulation was done using the corrected basic infection rate of Van der Plank (1963) for both aphids and BMV.

3.6.3 Effect of *D. noxia*-transmitted BMV on wheat in the greenhouse

Wheat (cv. Betta), from a virus free source, was grown in pots in the greenhouse, using the method described in 3.4.2. Three randomly chosen groups of 50 plants each were used, and treatments were applied to each group at a different growth stage, namely GS 15 , GS 30 and GS 45 respectively.

Ten plants in each group were randomly selected to serve as controls. The other 40 plants were removed from the greenhouse at the appropriate growth stage and transferred to a greenhouse set at similar temperatures and photoperiods. Approximately ten *D. noxia*, which had fed on wheat mechanically infected with BMV, were transferred to the experimental pots. The aphids were allowed to infest the wheat for two weeks after which they were killed by spraying with insecticide, and the plants returned to the original greenhouse. Different greenhouses were used to ensure that unwanted aphid infestation did not occur.

Sections of the lower two leaves of each plant were removed at four stages during the season and tested for BMV with the ELISA. The leaf sections were removed two weeks after the end of each treatment and again at harvest.

3.6.4 Effect of *D. noxia*-transmitted BMV on wheat under field conditions

Twenty four plots of 10m x 2m, separated by 2m spaces, were laid out in a wheat field which had a low *D. noxia* infestation level. Six randomly selected plots were sprayed for aphid control at weekly intervals from GS 30 onwards. The remainder were sprayed at weekly intervals from GS 30 until one week prior to artificial infestations. The treatments were as follows: a) Six plots were infested at GS 30 with 200 greenhouse reared viruliferous *D. noxia* for two weeks. After infestation the plots were sprayed and thereafter at weekly intervals until harvest, b and c) Infested at GS 45 and GS 55 respectively in the same manner as above.

A sample of the aphids used for the treatments was tested with the ELISA for BMV prior to the treatments to ensure that they were indeed viruliferous. Three weeks after the treatment 40 randomly selected plants from each plot were tested for virus and the percentage of infected plants per plot was determined. At the end of the season the wheat was harvested and the yields of the different plots determined.

3.6.5 Effect of seed- and *D. noxia*-transmitted BMV on wheat under field conditions

Wheat was planted over two seasons in plots of 10m x 2m with five rows of plants per plot, using a 6 x 6 Latin square design. Half of the plots, which had been randomly selected,

were planted with virus-free seed, and the other half with seed with a BMV infection rate of 25 %.

The plots were subdivided and the following treatments applied: Virus free seed: a) Six plots sprayed at weekly intervals to control aphids, b) Six plots naturally colonized by *D. noxia* up to GS 40 and then sprayed at weekly intervals, and c) Six plots colonized with greenhouse reared BMV infected *D. noxia* at GS 10 up to GS 40 and then sprayed with insecticide at weekly intervals. The same treatments were applied to the plots planted to BMV infected seed.

Sixty randomly selected plants in each plot were marked at the start of the season. They were harvested individually and plant length, number of ears, seeds and seed weight were recorded. The remaining plants in the middle three rows were harvested with a combine harvester and the yield was added to the yield of individually removed plants.

The sixty plants from each plot were individually tested with the ELISA for the presence and concentration of BMV. During the season records were also kept of aphid infestation on the sixty marked plants per plot.

The number of plants per running metre was also recorded to determine if the loss of seed viability observed in BMV infected seed also occurred under field conditions. The number of ears formed per running metre was also recorded. A count was also made at GS 40 of the total number of plants

germinated, of plants showing signs of aphid infestation, and plants showing an intense yellowing and stunted growth, indicative of viral infection.

3.7 COMPARISON OF THE EFFECT OF *D.noxia* FEEDING AND BMV INFECTION ON WHEAT UNDER FIELD CONDITIONS

Virus-free wheat seed was planted over two seasons in 6 x 6 Latin square design in plots of 10m x 2m with 2m spaces separating the plots on all sides. The following treatments were used, a) Six plots were sprayed for aphid control once weekly, b) Six plots were sprayed once weekly after GS 30, c) Six plots were sprayed once weekly up to two weeks prior to GS 30. They were then infected with laboratory reared virus free *D. noxia* for two weeks, after which normal spraying was resumed, d) Six plots where 10 % of the plants in the middle three rows were mechanically infected with 5 ml of BMV solution at a concentration of 1 mg of virus/ml of inoculation buffer, e) Six plots were infected using the virus concentration given in (d), but only 30 % of the plants in the three middle rows of a plot were infected, and f) Six plots were treated in the same manner as in (e), but with 50 % of the plants being infected.

A count was made at GS 40 of the total number of plants, of plants showing signs of aphid infestation, and of plants showing an intense yellowing and stunted growth, indicative of BMV infection. Samples of the plants showing these symptoms were tested for both BMV and CMV. Weekly counts of *D. noxia* infestation were also made throughout the season, to ensure

that infestation had occurred in the treatments intended for natural colonisation.

At the end of the season the plots were harvested individually and the yields determined. The yields were then correlated with the aphid counts made throughout the season and a comparison made between the *D. noxia* and BMV effect on yield.

3.7.1 Effect of BMV concentration on yield

Approximately four thousand wheat plants from the experiment described in 3.7 were randomly chosen from the plots at GS 40 and marked. At the end of the season these plants were removed from the field and BMV isolated using the method described in 3.2. The concentration of BMV in individual plants were determined with the method described in 3.3.1. Since the plants originated from plots subjected to specific treatments, the infection history by *D. noxia* for every individual plant was known. The BMV concentrations for every plant were then correlated with the yield of the plant.

3.8 EPIDEMIOLOGY OF BMV IN THE SUMMER RAINFALL AREA

A wheat field was divided into 36 10m x 10m plots with 10m separating the plots in all directions. The wheat between the plots was not removed to ensure a natural colonisation of the field by *D. noxia*. The object of this experiment was to determine the following:

3.8.1 Role of alternate hosts in the epidemiology of BMV

Volunteer wheat plants in the field, as well as *Bromus* grass and volunteer wheat surrounding the field, was tagged at the start of the season. Random samples of the volunteer wheat plants within the field and surrounding the field were tested for BMV and CMV with the ELISA at the start of the season. Samples of *Bromus* spp. were also tested in a similar manner.

3.8.2 Role of seed-transmitted BMV in the epidemiology of this virus

Samples of the seed used to plant the experiment were tested with the ELISA to determine the presence of seed-borne BMV. Random samples of plants in the field were also tested (1000 in all) two weeks after most seeds were fully germinated to determine the presence of seed-transmitted BMV.

3.8.3 Role of *D. noxia*-transmitted BMV in the epidemiology of this virus

Every plant in all the plots was monitored on a weekly basis, and records kept of *D. noxia* colonisation throughout the season. In this manner a comprehensive infestation record of all plants in the field was obtained. One hundred of the plants which had become infested during a specific week were randomly selected at the end of the season and harvested individually. The TKW of these plants was determined.

The presence and concentration of BMV in all plants that had become infested with *D. noxia* during the season was determined with the ELISA at the end of the season. Once every four weeks 100 plants, selected randomly, that had become infested by *D. noxia* during the preceding month were tested with the ELISA as well. Samples of *D. noxia* were also removed from infested plants during the course of the season and tested for the presence of BMV.

3.8.4 Natural dissemination of BMV under field conditions

The plots were subdivided into m^2 blocks and record was kept of the position within a plot of plants that were infested with *D. noxia*. As soon as a plant was infested with *D. noxia* the plant was tagged to ensure that the plant was not rerecorded at a later date. Record was also kept of the GS of a plant during initial colonisation.

The spatial distribution pattern of *D. noxia* was determined. Use was made of the Poisson series for random distributions. Since this method soon proved to be inadequate, use was made of the ordinary run technique.

Ordinary run analyses were done in the plots as was described by Madden *et al.*(1982). A run is defined as a succession of one or more infected or healthy plants. In - - + + - + - +++ there are six runs (U). The runs are"- -"+ +"-"+"-"+++". The expected number of runs under the hypothesis of randomness is given by:

$$E(U) = 1 + 2m(N-m)/N$$

Where E(U) = Expected number of runs

m = Number of infected plants

N = Total number of plants

The observed number of runs will be less than E (U) if there is clustering of infected plants. The standard deviation of U is given by:

$$S_u = \{ 2m(N-m)[2m(N-m)-N]/[N^2-(N-1)] \}^{\frac{1}{2}}$$

The standardised U is given by:

$$Z_u = [U + 0.5 - E(U)]/S_u$$

The constant 0.5 is the correction for continuity. The value of Z_u ($-Z_u > 1.64$ under a one sided left tail probability) will be a large negative number if there is clustering.

3.8.3 Effect of chemical control of *D. noxia* on BMV dissemination under field conditions

Half of the plots, selected randomly, and wheat surrounding the plots were sprayed for aphid control at GS 32 and GS 45. The aphid infestation in the other 18 plots was allowed to continue nat.. lly.

3.9 COMPUTER SIMULATION OF THE EPIDEMIOLOGY OF BMV

3.9.1 Model structure

The model structure was determined by the availability of data, and for certain factors an approximation of possible effects were done.

Factors influencing *D. noxia* populations were considered to be the following a) temperature, b) wind, c) rainfall and d) number of initial infestations. Parasites and predators were not considered to be important (Prinsloo, 1990).

Factors influencing BMV were considered to be a) growth stage of host plant, b) virus concentration in infected plants, c) efficiency of *D. noxia* vectoring and d) seed-transmitted BMV. The model structure is illustrated in Figure 7.

Based on these assumptions a structure was formulated, taking the various factors into consideration. The model was structured according to the method described by De Wit and Goudriaan (1978), as follows:

i) State variables:

Total plants infected, Total plants infested, Days elapsed, Percentage seed infection, Total number of plants.

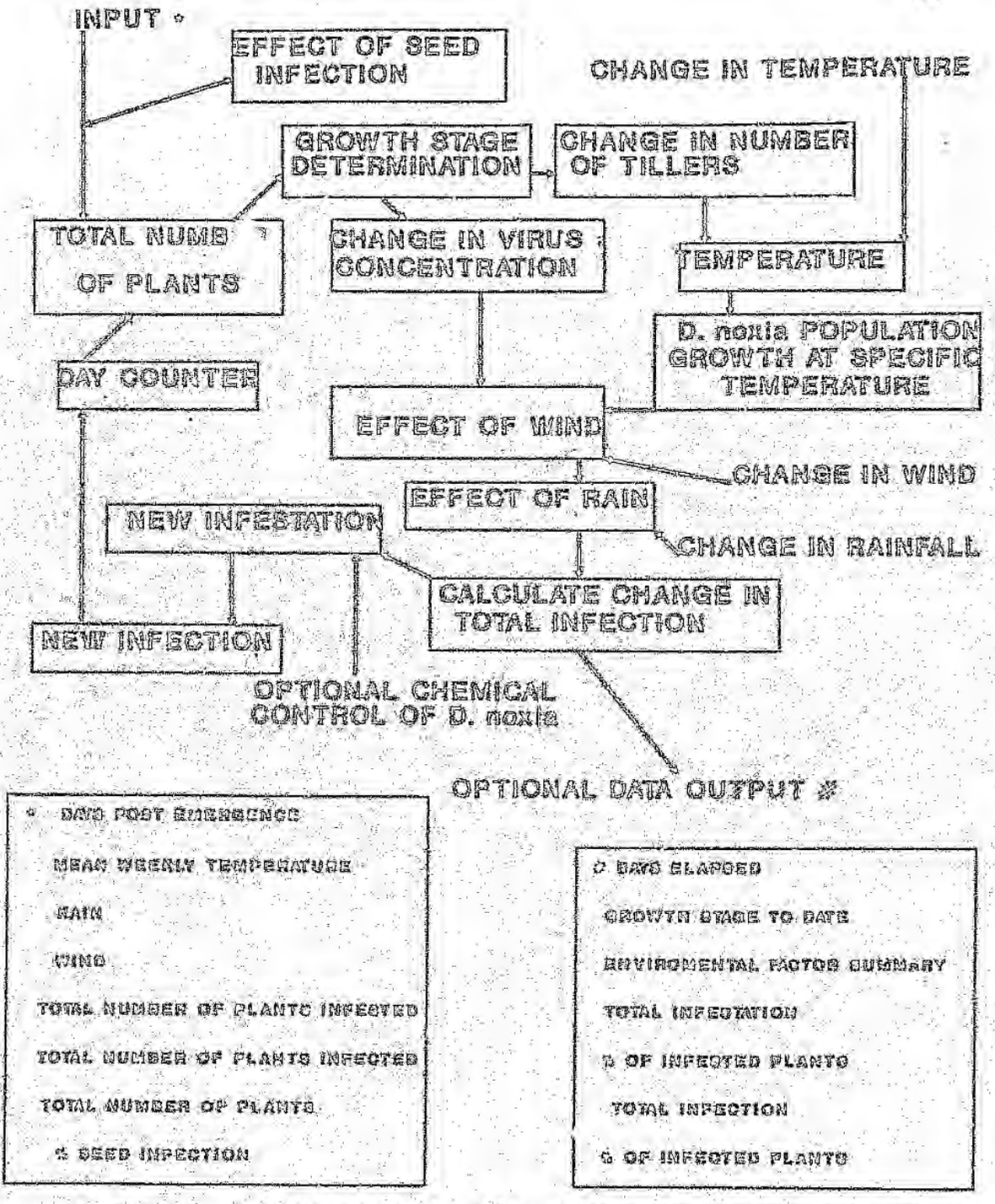


Figure 7. A flow chart to illustrate the program structure of SIMNOX, a computer program created to simulate the epidemiology of BMV and *D. noxia* in the summer rainfall area.

The formula was adapted from :

$$dx_t/dt = R_c\ (x_{t-p} - x_{t-p-i})\ (1-x_t)$$

where dx_t/dt = The change in number of infected plants with time

R_c = Increase per day at specific temperature (Polynomial regression, Aalbersberg ,1987) x reproductive period at specific temperature/ Total life span at specific temperature

x_t = Total life span at specific temperature

x_{t-p} = Duration of instars i-iv at specific temperature

x_{t-p-i} = Total life span - post reproductive period -duration of instars i-iv at specific temperature

And for BMV:

R_c = Concentration increase per day during specific growth stage / threshold for infectivity

x_t = Total life span (estimated)

x_{t-p} = Latent period at specific growth stage

x_{t-p-i} = Total life span - concentration threshold - latent period

For aphid populations a wind factor was added and a rainfall factor subtracted, using estimated factors. An estimate of vector efficiency, based on experimental data, was multiplied with the basic BMV increase rate.

The growth stage of a plant at specific dates was determined according to an approximate duration of growth stages during several seasons. Zhou *et al.* (1989) give a formula for a crop growth model based on the decimal code (Zadoks *et al.*, 1974), which was not incorporated into this model, but should be considered in future adaptations.

The time step used in the model was based on real-time to enable the author to combine research done by himself with the

work done by Aalbersberg (1987). The life-table method requires different methods, and this was considered to be too complex for the first model. Although the model works on a day to day step, an option to change mean temperatures, wind factors and rain factors as well as chemical control of aphids at various estimated efficiencies on a weekly basis, have been built into the model.

3.9.3 Model verification and validation

The model was verified against the data used to construct the model. Validation was based on data of other researchers, using the same data for the year in which the work was done. Data for *D. noxia* population simulation validation was obtained from Du Toit (1986(b)), Aalbersberg (1987), (eastern OFS), and Kriel (1984), (western OFS). Data for the validation of BMV infections were based on work done by the author, but which was not used in the construction of the model.

Several runs using various parameter changes were done to test the various aspects of the model but more possibilities, which have not yet been explored, exists.

Estimates of the effect of rainfall and wind on *D. noxia* populations had to be made as no data on these factors are available at this stage. These estimations are presented in Table (2) and (3).

Table 4. Comparison of the summer rainfall area isolate of BMV with other isolates

Isolate	A max/A min	A_{260}/A_{280}	
BMV	1.49	1.70-1.73	Lane (1974)
BMV	1.47	1.76	Lane (1974)
BMV uct	1.28	1.41	Rybicki (1984)
BMV sra	1.27±0.03	1.65±0.05*	

uct = eastern OFS isolate
sra = summer rainfall area isolate
* = Mean values of 30 determinations

4.2.2 Rate-zonal Centrifugation

The band position, after rate-zonal centrifugation of the isolates, did not differ significantly from that found by Rybicki (1984). After the centrifugation runs the virus suspensions migrated approximately half-way do the centrifuge tubes. No contaminants were found in the samples after fractionation of the sucrose gradients ar ultraviolet scanning at 254 nm in a spectrophotometer (Fig. 9).

The sedimentation coefficient ($s_{20,w}$) of the isolates was calculated and compared to coefficients reported by other researchers (Table 5). No significant difference in $s_{20,w}$ for the summer rainfall area isolate of BMV could be indicated

culated to be 1.43 g of virus per kg of plant tissue (wet mass).

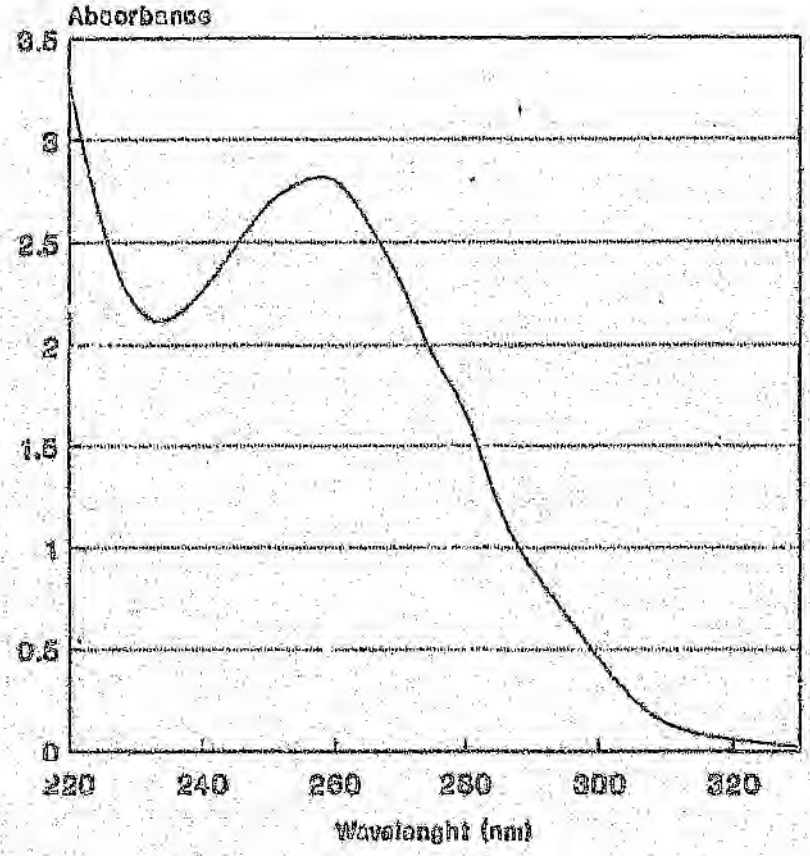


Figure 8. A scan of an acetate extraction (pH 4) of BMV from diseased field material.

The scans of several isolates were compared to data published by other researchers (Table 4). From this data it can be seen that the summer rainfall area isolate compares well with the eastern OFS isolate (Rybicki, 1984), with only slightly higher A_{260}/A_{280} absorbance ratios. The A max/A min of these isolates are consistently lower than the values reported by overseas researchers.

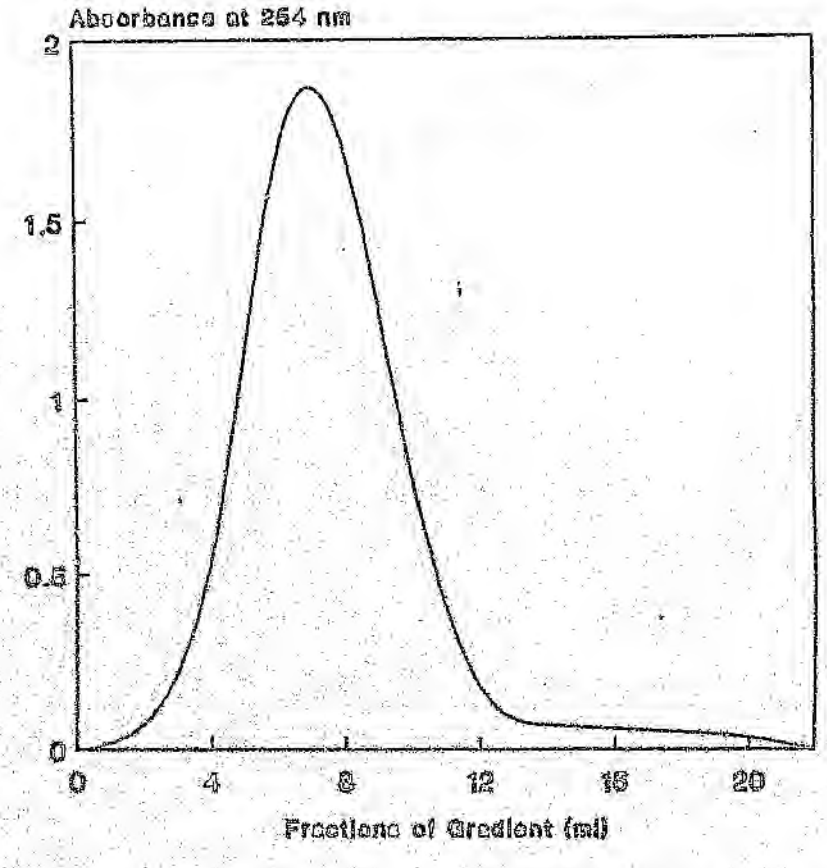


Figure 9. Sucrose density gradient fractionation of acetate extracted BMV at pH 6 after rate zonal centrifugation.

Table 5. Comparison of the calculated sedimentation coefficient ($s_{20,w}$) of the summer rainfall area isolate of BMV with other isolates

Isolate	$s_{20,w}$	
BMV	92 S	Lane (1974)
BMV	86.2 S	Lane (1974)
BMV	86 S	Lane (1974)
BMV	87.3 - 0.47c	Lane (1974)
BMV	84.05 S	Lane (1974)
BMV^{uct}	87 S	Rybicki (1984)
BMV^{sra}	86.8 S*	

uct = eastern OFS isolate
sra = summer rainfall area isolate
* = Mean of 24 determinations

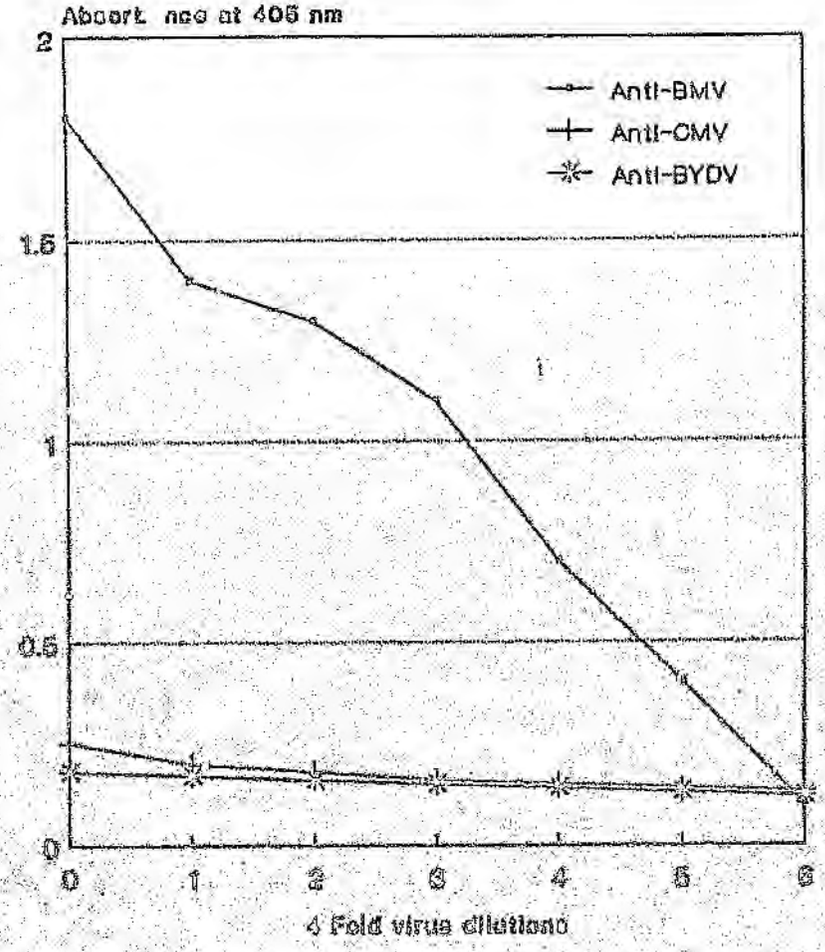


Figure 10. DAS-ELISA of BMV isolates against anti-BMV, CMV and BYDV globulins.

Crude extracts of plant sap from diseased field material reacted with anti-BMV serum but no reactions were found with anti-CMV and anti-BYDV serum.

4.2.6 Electron microscopy

Regular isometric particles were observed in the isolates examined with the electron microscope (Fig. 11). The particles were measured and had an apparent diameter of 24.8 ± 0.3 nm (Result of 20 measurements). No other regular or characteristic particles appeared with several isolates ex-

amined. These results compared well with those reported by Rybicki (1984).

