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REOVIRUSES OF GRASSES AND CEREALS AND THEIR VECTORS

M. CONTI

Istituto di Fitovirologia Applicata, National Research Council, Torino, Italy

ABSTRACT

Leafhoppers and planthoppers transmit plant-infecting viruses of at least six taxonomic groups including that of the Plant Reoviruses (PRVs). These have double-shelled 65-70 nm icosahedral particles containing a 10-12 segment ds-RNA genome, and are transmitted propagatively by virus-specific leafhopper or planthopper vectors. PRVs are not transmissible by sap inoculation nor through the seed or pollen of their host plants. They are restricted to the phloem tissues and cause the formation of typical intracytoplasmic virus matrices, or "viroplasms". Typical symptoms in plants consist of dark green pigmentation, dwarfing or stunting, and hyperplasia of the phloem, resulting in the appearance of typical vein enations, or tumors, on leaves, and pronounced splitting of the roots. the spred of PRVs in the field is completely dependent on their phloem-sucking hopper vectors whose biology, host preference and seasonal migrations greatly influence virus epidemiology and incidence in susceptible crops. The vectors of PRVs in Subgroup 1 (Phytoreovirus) are all leafhoppers (Cicadellidae) while those of members in Subgroup 2 (Fijivirus) are all planthoppers (Delphacidae). Subgroup 1 viruses pass through the eggs of leafhoppers to a high proportion of their progeny. the same phenomenon has been reported for some Subgroup 2 viruses but these results are controversial. The only member in Subgroup 3, rice ragged stunt virus (RRSV), resembles Subgroup 2 viruses in many aspects but its particle structure and the size of its genome segments are unlike those of other PRVs. In the last few years severe epidemics and new outbreaks of PRVs have been reported from different parts of the world. In most instances studies have revealed that epidemics were due mainly to changes in local cropping systems or agricultural practices or both.

INTRODUCTION

The Auchenorrhyncha include a number of species which transmit plant viruses and intracellular prokaryotes such as spiroplasmas, mycoplasmas (MLOs) and bacteria. The great majority of vectors are in the families Cicadellidae (leafhoppers) and Delphacidae (planthoppers). In this paper, I shall use "leafhoppers" and "planthoppers" when referring strictly to species of either one or the other corresponding taxonomic groups, and the term "hoppers" when referring to both.

Among the various groups of hopper-borne viruses, Plant Reoviruses (PRVs) seem to be those most specifically associated with their insect vectors, as all presently known members are hopper-borne and none is known to have a different type of vector or any alternative means of natural spread. The recently discovered thin, possible circular, filamentous viruses, such as maize stripe and rice stripe viruses (Gingery et al. 1981, Toriyama 1983) are the only others which seem to depend entirely on hoppers for thie field spread. All the other hopper-borne viruses either belong to large clusters which include members transmitted by vectors other than Auchenorrhyncha (e.g.- besides hoppers- aphids, tingid bugs and mites for Plant Rhabdoviruses, and whiteflies for Geminiviruses) or constitute small taxonomic entities, often still unofficial, including only two of three viruses (Conti 1985). Moreover, even the phytopathologenic MLOs are not transmitted exclusively by hoppers, as some have other types of vectors, such as psyllids.

The close, specific association of PRVs with hoppers has led to the hypothesis that such viruses originated as hopper-infecting viruses that secondarily developed the capability to multiply in plants, and cause plant diseases. The several points supporting this hypothesis have been discussed in a previous paper (Conti 1984). In connection with this, it is worth mentioning here that, with only one exception, all presently known PRVs infect cereals and grasses, that are the preferred breeding and feeding hosts of their hopper vectors. The above mentioned exception is the wound tomor virus (WTV) that infects dicotyledonous hosts. However, WTV only has been transmitted experimentally to plants using field-collected leafhoppers. The virus never has been found infecting plants in the field.

Properties of PRVs particles

The name reoviruses was originally given to viruses infecting animals. The viruses were found in their respiratory (R) or enteric (E) organs without showing, in most cases, a clear association with disease symptoms, hence the term "orphan" (O). The first PRVs to be purified and observed with the electron microscope were WTV in the USA and rice dwarf virus (RDV) in Japan (Brakke et al. 1954, Fukushi & Kimura 1959). These studies also revealed for the first time the close similarity of both the the WTV and RDV particles to those of the Reoviruses. The similarity was further strengthened when it was demonstrated that these two viruses also contained double stranded RNA genomes, as is the case for animal Reoviruses (Francki & Boccardo 1983).

