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VECTOR-INDUCED MODIFICATIONS IN A PLANT VIRUS

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The planthopper-borne rough dwarf virus disease manifests itself on maize plants in two different groups of symptoms presumably caused by two distinct strains of the virus. The severe, dwarfing strain is transmitted to maize plants only by *Laodelphax striatellus* and *Javesella pellucida*. The milder, non-dwarfing strain is transmissible by two additional delphacid species, namely *Delphacodes propinqua* and *Sogatella vibix*, which are unable to provoke the dwarfing-strain syndrome on maize test plants. However, when the dwarfing strain was injected into the haemocoel of these two non-vectors, the virus, upon replication and circulation in this unnatural medium, was modified into a novel strain inducing hermaphroditism in the vector-inoculated maize test plant.

Modification of the dwarfing strain into the non-dwarfing one also occurs in the body of the natural vector (*L. striatellus*) when the insect acquires the former strain in an imperfect manner. This happens when the vector feeds on infected maize which is an unsuitable host species for this planthopper, or when the virus is acquired by trans-ovum transmission. All above-mentioned modifications are irreversible.

The maize rough dwarf virus disease (MRDV) of maize plants in Israel gives rise to two different sets of symptoms, presumably caused by two distinct strains of the virus. The one set, described by Grancini (1958), comprises dwarfing of the infected plant accompanied by the appearance of numerous minute swellings along the veins of the lower surface of the leaf. Plants affected by this strain (hereinafter referred to as "dwarfing strain") usually die before flowering. The second type of syndrome is noticeably milder, infected plants being almost as tall as non-infected ones, and the number of vein swellings much smaller. This non-dwarfing form of the disease also affects the reproductive organs of the plant resulting in either partial sterility of both male and female flowers, or other floral malformations as described by Grancini (1958), Harpaz (1959) and Blattny, Pozdena & Prochazkova (1965).

The vectors of MRDV are taxonomically-related planthoppers of the delphacid (= araeopid) family, namely *Laodelphax striatellus** (Fallén) and *Javesella** *pellucida* (Fab.) (Harpaz, 1966). The latter species is not found in Israel, but occurs in Europe and North America. In addition, two other delphacid planthopper species, *Delphacodes propinqua* (Fieber) and *Sogatella** *vibix* (Haupt), were later found in

* For generic and specific synonymy see Fennah (1963, 1963a).

our laboratory to be capable of transmitting MRDV, but only the non-dwarfing form of the disease. As regards *D. propinqua*, however, transmission of the virus could be accomplished only under unnatural circumstances as described on page 102.

Results

None of the above-mentioned planthoppers can normally breed on maize plants, though they can survive on this plant for short periods of 2—7 days. *L. striatellus* breeds in nature on wheat, barley, oats, rice, *Panicum* spp., *Agropyron repens* P.B., and related grasses; *J. pellucida* — on wheat, barley, rye, oats, *Lolium* spp., and other wild grasses; *D. propinqua* — on Bermuda grass (*Cynodon dactylon* L.); *S. vibix* — on *Panicum repens* L., *Panicum crus-galli* (L.) P.B., wheat, barley, and oats.

Our early experiments on the virus/vector relationships of MRDV showed that although an inoculative specimen of *L. striatellus* can readily infect a susceptible maize seedling, this planthopper can only in an extremely small number of instances (45 out of almost 5000 tests) acquire the virus from an infected maize plant. Moreover, in every one of these 45 cases of transmission, only the virus strain giving the non-dwarfing type of symptoms was transmitted. This means that maize can hardly serve as a source of virus inoculum for the spread in the disease in the field. In other words, the communication of MRDV between maize and other hostplants of the virus in nature is of a "one-way-traffic" character. Incidentally, alternative hostplants of MRDV are oats (Lovisolo, Vidano & Conti, 1966) wheat (Vidano, Lovisolo & Conti, 1966), barley, *Panicum crus-galli, Panicum phyllopogon* (Stapf.) Koss. (Vidano *et al.*, 1966a), as well as *Cynodon dactylon, Hordeum bulbosum* L., *Agrostis verticillata* Vitt., *Oryzopsis miliacea* (L.) Asch.-Schw., *Lolium perenne* L., *Panicum repens* L., *Setaria verticillata* (L.) P.B., *Cenchrus echinatus* L., and *Sorghum sudanese* (Piper) Stapf. (Klein & Harpaz, unpublished).