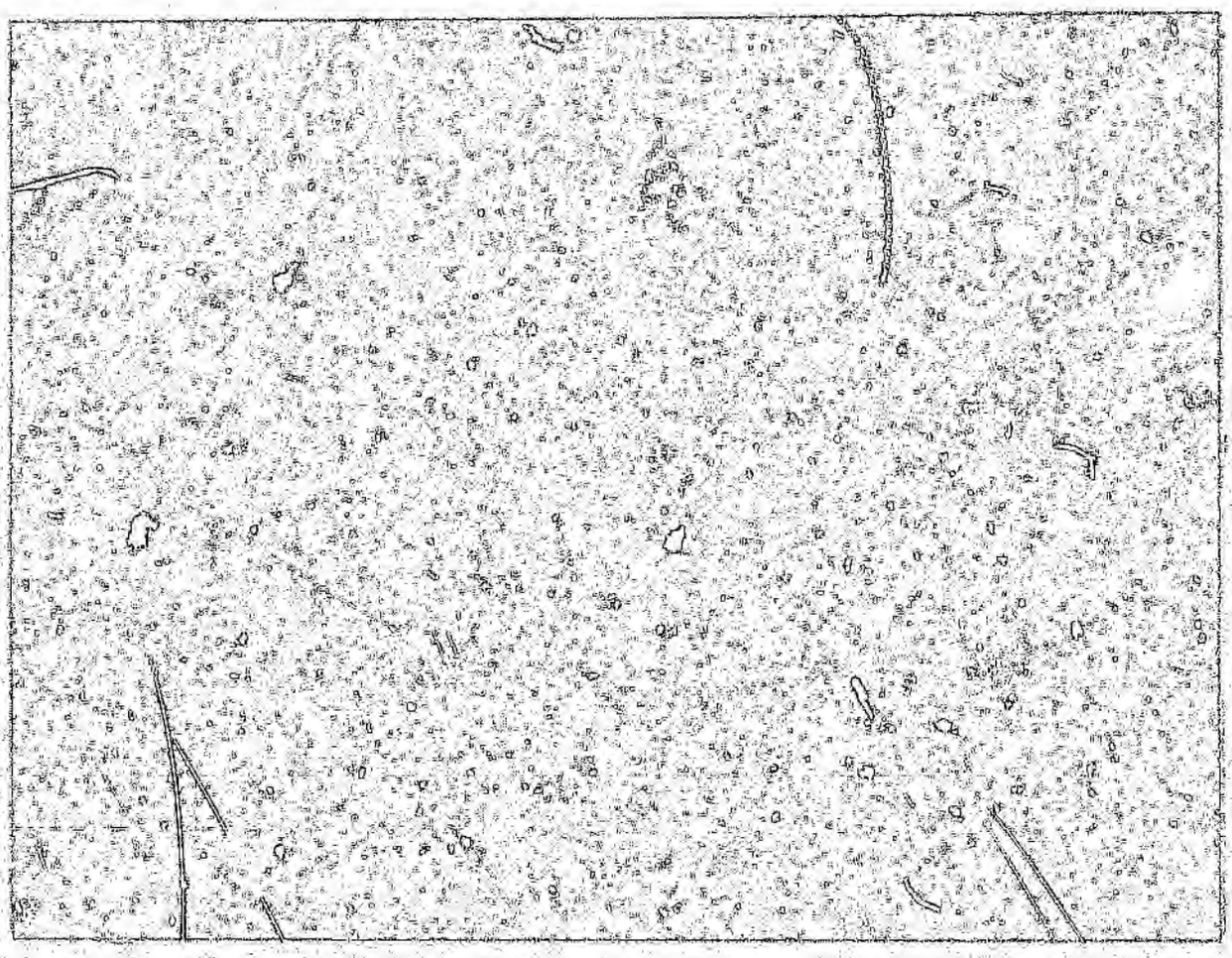


Figure 11. Electronmicrograph of a summer rainfall area BMV isolate.

4.2.7 Isolation and identification of other viruses occurring simultaneously with BMV under field conditions

The initial attempts to isolate CMV from the field collected material failed. Isolations from wheat, mechanically infected with a crude CMV extract from lupin leaves, yielded a homogenate with a faint green colour. The PEG precipitate

average 14 % of the aphids found on wheat and *Bromus* spp. in the eastern OFS were also viruliferous.

Table 7. Occurrence of BMV in *D. noxia* and plants in the summer rainfall region

LOCALITY	*D. noxia*			*Bromus* spp.			*Eragrostis* spp.			Volunteer wheat		
	'85	'86	'87	'85	'86	'87	'85	'86	'87	'85	'86	'87
EASTERN OFS	21	15	12*	9	12	13	2	3	4	25	42	38
CENTRAL OFS	6	13	4	5	7	6	0	0	0	15	11	05
WESTERN OFS	1	1	0	0	1	1	0	0	0	1	1	0
NATAL	1	1	0	0	0	0	0	0	0	0	1	0

* Numbers expressed as average % positive tests out of 50 random samples at each locality)

Brome mosaic virus could be transmitted to virus free wheat by sap inoculation with sap from *Bromus* spp., *Chenopodium* spp. and volunteer wheat collected at different localities in the eastern OFS. It was found that *Bromus* spp., *Eragrostis* spp. and volunteer wheat in the eastern OFS have a higher natural incidence of BMV than plants from other regions.

4.2.9 Natural occurrence of BMV in the summer rainfall area

Field collected samples of *D. noxia* were tested for the presence of BMV and the results (Table 7) indicated that the main distribution of BMV both in natural plant hosts and *D. noxia* was localised mainly in the wheat producing regions of the eastern OFS (See Fig. 2).

Table 2. Wind factor estimation for use with the SIMNOX model

Program input	Mean weekly wind speed	
0	0	Km/h
1	1-3	Km/h
2	>3-5	Km/h
3	>5-7	Km/h
4	>7-9	Km/h
5	>9-11	Km/h
6	>11-15	Km/h
7	>15-20	Km/h
8	>20-30	Km/h
9	>30 +	km/h

Table 3. Rain factor estimation for use with the SIMNOX model

Program input	Mean weekly rainfall	
0	0	mm
1	1-3	mm
2	>3-6	mm
3	>6-9	mm
4	>9-12	mm
5	>12-15	mm
6	>15-20	mm
7	>20-30	mm
8	>30-40	mm
9	>40+	mm

A polynomial regression equation for the effect of temperature on the basic infestation rate of *D. noxia* was determined and used in the program:

$$Y = 0.75 X^2 - 19.5 X + 95$$

where Y = Daily population increase factor

X = Mean temperature for the week

Growth stage determination for the first 112 days post emergence was determined according to the following:

$$Y = 2 + 0.29048 X$$

where Y = Growth stage

X = Days post emergence

The following equation was used for growth stage determination for the rest of the season after 112 days post emergence:

$$Y = -37.8 + 0.70477\ X$$

where Y = Growth stage

X = Days post emergence

CHAPTER 4

RESULTS

4.1 VIRUS ISOLATION FROM WHEAT IN THE SUMMER RAINFALL AREA

The acetate (pH 4.0) BMV isolation method consistently yielded a clear and straw-coloured clarified homogenate. The PEG precipitate was small and resuspended easily and the final high-speed pellets were light brown and translucent.

4.2 CHARACTERIZATION OF VIRUS ISOLATED FROM WHEAT IN THE SUMMER RAINFALL AREA

4.2.1 Ultraviolet Spectroscopy

In the case of the pH 4.0 acetate isolate, UV absorbance was used to calculate the yield of BMV from infected tissue. The UV spectrum was typical of a spherical virus (Fig. 8) (Rybicki, 1984).

The A_{260}/A_{280} values were 2.85/1.73 respectively, giving a ratio of 1.65 for the virus isolate. Based on an extinction coefficient of 5.2 (Lane, 1974), the concentration of the virus preparation was calculated to be 4.29 mg/ml. The virus, which had been isolated from 300g of plant material, was suspended in 100 ml of buffer. The yield was then cal-

4.2.3 Infectivity Assays

Isolates of BMV that were mechanically inoculated into wheat seedlings gave rise to the normal mosaic symptoms. The yellowing syndrome, where inoculated leaves turned yellow after infection (Rybicki, 1974), rather than displaying mosaic symptoms, was not observed. Brome mosaic virus isolates inoculated into *Chenopodium hybridum* produced distinct necrotic local lesions typical of BMV infection (Rochow, 1959). Plants mechanically inoculated with celite and acetate buffer (0.3M, pH4) produced no symptoms.

4.2.4 Anti-body production

The goat IgG prepared with the method described in 3.3.4 was of similar quality compared to rabbit sera obtained from UCT. The titer end-points of the sera, as well as serum quality (as far as non-reaction with host protein was concerned) were also similar. As a large amount of serum was needed, it was reasoned that goats would be the better experimental animal to use.

4.2.5 Serological tests

Only BMV was found in the isolates with the ELISA and it was therefore assumed that the samples contained pure BMV (Fig. 10).

was very small and resuspended easily. The final high-speed pellets were light green and translucent.

It was found that CMV occurred simultaneously in plants with BMV infection in only 2 % of the plants tested (Table 6). Although CMV was found to be present, the effect on the plant and possible effect in conjunction with BMV remains to be determined.

Table 6. The frequency of the simultaneous occurrence of BMV and CMV in wheat in the summer rainfall area over two seasons (1986-87)

YEAR	NUMBER OF PLANTS TESTED	BMV (%)	CMV (%)	BMV,CMV (%)
1986	4600	2769[x] (60.2)[y]	138 (3)	92 (2)
1987	1700	918 (54)	40 (2.4)	16 (1)

[x] Numbers indicate positive tests for the virus concerned.
[y] Numbers in brackets indicate the percentage

Wheat inoculated with crude extracts of CMV - infected lupin leaves caused yellowing of the leaf as described by Rybicki (1984). Only CMV could be detected serologically in these plants with the ELISA.

4.2.8 Transmission of BMV from alternate hosts to wheat

BMV was isolated from *Eragrostis* spp., *Bromus* spp., *Chenopodium* spp. and volunteer wheat in all areas in the eastern OFS (Table 7). Similar plants collected in the western OFS and Natal tested negatively in most cases. On

Both *D. noxia* reared in the greenhouse and field collected individuals were able to transmit BMV from mechanically infected wheat and infected field collected volunteer wheat and *Bromus* to virus-free wheat grown in the greenhouse (See 4.5.1).

4.3 EFFECT OF BMV ON THE WHEAT PLANT

4.3.1 Spread of BMV through infected plants

The systemic spread of BMV in mechanically infected host plants is illustrated in Figure 12. It was found that BMV became systemic after the initial mechanical infection of the bottom leaves of the plant (Table 8). Leaves were counted from the top down i.e. flag-1 was the leaf directly below the flag leaf. The effect of mechanical infection of the flag-5 with BMV during different growth stages is illustrated in Figure 13, and the effect of different inoculum concentrations in Figure 14.

These results correlated well with those of Proll (1967 (b)). The virus was translocated from the primary infection site in the primary (bottom) leaves of the infected plants and at the end of the season the virus could be found in the flag leaves. It was found, however, that a decrease in concentration also occurred, and the concentration of virus in the flag leaves were approximately halved at the end of the season (Table 8). Proll 1967(b) also found a decrease

in the multiplication capacity of the virus with increasing age of the leaves, but suggests no reasons for this.

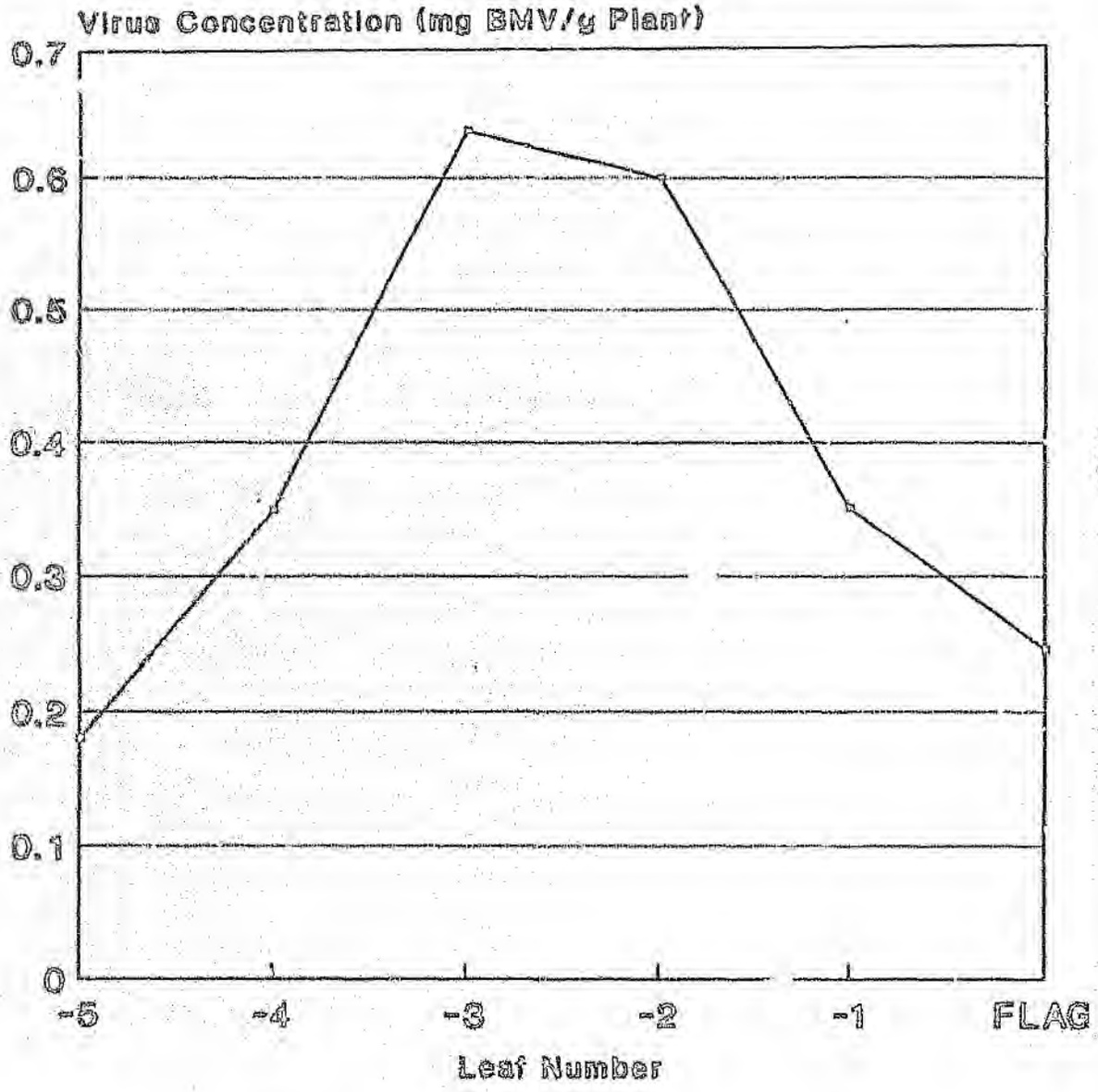


Figure 12.The systemic spread of BMV in leaves of plants after mechanical infection of the flag-5 leaf.

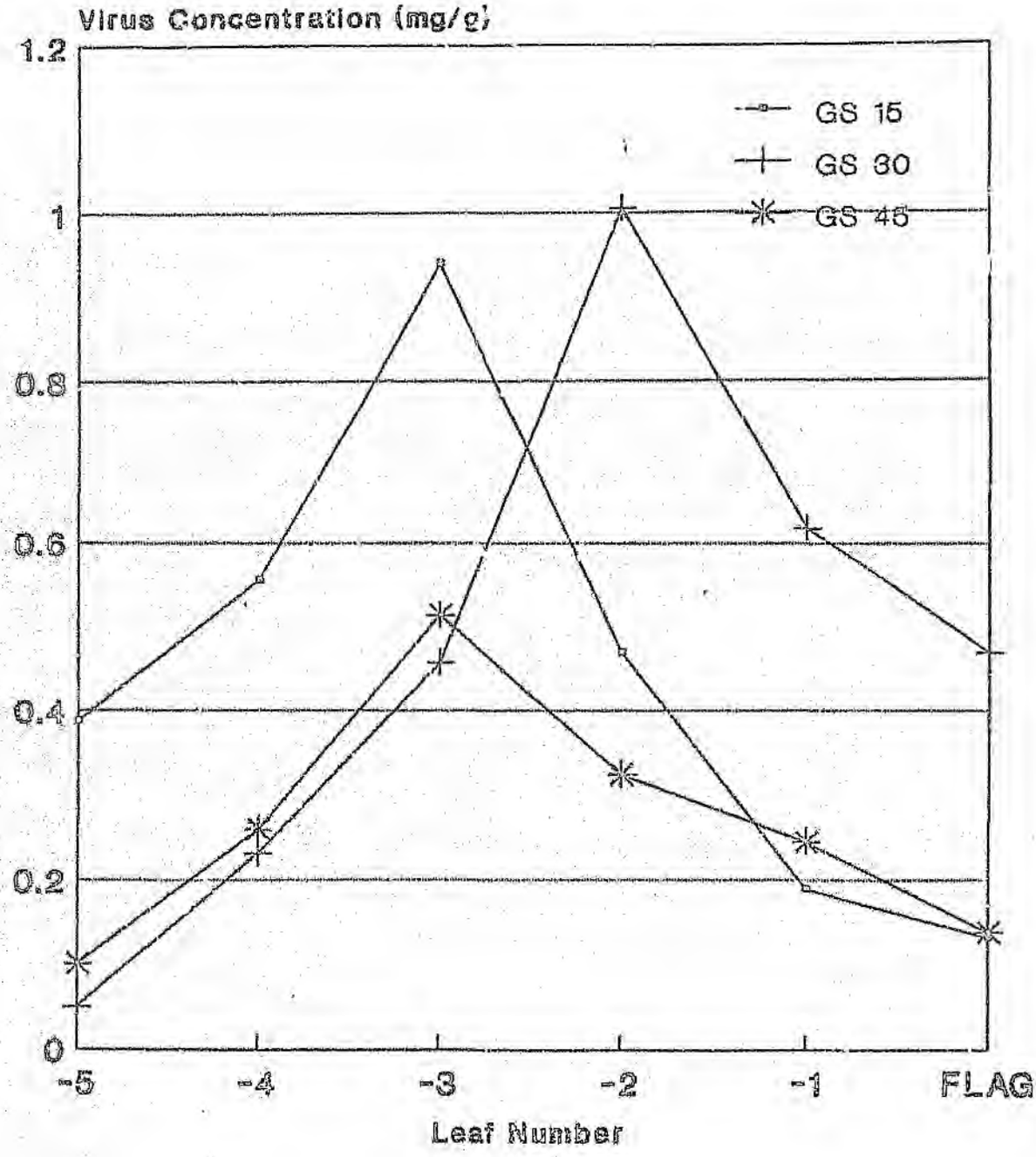


Figure 13.The systemic spread of BMV in leaves of plants after mechanical infection of the flag-5 leaf during different growth stages.

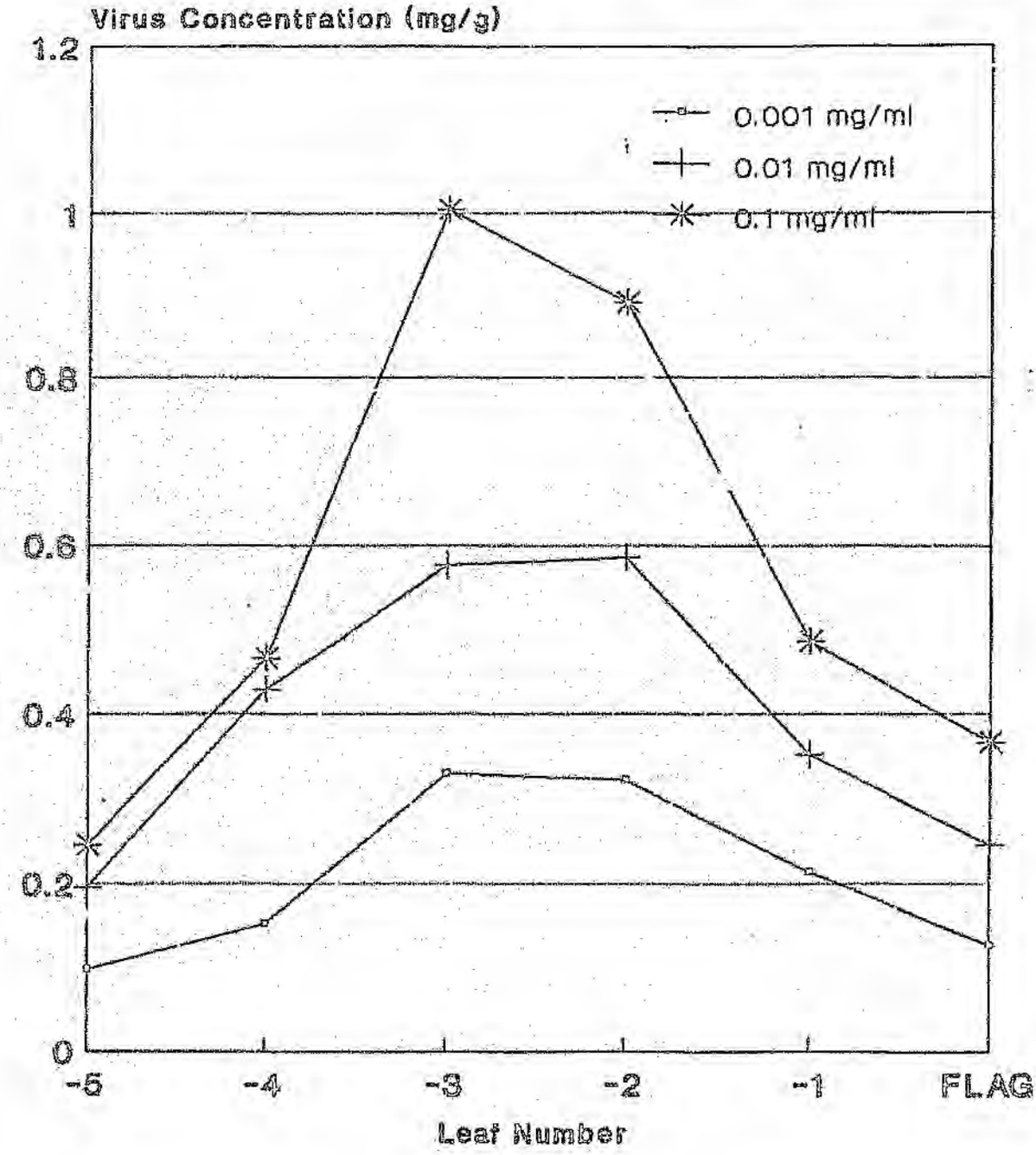


Figure 14.The systemic spread of BMV in leaves of plants after mechanical infection of the flag-5 leaf with different inoculum concentrations.

Table 8. The spread of BMV in the wheat plant after mechanical infection of the bottom leaves (flag-5) with BMV during different growth stages and with different inoculum concentrations

GROWTH STAGE	INOCULUM (mg/ml)	LEAVES (mg BMV/g) -5	-4	-3	-2	-1	FLAG
15	0.001	0.196b*	0.175c	0.353ef	0.190d	0.144d	0.072f
	0.01	0.453a	0.784a	0.913b	0.380cd	0.207d	0.154de
	0.1	0.515d	0.706a	1.544a	0.838b	0.218d	0.169cde
30	0.001	0.033d	0.157c	0.245f	0.547c	0.351c	0.229c
	0.01	0.048d	0.192c	0.318ef	1.063b	0.639b	0.465b
	0.1	0.070cd	0.349b	0.810bc	1.406a	0.865a	0.714a
45	0.001	0.066cd	0.124c	0.391ef	0.233d	0.146d	0.079f
	0.01	0.085cd	0.312b	0.501de	0.314cd	0.211d	0.188ef
	0.1	0.150bc	0.343b	0.647cd	0.428cd	0.377c	0.216cd

* = Numbers within a column followed by the same letters are not significantly different (P=0.05)

It is of interest to note that the highest concentration of BMV in leaves was reached in all treatments in the leaves that emerged during GS 30-35 (Fig 13, 14). Data of aphid colonisation of wheat under field conditions indicate that this is the new growth (flag-2 and flag -3) that is emerging at the time when *D. noxia* populations enter a logarithmic phase in their reproduction and colonisation. This would mean that during these growth stages a high virus concentration is present in leaves being colonized. The change in GS during this period is rapid (Table 1). Since *D. noxia* tend to migrate upwards on the plant (Aalbersberg, 1987), circumstantial evidence would suggest, that if this aphid proved to be an effective vector of BMV, high concentrations of virus would reach the flag leaves within approximately a week. This could cause a detrimental effect on yield. In

the absence of aphids, however, the concentrations in the flag leaf and flag-1 tend to be low.

The internodes of tillers also tested positively for BMV. The same decrease in concentration, noted in the case of leaves, was found here. The concentration of BMV in the internodes between, for instance, the flag leaf and flag-1 as compared to the internodes between flag-4 and flag-5, was significantly lower. This might be explained by a lowering of the metabolic activity in the plant with increased age, but no direct proof for this was found.

4.3.2 Effect of BMV in the greenhouse

The only macroscopic symptom developing on plants, infected during the seedling stage, was a faint mosaic in those plants infected with the high titers (0.1 mg\ml) of virus. These symptoms disappeared after GS 20, but a fast die-back of the lower leaves was observed in all the plants which had been infected with virus (0.1, 0.01 and 0.001 mg/ml). Only the plants infected with the high titers of BMV (0.1 mg/ml) in the other growth stages (GS 30 and GS 45) developed visible mosaic symptoms.