Subsequent studies led to detection and characteristion od several other viruses of plants, vertebrates and insects having the same reovirus-like properties, and to their classification into the Family Reoviridae (Matthews 1982).

The particles of PRVs have icosahedral symmetry and appear either in ultrathin sections of plant and insect tissues or in crude plant sap and purified preparations as isometric entitities, 65-70 nm in diameter, with an inner dark core surrounded by a less opaque outer layer. This is because they have a double protein shell consisiting of an inner capsid 45-50 nm in diameter, and an outer capsid, 65-70 nm in diameter. The latter can bear (Subgroup 2: Fijivirus) or not bear (Subgroup 1: Phytoreovirus) an external spike or knob at each 5-fold vertex (A-spike). the structure of the outer shell of Phytoreoviruses has not been clearly determined. The outer shell of Fijiviruses is about 8 nm thick and probably consists of 92 morphological subunits of two types: (i) the B subunits, occurring at the 5-fold vertices, each capped in intact particles by an A-spike; (ii) the C subunits, composing the rest of the outer shell. When both the A-spikes and C subunits are missing, what results is the so called "B-spiked subviral particle" which consists of the inner protein shell with the subunits now protruding from it as B-spikes (Boccardo & Milne 1984).

Subgroup 3 of the PRVs includes only one member, rice ragged stunt virus (RRSV), whose particles resemble the B-spiked subviral particles of Fijiviruses.

The PRVs all have double-strande RNA genomes consisiting of 12 segments in Subgroup 1 members, and of 10 segments in members in members of Subgroups 2 and 3.

PRVs in plant and insect host

In infected plants, PRVs are restricted to the neoplastic tissues derived from the phloem although, occasionally, they have also been observed in some tracheids (Appiano & Lovisolo 1979). This explains why PRVs are so specifically transmitted by phloem-feeding vectors, and although very erratically, by needle punctures but not by animal sap inoculation (Milne & Lovisolo 1977). RDV constitutes an exception in that it does not cause neoplasia but induces stunting

and chlorotic flecks in plants. Its particles can be found not only in phloem tissues but also in the parenchyma cells, where the chloroplasts appear badly damaged.

The hopper vector's body is invaded more extensively by PRV particles: they have been detected in the gut, fat body, mycetome, salivary glands, nervous system, ovaries, and other organs and tissues of viruliferous insects. The sequential stages of development of PRV infection in vector cells have been studied in detail by Shikata & Maramorosch (1967) for WTV in Agallia constricta Van Duzee, and by Vidano (1970) for maize rough dwarf virus (MRDV) in Laodelphax striatellus Fallen. They were, in order: (a) the accumulation of granular electrodense material in typical aggregates, or viroplasms; (b) the appearance, at the periphery of such aggregates, of a few virions; (c) the formation of increasing numbers of individual virions, not only at the periphery but also within the viroplasms; (d) the engulfing of virions within multimembranous structures; and (e) the formation of virus microcrystals either at the sites of former viroplasms, or at some distance.

To summarize, three types of intracellular structures appear most typical of PRV infection in both plants and insects: (a) viroplasms, which are about the size of the cell nucleus, are not membrane-bound and, at least with viruses of Subgroups 2 and 3, contain zones of different appearance and consistency (Milne 1977); (b) tubular structures which contain single rows of PRV particles, are unbranched and sometimes incompletely closed, forming scrolls. Such tubules have never been seen in sugarcane infected with FDV, or in rice infected with RRSV. With MRDV they consist of protein apparently serologically unrelated to virus particle antigens. Fragments of such tubules, with virus particles inside, can often be observed in crude plant sap preparations from neoplastic tissues (Conti & Lovisolo 1971); and (c) crystalline PRV particle aggregates of variable size, frequently invading most of the cytoplasm of infected cells.

All the PRVs replicate in the cytoplasm, where the viroplasm are the sites of virus synthesis in both plant and insect cells.