However, the inability of the planthopper to acquire the virus from infected maize plants by means of natural feeding could still be overcome by injecting infective sap directly into the vector's haemolymph, of by puncturing its intestine immediately after feeding on a MRDV-infected maize plant. The present paper reports the results of introducing dwarfing strain inoculum by means of these artificial methods into the bodies of vectors which normally do not transmit this strain. It likewise deals with the change in the properties of the virus upon passage from mother planthopper to her progeny during trans-ovum transmission.

METHODS AND MATERIALS

Virus-free cultures of *L. striatellus* and *S. vibix* were maintained on wheat plants under lamp chimney glass cages, and those of *D. propinqua* on Bermuda grass plants.

Maize plants of the hybrid variety "Névé Yaar 180", and wheat plants of the variety "Dwarf 46" served as sources of dwarfing strain inoculum. Both were in-

oculated in the laboratory by means of *L. striatellus*, which had previously acquired the virus from MRDV-infected wheat plants.

Injection

Infective sap for vector injection was prepared by grinding 250 mg of fresh MRDV-infected plant tissue in 1 ml of phosphate buffer solution pH 6.2. The ground tissue was then centrifuged for 5 min at 2500 r.p.m., filtered through paper and kept in the cold during the subsequent injection procedure. The sap was injected into the thoracic cavity of 4th- or 5th-stage larvae of the planthopper. The larvae were anaesthetized with carbon dioxide for 1.5—2 min and injected under a stereoscopic microscope with a very fine glass capillary inserted between the middle and hind pair of legs. Once the capillary was well inserted in the sternum the fluid was gently pressed in by help of a rubber bulb connected to the capillary by means of a rubber tube and a hypodermic needle. Soon after injection the insects were placed on healthy wheat plants at 20° for recovery and subsequent virus incubation in their bodies. At this temperature, the injected vector requires 14—23 days to become inoculative. About 25% of the injected planthoppers died within 24 hrs as a result of the injection injuries, but the remainder recovered and survived for a fairly long time. Some of the survivors even reproduced normally.

Intestinal punctures were performed by means of a sterilized entomological pin (No. 00) pushed into the centre of the metasternum.

Virus acquisition by normal feeding was obtained at a constant temperature of 20°, followed by a virus incubation period of varying length on healthy wheat seedlings.

Inoculation feeding on test plants was done at 24° and lasted 24 hrs. The inoculated test plants were at their coleoptile stage, i.e. 1—2 days after emergence, and one planthopper was employed for each test seedling.

Following the inoculation feeding the test plants were placed under insect-proof conditions at 24°, which is the optimum temperature for symptom development (Harpaz, 1964).

Trans-ovum transmission

Ten injected *L. striatellus* females, previously tested for transmission of the dwarfing strain of MRDV, were allowed to oviposit on healthy wheat seedlings. Every two days these females were transferred to new wheat seedlings in order to obtain uniformity in egg age. One day before hatching, the eggs were dissected out of the plant tissue by aid of fine needles under a stereoscopic microscope. The eggs were then placed on moistened filter paper and incubated at a constant temperature of 25°. Upon hatching the neonate larvae were transferred to healthy wheat seedlings where they developed up to their 4th instar. Only some 500 out of a total of 1000 incubated eggs hatched, and only 20% of these reached the 4th instar which was used for virus-inoculation feeding on maize test seedlings. The same planthopper

specimens were in fact serially transferred to additional maize test plants, but every 1—2 days they had to be returned to wheat for 3—4 days of feeding since they cannot survive on maize for longer periods. Many of them matured in the course of these transferences and even reproduced.