A slight difference in the height of the infected plants was also observed later in the season. The growth appeared to be stunted compared to the control plants (Table 9). During ear emergence a slight mottling of the flag leaves was

Table 9. The effect of BMV infection on plants after infection at different growth stages and inoculum concentrations

GROWTH STAGE DURING INOCULATION	INOCULUM CONCENTRATIONS (mg BMV/ml 0.03M pH 4 ACETATE BUFFER)			
GS 15	0.0mg/ml	0.001mg/ml	0.01mg/ml	0.1mg/ml
TILLERS	7.67(ab)*	6.63(ab)	7.17(ab)	7.37(ab)
EARS	6.10(ab)	4.63(abc)	5.00(bc)	4.20(c)
LENGTH(cm)	82.32(a)	78.67(ab)	76.97(abc)	74.20(bc)
SEEDS	204.53(ab)	157.77(cd)	144.83(d)	96.70(e)
YIELD(g)	8.23(a)	6.52(bc)	5.56(de)	3.38(g)
TKW(g)	41.21(a)	42.16.79(ab)	39.73(bc)	34.81(cd)
VIRUS(mg/g)	0.00(f)	0.37(e)	0.86(bc)	1.67(a)
GS 30	0.0mg/ml	0.001mg/ml	0.01mg/ml	0.1mg/ml
TILLERS	7.23(ab)*	6.50(b)	7.33(ab)	7.87(a)
EARS	5.97(a)	4.93(ab)	4.60(bc)	4.23(c)
LENGTH(cm)	79.92(abc)	78.64(abc)	78.00(ab)	72.20(c)
SEEDS	196.09(a)	157.73(bc)	138.63(d)	90.00(e)
YIELD (g)	8.10(a)	5.77(b)	4.65(d)	2.59(f)
TKW(g)	40.32(cd)	36.62(cd)	34.79(de)	30.30(e)
VIRUS(mg/g)	0.00(f)	0.57(de)	1.05(b)	1.62(a)
GS 45	0.0mg/ml	0.001mg/ml	0.01mg/ml	0.1mg/ml
TILLERS	8.10(ab)*	7.40(ab)	6.00(b)	7.33(ab)
EARS	6.27(a)	5.50(abc)	4.60(abc)	4.33(bc)
LENGTH(cm)	79.69(ab)	80.93(ab)	79.67(abc)	75.40(abc)
SEEDS	212.90(a)	177.03(bcd)	141.07(cd)	102.23(e)
YIELD (g)	8.49(b)	6.26(cd)	4.99(ef)	3.26(gh)
TKW(g)	40.35(cd)	35.84(cd)	35.61(d)	33.71(de)
VIRUS(mg/g)	0.00(f)	0.36(e)	0.54(e)	0.79(cd)

* Numbers within a row followed by the same letters, and for the same treatments, are not significantly different (P=0.05)

observed. This intensified and the leaves died prematurely. The virus concentrations in the monitored plants showed that the highest concentrations were reached after infection during GS 30.

The highest yield loss was also associated with infection during this growth stage, the smallest loss being associated with GS 15 infection (Fig 15).

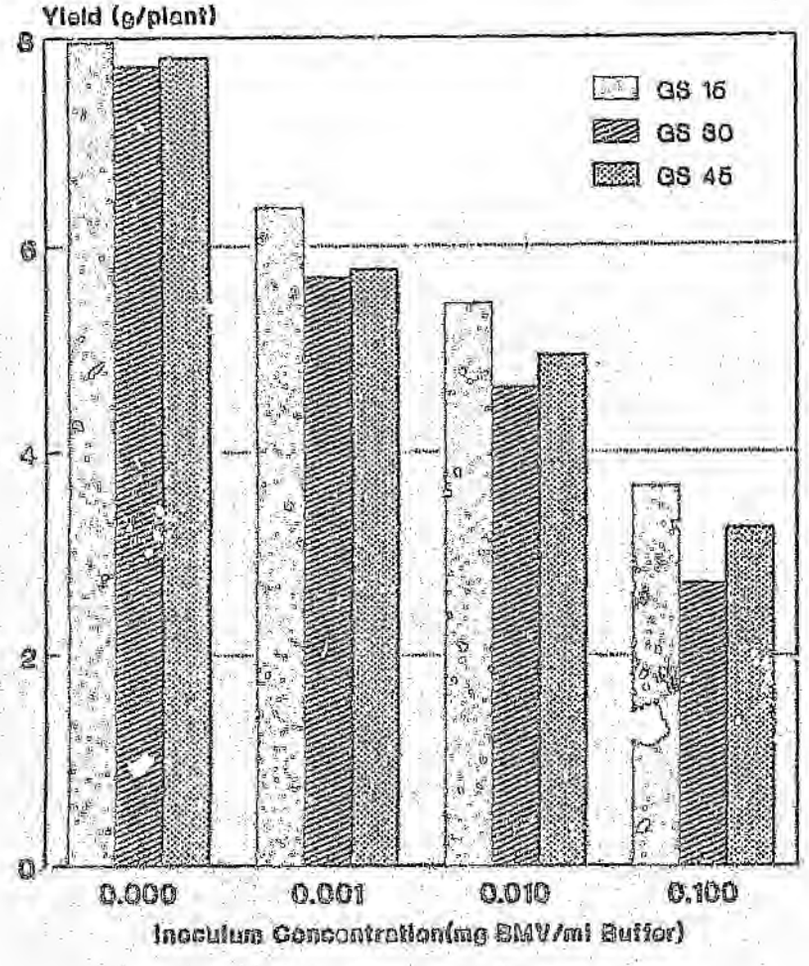


Figure 15. The effect of BMV on yield after mechanical infection during different growth stages and inoculum concentrations.

It was found that infection early in the life cycle (GS 15) resulted in a degree of compensatory growth (Table 9). On average these plants yielded a higher TKW than plants infected during other growth stages. The number of tillers formed, was not affected significantly by either the titer or GS during infection with BMV.

Ear development was affected significantly by the inoculum concentration. The control plants formed significantly more ears than all infected plants. Plant height was not significantly affected by BMV but infected plants appeared to be slightly stunted. The number of seeds per plant was significantly affected by BMV. It was found that the largest decrease of a production component of the plant occurred in seed formation.

Yield was significantly affected by both the stage of infection and the inoculum concentration. The yields of all plants treated with the different inoculum concentrations differed significantly, with the control plants producing the highest and the highest inoculum concentration the lowest yield per plant. It was also found that plants infected during GS 30 (stem elongation) had a significantly lower yield than the other infection times. When the multiplication of virus in infected plants was examined (Fig. 13) it was found that during the stem elongation phase, multiplication of virus infection was the highest. This could explain the yield reduction associated with infection during this growth stage. It was noted that virus multiplication of the highest inoculum titre (0.1 mg/ml) of the infection done during GS 15 also peaked at GS 30. This could indicate that the normal rapid growth of the plant during this stage facilitated virus multiplication.

The TKW was significantly affected by both the growth stage during infection and the inoculum concentration. The effect

of infections done during different growth stages differed significantly, with plants infected during GS 30 showing the largest reduction in TKW, followed by GS 15 and GS 45 infected plants (Table 9).

The inoculum concentration, rather than the growth stage during infection, had a greater effect on plant tillering, ear formation and height. It was found on the other hand that yield was mainly affected by the growth stage of the plant during infection.

4.2.3 Effect of BMV under field conditions

With field infected wheat, the effect on plant components not directly involved in yield, was more drastic than the effect found in the greenhouse (See 4.3.2). Infected plants tillered poorly in comparison to uninfected control plants, and there was a more marked effect on plant height (Table 10). The main yield components, namely number of seeds per plant, seed mass and TKW, were all affected significantly by BMV. Again the main yield reduction occurred with infections during GS 30 with high titers of BMV.

The overall yield reduction under field conditions, however, was less than the reduction found in the greenhouse (68% reduction in yield between controls and highest yield loss,

Table 10. The effect of BMV infection on plants under field conditions after infection at different growth stages and inoculum concentrations

GS	INOCULUM CONCENTRATION*	LENGTH	TILLERS	SEEDS	MASS(g)	TKW(g)
15	0.000	49.61a**	16.8a	520.6a	11.50bc	22.94a
	0.001	50.08b	14.4abc	442.3b	9.60d	22.39ab
	0.01	44.91cd	12.5cde	385.5cd	7.99f	21.25abc
	0.1	43.59de	10.9ef	309.3ef	6.33g	21.05abc
30	0.000	49.41a	17.6a	549.3a	1..11ab	22.44ab
	0.001	46.99bc	13.4cd	403.3bc	8.79e	22.36ab
	0.01	45.37cd	11.3def	325.6e	6.97g	22.16ab
	0.1	41.79e	13.0f	262.2f	5.14h	20.12c
45	0.000	54.68a	17.2a	562.0a	12.50a	22.42ab
	0.001	47.91bc	16.1ab	527.0a	11.17c	21.51abc
	0.01	45.31cd	13.3cd	402.9bc	8.40ef	21.24abc
	0.1	43.04de	11.4def	341.6de	6.99g	20.76bc

* The concentrations are expressed as mg of virus/ml of inoculation buffer

** Numbers within a row followed by the same letters, and for the same treatments, are not significantly different (P=0.05)

compared to 57% under field conditions). This might be explained by the generally more vigorous growth of wheat under natural conditions.

The virus concentrations in infected plants tended to be lower than in greenhouse material, but concentrations peaked after infection during GS 30, the same was found with previous experiments (see 4.3.1 and 4.3.2).

4.3.4 Yield losses associated with BMV in different cultivars

A significant correlation (r^2 =0.693) was found between the percentage of BMV infected plants and the yield of plots in all cultivars tested. There was a considerable difference in the degree to which the different cultivars were affected by the virus. The high yielding cultivars (Tugela, SNK 108) had the largest reduction in yield with increased BMV infection. The lower yielding cultivars (Betta, Molen and SST 107) were affected to a lesser extent by the presence of the virus (Fig. 16).

Comparison of the hectolitre masses of the different cultivars showed that the effect of the virus on yield was not always the same (Table 11).

Table 11. The effect of BMV infection on the hectolitre mass of a number of cultivars.

	HECTOLITRE MASS (hl mass) AND GRADE			
	CONTROL		BMV INFECTED	
CULTIVAR	hl mass	GRADE	hl mass	GRADE
BETTA	80.14	SUPER[X]	77.94	A 1[R]
TUGELA	76.78	A 1	73.65	U 1
MOLEN	77.10	A 1	73.66	U 1
SNK 108	75.74	A 2	73.01	U 1
SST 107	71.85	U 2	70.22	U 2

[X] Prices paid for grades (based on the 1988 price list) was as follows: Super R394.84/t, A1 R393.50/t, A2 R385.92/t, U1 R364.25/t, U2 R339.91/t

The virus also had a secondary effect on the cultivars in that an effect on the grading of infected and control seeds

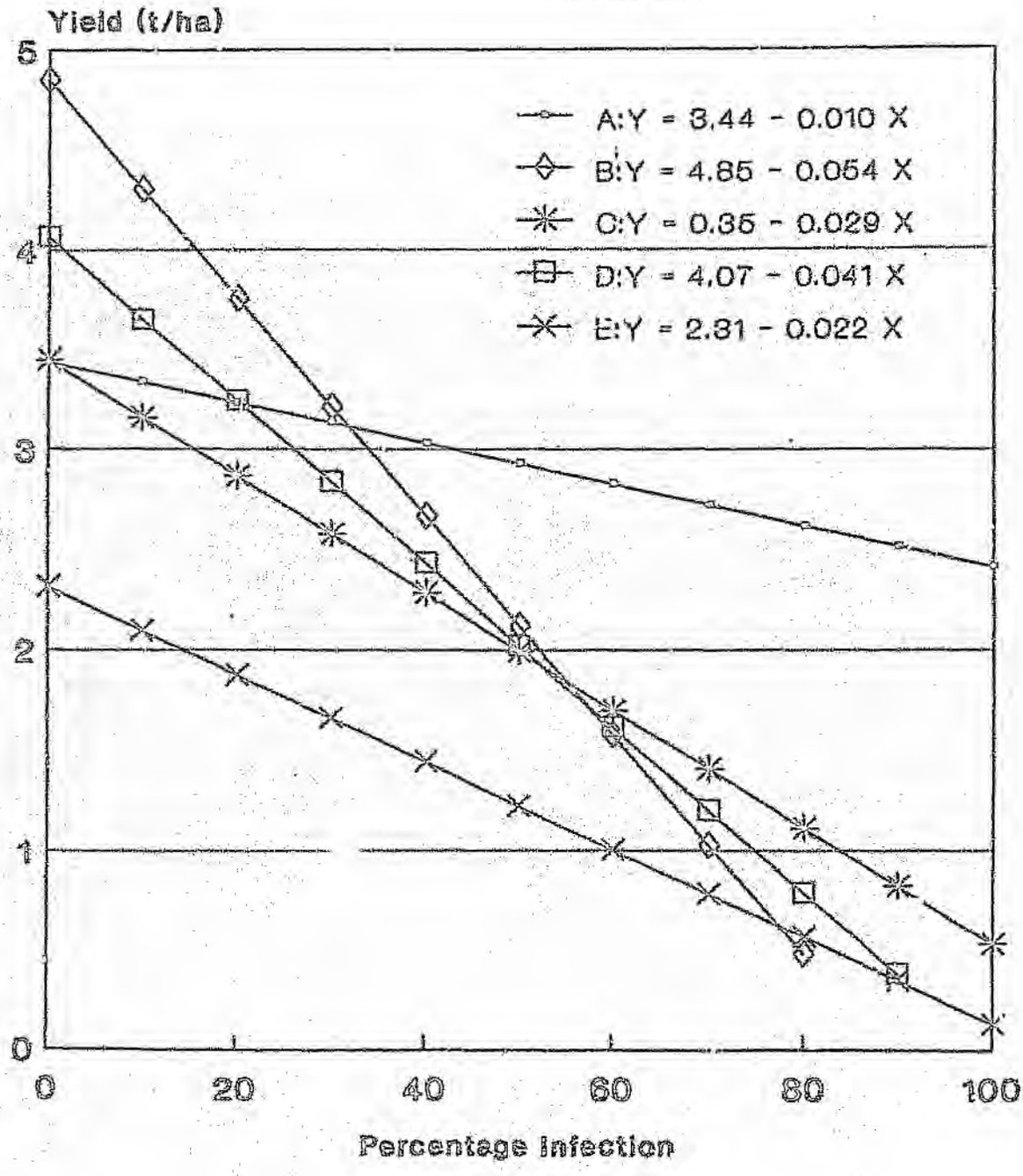


Figure 16. The effect of BMV infection on the yield of a number of selected cultivars. A) Betta, B) Tugela, C) Molen, D) SNK 108, E) SST 107

was found. The grade obtained for a wheat crop is determined by the quality of the seed harvested, based on the percentage of healthy seed, and is determined by the co-operative where the crop is sold. Betta had the lowest reduction in hectolitre mass and grain grading (Table 11).

Although BMV infection caused the greatest yield reduction in Tugela, this cultivar nevertheless gave the highest yield at low infection percentages (Fig. 16).

The percentage of plants infected with BMV in the different cultivars did not differ significantly. The seed infection percentages of all the cultivars tested, were also not statistically different. Aphid control on all plots was satisfactory, indicating that the differences in the yields between infected and control plots of the cultivars were due to the effect of BMV infection.

4.4 SEED TRANSMISSION OF BMV

4.4.1 Localisation of the site of BMV infection on seed

The embryos of BMV infected seeds (n = 400) contained a significantly higher concentration of virus than the seed coat or endosperm (Table 12).

Table 12. The site of BMV infection in wheat seeds as shown with the DAS-ELISA

VIRUS CONCENTRATIONS (mg/g) IN THE:		
EMBRYO	SEED COAT (Epiderm)	ENDOSPERM
0.404mg/g(a)[x]	0.243mg/g(b)	0.151mg/g(c)

[x] Means followed by the same letter are not significantly different (P = 0.05)

These results confirm that BMV is a seed-borne virus, and not just a seed contaminant. The localisation of the BMV in the embryo of infected seeds also suggests that a detrimental effect on germination is possible, as was confirmed in 4.4.3.

4.4.2 Effect of surface-sterilisation of BMV infected seeds

The surface-sterilisation of the 60 BMV infected seeds with sodium hypochlorite had no effect on the virus content of the infected embryos and endosperm (Table 13). The seed-coat showed a significantly lower BMV concentration. Germination tests on surface-sterilized seeds indicated that the det-

rimental effect of BMV or seed viability was not lessened by this sterilisation method.

Table 13. The effect of surface-sterilisation on the BMV content of infected wheat seeds.

DISSECTED SECTIONS TESTED	CONTROL	STERILIZED
EMBRYO	0.404mg/g[x] (a)[y]	0.410mg/g (a)
SEED COAT	0.210mg/g (b)	0.120mg/g (d)
ENDOSPERM	0.150mg/g (c)	0.152mg/g (c)

[x] Figures expressed as mg BMV/g seed material
[y] Means followed by the same letter are not significantly different(P = 0.05)

There was a significantly higher concentration of BMV in the embryos of both the controls and sterilised seeds, than in the seed coat or endosperm of the relevant treatments.

4.4.3 Effect of BMV seed-infection on germination

Germination rate was significantly correlated with the percentage of seed infected with BMV (r^2=0.88, $P < 0.05$), (Fig. 17).

4.4.4 Effect of inoculum concentration and time (growth stage) of infection with BMV of the seed parent on the seed produced

It was found that BMV infection of wheat at different growth stages and virus concentrations had an effect on the viability of the seed formed by such plants (Table 14).

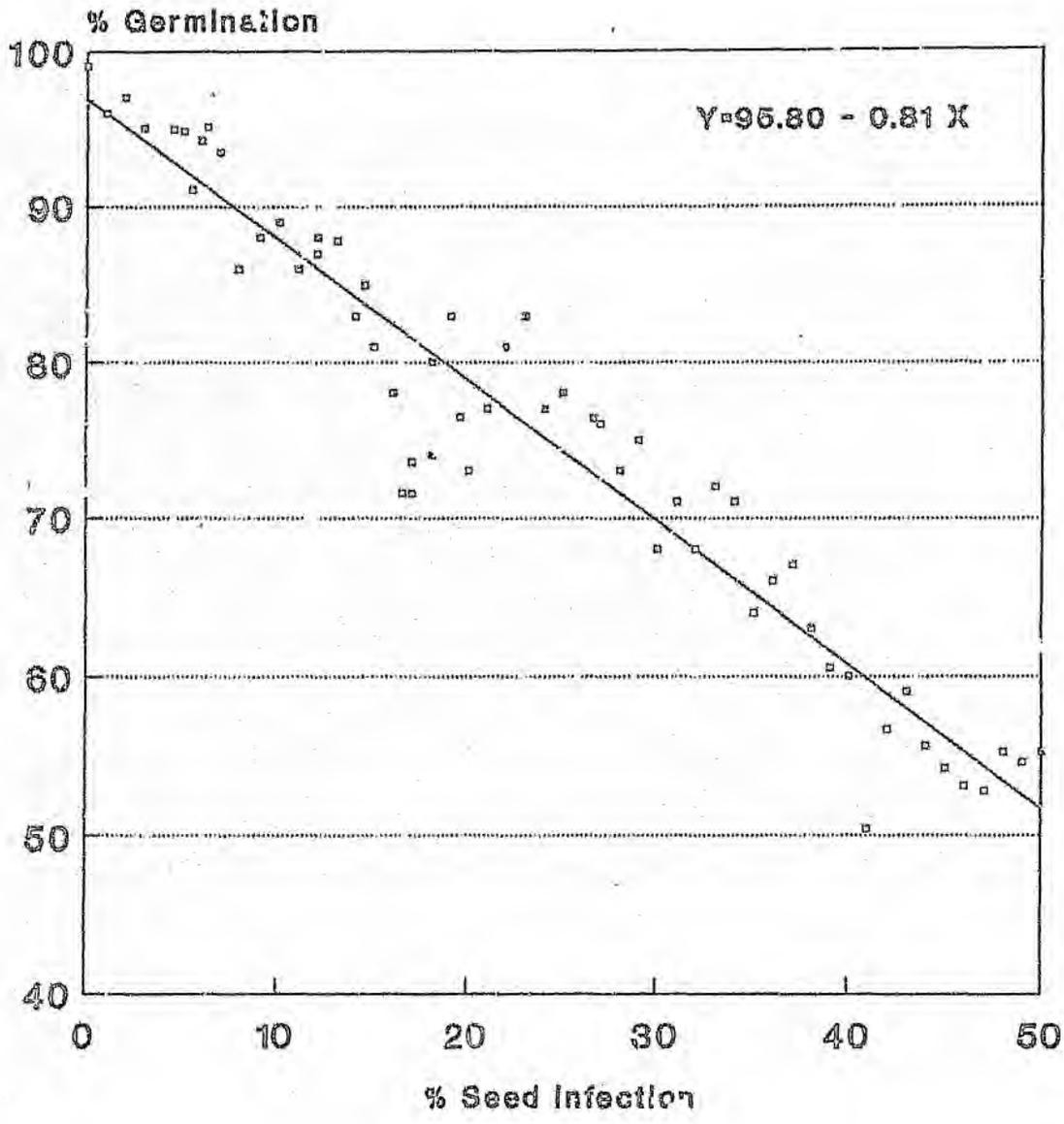


Figure 17.The correlation between BMV infection and the viability of wheat seeds.

Table 14. The effect of inoculum concentration and time (growth stage) of BMV infection of seed parents on the germination of progeny seed, and the transmission of virus to progeny plants

INITIAL INFECTION OF SEED PARENT	INOCULUM CONC. (mg BMV/ml buffer)	% PROGENY SEED INFECTED[X]	% GERMINATION OF PROGENY SEED	%TRANSMISSION OF VIRUS TO PROGENY PLANTS
G.S. 15	0.000	9.5 (fg)	90.3 (ef)	1.1 (de)
	0.001	18.5 (d)	83.2 (cd)	1.8 (de)
	0.01	23.6 (bc)	73.5 (b)	1.5 (de)
	0.1	39.6 (a)	61.0 (a)	2.2 (de)
G.S. 30	0.000	12.6 (ef)	88.0 (e)	0.7 (e)
	0.001	22.3 (c)	86.8 (de)	9.9 (ab)
	0.01	26.3 (b)	80.5 (c)	6.8 (bc)
	0.1	36.6 (a)	75.6 (b)	11.2 (a)
G.S. 45	0.000	6.3 (gh)	93.0 (fg)	1.0 (e)
	0.001	5.6 (h)	95.5 (g)	2.0 (de)
	0.01	6.2 (gh)	94.8 (g)	2.0 (de)
	0.1	14.4 (e)	92.0 (fg)	4.9 (cd)
MEAN % :		18.7	84.5	3.8

[X] Values within a column followed by the same letters are not significantly different (P = 0.05)

Some infection occurred in plants originating from virus-free seed, probably due to crowding of plants in the greenhouse. The progeny seed of plants infected during GS 15 (seedling) had a high incidence of seed infection. The infected seeds germinated poorly and only very few cases of virus trans-mission to plants from these seeds were found. Seed from plants infected at GS 30 (stem elongation) had the highest seed infection level. The transmission of the virus through seed to progeny was the highest in this group.

The seeds from plants infected at GS 45 (flag leaf) had significantly lower BMV seed infection, and the germination

of this group was significantly higher than the other groups. The number of progeny plants infected by BMV in this group was low.

4.4.5 Transmission of BMV through seed to progeny plants

It was found that only a low percentage of virus infection on seed was successfully transmitted to progeny plants, and that the growth stage during infection of the seed parent, as well as the virus concentration, played a part in the level of seed transmission of BMV (Table 15 and 16).

Table 15. The effect growth stage during infection with BMV on the germination and transmission of virus through seed

GROWTH STAGE OF SEED PARENT AT INFECTION	%INFECTION OF PROGENY SEED	%GERMINATION OF PROGENY SEED	%TRANSMISSION THROUGH SEED TO PROGENY PLANTS
GS 15	22.80 (b)[x]	77.00 (a)	1.65 (b)
GS 30	24.54 (a)	82.73 (b)	7.15 (a)
GS 45	8.13 (c)	93.82 (c)	2.47 (c)

[x,y] Values within a column followed by the same letters are not significantly different (P = 0.05)

Table 16. The effect of inoculum concentration on the germination and transmission of virus through seed

INOCULUM CONC. USED TO INNOC. SEED PARENT	%INFECTION OF PROGENY SEED	%GERMINATION OF PROGENY SEED	%TRANSMISSION THROUGH SEED TO PROGENY PLANTS
0.000 mg/ml	9.47 (d)[y]	90.43 (d)	0.93 (c)
0.001 mg/ml	15.47 (c)	88.50 (c)	4.57 (ab)
0.010 mg/ml	18.70 (b)	82.93 (b)	3.43 (b)
0.100 mg/ml	30.20 (a)	76.20 (a)	6.10 (a)

[x,y] Values within a column followed by the same letters are not significantly different (P = 0.05)

Infection of the seed parent during GS 30 (stem elongation) resulted in a significantly higher transmission of virus to progeny plants than the other growth stages. The highest virus inoculum concentration (0.1 mg/ml) of seed parent also resulted in the highest transmission of the virus to the progeny plants.

4.4.6 Seed-transmission of BMV in different wheat breeding lines

The ercentage of BMV infection of the seed and the germination of infected seed was correlated between the different winter and intermediate breeding lines tested (Fig. 18).