PRVs of grasses and cereals

Eight PRVs are presently known to infect Gramineae but because some of them were named before their characterisation was complete some viruses were locally indicated by different names (e.g. OSDV and MRDV, see below). The PRVs of gramineae are listed hereafter, summarizing for each of them the most important characteristics, namely: (a) geographic distribution; (b) main host plants; (c) vectors; (d) symptoms and virus strains or synonyms (Boccardo & Milne 1984, Conti 1984, 1985, Milne & Lovisolo 1977).

Subgroup 1 (Phytoreovirus)

Rice dwarf virus (RDV)

(a) China, Japan, Korea.
(b) Rice (Oryza sativa L.), barley (Hordeum vulgare L.), oats (Avena sativa L.), rye (Secale cereale L.), wheat (Triticum aestivum (L.) Desf.), several grasses of the genera Alopecurus, Echinochloa, Panicum, Paspulum, and others.
(c) Nephotettix cincticeps (Uhler), N. nigropictus (Stal), Recilia dorsalis (Motschulsky) (Cicadellidae).
(d) Rice is the crop most severely affected by RDV. Early symptoms consist of white-yellowish specks along the veins of newly unfolded leaves. Subsequently, these specks spread along the leaf lamina, parallel to the midrib, to form fine, discontinous streaks. The plants become dwarfed, develop dark-green foliage, and produce an excessive number of stunted tillers.

Rice gall dwarf virus (RGDV)

(a) Malaysia, Thailand
(b) Rice
(c) Nephotettix cincticeps, N. malayanus Isihara & Kawase, N. nigropictus, N. virescens (Distant, Recilia dorsalis (Cicadellidae).
(d) The symptoms on infected rice are: severe stunting, dark-green colour of the leaves and whitish vein swellings - or enations - on the outer surface of the leaf blades and sheaths. Sometimes, the tip of leaves appears slightly twisted.

SUBGROUP 2 (Fijivirus)

Fiji disease virus (FDV)

(a) Australia, Fiji, Madagascar, New Britain, New Guinea, New Hebrides, Philippines, Samoa.
(b) Sugarcane (Saccharum officinarum L.), maize (Zea mays L.), Sorghum spp.
(c) Perkinsiella saccharicida Kirkaldy, P. vastatrix Breddin, P. vitiensis Kirkaldy (Delphacidae).
(d) Sugarcane, in which the virus causes "Fiji disease", is the crop suffering the most serious damage from FDV. Infected plants are stunted, with conspicuous swellings along the veins of the leaves and leaf sheaths. The deformation and "bitten" appearance of the leaves unfolding from the spindle is also a typical field symptom. Growth is severely retarded, and plants remain stunted. Distinct virus strains have not been recognized so far but sugarcane varieties which are virus resistant in one country may not be resistant in others. Whether this is due to different virus strains, different local vectors, or other factors is not yet known.

Oat sterile dwarf virus (OSDV)

(a) Northern and Central Europe: Britain, Czechoslovakia, Germany, Scandinavia, Poland.
(b) Oats, barley, rye, wheat, and many grasses of the genera Arrhenatherum, Cynosurus, Poa, Lolium, and others.
(c) Javesella pellucida (Fabricius), J. dubia, (Kirschbaum), J. obscurella (Boheman), Dicranotropis hamata (Boheman) (Delphacidae).
(d) Infected plants exhibit dwarfing, excessive tillering and suppression of flowers. Leaves become dark-green and twisted, and develop typical vein enations when plants are kept at temperatures around 18°C. Wheat and barley show less severe symptoms than oats, the natural host of OSDV. Lolium spp. are severely affected by the virus, and develop conspicuous enations. In some cases, e.g. L. perenne L. and L. multiflorum Lam., plants can be killed by OSDV infection. Arrhenatherum blue dwarf virus (ABDV) and Lolium enation disease virus (LEDV) are serologically indistinguishable from OSDV, and are now considered as its symptoms.

"Maize rough dwarf virus cluster". The following three viruses are treated separately but it is not clear whether they should in fact be considered strains of the same virus, as comparative work on serology, host-range and vectors is incomplete.

Maize rough dwarf virus (MRDV)

(a) Southern and Central Europe (France, Italy, Portugal, Spain, Switzerland), Sweden, Iran, Israel, Argentina, and possibly China.
(b) Maize, barley, oats, rye, wheat and several grasses of the genera Digitaria Echinochloa, Cynodon, and others.