RESULTS

L. striatellus

When the planthopper intestine was needle-punctured prior to a 24-hour-long acquisition access to an infected maize plant, only the non-dwarfing strain of MRDV could be transmitted. Transmission rate was 16 plants infected out of 1000 plants fed on by intestine-punctured planthoppers. However, in the cases of a puncture made immediately after an acquisition access of the same length, both strains of the virus were transmitted, though at a rate of 31/1000 for the non-dwarfing strain, and 3/1000 for the dwarfing strain. No differences in this respect could be noticed among 2nd to 5th stage larvae or adult planthoppers.

D. propinqua

Planthoppers of this species, whether in the larval or adult stage, could not transmit any of the virus strains by natural feeding on infected maize plants. Nor could they transmit the virus by feeding on infected wheat or Bermuda grass plants. Post-acquisition puncturing of the intestine also did not help in this matter.

Incidentally, in 1966 Harpaz cleared *D. propinqua* from an earlier implied suspicion (Harpaz, 1961) of being a vector of MRDV. This dismissal is still valid as long as acquisition of the virus is attempted through natural feeding on an infector plant.

On the other hand, when infective sap, extracted from a maize plant showing dwarfing symptoms, was injected into the thoracic cavity of the planthopper, only the non-dwarfing syndrome was provoked in the maize test plants.

However, some of the injected specimens produced an additional, previously unknown manifestation in the maize test plants besides the anticipated vein swellings which in this case were rather scant. The new syndrome was marked by the development of pistillate inflorescences on the staminate tassels. Such floral characters are neither novel nor rare in maize plants, but result from causes other than MRDV, e.g. infection by the maize smut fungus *Ustilago maydis* (De Candole) Corda (Fischer & Holton, 1957). This induced hermaphroditism, or sex inversion, was also noticed with regard to *Sogatella vibix*, though to a much lesser extent.

Sogatella vibix

By natural feeding on infected maize or wheat plants this planthopper could only produce the non-dwarfing syndrome in maize plants, at a rate as high as 20 percent. Postacquisition punctures of the intestine, or injection of infective sap did not

increase the transmission rate above the aforementioned 20% level. Nor could these techniques produce any dwarfing type transmissions.

Trans-ovum transmission

Only 4% of the offspring of inoculative *L. striatellus* mothers that reached the 4th instar could transmit the virus to maize test plants. All these infections were always of the non-dwarfing sort. The wheat plants on which these larvae developed did not show any MRDV symptoms. Sixteen subsequent generations of these planthoppers were maintained on apparently healthy wheat plants without recourse to a source of MRDV infection. However, when these 16th generation planthoppers were allowed to feed on maize test seedlings, they occasionally produced MRDV symptoms, always of the non-dwarfing kind.

DISCUSSION

The dwarfing strain of MRDV is transmissible only by two delphacid planthopper species, namely *L. striatellus* as reported above, and *Javesella pellucida* as found in Italy by Harpaz *et al.* (1965). Once the virus is introduced (whether by means of intra-haemocoelic injection, or even by normal feeding) into the body of delphacid planthoppers which are not the natural vectors of this virus, then the latter apparently undergoes certain changes during its latent period of propagation and concentration within the body of the unnatural vector. The principal modification is a general attenuation of the virus, resulting in a much milder symptom expression, namely the absence of the stunting effect on the infected maize plant. However, these vector-induced modifications are not restricted solely to an attenuation effect, since both these unnatural vectors (*S. vibix* and *D. propinqua*) "converted" the virus, artificially introduced into their body, into a novel strain that provoked sex inversion in the inoculated maize test plants. This particular virus-induced hermaphroditism has not yet been encountered in maize plants in the field. Another feature of this vector-modified virus is its irreversibility, i.e. its inability to be retransmitted by the same vectors. In other words, once the virus enters the host plant it finds there a "dead end" whence it cannot be recovered, not even by aid of the vector injection technique.

Even in cases where the natural vector (*L. striatellus*) is involved, but the mode of virus acquisition is imperfect, the symptoms produced on the maize test plants were invariably of the non-dwarfing form. This refers to the passage of dwarfing-strain MRDV from the mother *L. striatellus* to her progeny through the egg stage, resulting in only non-dwarfing symptoms transmitted by the progeny. Similarly, when *L. striatellus* planthoppers acquired the virus imperfectly by feeding on MRDV-infected maize which is an unsuitable host species for this insect, the symptoms provoked on the inoculated maize test plants were exclusively of the non-dwarfing type. Incidentally, this is the reason why infected maize plants do not

serve as a source of spread of dwarfing MRDV in the field, the virus being able to readily move into these plants, but it can hardly be picked up therefrom by its vectors.