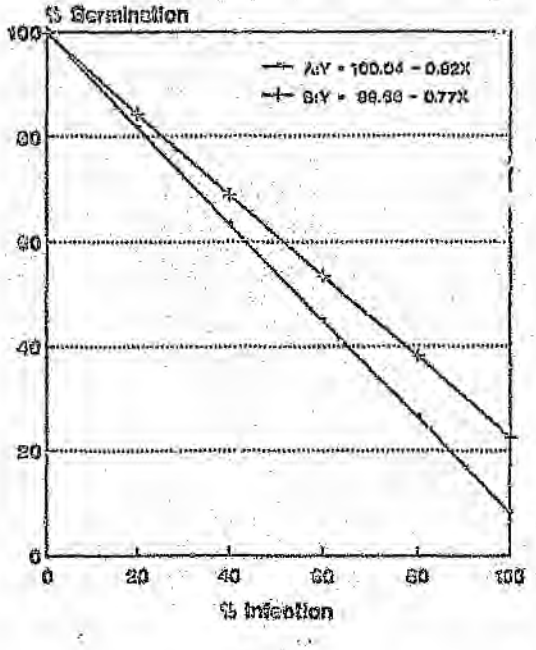


Figure 18. The effect of BMV seed-infection on the germination of A) 36 winter and B) 36 intermediate breeding lines

The intermediate breeding lines had a better ability to transmit BMV (6 %) to progeny plants through infected seed than the winter lines (4 %).

4.4.7 Economic importance of seed transmitted virus in wheat

It was found that there was a significant correlation between seed infection and the number of plants that failed to germinate (r^2= 0.9866)(Fig.19). The number of plants per running metre, from infected seed, differed significantly from virus free controls. The number of plants per plot, yield per plot, tillers per plant and TKW also differed. The percentage of plants per plot with visible BMV infection symptoms (Table 17) in the surviving plants did not differ statistically from each other.

Table 17. The effect of % BMV seed-infection on plant performance

% SEED INFECTED	PLANTS/ METRE	PLANTS/ PLOT	TILLERS/ PLANT	% BMV SYMPTOMS[Y]	TKW	YIELD (t/ha)
0	10.95a[X]	315.3a	13.69b	0.95	39.55a	5.05a
10	9.89ab	300.1a	16.07b	0.99	37.70b	4.92a
20	8.81bc	270.1b	19.54a	0.98	37.44b	4.68ab
30	8.32c	252.2b	19.62a	0.98	35.72c	4.45bc
40	7.77c	245.6b	20.62a	1.07	34.84c	4.17c

[X] Numbers within a row followed by the same letters are not significantly different (P=0.05). Letters are omitted when no significant differences exist across an entire row.

[Y] Values calculated as a percentage of plants per plot with visible BMV symptoms. Infections were confirmed by ELISA, with symptomless plants testing negatively.

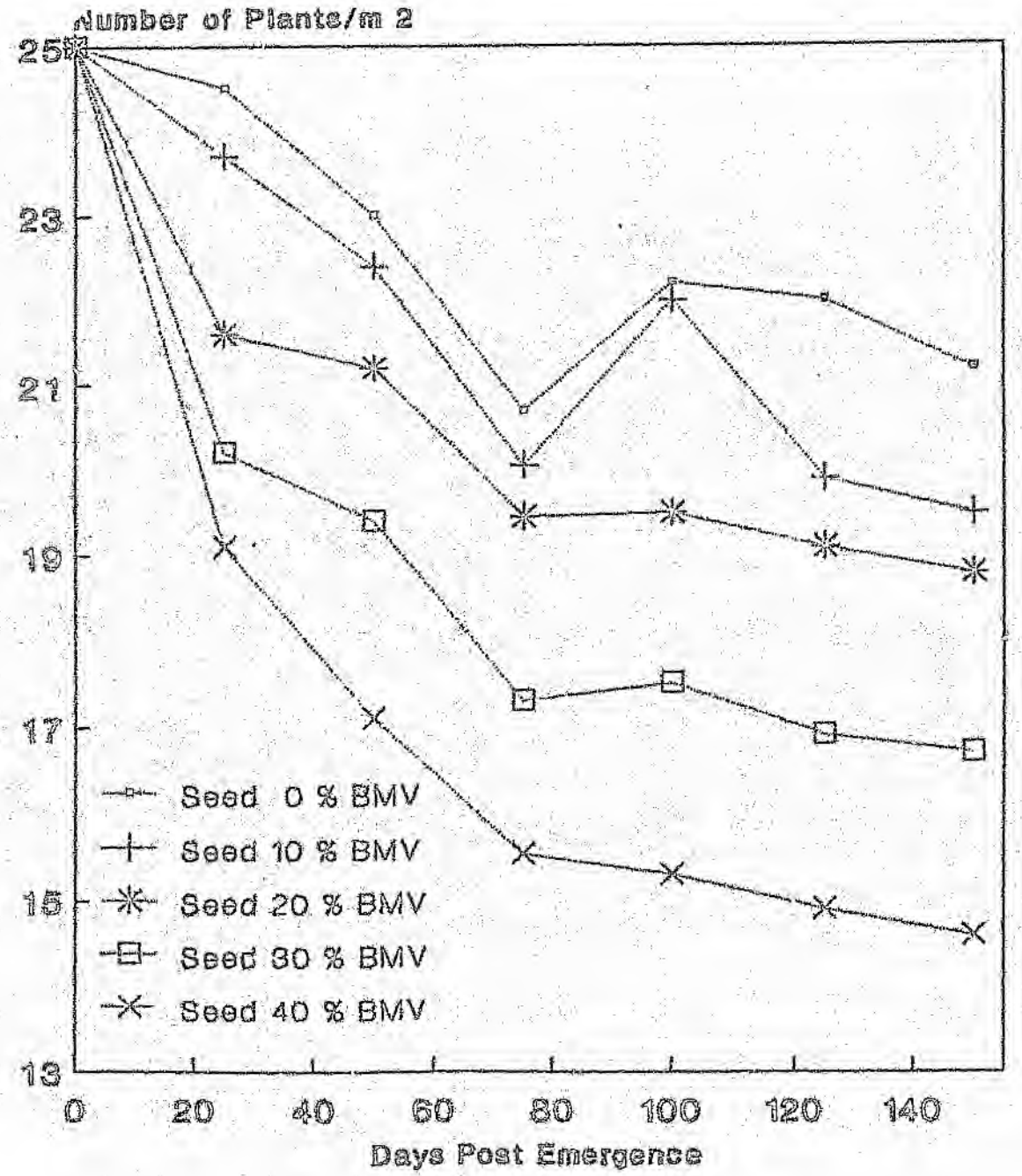


Figure 19. The number of plants per m^2 in plots planted with different levels of BMV seed infection.

The yield of the plants grown from virus-free seed (0%) and the lower percentages of seed infection (10%, 20%) did not differ significantly, but the plants grown from the 30% and 40% infected seed batches gave significantly lower yields (Figure 20).

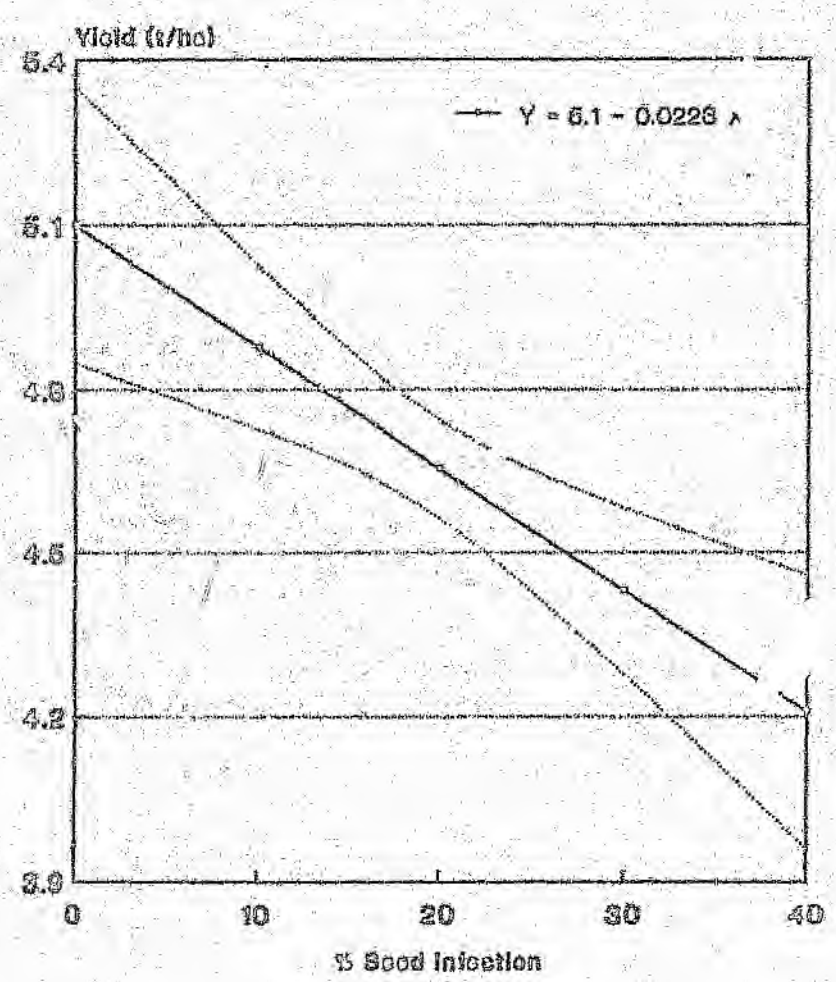


Figure 20. The correlation between percentage of BMV seed infection and yield loss. Dotted lines indicate the 95 % confidence interval.

4.5 APHID TRANSMISSION OF BMV

4.5.1 Incidence of naturally occurring viruliferous *D. noxia*

The results of field collected *D. noxia* tested for BMV with the ELISA showed that 14 % of the aphids were virus-infected. Only 5 % of the virus-free wheat exposed to field-collected *D. noxia* became infected with BMV.

4.5.2 Transmission method of BMV by *D. noxia*

4.5.2.1 Nonpersistent transmission

The greenhouse reared viruliferous aphids were not able to transmit BMV to virus-free plants effectively when a short feeding period was permitted (Table 18).

Table 18. Transmission of BMV by *D. noxia* after plants had been exposed to viruliferous *D. noxia* for different feeding periods

DURATION OF EXPOSURE TO VIRULIFEROUS *D.noxia* (min)	DURATION OF PRE-FEEDING FASTING PERIOD (min)										X
	5	10	15	20	25	30	35	40	45	50	55
0 (Control)	-Y	-	(3)	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	(1)	-	-	-	-	-	(1)	-
25	-	(2)	(3)	-	-	(1)	-	-	-	-	-

X Repetitions contained 10 plants in each exposure period

Y (2) Numbers of plants infected with BMV (-) Plant not infected with BMV.

Only a low percentage of successful BMV transmissions occurred. The variation in preacquisition fasting times had no effect on the rate of successful transmissions.

4.5.2.2 Persistent transmission

The experiment proved that *D. noxia* was able to transmit BMV serially from one host to the next, after the host had been colonised by the aphids for 48 hours at a time, before the aphids were transferred to the new batch of plants (Table 19).

Table 19. Serial transmission of BMV by *D. noxia* in the greenhouse

VIRUS INFECTED *D. noxia* PRIOR TO FIRST TRANSFER	PRE-FEEDING FASTING PERIOD (min)	NUMBER OF TRANSFERS TO NEW PLANTS				
		1	2	3	4	5
45%	10	9%[X]	3%	1%	0%	0%
32%	20	6%	4%	1%	0%	0%
37%	30	8%	2%	1%	0%	0%
21%	40	3%	1%	0%	0%	0%
29%	50	5%	2%	0%	0%	0%

[X] Figures expressed as percentage of plants with BMV infections.

It was found after the second transfer that a decrease in the number of plants with BMV infection had occurred.

4.5.2.3 Influence of the GS of the target plant and duration of exposure to viruliferous *D. noxia* on BMV transmission

It was found that infections occurring two weeks after initial colonisation by *D. noxia*, resulted in the highest virus concentrations in infected plants. The highest rate of transmission also occurred during this period (Table 20).

Table 20. Transmission of BMV by *D. noxia* after different exposure periods and during different growth stages

GROWTH STAGE	PLANTS INFECTED	AVERAGE BMV CONCENTRATION	DURATION OF EXPOSURE
25	17%	0.047 mg/g[x]	7 days
30	83%	0.598 mg/g	14 days
35	50%	0.390 mg/g	21 days
40	23%	0.110 mg/g	28 days
45	27%	0.075 mg/g	35 days
50	22%	0.039 mg/g	42 days

[x] = Concentrations of BMV in mg virus/g plant material

Although high percentages of transmission occurred, it was found that only plants infected with virus concentrations in excess of 0.4 mg/g were prone to yield loss (See 4.6.1). The apparent transmission ability of *D. noxia* is also misleading. It was found that it is the host susceptibility to BMV during GS 30 (See 4.3.2 and 4.3.3), rather than the ability of *D. noxia* to transmit BMV, that caused high percentages of effective transmission.

4.5.2.4 Vertical transmission of BMV in *D. noxia*

No vertical transmission of BMV occurred, even if the new aphids were the offspring of aphids that were BMV infected and had a proven ability to transmit BMV (See 4.5.2.3) (Table 21).

Table 21. The vertical transmission of BMV in *D.noxia*

PARENT APHIDS	OFFSPRING	WHEAT PLANTS[b]
.219(a)[a,c]	.001(b)	.001(b)
.221(a)	.002(b)	.003(b)
.211(a)	.001(b)	.015(b)
.199(a)	.001(b)	.003(b)
.192(a)	.001(b)	.004(b)
.108(a)	005(b)	.003(b)
.121(a)	.002(b)	.001(b)

a = The values indicate the absorbance at 405 nm in a test for BMV with the ELISA
b = Host plants for the offspring aphids
c = Numbers within a column followed by the same letters are not significantly different (P = 0.05)

Different instars of *D. noxia* might have differing abilities to transmit BMV, but this was not tested as populations used in experimentation consisted of random samples of all instars, and no significant difference in transmitting ability of populations was found during the course of this study.

4.5.2.5 Effect of BMV on the longevity of *D.noxia*

No significant difference in the longevity of *D. noxia* feeding on BMV infected and virus-free plants was found (Table 22).

The slight lengthening of the nymphal stages of the aphids feeding on BMV infected plants might be due to a lowering of the available nourishment caused by the virus infection of the host. The reproductive period was also shorter, but the mean number of offspring produced did not differ significantly.

Table 22. Life span of D. noxia at 14 °C feeding on virus infected and virus free host plants.

HOST PLANT	INSTARS I - IV	REPRODUCTIVE PERIOD(Days)	POST REPRODUCTIVE PERIOD(Days)	TOTAL LIFESPAN
Virus free	11.29	42.00	24.13	77.42
BMV infected	11.33	41.26	24.44	77.03

4.5.3 Effect of *D. noxia*-transmitted BMV on wheat in the greenhouse

The effect of aphid-transmitted BMV on plant development was determined in the greenhouse (Table 23). It was found that infection at an early stage resulted in some compensatory growth in the infected plant because of the increase in the number of tillers per plant. However, the effect of this stimulation in vegetative growth did not compensate for the loss of yield.

Infection during GS 30 had the greatest influence on plant yield, which was the same as was found with mechanical infection mentioned previously (Table 9).

Table 23. The effect of BMV transmitted by *D.noxia* on plant development after infection at three different growth stages during the season

INFECTION DURING	TILLERS	EARS	LENGTH (cm)	SEEDS	YIELD (g)	TT (g)
G.S. 15	11.9(ab)*	7.3	66.4(a)	177(b)	5.6(bc)	32.4(a)
G.S. 30	13.9(a)	7.3	66.4(a)	170(b)	4.7(c)	27.5(b)
G.S. 45	10.9(b)	7.5	61.0(b)	186(b)	6.0(b)	31.9(a)
CONTROL	10.7(b)	8.1	65.8(a)	230(a)	7.9(a)	34.4(a)

* Numbers within a column followed by the same letters are not significantly different (P=0.05). Letters are omitted when no significant differences exist.

It was also found that the virus concentration in plants was higher in those plants infected by aphid-transmitted virus as compared to mechanical infection. The virus multiplied rapidly in the plant for the first 50 days post infection. With mechanical infection the concentration peaked at an average of 0.64 mg BMV/g of plant tissue at 60 days post infection. With aphid-transmitted BMV the concentration peaked at an average of 1.0 mg/g at 70 days post infection (Fig. 21).

It was, however, also found that the variation in virus concentration was larger with aphid-transmitted virus, and that single plants in the same treatments had high virus concentrations, while others were essentially virus-free. This might be due to the variation in the ability of *D. noxia* as a vector of BMV, and the relatively low percentages of aphids that were able to successfully transmit the virus.

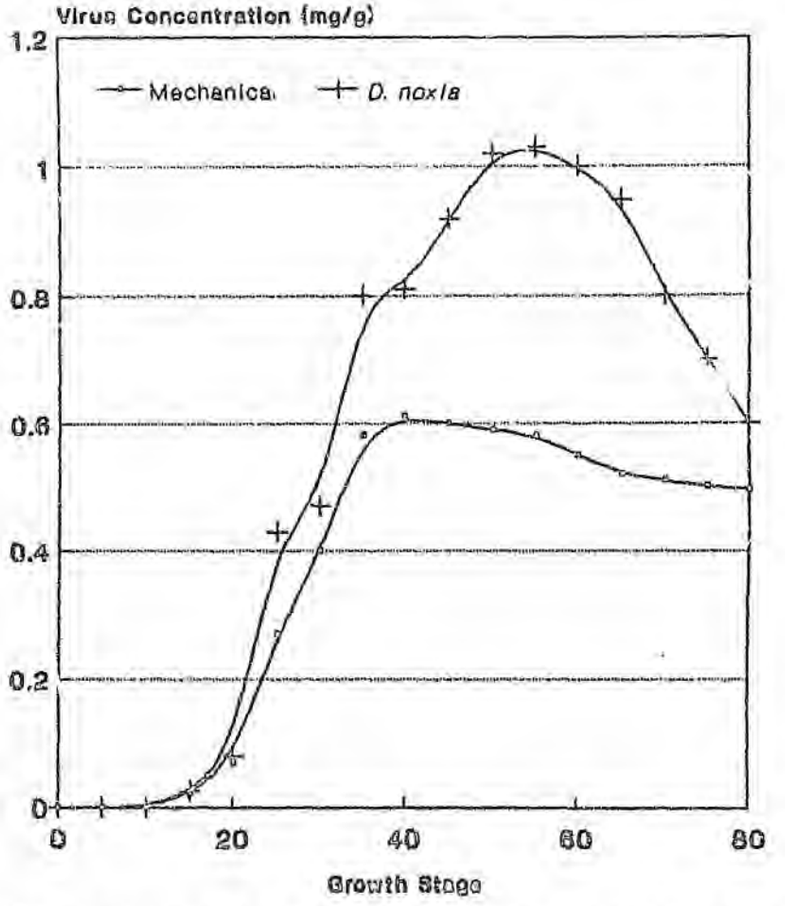


Figure 21.A comparison between the average increase of BMV concentration in plants after mechanical infection and infection by *D. noxia* transmitted virus

4.5.4 Effect of *D. noxia*-transmitted BMV on wheat under field conditions

The results showed that BMV could only cause a significant loss in yield in the absence of *D. noxia* if high percentages of plants were infected during GS 30. The greenhouse reared *D. noxia*, which had previously fed on BMV infected plants were allowed to infest the plots for one week only. A strict spraying programme for aphids was followed both before and after treatments. *D. noxia* feeding for such a short period

has no measurable effect on yield (Hewitt *et al.*, 1984; Kriel *et al.*, 1984; Fouché *et al.*, 1984) (Table 24).

Table 24. Yield loss caused by *D. noxia*-vectored BMV after infection at three different growth stages.

CONTROL	GS 30	GS 45	GS 55
4.358 t/ha(a)[y]	3.676 t/ha(b)	4.153 t/ha(a)	4.346 t/ha(a)
(0.7%)[x]	(85%)	(60%)	(48%)
% YIELD LOSS	15.6%	4.7%	0.3%

[x] Numbers in brackets indicate percentage of plants per plot testing positive for BMV.

[y] Numbers within a row followed by the same letters are not significantly different (P=0.05)

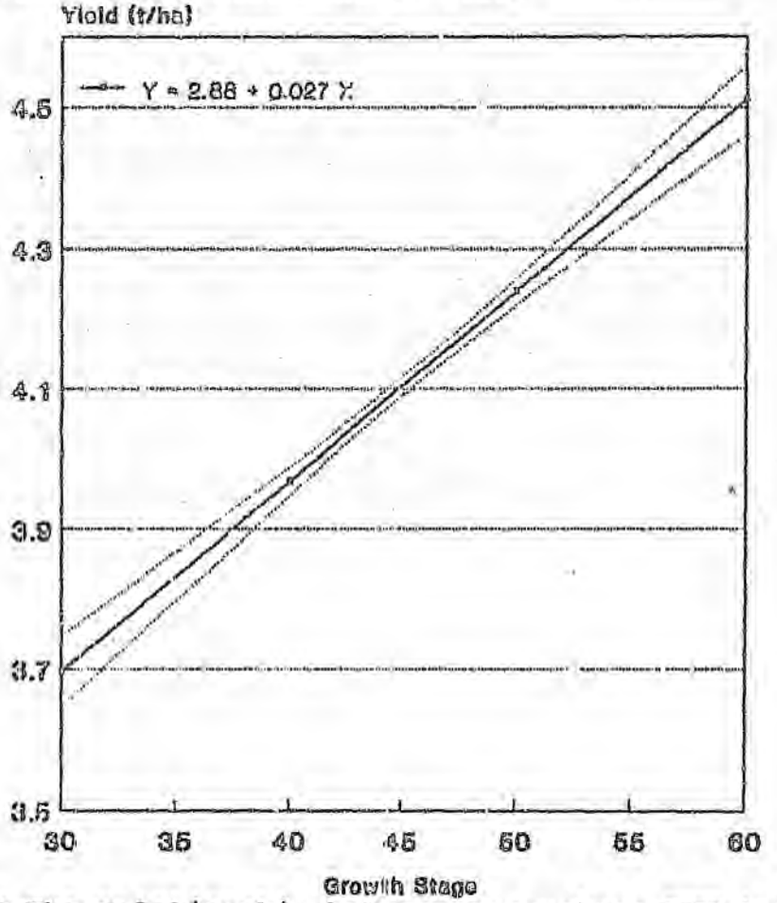


Figure 22. The relationship between percentage BMV infection, as determined with the ELISA, and yield after *D. noxia*-vectored infection at GS 30. Dotted lines indicate the 95 % confidence interval.

Only the plants treated at GS 30 yielded significantly less at the end of the season (Figure 22). This could be because virus infection occurred at a late stage in the plant's development and processes such as tiller formation and general plant growth could not be affected, since tillering was already complete and ears had already started to form. In addition there were no aphids present which would otherwise migrate from leaf to leaf and spread the infection throughout the plant.

4.5.5 Effect of seed- and *D. noxia*-transmitted BMV on wheat under field conditions

Neither BMV infection nor *D. noxia* infestation had any effect on the number of plants per metre, ears per metre or ears per plant (Table 25).

Table 25. The effect of BMV infected seed and BMV transmitted by *D. noxia* on wheat under field conditions

TREATMENT	PLANTS/m	EARS/m	EARS/PLANT	YIELD(t/ha)
SEED 0% BMV	8.78	72.34	10.04	5.35(a)[x]
SEED 25% BMV	8.46	72.86	9.93	5.00(b)
SEED 0% + *D.noxia*	8.14	66.26	8.95	4.96(b)
SEED 25% + *D.noxia*	8.04	63.00	8.72	4.60(c)

[x] Numbers within a column followed by the same letters are not significantly different (P=0.05). Letters are omitted when no significant differences exist.

The number of plants per running metre in plots planted with the infected seed did not differ significantly from

control plots. Effects on the wheat plant attributable to virus and virus/aphid infections were: The plant length, number of ears, seed mass and number of seeds (Table 26). The average yield loss attributable to seed infection was 7%, while aphids accounted for a loss of 8%, with a combined virus and aphid reduction of 14.4% in yield.

Table 26. The effect on plant development after being exposed to both seed- and aphid-borne BMV.

TREATMENT	TILLERS	LENGTH	SEEDS	MASS	YIELD(t/ha)
0%Seed infection					
control	25.62ab[x]	51.21a	482.3a	11.54a	5.35a
D noxia					
natural	28.44ab	45.89ab	447.5a	8.46bc	4.96b
viruliferous	21.22b	43.83ab	356.4bc	8.27bc	4.61c
25%Seed infection					
control	21.94b	45.58ab	445.6a	10.73ab	5.00b
D. noxia					
natural	33.43a	43.38ab	432.6ab	6.97c	4.60c
viruliferous	18.17b	38.35b	318.4c	6.17c	4.28d

[x] Numbers within a row followed by the same letters are not significantly different (P=0.05). Letters are omitted when no significant differences exist.

Additional losses for simultaneous seed-infection and *D. noxia* colonisation were found. Natural *D. noxia* infestation on plants originating from 25% infected seed indicated an additional 7.3% loss and virus infected *D. noxia* on the same plants, an additional 7.2% loss. This could be explained by the lower plant density, and resultant higher percentage of plants infested by the aphid.

4.6 COMPARISON OF THE EFFECT OF *D.noxia* FEEDING AND BMV INFECTION ON WHEAT UNDER FIELD CONDITIONS

The effect of *D. noxia* and that of BMV on field grown wheat plants are given in Table 27.

Table 27. Comparison of yield losses caused by virus free *D. noxia*, viruliferous *D. noxia* and mechanical BMV infection on wheat under field conditions

TREATMENT	YIELD (t/ha)	YIELD LOSS
CONTROL	6.78a*	
VIRUS FREE *D. noxia*	5.91ab	12.83%
VIRUS INFECTED *D. noxia*	5.73b	15.49%
10 % MECHANICAL BMV INFECTION	5.28b	22.12%
30 % MECHANICAL BMV INFECTION	5.18b	23.60%
50 % MECHANICAL BMV INFECTION	5.11b	24.63%

* Numbers within a column followed by the same letters are not significantly different (P=0.05)

In interpreting these results it must be remembered that mechanical infection of the plants resulted in higher effective infection than when the virus had been transmitted by *D. noxia* only (See 4.5.3).