(c) Laodephax striatellus (Fallen), Toya propinqua (Fieber), D. hamata J. pellucida, Sogatella vibix (Haupt), Ribautodelphax notabilis Logvinenko (Delphacidae).

(d) Maize. The early symptoms are dwarfing, dark-green colour of the leaves and leaf sheaths. Subsequently, typical whitish enations or tumors develop on the veins. The whole plant then becomes progressively purple-reddish colour, starting from the leaf margins. The roots also develop typical symptoms, consisting of longitudinal splitting or cracking. Barley, oats and wheat plants inoculated at germination stop growing at the 3rd leaf stage, become dark green, and produce an excessive number of tillers which remain stunted and confer on plants a characteristic bushy appearance. Cereal tillering disease virus (CTDV), which naturally infects barley, wheat and oats in Sweden, and the "Mal de Rio Cuarto" (=Rio Cuarto disease) of maize in Argentina are serologically indistinguishable from MRDV, and can be considered as geographical 'races' of this virus. However, to confirm this, serological, host-range and vector studies on these viruses should be completed. The Iranian isolate of MRDV has not yet been fully characterized; its local, natural vector is R. notabilis and not L. striatellus. The virus described in China and considered to be MRDV may instead be closer to RBSDV. The B-spiked subviral particles of MRDV, RBSDV and PaSV (see below) are serologically related, although not identical.

Rice black streaked dwarf virus (RBSDV)

(a) Japan, Korea, and possibly China.

(b) Rice, maize, barley, oats, wheat, and several grasses of the genera Alopecurus, Cynosurus, Digitaria, Echinochloa, Lolium and few others.

(c) L. striatellus, Unkanodes albifascia Matsumura, U. sapporona Matsumura (Delphacidae).

(d) Symptoms on infected rice consist of whitish waxy swellings along the veins on the outer surface of leaves, leaf sheaths and culms, which later become dark brown, forming black-streaked enations of various lengths. Infected plants are stunted and do not yield, or produce poor heads. Dark brown blotches may appear also on some grains. Symptoms on maize, barley, oats and wheat closely resemble those described for MRDV.

Pangola stunt virus (PaSV)

(a) Brazil, Fiji, Guyana, Peru, Taiwan.

(b) Pangola grass (Digitaria decumbens Stent) and other Digitaria species.

(c) Sogatella furcifera (Horvath) (Delphacidae).

(d) Crops of pangola grass affected by PaSV are severely stunted and regenerate only poorly after cropping. Individual plants exhibit yellowing, kinking or twisting of young leaves and inflorescences, minute vein swellings, excess tillering, and purpling of the leaf margins.

SUBGROUP 3

Rice ragged stunt virus (RRSV)

(a) China, India, Indonesia, Japan, Malaysia, Philippines, Sri Lanka, Taiwan, and Thailand.

(b) Rice.

(c) Nilaparvata lugens (Stal) (Delphacidae).

(d) Infected plants are stunted, with whitish spindle-shaped enations, something turning brown as plants age. Leaves are twisted and ragged, and may show a green colour darker than normal. Infected mature plants also remain after heading while healthy plants start to turn yellow. Excess branching may

occur at the nodes. Delayed flowering, incomplete panicle emergence, and unfilled grains are additional, typical symptoms which cause severe yield reduction.

Transmission and epidemiology of PRVs

The only natural means of transmission of PRVs is by their hopper vectors, which are leafhoppers for viruses of Subgroup 1, and planthoppers for viruses of Subgroups 2 and 3. The virus-vector relationships are of the propogative type, meaning that PRVs are acquired by hoppers through prolonged acquisition feeding periods on infected plants, retained in the vector's body where they multiply in the insect cells, and are transmitted with inoculation feeding periods of approximately the same duration necessary for virus acquisition. With the only exception of RDV, both the acquisition and inoculation of PRVs occur exclusively in the plant phloem. An alternative means of virus acquisition by hoppers is, in some cases, by transovarial transmission from the infected females to their offspring. To the contrary, infected males do not transmit PRVs to their descendants via congential passage of virus to females during mating.