As regards hosts other than maize, no plant has yet been found that reacts with any visible symptoms to the vector-modified MRDV. This also explains our failure to carry out any cross protection tests to determine the exact relationship between the dwarfing strain of MRDV and the vector-modified non-dwarfing one. The absence of an adequate method for purification of this virus likewise makes it impossible for the time being to determine the serological relatedness of these two strains.

Apart from differences in symptom expression, the only other indication we so far have for the existence of more than one strain of this virus is the observation under the electron microscope of two distinct types of MRDV particles in infected maize tissue (Kislev, Harpaz & Klein, 1968). However, although two kinds of particles were distinguished, with diameters of 50 and 70 mμ respectively, this can still be ascribed to reasons other than strainal varietion (Gerola & Bassi, 1966).

It should, however, be pointed out that the above-reported results cannot, in our view, be interpreted as merely a case of a mixture of existing strains simultaneously infecting the same host plant and the vector sifting out one or more of them at a time. The virus is definitely not seed-borne, and we made reasonably sure that the maize seedlings serving as initial experimental stock were inoculated with a single strain, the dwarfing one. Hence, the modified strains obtained in the course of this study apparently resulted from replication of the virus in an atypical medium, namely the body tissues of a strange vector, or from imperfect acquisition of the virus by the natural vector. The non-dwarfing strain presumably deriving from imperfect acquisition is quite prevalent in nature, whereas the sex inversion strain originating from unnatural vectors has so far occurred only under experimental conditions in the laboratory.

The phenomenon whereby viruses change their properties when they are mechanically transferred between different host-plants has already been described and discussed by Bawden (1958). However, to the best of our knowledge this seems to be the first report of an insect-borne virus being altered as it passes through the body of a vector which is different from the natural one. Unfortunately the nature of this modification is irreversible, so that our case cannot be as fully proven as Bawden's one. Yet, we consider the vector-induced modifications as an additional, hitherto overlooked mechanism in the evolution of new strains of plant viruses in nature.

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ZUSAMMENFASSUNG

VEKTOR-BEDINGTE MODIFIKATIONEN IN EINEM PFLANZENVIRUS

Die durch Langkopfzirpen übertragene Rauhverzwergungsvirose zeigt sich auf Maispflanzen in zwei verschiedenen Symptomgruppen, die vermutlich durch zwei getrennte Virusstämme verursacht werden. Der schwere, Verzwergung erregende Stamm wird auf die Maispflanzen nur durch *Laodelphax striatellus* und *Javesella pellucida* übertragen. Der mildere, nicht Verzwergung erregende Stamm ist auch durch zwei andere Langkopfzirpenarten (*Delphacodes propinqua* und *Sogatella vibix*) übertragbar, die aber unfähig sind, das Verzwergungssyndrom an Maistestpflanzen hervorzurufen. Jedoch, wenn der Verzwergungsstamm in das Haemocoel dieser zwei Nichtüberträger injiziert wurde, modifizierte das Virus infolge der Vermehrung und Zirkulation in diesem unnatürlichen Medium der Zirpenkörper in einen neuen Stamm. Dieser neue, vorher nicht vorhandene Stamm führte Hermaphroditismus in die vektor-geimpfte Maistestpflanze ein.

Modifikation des Verzwergungsstammes in einen nicht Verzwergung erregenden Stamm kommt auch im Körper des natürlichen Vektors (*L. striatellus*) vor, wenn dieser Überträger den erstgenannten Stamm auf unvollkommene Weise aufnimmt. Dies geschieht, wenn der Vektor auf infiziertem Mais saugt, der eine ungeeignete Wirtsart für diese Zirpe ist, oder wenn das Virus durch eine transovum-Übertragung von der Mutterzirpe auf ihre Nachkommen aufgenommen wurde. All diese genannten Modifikationen sind nicht umkehrbar.

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