D. noxia had a far more detrimental effect on yield than BMV, but with the simultaneous occurrence of BMV and *D. noxia* the effect on yield was increased (Fig. 23).

4.6.1 Effect of BMV concentration on yield

A significant correlation between virus concentration in a plant and the same plant's yield was found in all of the ap-

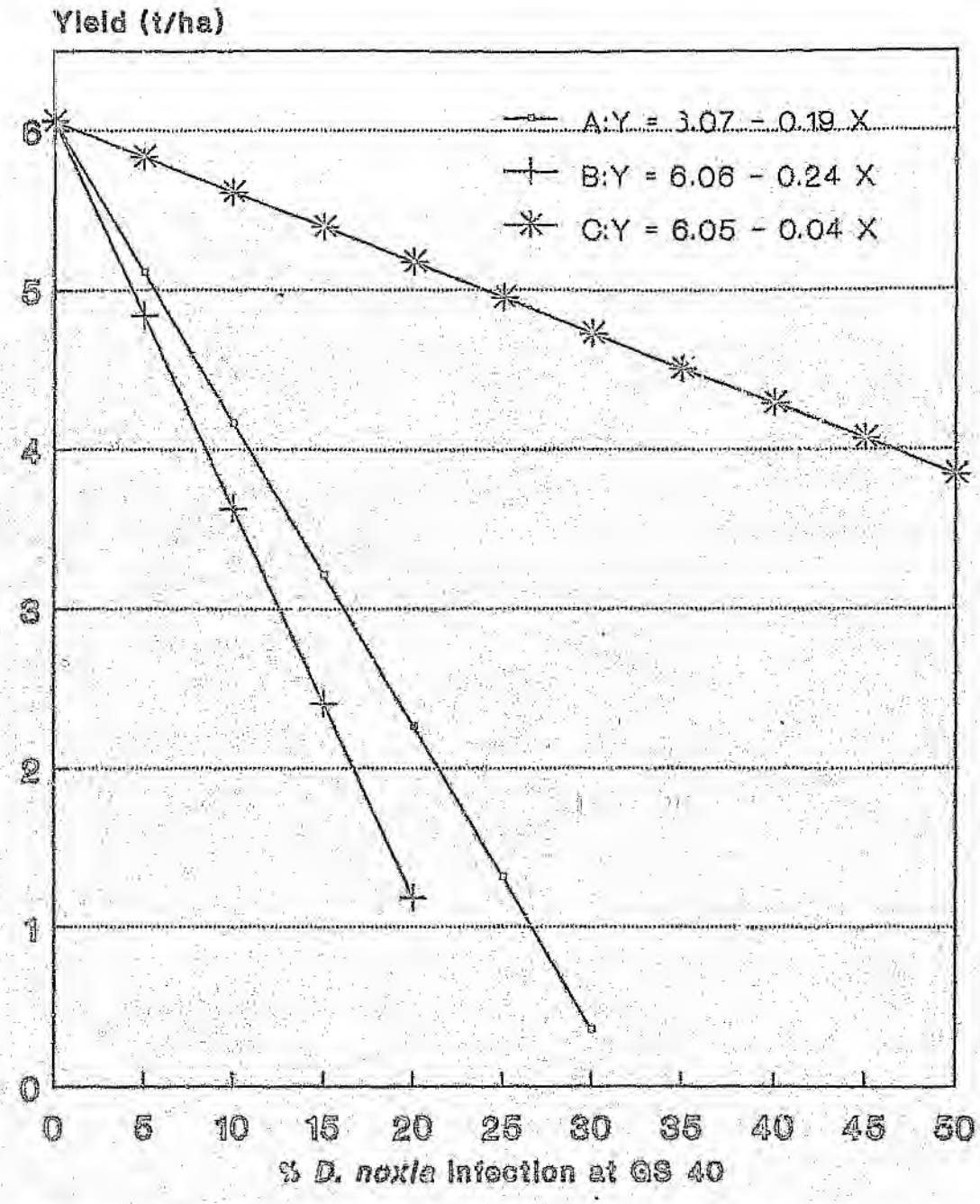


Figure 23. The effect of percentage of plants infested with *D. noxia* at G S 40 and BMV on the yield of wheat under field conditions. A) *D. noxia* infestation, B) *D. noxia* and BMV and C) BMV infection.

proximately 4000 plants tested individually (Table 28). Results showed that only after a titer of approximately 0.4 mg virus/g of plant material was reached, did the yield loss become significant (Fig. 24).

It was also found that the variation around the line decreased significantly with higher virus levels, which indicated that the higher the virus concentration, the more the yield of the plant was influenced (Table 28).

Table 28. The relationship between virus concentration /plant and average yield per plant

VIRUS CONCENT. INTERVALS (mg/g)	AVERAGE YIELD PER PLANT	NUMBER OF PLANTS PER CATEGORY	CORRELATION (r^2)	% OF TOTAL POPULATION IN CATEGORY
0 - 0.4	16.51g	2024	0.014	60
>0.4 - 0.8	6.02g	708	0.472*	21
>0.8 - 1.2	3.51g	436	0.634*	13
>1.2 - 1.6	2.51g	64	0.641*	2
>1.6 - 2.0	2.09g	36	0.653*	1
>2.0 - 2.4	1.37g	92	0.645*	3

* Denotes a significant correlation (P=0.001)

When examining the yield reduction of the virus infected plants one must bear in mind that they were also colonised by aphids which can also cause a yield reduction of up to ± 40 %. It must also be stressed that only about 40 % of nearly 4000 plants, tested individually for virus, reached concentrations equal to or higher than 0.4 mg/g, and BMV could therefore only be deemed to be a contributing yield-

limiting factor in this portion of the plant population (Table 28).

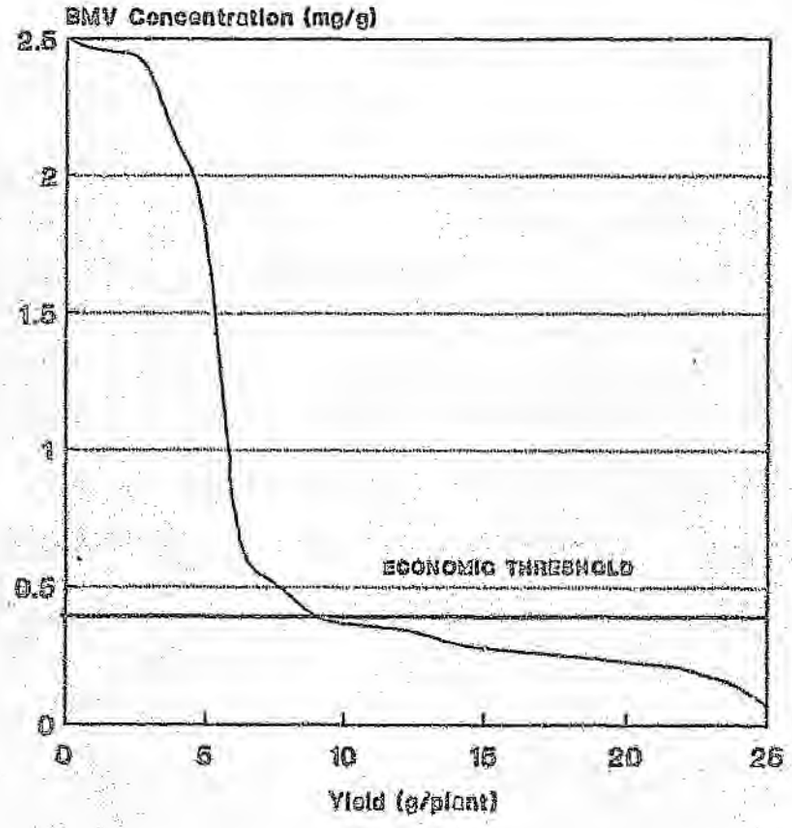


Figure 24. Yield loss associated with BMV in infected wheat, indicating the threshold for BMV concentrations of yield reducing potential.

A Poisson distribution analysis was done to determine if the distribution of plants in the different concentration intervals was random. It was found that the number of infections of a concentration equal to or lower than 0.4mg/g was significantly higher, and a normal distribution could not be indicated. There was a significant correlation be-

tween virus concentration and yield reduction only after a titer higher than 0.4 mg/g was reached in the infected plant.

4.7 EPIDEMIOLOGY OF BMV IN THE SUMMER RAINFALL AREA

A single experiment, incorporating 110000 plants, was designed to investigate, in a single season, all the previously studied epidemiological aspects in order to validate the results found in the previous seasons. Since the results did not differ significantly from previous findings, this experiment was undertaken during one season only. This experiment served a further purpose in that it formed the basis for the verification of the epidemiological model results (See 4.8).

4.7.1 Role of alternate hosts in the epidemiology of BMV

The volunteer wheat in the field and *Bromus* spp. and volunteer wheat surrounding the field were all heavily infected with BMV (Table 29).

These plants acted as inoculum sources of BMV for further dissemination by *D. noxia*. Results at the end of the season proved that BMV infection of the newly sown crop were initiated from "point" sources, within the field and surrounding the field where volunteer wheat occurred. The *Bromus* grass were not colonised by *D. noxia* during the month preceding planting and subsequently. Some of these plants

over-seasoning hosts for *D. noxia*, and could therefore have served as inoculum sources for the volunteer wheat.

Table 29. The incidence of BMV in alternate host plants surrounding and within a newly sown wheat field

SOURCE PLANTS	MAY	JUNE	JULY
Volunteer wheat:			
surrounding field	0.6-2.4[a]	1.1-2.7	0.6-0.9
within field	0.4-0.6	0.9-1.4	1.3-1.9
Bromus spp.	0.3-0.4		
% Volunteer wheat infected:			
surrounding field	64%	24%	4%
within field	14%	100%	100%

[a] = Virus concentration in mg BMV per g plant material

Samples of *D. noxia* found on the volunteer wheat were tested with the ELISA and 40% of the samples were found to be infected with BMV.

4.7.2 Role of seed-transmitted BMV in the epidemiology of this virus

The seed samples tested before planting were found to be 7% infected with BMV. This is within the normal range for commercial seed, and with this percentage, no significant effect of seed-transmitted was found.

Approximately 0.1% of the newly sown crop tested positively for BMV before *D. noxia* colonisation commenced. The plants testing positively were never nearer than 5 m from the nearest volunteer wheat plants, and no indication of aphid feeding or other insect damage and leaf or stem rust were

feeding or other insect damage and leaf or stem rust were found on these plants. It was therefore reasoned that this infection had been caused by seed-transmitted BMV. The colonisation pattern of *D.noxia* later in the season also indicated that these plants were not preferentially colonised. These plants therefore did not constitute a primary virus reservoir (Von Wechmar, 1980), and were not considered to be important in the epidemiology of BMV.

4.7.3 Role of *D.noxia*-transmitted BMV in the epidemiology of this virus

Although *D. noxia* was found to be an ineffective vector of BMV (See 4.5.2.1-3), the enormous population build-up of this aphid during the wheat season would suggest that even if BMV transmission at low levels occurred, the number of plants infected would be large.

Contrary to the findings of Von Wechmar and Rybicki (1981), BMV was transmitted by *D. noxia* in a semipersistent manner under field conditions. These findings agree with those of Damsteegt and Hewings (1987). This manner of transmission of BMV would limit the dissemination of BMV under field conditions.

It was found (Fig. 25) that even with the enormous aphid population build-up during GS 20-35, very few plants became infected with BMV. Most of these infections also fell in

The growth stage during which plants became infected with BMV also had an effect on the virus concentration reached in such plants (Table 30).

It was also found that effective transmission of BMV decreased during the season up to GS 45 when all effective transmission ceased. A slight increase was noted during GS 30 (Table 31). The high effectivity at the beginning of the season was probably due to aphids colonising wheat directly from the highly infected volunteer plants. These aphids were exposed to high concentrations of BMV (See Table 29), and the transmission was effectivity high (Table 31). Subsequent transfers were from plants with lower virus concentrations (Table 30) causing the rate of effective transmission to drop rapidly.

Table 30. The effect of growth stage on virus concentration in number of plants infected with BMV under field conditions

	VIRUS CONCENTRATIONS (mg/g)				
GS	0.0-0.1	>0.1-0.2	>0.2-0.3	>0.3-0.4	0.4 +
15	0.0 g[x]	0.0 g	0.0 g	1.0 g	2.0 g
20	22.0 fg	14.0 fg	2.0 g	3.0 g	1.0 g
25	100.0 efg	21.0 fg	3.0 g	2.0 g	2.0 g
30	512.0 c	114.0 ef	13.0 fg	1.0 g	11.0 fg
35	570.0 c	149.0 e	11.0 fg	3.0 g	3.0 g
40	783.0 b	2.0 g	3.0 g	0.0 g	9.0 fg
45	958.0 a	13.0 fg	5.0 g	5.0 g	9.0 fg
50	564.0 c	3.0 g	0.0 g	0.0 g	0.0 g
55	336.0 d	0.0 g	0.0 g	0.0 g	0.0 g

[x] Numbers within a column followed by the same letters are not significantly different (P=0.05)

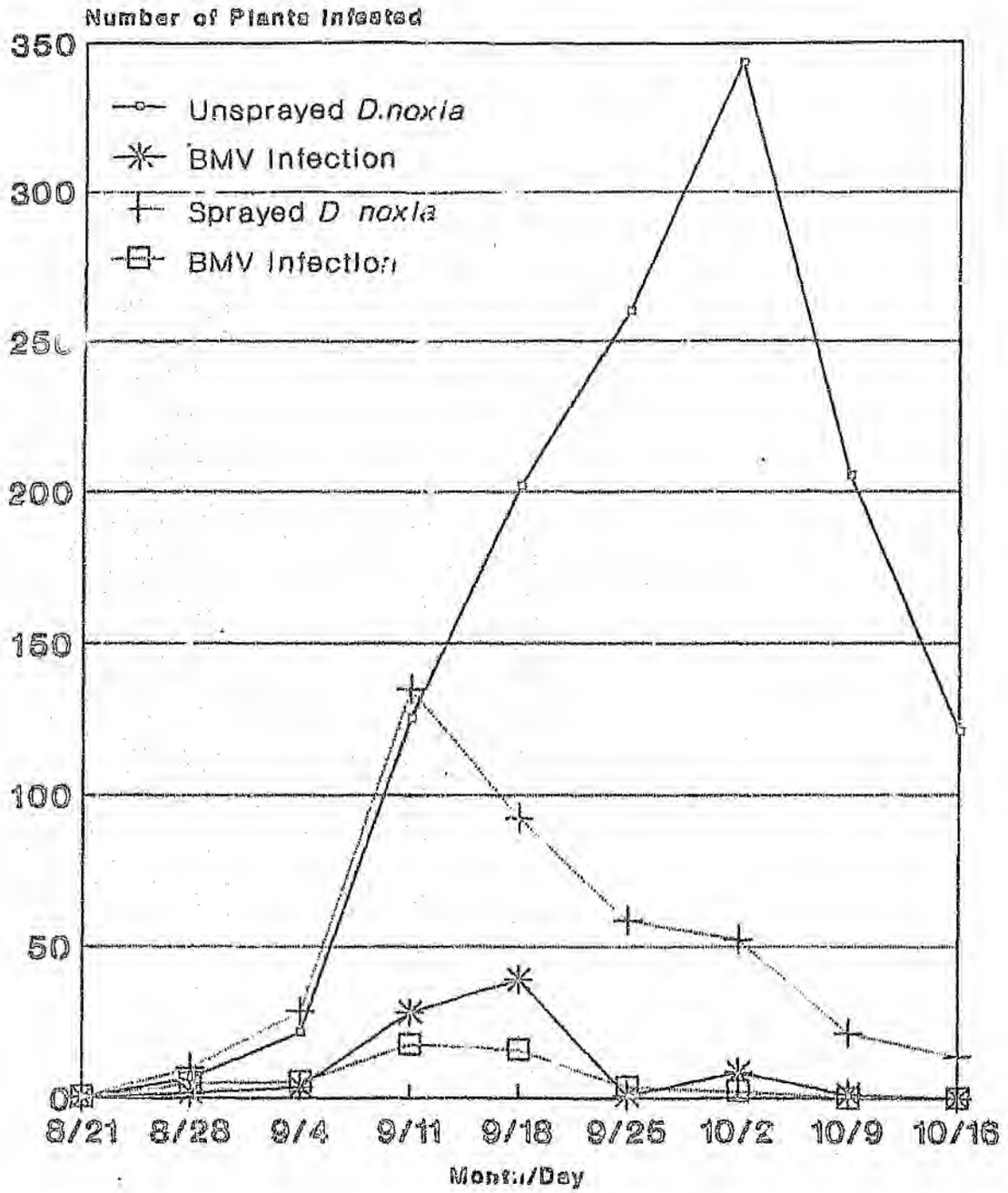


Figure 25. The build up of a *D. noxia* population during the a wheat season, and the associated transmission of BMV.

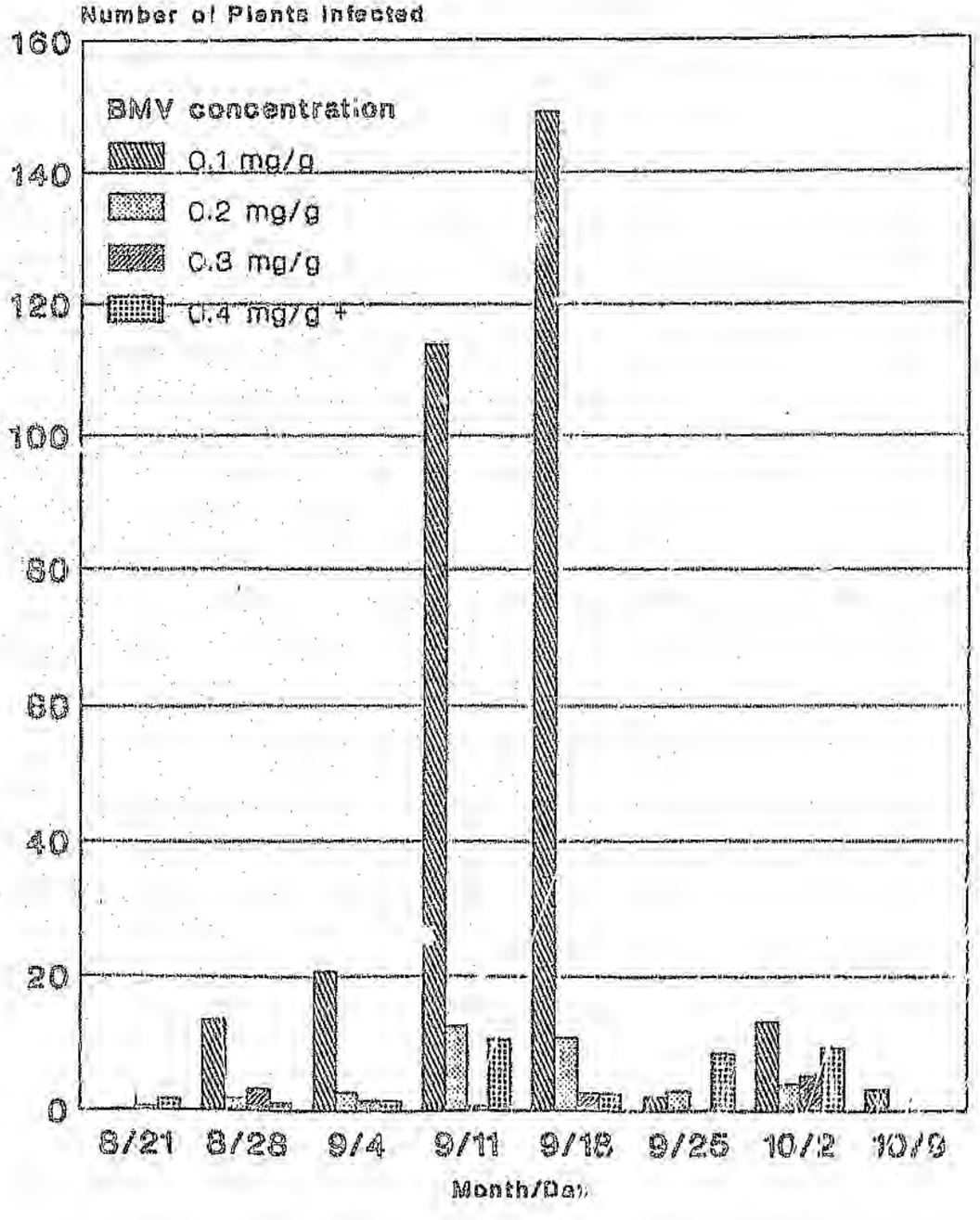


Figure 26. The virus concentrations in plants infected by aphid-transmitted BMV under field conditions.

It was found that other factors such as the GS of the host and target plant also influenced the transmission of the virus.

Table 31. The effect of GS of the host plant on the transmission of BMV by *D. noxia* under field conditions

GS OF TARGET PLANT	EFFECTIVE TRANSMISSION
15	73.9%
20	6.0%
25	1.6%
30	2.1%
35	0.4%
40	1.1%
45	0.9%
50	0.0%
55	0.0%

The effect of virus concentration and GS during infection could also be seen in the yield (TKW) at the end of the season (Fig. 27).

The results presented in 4.6 were also confirmed in this experiment. The additional loss caused by BMV in the presence of *D. noxia* was found to be low (Fig. 28).

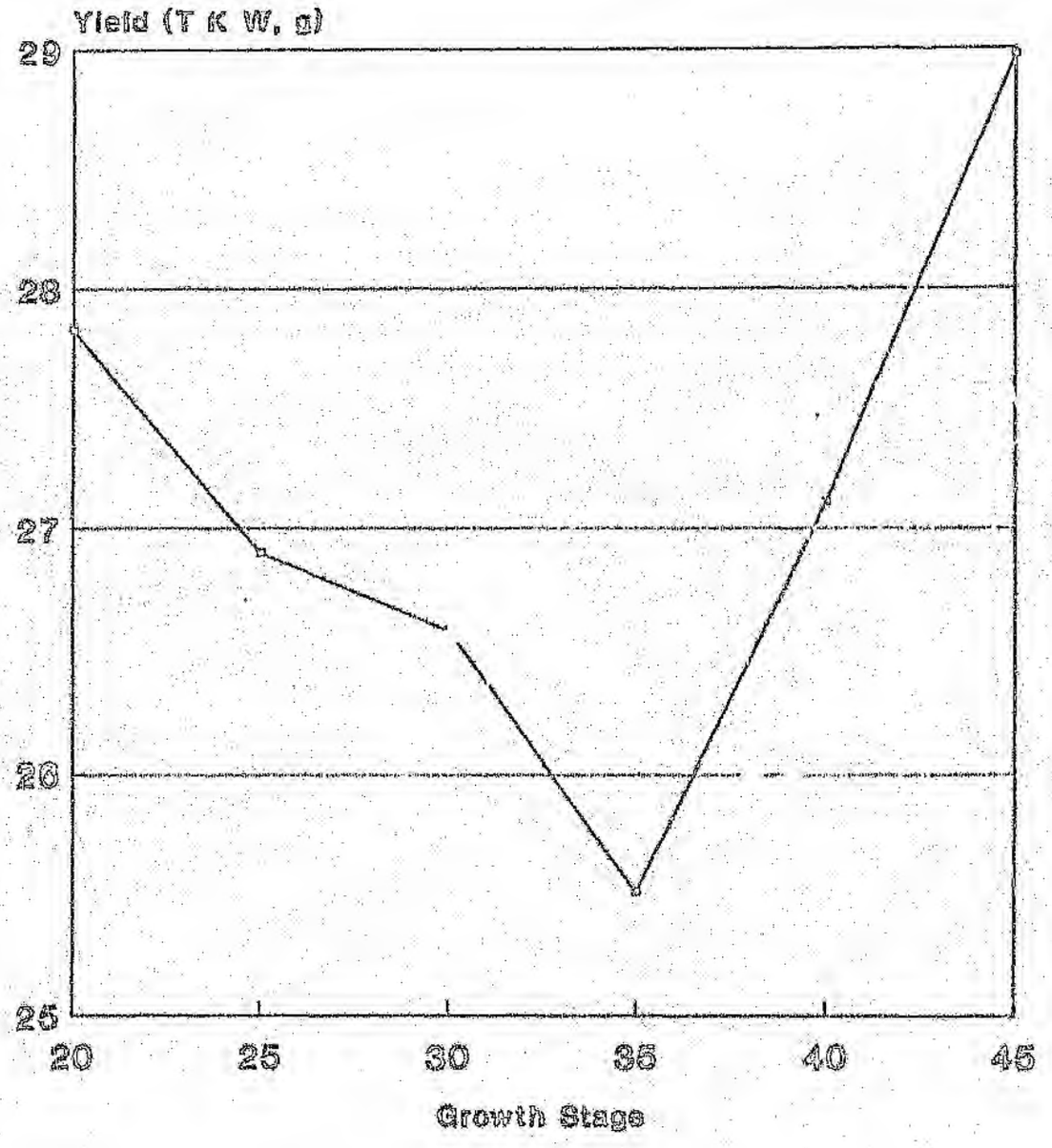


Figure 27. The effect of aphid vectored BMV infection during different growth stages on the TKW of infected plants.