The process of transmission of PRVs by hoppers includes three phases: acquisition, latent (or incubation) period, and inoculation. The virus acquisition is accomplished with feeding periods ranging from 15 min. to some hours, while the inoculation can take place is as short as 5 min. or as long as 5 hr feeding. RDV, which invades also plant tissues other than the phloem, can be acquired and inoculated by its main vector, N. cincticeps, in 1-3 min feeding. In general, when the acquisition is prolonged beyond its minimum efficient value, the proportion of infective hoppers increases, although only up to a certain level which may be different for each virus-vector combination. Similarly, by increasing the duration of the inoculation feeding period, the percentage of virus-transmitting hoppers can be increased up to a certain limit (Conti 1984).

In laboratory work, the age and temperature at which test plants are inoculated greatly influence the transmission results. In general, the resistance of plants to virus infection increases with plant age. For this reason, it is a common practice, in experimental work with PRVs of grasses and cereals, to inoculate plants at the coleoptile stage, when they are extremely susceptible. The best results for MRDV transmission, for example, are obtained by keeping both maize and barley seeds, at 25-28^{o}C, on wet substrata. The emerging plants are then transplanted to pots as etiolated coleoptiles, 2-3 cm long, and exposed to infected hopper feeding for at least 3 days.

The temperature at which PRVs are best transmitted by hoppers and symptoms develop most successfully on plants, is between 18 and 20^{o}C. When the incoulated plants are grown at temperatures over 25^{o}C, typical PRV symptoms, particularly the characteristic phloem enations, develop poorly.

The latent period of PRVs in their vectors is generally 1-3 weeks, during which time virus multiples in the hoppers invades most tissues, including the salivary glands (Shikata & Maramorosch 1967, Vidano 1970). After the latent period is completed, the hoppers become infective or "inoculative". Vectors retain and transmit these propagative viruses for their entire life span without further feeding on infected plants.

The transovarial transmission of PRVs has been demonstrated for all the members of Subgroup 1 (Phytoreovirus) in their leafhopper vectors. It may be very high for RDV in both N. cincticeps (32-100%) and R. dorsalis (up to 64%), and for RGDV in N. nigropictus (66.7-92.8%). Virus transmission through the vector's egg has also been reported for some Fijiviruses, e.g. MRDV and OSDV, but the data from different studies are not in agreement and need to be clarified (Conti 1984, Milne & Lovisolo 1977).

PRVs have restricted or moderately wide host ranges, including several species which are only experimental hosts. So, in general, wild plant sources of PRVs are not common in the field. Because PRVs multiply also in their hopper vectors and some are transovarially transmitted, such insects constitute an additional natural source of virus which may be more important than plant hosts in preserving viruses from one vegetative cycle to another. OSDV, for example, can survive during winter in its vector J. pellucida, and CTDV in Sweden and MRDV in Italy can survive as well in L. striatellus. Both these planthoppers overwinter as diapausing young nymphs, and can acquire PRVs from plants before entering into diapause. In these situations, the incidence of PRVs on annual crops sown the next spring greatly depends on the stage of growth that plants reach when planthoppers leave their overwintering sites and invade the surrounding crops.

The epidemiological cycle of some PRVs, e.g. FDV and the rice-infecting viruses - namely RDV, RBSDV, RGDV and RRSV - develop mainly, if not exclusively, between the susceptible crop and the hopper vector(s), since vectors both feed and breed on the rice crop. In some cases, other cultivated species can be infected by hoppers that occasionally feed on them, for example maize with RBSDV in Japan, by insects migrating from the rice crops.

Other PRVs develop their cycles between crops, insect vectors and wild plants. Wild plants may serve as winter virus reservoirs or represent the preferred feeding hosts for the hopper vectors during the vegetative season. In the case of MRDV, for example, Cynodon dactylon Pers. is a winter host of the virus in Israel while, in Italy, Digitaria sanguinalis (L.) Scop. and Echinochloa crusgalli (L.) P.B. are the most important sources of virus for the second annual generation of vectors. This is the generation which enters into diapause and overwinters.

As far as control of PRV infections is concerned, there are various possibilities, depending on the virus epidemiology. When winter virus reservoirs are cultivated plants, virus spread can be limited by substituting the susceptible species with other, non-susceptible species; when PRVs overwinter prevalently in their hopper vectors, their spread can be controlled by spraying the specific overwintering sites of vectors with insecticides; when weed species are important virus sources, PRV spread can be controlled with both herbicides or roguing.

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