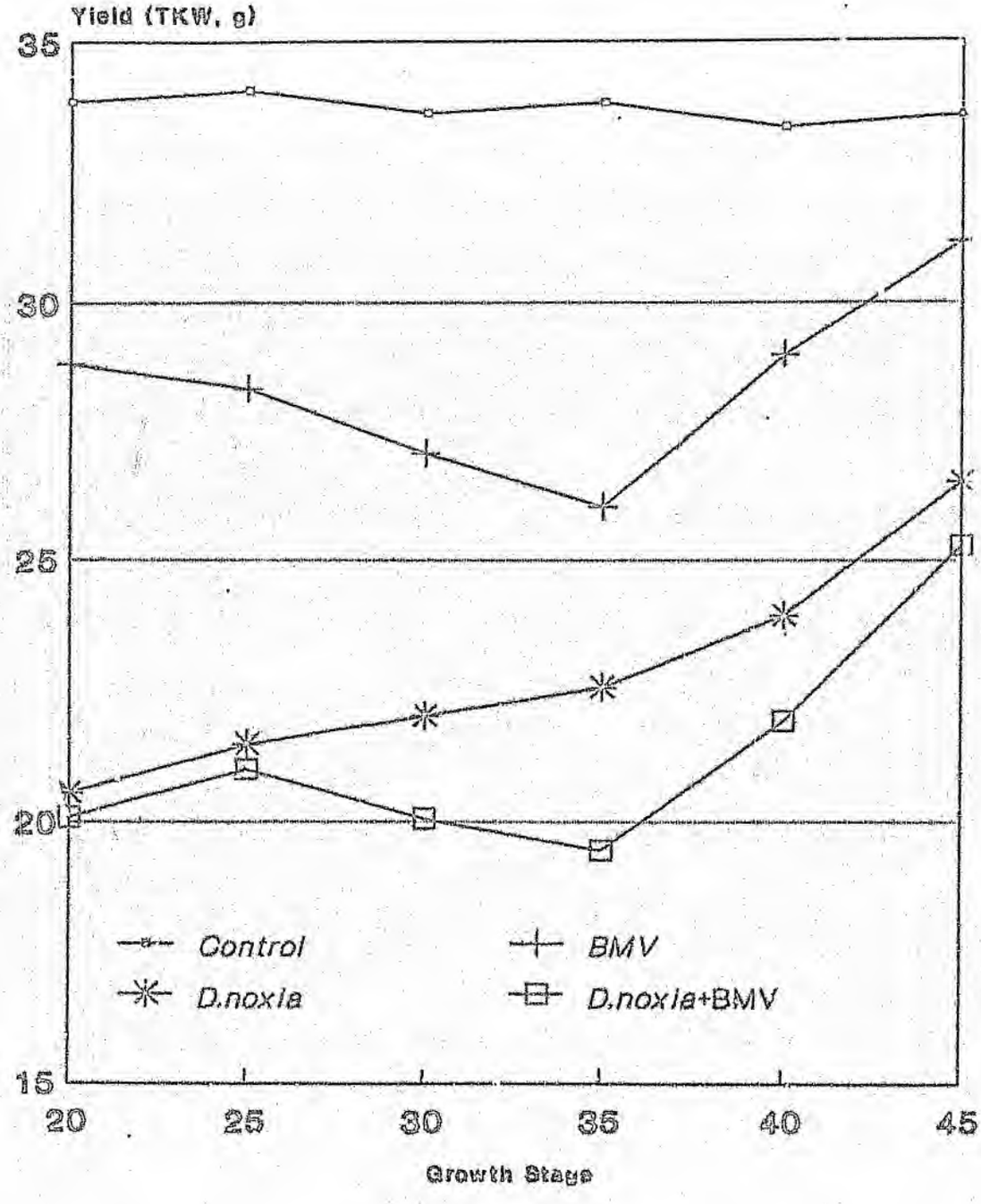


Figure 28. The effect of BMV in the presence and absence of *D. noxia* on the TKW of infected plants.

4.7.4 Natural dissemination of BMV under field conditions

In the two weeks following initial colonisation (during GS 12) of the field by *D. noxia* analyses of the spatial distribution with the ordinary run method indicated a normal distribution of infested plants. This also held true for BMV infection (Fig. 29, 30). The pattern of *D. noxia* colonisation changed during the following three weeks (up to GS 30), when the pattern gradually changed from random to clustered in all plots (Fig 31, 32). The spatial distribution of BMV infection remained random up to the week before GS 30, when it changed to clustered. The effect of wind could be seen during this period, in that *D. noxia* infested plants formed a triangular pattern with the volunteer plants (infestation focal points) being roughly halfway down the base line of the triangle, and infested plants forming a triangle in the direction of the prevailing daily wind direction.

During the final phase of *D. noxia* colonisation the distribution of aphids became epidemic, with nearly 100% of the plants in plots where no chemical control was used, being infested (Fig. 33, 34). The spread of BMV slowed down during this period, and remained essentially the same as during the previous three weeks.

. = UNINFECTED PLANT

i = D. noxia + BMV ABOVE 0.4 mg/g

o = D. noxia + BMV BELOW 0.4 mg/g

d = D. noxia INFESTED PLANT

s =POSSIBLE SEED-TRANSMITTED BMV (BELOW 0.4 mg/g)

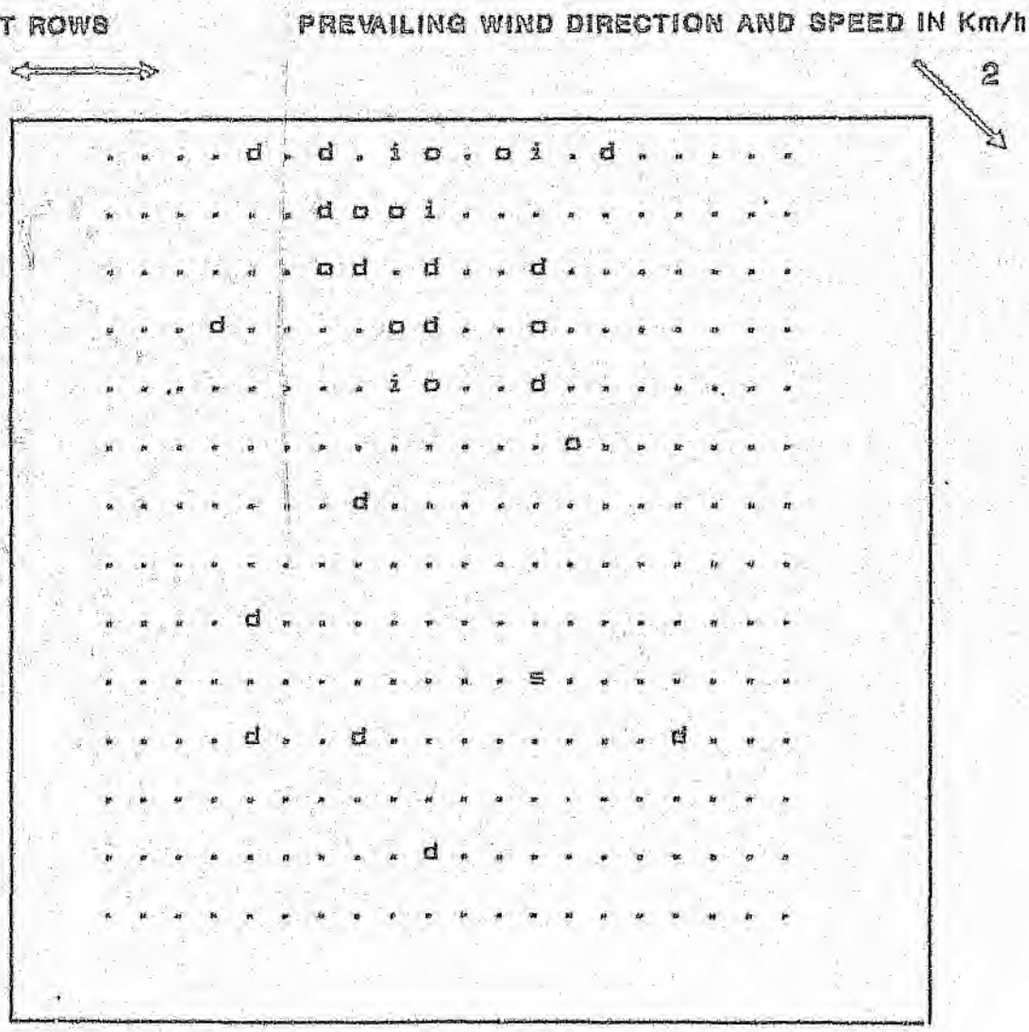


Figure 29.A generalised distribution map of *D. noxia* infested and BMV infected plants in a plot during the early stages of the wheat season.

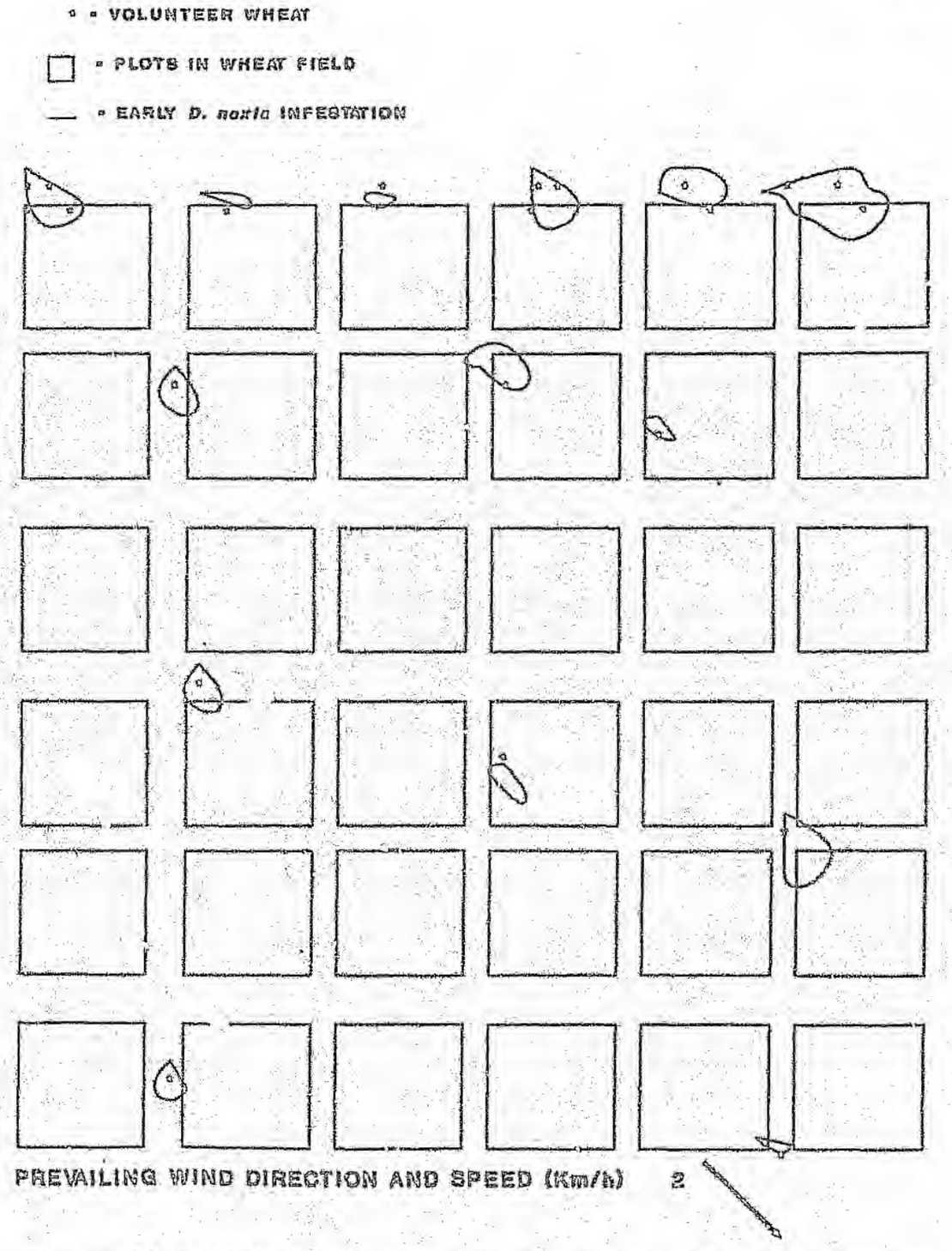


Figure 30.A distribution map of *D. noxia* infestation within a field during the early stages of the wheat season.

. = UNINFECTED PLANT

i = D. noxia + BMV ABOVE 0.4 mg/g

o = D. noxia + BMV BELOW 0.4 mg/g

d = D. noxia INFESTED PLANT

s =POSSIBLE SEED-TRANSMITTED BMV (BELOW 0.4 mg/g)

PLANT ROWS ⟺

PREVAILING WIND DIRECTION AND SPEED IN Km/h 8 ↘

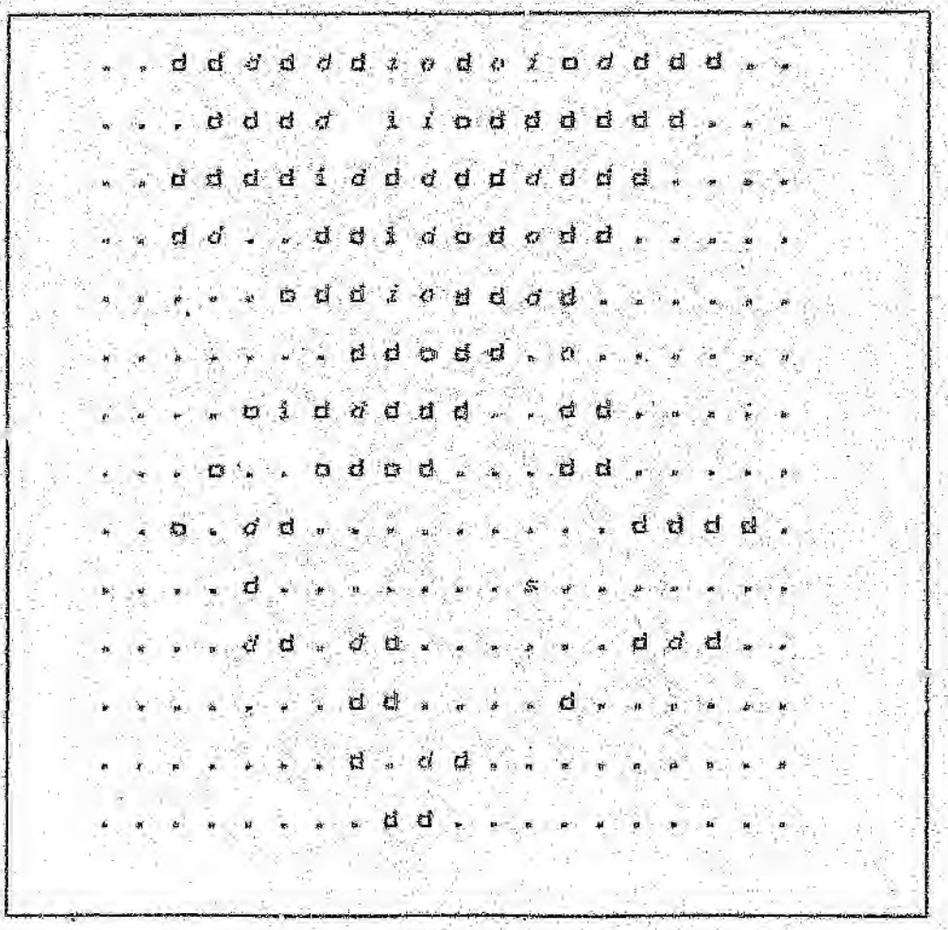


Figure 31.A generalised distribution map of *D. noxia* infested and BMV infected plants in a plot during the middle stages of the wheat season. New infections are indicated in bold paint.

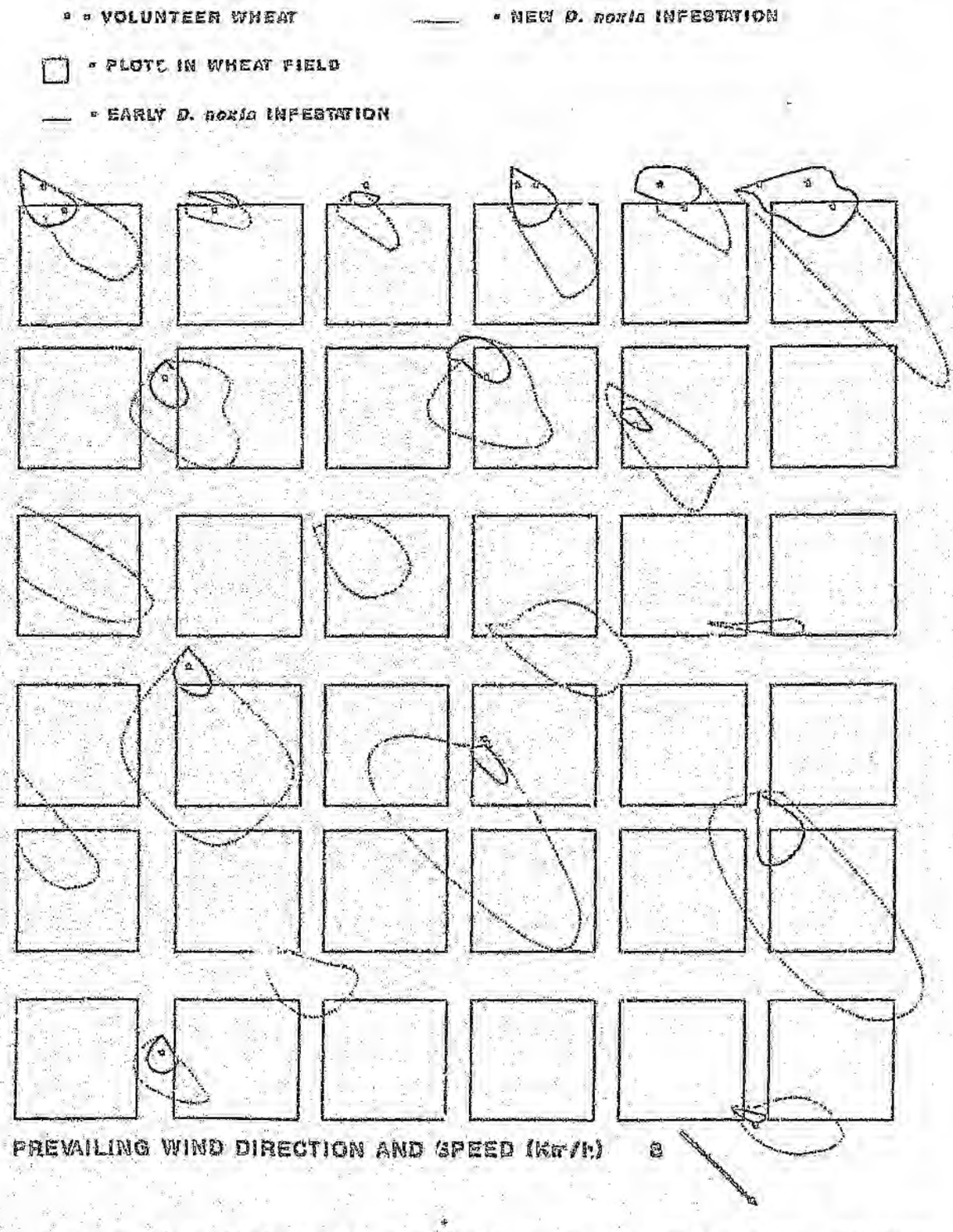


Figure 32. A distribution map of *D. noxia* infestation within a field during the middle stages of the wheat season.

. = UNINFECTED PLANT

i = D. noxia + BMV ABOVE 0.4 mg/g

o = D. noxia + BMV BELOW 0.4 mg/g

d = D. noxia INFESTED PLANT

s =POSSIBLE SEED-TRANSMITTED BMV (BELOW 0.4 mg/g)

PLANT ROWS ⟺

PREVAILING WIND DIRECTION AND SPEED IN Km/h

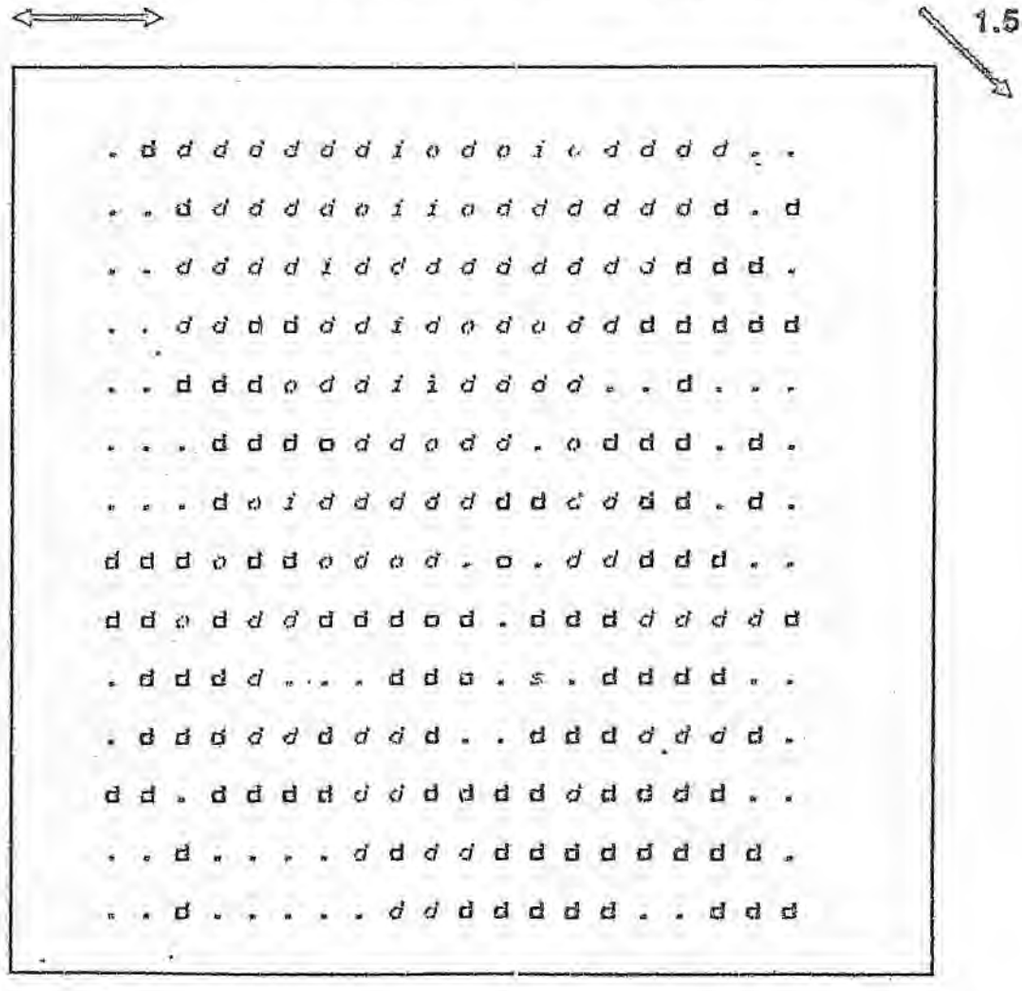


Figure 33.A generalised distribution map of *D. noxia* infested and BMV infected plants in a plot during the late stages of the wheat season. New infections are indicated in bold print.

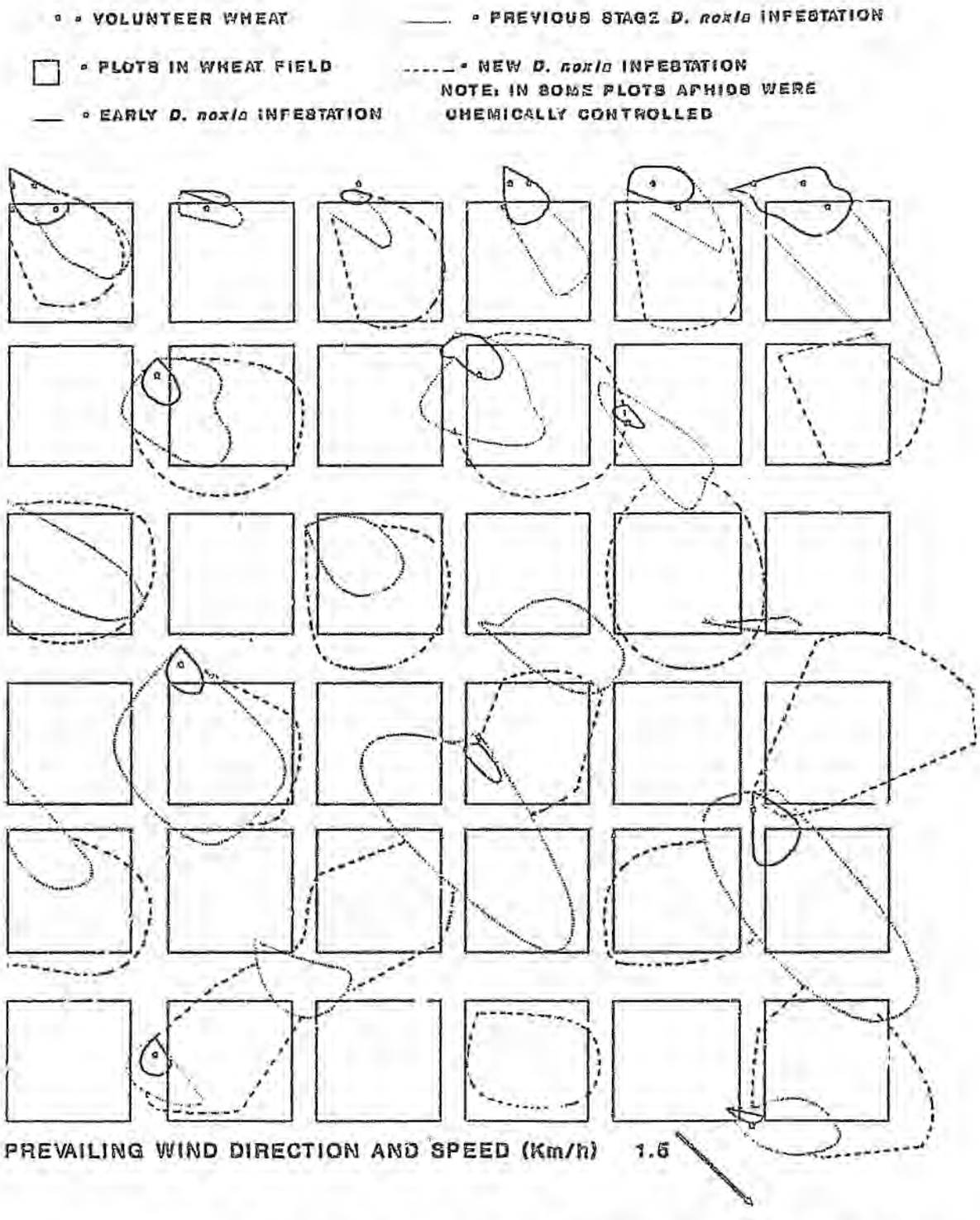


Figure 34.A distribution map of *D. noxia* infestation within a field during the late stages of the wheat season.

4.7.5 Effect of chemical control of *D. noxia* on BMV dissemination under field cordicions

Chemical control of *D. noxia* at GS 30 caused a marked decline in new plants becoming infected with BMV (Fig. 35). The first control attempt was unsatisfactory, and control was repeated a week later. The unsatisfactory control during the first attempt was due to the dry period experienced during this part of the season.

The effect of chemical [illegible] also seen in the yield data (Table 32). The [illegible] infected with virus concentrations in excess of [illegible] topped and a lower yield loss was recorded. The [illegible] *D. noxia* at GS 30 also caused virus infected plants to recover from the detrimental effects of BMV to a certain degree.

Table 32. The effect of aphid control on virus concentration in infected plants, and the effect on yield (TKW)

BMV CONCENTRATION(mg/g)	UNSPRAYED	SPRAYED
0.0-0.1	33.562a*	33.368a
>0.1-0.3	23.011c	30.932a
>0.3-0.4	22.530c	28.085b
0.4 +	19.810d	25.884bc

* Numbers within a column followed by the same letters are not significantly different (P=0.05)

CHAPTER 5

CONCLUSIONS AND DISCUSSION

5.1 VIRUS ISOLATION FROM WHEAT IN THE SUMMER RAINFALL AREA

Extraction of virus from diseased plants collected in the summer rainfall area resulted in the recovery of large amounts of BMV with the pH 4 acetate/PEG procedure. Similar plants were used in the isolation procedure for CMV. No CMV could be recovered from field-infected plants, but the ELISA confirmed the simultaneous presence of this virus in 1.5% of BMV infected plants tested. No BYDV was isolated from diseased plants in the summer rainfall area, and no positive ELISA results were obtained when testing for BYDV.

5.2 CHARACTERISATION OF VIRUS ISOLATED FROM WHEAT IN THE SUMMER RAINFALL AREA

The various analytical procedures used showed the BMV to be "pure" using ultraviolet spectroscopy, sucrose density gradient banding, electron microscopy and serological techniques. Electron microscopy of purified BMV did not reveal any detectable contamination with other viruses.

Comparison of the summer rainfall area isolate of BMV with the isolate characterised by Rybicki (1984), indicated no major differences. This strain is reported by him to be a

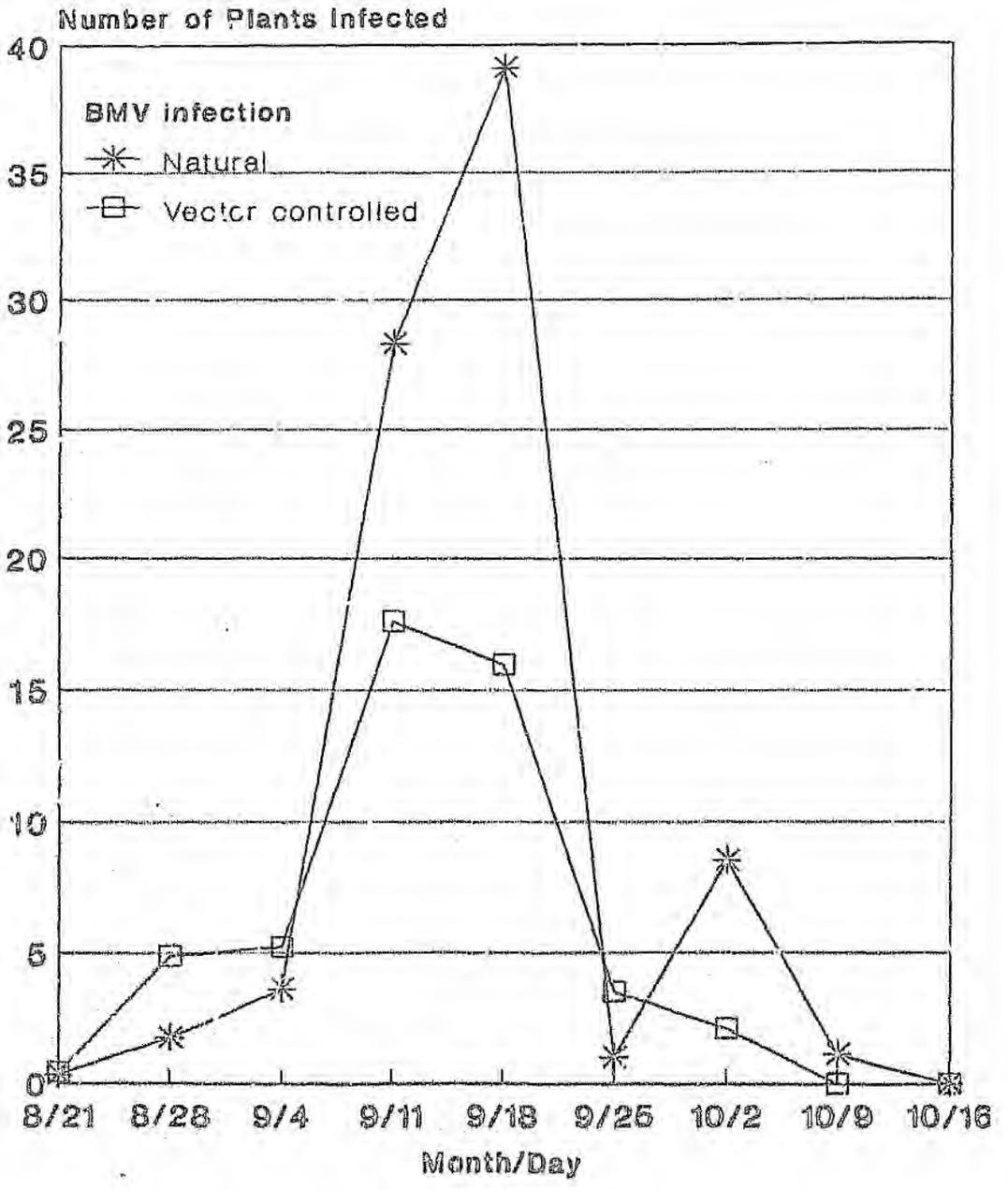


Figure 35. The effect of chemical control of the aphid vector on the dissemination of BMV under field conditions.

4.8 COMPUTER SIMULATION OF THE EPIDEMIOLOGY OF BMV

The model was run with weather data of the 1987 - 1989 seasons and the simulation compared to data reported by Aalbersberg (1987), Du Toit (1986(b)), and data of the author not used in constructing the model (Fig. 36). The model compared well with the 1987 and 1989 season, but could not accurately simulate the 1988 season. The reason for this could be the mild winter preceding the 1988 wheat season and the probably higher survival rate of *D. noxia*. This could have caused a higher number of plants to be colonised early in the season. A number of 10 plants initially infested were used in these runs, but this did not take the above mentioned factor into consideration.

A simulation of the 1989 season, using the data used to construct the model, was also run (Fig 37). It was found that the model simulated the actual data very well. This does not, however, invalidate the shortcomings of the model to be discussed later.

The effect of seed infection was also accurately predicted by the model (Fig 38). The number of plants decreased in the same order that as found with actual data recorded during the course of this study.

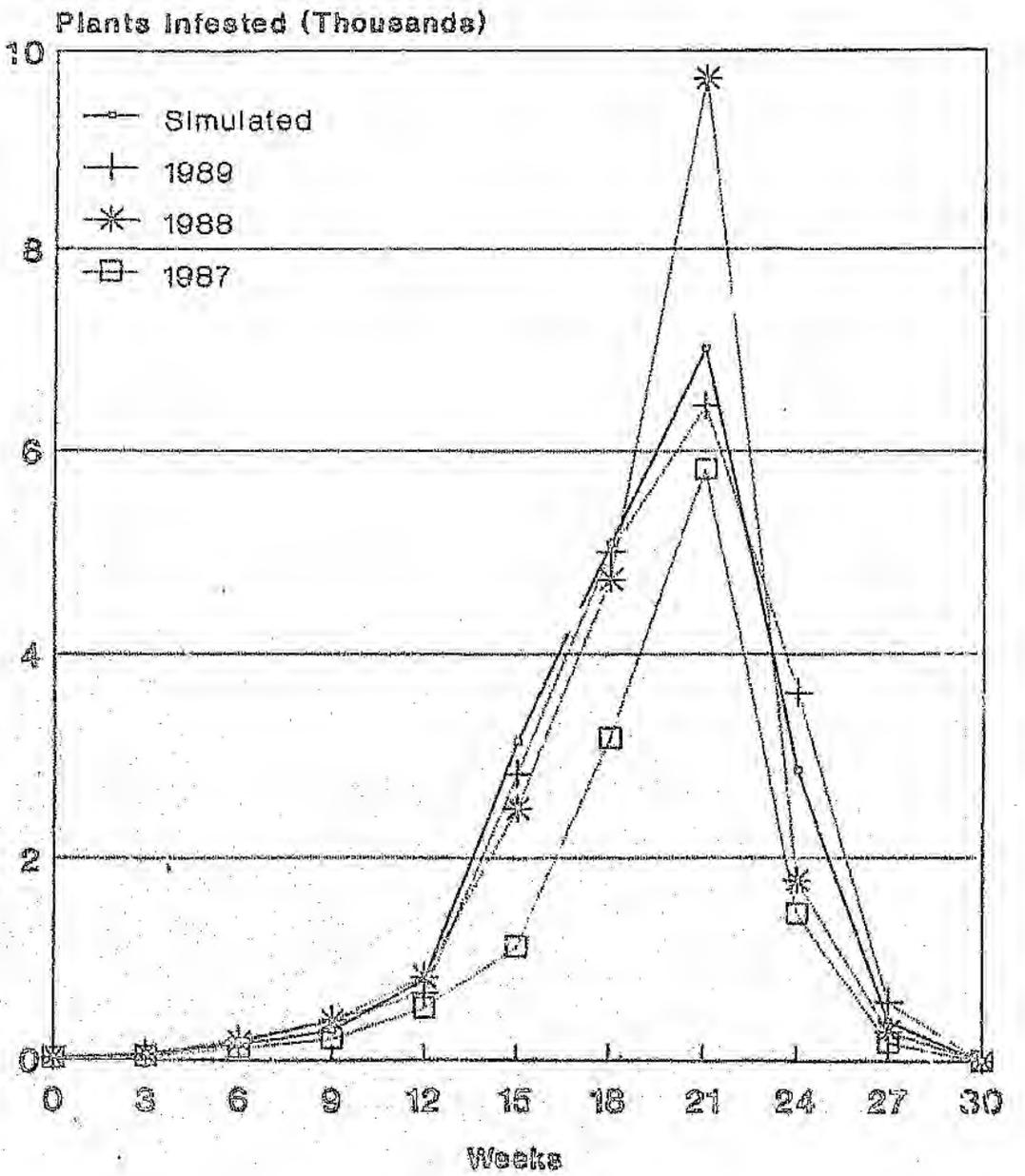


Figure 36.Validation of the simulated model against actual data recorded during the 1987-1989 wheat seasons.

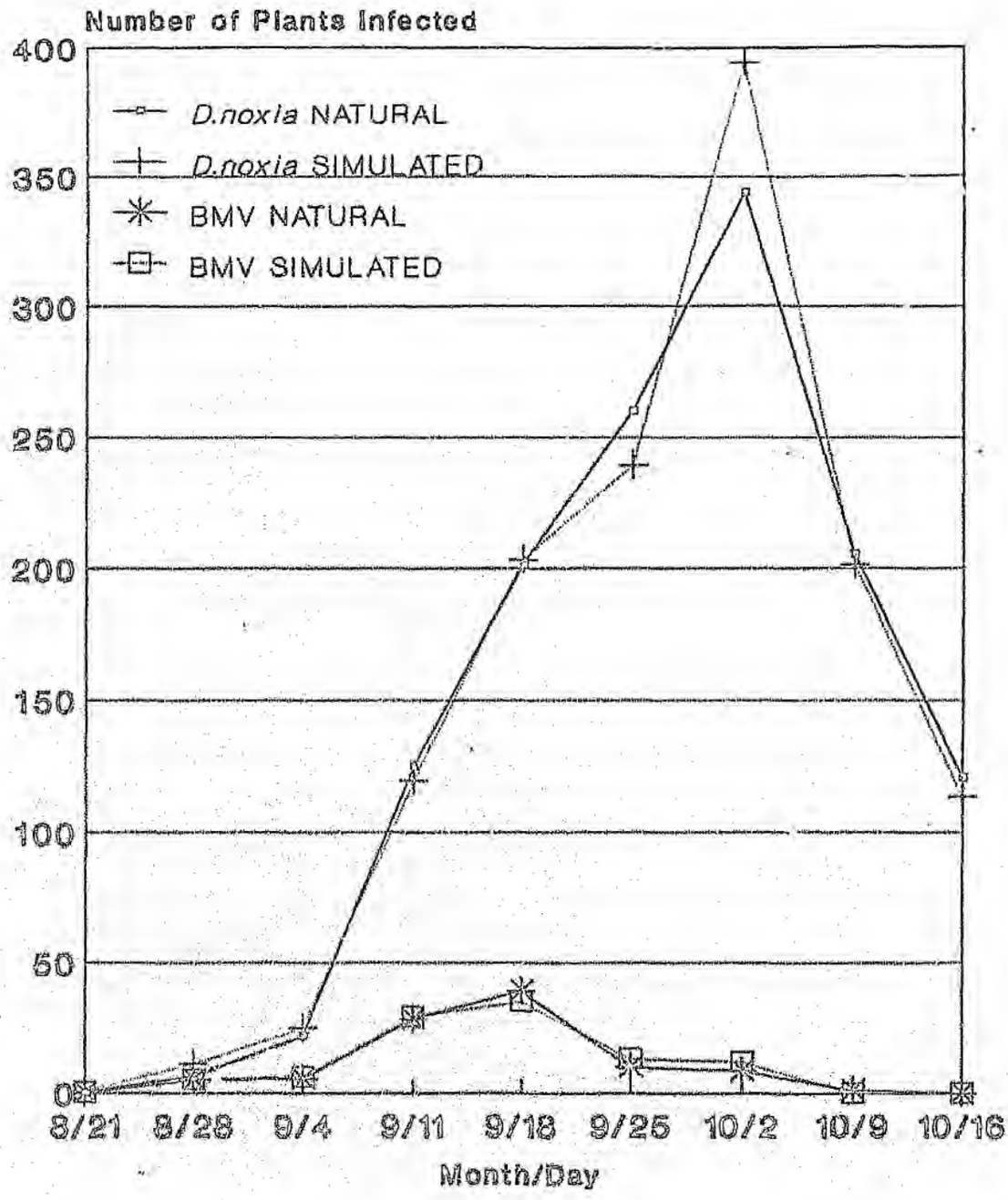


Figure 37.Verification of the model in a run with data used to construct the model.

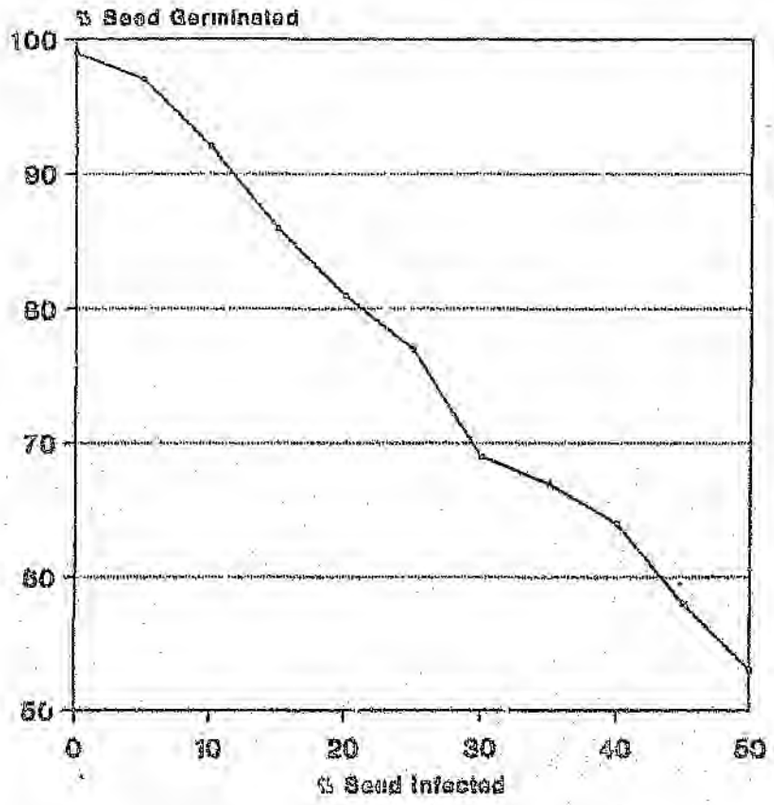


Figure 38.A simulation of the effect of various levels of seed infection on the number of plants germinated.

The effect of a constant mean temperature throughout the season was also simulated (Fig. 39). Although the data used to construct the model were based on the effect of growth stage on virus concentrations, it was found that the effect of mean temperature on *D. noxia* populations also affected transmission of BMV.

The model indicated that wind during August (normally a very windy month) had a marked effect on virus spread. Again this effect was only indirectly simulated in the sense that windy conditions caused a more rapid aphid colonisation of

new plants, and this resulted in an increase in virus dissemination (Fig. 40).

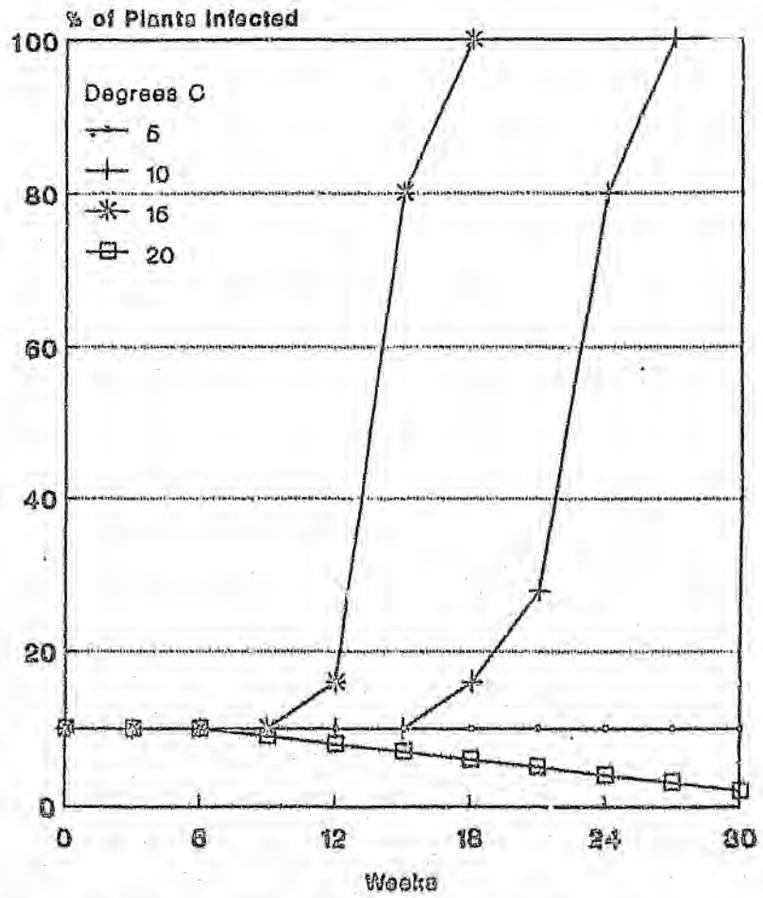


Figure 39.A simulation of the effect of various mean temperatures on the levels of BMV infection.

The limited or marginal spread of BMV can be negated by the effect of wind. Adlerz (1974(b)) found that wind caused an earlier and more extensive spread of virus to susceptible crops when the crop is situated downwind from the virus source than the crop located upwind. Aphids tend to be relatively immobile, most reproduce parthenogenetically and tend to form colonies (Gutierrez *et al.*, 1980). This

immobility is cancelled to a large extent by a wind blowing across infested plants.

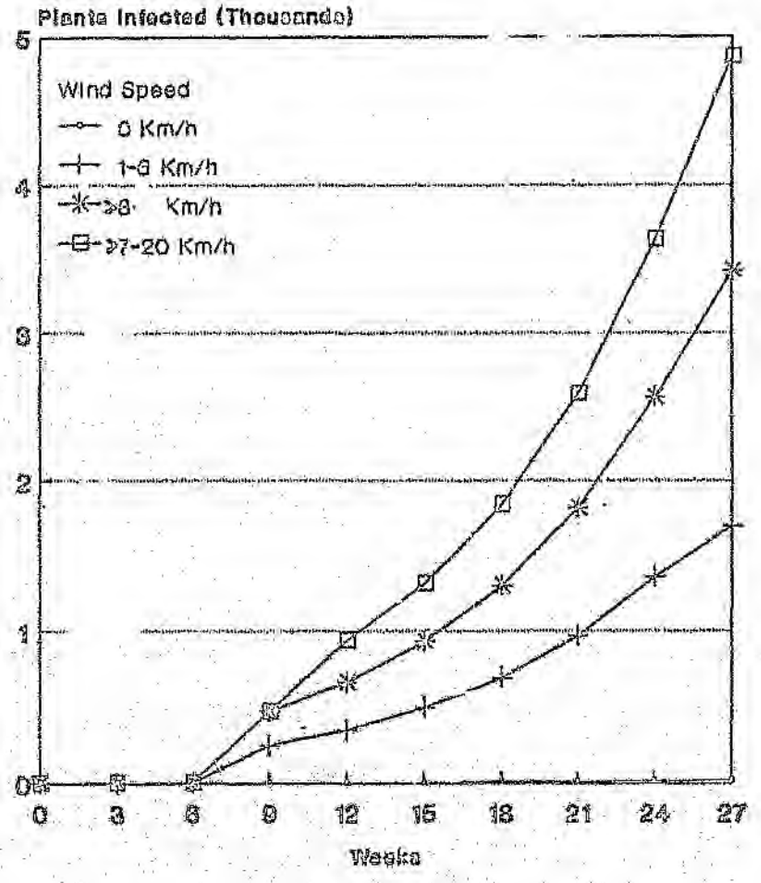


Figure 40.A simulation of the effect of various levels of wind speed on the dissemination of BMV.

The effect of the number of plants initially infected was also simulated (Fig. 41). This could be explained by the fact that pathogen source density has a direct effect on the number of transmissions occurring (Van der Plank, 1963).

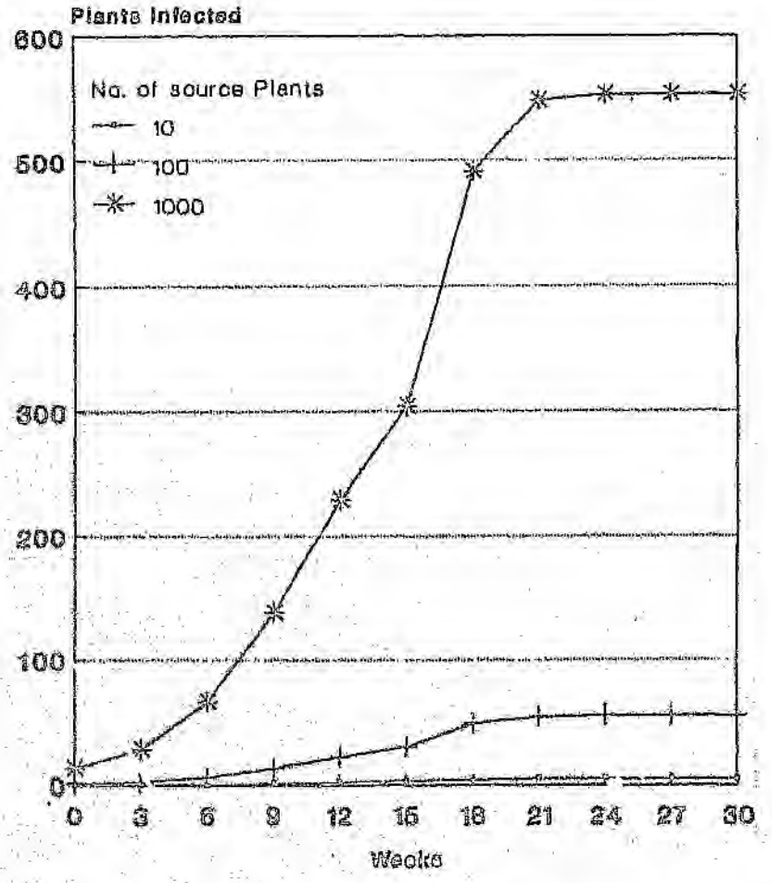


Figure 41.A simulation of the effect of various number of initially infected source plants infected with BMV on the dissemination of virus.

There are still numerous other possibilities and various simulations that can be attempted with the model, but to simulate all of them would take in excess of 2186 runs, which is clearly impractical here. The model is by no means complete, or completely tested at this stage, but it does have the following features: a) weekly adjustment of environmental factors are possible, leading to the possibility of accurate forecasting, and b) the effect of chemical control of *D. noxia* at specific stages with varying effectiveness can be simulated. This might prove to have

the greatest potential value in advising growers of the possible outcome of chemical control of *D. noxia* during various stages in the season.

The shortcomings of the conceptual program include the following: a) Data used to construct the model based on a single winter cultivar, and predictions could therefore deemed to be reliable for this cultivar only. b) The aphid ecology data used to construct the model are based on research done in the eastern OFS. Although eastern and western OFS data were used in validation of the model, the accuracy of the model for other wheat growing areas must still be determined. c) The virus development is based on growth stages, which is in itself only an approximation.

In spite of these negative factors this model does not represent an end in itself. It is a first attempt and can lead to many interesting future research possibilities. The basic reaction of the model however, is accurate, given the above-mentioned shortcomings.

distinct isolate or strain, at least to the same degree as natural isolates from the USA are different to the SA type strain (Rybicki, 1979).

Infectivity studies with the BMV extract inoculated into wheat seedlings gave rise to the normal mosaic symptoms. The yellowing syndrome described by Rybicki (1984) where inoculated leaves turned yellow after infection, rather than displaying mosaic symptoms, was not observed.

Rybicki (1984) stated that it is possible that another virus was present at very low concentrations in the original extract he used. He speculated that this was either diluted out or was not truly mechanically transmissible and was only inefficiently transmitted. Alternatively it was not detected because of phenotypic mixing or genomic masking. He stated that the latter speculation is supported by the results of Falk et al., (1979), who found that a luteo-virus viz beet western yellows virus (BWYV) was mechanically transmissible in extracts of plants also infected with lettuce speckle mosaic virus (LSMV), and that the normally non-aphid transmitted LSMV could be transmitted by aphids from plants also infected with BWYV.

As was stated previously a small number of plants with simultaneous infections of BMV and CMV was found. Wheat seedlings infected with CMV caused the leaves to turn yellow in the manner described by Rybicki (1984). As no evidence of BYDV infections was found in wheat in the summer rainfall

region, the yellowing syndrome could have been caused by CMV, rather than BYDV. Cucumber mosaic virus and BMV are known to share many chemical properties. The coat proteins showed little homology, but the coding genomes of the viruses showed over 35 % homology (Murthy, 1983). This would support the genomic masking theory postulated by Rybicki (1984) better than the presence of BYDV which he reported to be the second virus in a complex occurring in the eastern OFS.

The main distribution area of BMV was found to be the summer rainfall area of the OFS. Brome mosaic virus was isolated from *Eragrostis* spp., *Bromus* spp *Chenopodium* spp. and volunteer wheat in the eastern OFS. Plants collected in the western OFS and Natal were mostly uninfected. Brome mosaic virus could be transmitted to virus free wheat by sap inoculation with sap from *Bromus* spp., *Eragrostis* spp. and volunteer wheat, however, no evidence of aphid colonisation and therefore a viable method of virus transmission to wheat under field conditions from *Eragrostis* spp. and *Chenopodium* spp. was found.

It is concluded that a large number of natural hosts for BMV occur in the eastern OFS and that these hosts could be an important source of infection of wheat crops. The occurrence of *D. noxia* on volunteer wheat and *Bromus* spp. could form the most important method by which new crops become infected with virus. It is doubtful if all the alternate hosts form part of the epidemiology of the virus

as far as wheat is concerned. There seems to be no plausible method for BMV transmission to wheat from for instance *Chenopodium* spp. and *Eragrostis* spp. It might be possible that *D. noxia* or other wheat aphids probe on these plants under field conditions, but this could not be demonstrated under laboratory conditions.

5.3 EFFECT OF BMV ON THE WHEAT PLANT

Brome mosaic virus multiplied rapidly in infected plants during the first 50 days post-infection, and the concentrations peaked at an average of 0.64 mg of virus/g of plant tissue in the absence of aphids. With aphid transmitted virus the concentration peaked at an average of 1.0 mg/g at 70 days post infection.

It was found that BMV spreads systemically through the wheat plant after infection, but that some leaves showed consistently higher concentrations of BMV than others. This was also found by Simons (1958), Stimmann and Swenson (1967), and Zitter (1970) where CMV caused a peak concentration of virus in certain leaves, but that this concentration was not maintained in those or succeeding leaves.

The BMV concentrations during GS 15 and 45 peaked in the flag-3 leaves, and during GS 30 in the flag-2 leaves. The presence of high virus concentrations in the flag-2 leaves of wheat plants during the GS when the plants are especially susceptible to BMV infections, had no discernable effect on

aphid-transmission under field conditions (See 4.3.1). The higher concentrations of BMV in the flag leaf after infection during GS 30 might explain the negative effect on yield found after infections occurring during this growth stage, because of the involvement of this leaf in kernel development.

Brome mosaic virus infection had a detrimental effect on the development of wheat under both greenhouse and field conditions. Infected plants were stunted, fewer ears were formed per plant and fewer seeds were formed per ear. The TKW was also lower than that of the controls. These results correlated well with the results reported by Poscai (1987).

The inoculum concentration, rather than the growth stage during which infection occurred, had a greater effect on plant tillering, ear formation and plant height. It was also found that yield had been mainly affected by the growth stage of the plant during infection.

With the very high inoculum concentrations of BMV (0.1 mg/ml) used to infect field grown wheat during stem elongation (the most susceptible stage in the life cycle), the highest yield losses obtained were never in excess of 20 %, even when more than 60 % of the plants in a plot were infected. It must be kept in mind that such conditions in the field would be extremely rare. The inoculum concentrations of seed and aphid transmitted BMV are very low (approximately 0.001 mg/ml), and infection does not neces-

sarily occur during the stem elongation phase in the life cycle. It is therefore concluded that under normal circumstances the yield loss attributable to BMV infection is very low (±2 %).

The study further showed that although high yield losses were found with infection in the greenhouse (up to 40 %), only 20 % loss due to BMV infections could be found under field conditions. The difference in yield losses caused by BMV under greenhouse and field conditions is difficult to explain. One reason could be the ability of winter cultivars to tiller more profusely with the lower temperatures reached under field conditions.

Different wheat cultivars react differently to BMV infection. The high yielding cultivars were more prone to yield losses than lower yielding cultivars. Betta, for instance, had a yield loss of 18 % at high rates of BMV infection (80% +), compared to Tugela, which had a loss of 80 % at the same rate of infection. During the course of this study, however, the natural occurrence of BMV under field conditions seldom exceeded 5 %, and with such low infection percentages no economically important losses for any of the cultivars can be expected.

5.4 SEED TRANSMISSION OF BMV

The site of the infection on seed indicated that BMV was seed-borne and not just a coincidental contaminant and that

an external seed treatment did not rid the seed of BMV. The localisation of BMV in the embryo of infected seed also implied that the virus might have an effect on the viability of the seed itself, as was proven in the subsequent tests.

The effective transmission of virus was relatively low and was closely correlated to the infection time (growth stage of the seed parent) as well as the inoculum concentration. It was found that wheat (seed parents) infected with BMV during GS 30 (stem elongation) formed seeds with significantly higher BMV transmission ability than the other two stages tested.

Only a small number of plants were infected through seed infection, mainly due to the detrimental effect of seed infection on seed viability. Results indicated that this route of virus infection did not play any significant role in the final overall infection of a given field. No preferential selection of seed-transmitted BMV infected plants by *D. noxia* occurred under field conditions.

Results indicated that seed infection percentages as high as 20 % did not affect the eventual yield significantly, partly due to a better tillering of wheat with the lower number of plants per m^2. During 1987 and 1988 good seasons for wheat production were experienced due to a high rainfall, but this did not have an effect on the economically important level of seed infection. The 1989 season rainfall was lower, and

the tolerable seed infection level was somewhat lower, but still in excess of 15%.

Commercial seed sources rarely surpassed a 5 % BMV infection and in the light of present results, such a level is tolerable. Tests on the seeds of other cultivars showed that the true winter types reacted in essentially the same way as Betta, while the intermediate types transmitted BMV more effectively. This was probably due to the shorter growing season and a resultant faster metabolic rate in the intermediate cultivars, which might favour BMV infections.

5.5 APHID-TRANSMISSION OF BMV

Only 14 % of *D. noxia* collected at the start of the season (June) were viruliferous and only 5 % of virus free wheat exposed to field-collected *D. noxia* became infected with BMV. Viruliferous *D. noxia* was able to transmit BMV to virus-free plants at very low rates after 25 minutes of exposure to BMV infected wheat. Aphids were also able to transmit BMV serially from one host to the next after the plants had been exposed to viruliferous aphids for 48 hours, before the aphids were transferred to the next plant.

Diuraphis noxia was found to be able to transmit BMV under both greenhouse and field conditions, but preliminary results indicated that *D. noxia* was not an effective vector. Previous researchers reported a nonpersistent type of trans-

mission with *R. padi* and *D. noxia* (Von Wechmar, 1982), while Damsteegt and Hewings (1987) report persistent transmission.

It was found that BMV was transmitted by *D. noxia* in a semipersistent manner. The reasons for this being that the acquisition and inoculation were not accomplished in brief probes later in the season under field conditions and that the probability of acquisition increased with increased acquisition periods. Preliminary fasting did not enhance acquisition and no definite latent period before reinfectivity was found.

It must, however, be stated that a form of nonpersistent transmission did occur during the early stages of the wheat season. This was also found in the greenhouse when infected plants with high BMV concentrations were used as virus source plants in transmission studies.

The first report of a plant virus being transmitted in a unique way that did not fall directly into the categories of nonpersistent, semipersistent or persistent was that of the dahlia mosaic virus (Brierly and Smith, 1950). This virus was reported to be acquired in 15 min to 98 hr of acquisition feeds. Another virus reported to be transmitted atypically was cauliflower mosaic virus (CaMV). This virus was aquired by the cabbage aphid, *Brevicoryne brassicae* (L.) during a comparatively long (40 min) acquisition feed (Caldwell and Prentice, 1942). Further work (Van Hoof, 1954) indicated that preacquisition fasting had no effect on

the efficiency of short (2 min) or long (24 hr) acquisition feeds by *Myzus persicae* (Sulz.) and *B. brassicae* in transmitting CaMV. However. *B. brassicae* retained the virus longer than *M. persicae*. These interesting results were also obtained by Hamlyn (1955) who worked with the same two species of aphids and found that they did not seem to respond to preacquisition starvation for either long or short acquisition feeds. CaMV was aquired by both species of aphids in 30 sec to 24 hr.

This atypical mode of transmission was first referred to as a bimodal type by Chalfant and Chapman (1962), who published details of the transmission mechanism of CaMV by two species of aphids. Typically, aphid species transmitting viruses bimodally acquire virus during short probes of several seconds and also during longer probes of several hours to days (Lim and Hagedorn, 1977).

Chalfant and Chapman (1962) found that aphids transmitting pea-seed borne mosaic virus transmitted the virus in a bimodal manner. Aphids transmitting the first peak of infection behaved in a typical nonpersistent manner, acquiring virus during short probes and retaining virus for a comparatively short period, but with longer acquisition feeds, aphids appeared to lose the ability to transmit. Thereafter, there was a progressive increase in transmission as typified by semipersistent viruses, with a much longer period of retention. They found that preacquisition fasting

did not increase the efficiency of transmission at a second acquisition peak.

Diuraphis noxia exhibits a form of bimodal transmission under natural conditions. The virus concentration in infected volunteer wheat plants was found to be high, which in turn caused a high probability of *D. noxia* coming into contact with high concentrations of BMV. During this stage in the wheat season the new crop was emerging, and the colonization period of *D. noxia* on volunteer plants tended to be of short duration, before moving to the newly emerging wheat. High rates of BMV transmission occurred, but during the secondary colonization period within the field, transmission at effective levels only occurred in cycles of three weeks. These cycles were coupled to the GS of the host during such periods. The final cycle occurred just before migration of the aphids from the wheat commenced, which could explain the annual persistence of BMV in spite of low effectivity levels of both seed and aphid-transmission.

No vertical transmission of BMV occurred and no significant decrease in the total life span was found. A statistically insignificant decrease in multiplication rate was found which may allow predators and parasites to have a greater effect on *D. noxia* populations.

It was found that the efficiency of *D. noxia* was closely related to the GS of the target plant and that only transmissions occurring early in the season (GS 10-20) and during GS

30-35 caused effective concentrations of BMV to be transmitted. It was also found that BMV transmitted after GS 35 under field conditions had very small effects on yield.

It was found that the virus concentration was lower in plants infected by aphid-transmitted BMV as compared to mechanical infection. It was, however, also found that the variation in virus concentration was larger with aphid-transmitted virus, and that single plants in treatments where *D. noxia* had been used to transmit BMV, had high virus concentrations, while others were essentially virus-free. This might be due to the relative inefficiency of *D. noxia* as a vector of BMV, and the relatively low numbers of aphids that were able to successfully transmit the virus.

Although an apparently larger effect of BMV on yield was found in the treatments where infected seed had been used, it was found that this was more the impact of other factors, than directly linked to the presence of seed-transmitted BMV. The positive effect of the ability of wheat to tiller more profusely as a result of the lower plant density, caused by the loss in seed viability of BMV infected seed, is cancelled by the simultaneous presence of *D. noxia*. No compensatory growth takes place, and the apparent effect of BMV infected seed is amplified. This yield loss is therefore only secondarily attributable to the presence of BMV. In this context seed infection was found to be important. The fewer plants in plots planted with BMV infected seed also caused the remaining plants to be colonised faster by

D. noxia leading to more infestations and a larger yield loss.

5.6 COMPARISON OF THE EFFECT OF *D. noxia* FEEDING AND BMV INFECTION ON WHEAT UNDER FIELD CONDITIONS

When BMV and *I noxia* occurred simultaneously and no chemical control used, the virus reached higher concentrations than when it occurred alone. Once the virus had achieved a concentration of 0.4 mg/g, significant losses were caused without the further presence of aphids being necessary. It was also found that only 30 % of the total number of plants tested (4000) reached virus concentrations in excess of 0.4 mg/g.

Although high percentages of BMV infected wheat were found in commercial fields in some cases, the virus concentrations in the infected plants were below the 0.4 mg/g economically important threshold in more than 90 % of the cases tested. This is typical of the situation under normal farming practises. The mere presence of the virus in a plant is therefore not regarded as an important factor. The concentration of BMV in the infected plant is far more important in determining potential yield loss, and gives a more reliable estimate of disease incidence in this case.

Although the virus contributed to the damage caused, when BMV and *D. noxia* occurred together, it was only after a level of ± 0.4 mg virus/g plant material was reached, that

yield losses were closely correlated to virus concentrations. This concentration was seldomly reached in plants which were only infected by seed transmitted BMV.

The mere presence of BMV, as determined with the ELISA, did not constitute a threat to yield. Only BMV concentrations in excess of 0.4mg/g were considered to be of economic importance, and these concentrations have to be proved before any threat to wheat yield can be implied.

Although the ELISA is a rapid and sensitive method of identifying the presence of BMV under field conditions, it can also be misleading in the interpretation of results. Because of the proven ability of BMV to cause symptomless infections under field conditions, large incidences of BMV could be indicated with the ELISA, when in fact most of the plants are only latently infected. Caution should therefore be used not to use the ELISA solely as a quantitative test for virus incidence. Virus isolation and BMV concentration determination by ultraviolet spectroscopy or other methods should be standard practice in determining disease occurrence. In this manner both quantitative and qualitative data on BMV infection are obtained, and more precise and correct interpretation of data ensured.

5.7 EPIDEMIOLOGY OF BMV IN THE SUMMER RAINFALL AREA

Among the natural grasses occurring in the eastern OFS, only *Bromus* spp. has been reported as a natural host for both

and *D. noxia*. Although *Eragrostis* spp. and *Chenopodium* spp. also act as alternate hosts for BMV, evidence of *D. noxia* colonisation on these plants was not found.

Volunteer wheat and *Bromus* spp.were found to be the main inter-seasonal hosts of both BMV and *D. noxia*. Especially volunteer wheat is directly involved in the epidemiology of this virus, and forms the focal points in new infection and infestation. The high concentrations of BMV in these plants are the inoculum sources of the virus, and during the early part of the wheat season BMV is transmitted from these plants in a nonpersistent manner by *D. noxia*.

Seed transmitted BMV played a very minor role as a virus source, due to the low virus concentrations in such plants, and because no preferential colonisation of these plants by *D. noxia* was found.

The aphids transmitted BMV in a semipersistent manner during the later stages in the season, albeit at low levels of efficiency. Only small numbers of plants were infected with BMV concentrations in excess of 0.4mg/g. Of all plants infested by *D. noxia* during the season only 0.9% were infected with BMV in concentrations able to cause yield losses. Of all plants that were naturally infected with BMV on the other hand, only 9.7% of the virus concentrations were high enough to cause yield loss.

Primary colonisation of the new field by *D. noxia* started during GS 10 to 15. The infestation levels remained low until the onset of the windy period during August, when colonisation was rapid. It was during this period, when the wheat was approaching GS 30 and secondary colonisation in process, that the mode of BMV transmission changed to semipersistent, and the first peak of higher BMV concentrations was seen. The rapid colonisation of new plants by *D. noxia* lasted for a further three weeks, after which then the aphids started to migrate. It was during this part of the season that a second peak of BMV infected plants in excess of 0.4mg/g was seen.

The first BMV concentration peak coincided with the most susceptible GS of wheat for the virus, it could, however, not be directly determined if this was due to a change in vector efficiency or host plant susceptibility. The second peak in virus concentration was probably caused by aphid transmitted BMV originating from GS 30 infected plants.

As was mentioned earlier this second peak could explain the survival of BMV in spite of ineffective transmission methods. This second peak coincides with the onset of *D. noxia* migration to alternate hosts for the over-wintering period. The high BMV concentrations would therefore lead to effective aphid- transmission taking place and this would constitute a plausible survival mechanism.

The chemical control of *D. noxia* depressed the total number of BMV infections to a large extent. Not only was the number of plants with BMV infections smaller, but no second concentration peak occurred. It is almost standard practice for wheat growers to control *D. noxia* populations chemically during the wheat season. This could explain the relatively low incidence of BMV in wheatlands, in spite of large *D. noxia* populations occurring annually. The survival mechanism of the virus apparently depends on being transmitted to alternate hosts during the second peak period, and in the absence of aphid vectors, or if only low numbers of vectors are present, low transmission efficiency could result in BMV control.

The second concentration peak leads to the possibility of aphids such as *R. padi*, with a proven BMV transmission ability (Von Wechmar, 1982), and which only effectively colonise wheat after the departure of *D. noxia*, being involved in the epidemiological cycle of BMV as well. The transmission method of BMV by these aphids has not yet been studied under field conditions, and could be an interesting future project.

5.8 COMPUTER SIMULATION OF THE EPIDEMIOLOGY OF BMV

Although computer simulation of epidemiology is a useful method to study epidemiology, and instrumental in understanding some of the complexities involved, it cannot replace good fundamental research. It does, however, serve as

an excellent method of determining research direction and to ensure that relevant data are obtained. Modelling at an early stage in virus epidemiology and a well thought out strategy could prevent virologists falling into some traps, which have ensnared their colleagues working on pests and fungal diseases, thereby leading to quicker development of useful models (Carter, 1986).

The model indicated that research on the effect of temperatures on the systemic infection and the transmission of BMV is required. Exact data on the effect of wind and rainfall on *D. noxia* populations was another research topic in which data were found to be inadequate.

The model developed during the course of this study is a conceptual model only, and interesting routes for future development could include an automated growth stage determination subroutine, and a subroutine simulating the effect of predators and parasites on *D. noxia* populations.

5.9 SUMMARY

The eastern OFS strain of BMV is a distinct strain or biotype, and occurs in such high concentrations in some infected plants that any other viruses are either not detectable in the presence of this virus, or the frequency of simultaneous occurrence is so low (i.e., CMV) that the other virus could not be regarded as economically important.

Although seed transmission of BMV was reported by other researchers (Von Wechmar *et al.*, 1984) to be of major importance in the epidemiology of the virus, no evidence could be found to support this statement. Not only is the virus eliminated to a large extent by a loss in viability of infected seed, but also only very high percentages of seed infection (> 20 %) caused an economically important yield loss, because of the ability of especially winter cultivars to tiller more profusely under conditions of lower plant density due to non-viable seed.

Von Wechmar reported (1981) that tests on commercial seed sources indicated the presence of BMV. Repetitions of such tests done during the course of this study also indicated the presence of this virus, but the percentages of infections were seldom higher than 5 %. In the light of the results obtained during this study such low percentages would have no effect on the yield of plants germinated from the seed.

Brome mosaic virus infection had a detrimental effect on the development of wheat under both greenhouse and field conditions, and yield was mainly affected by the growth stage of the plant during BMV infection.

With very high inoculum concentrations of BMV (0.1 mg.ml) used to infect field grown wheat during stem elongation (the most susceptible stage in the life cycle), the highest yield losses obtained were never in excess of 20 %. The inoculum

concentrations of seed and aphid transmitted BMV are very low (approximately 0.001 mg/ml), and infection does not necessarily occur during the stem elongation phase in the life cycle. It was found that under normal circumstances the yield loss attributable to BMV infection is very low.

Although the virus forms part of a wheat disease/major pest complex, (Rybicki, 1984), the effect of the virus was negligible when compared to the phytotoxic effect of *D. noxia*. This aphid alone can cause yield losses of up to 50 %. The present chemical control strategy for *D. noxia* (Du Toit, 1986(a)) is based on aphid control before GS 31 in the case of severe early infestations. It was found that under greenhouse conditions *D. noxia*-transmitted BMV attained slightly higher virus titers than mechanically transmitted infections in single plants, but that the overall *D. noxia*-transmitted virus concentrations were lower. Effective aphid control under field conditions following the recommended procedures, did control the inoculum concentration of virus and reduced the number of source plants.

Diuraphis noxia is not a very effective vector of BMV. Only 20 % successful transmissions of BMV with field collected *D. noxia* occurred under controlled conditions and this level dropped even further with subsequent transfers using the same colony of aphids. It was found that *D. noxia* transmits BMV in a bimodal manner under field conditions

Stem and leaf rust are presently under good genetic control due to the use of resistant cultivars and this route of BMV transmission is considered to be of minor importance. Although many possible BMV transmission routes to newly sown wheat crops exist i.e., seed and *D. noxia*, none of these routes were found to be effective.

This fact would explain the low percentages of field infections of BMV inoculum concentrations high enough to cause yield losses. Although heavy latent infections are sometimes found with the sensitive ELISA, the mere presence of BMV at low concentrations does not imply that there will be any effect on yield. This only occurs if BMV concentrations exceed the 0.4 g BMV/kg plant tissue threshold. This seldom happens with even severe infections with both BMV and *D. noxia*.

Taking these facts into consideration it would be unfounded to consider BMV as a major wheat disease. The potential for an epidemic is small and even in the event of such an outbreak, the ability of BMV to cause large crop losses is limited.

Although RhPV (Von Wechmar and Rybicki, 1981) and aphid lethal paralyses virus (APLV), an aphid pathogenic virus recently reported by Williamson *et al.* (1987), was reported to be possible natural control measures of *D. noxia* populations under field conditions, no marked effect of these viruses were noted during the course of this study. These viruses

would have to have a drastic effect on the aphid to be of any importance under field conditions.

Field populations, collected in the same area where RhPV is reported to occur, were used frequently during this study and most of these aphids completed normal life cycles, and more importantly, became reproductive. If ALPV or RhPV were present in these aphids, the pathogenic effect of these viruses was to small to influence *D. noxia* populations. To have a major effect on *D. noxia* these viruses would have to influence the life cycle of the aphid by either preventing the aphid from becoming reproductive, or to cause death before reproduction could commence.

An alternative, more viable, control measure of *D. noxia*, is genetic host plant resistance. Genetic resistance to *D. noxia* in South African wheat lines was dicovered by Du Toit (1987). He also pioneered the incorporation of the resistant gene into most of the popular cultivars presently being planted in the summer rainfall area.

With cultivars resistant to *D. noxia* soon to become a reality, the epidemiology of BMV in the summer rainfall area might change. The presence of BMV in wheat seeds predating the arrival of *D. noxia* in South Africa (Von Wechmar *et al.*, 1984) indicates that some other vector might have been operating. The wide scale planting of resistant cultivars might lead to the population increase of other aphid species which could be more effective vectors of BMV. On the other hand

the increased mobility of aphids caused by host characteristics, such as a repellent or poor nutritional status, may provoke increased dispersal with consequent virus spread. It is important not to substitute insect with virus problems (Gibson and Plumb, 1977).

Resistance to *D. noxia* in wheat is based on two separate single dominant genes. Bearing in mind the enormous reproductive potential of this aphid, the possibility of this resistance being overcome in the near future seems very real. To such a genetic change in *D. noxia* could be coupled a better vectoring ability for BMV. This in turn might lead to a whole different effect of BMV on wheat in the summer rainfall area. The fact that this virus is widespread in alternate natural hosts in the eastern OFS is beyond doubt. An effective vector could therefore change the present situation.

In the event of a large decrease of *D. noxia* populations, the other aphids involved e.g. *S. graminum*, *R. padi* and *S. avenae* could undergo a change in their colonisation patterns and population sizes. Under the present circumstances they are both too few and colonise too late in the season to be seriously considered as major vectors of BMV under field conditions. Should the situation change, however, a serious attempt should be made to learn more about the biology of these species. The vectoring abilities of both *R. padi* and *S. graminum* were proven under laboratory conditions (Rybicki, 1984). Especially since these vectors are

reported to transmit BMV in a nonpersistent, nonspecific manner, the possible impact on the epidemiology of BMV could be important.

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