IV

The above-mentioned possible function of the salivary secretions may explain the sucking of the insects which are often toxicogenic to the host plant. *N. cincticeps* is, however, not always severely toxicogenic. It is probable that the incidence and severity of the phytotoxemias are closely related not only to the toxic effect of the salivary secretions, but also to the feeding behavior of the insect, and the physiological and developmental status of the host plant. Otherwise, the plant hormone may have no effect on the metabolism of insects. According to NUORTEVA (1956), IAA can be transferred in the active form from a nutrient medium to the salivary glands of *Stenodema calcaratum* FALL. Also the invertase activity of *Tettigella viridis* LINNÉ was little affected by IAA (the author's unpublished data). Careful studies are in progress on the function of the salivary glands with special relations to the phytotoxemia and the role in the digestive process of the insect.

The author appreciates Prof. K. IYATOMI for

his assistance in the preparation of the paper.

REFERENCES

- ALLEN, T. C. (1947) J. Econ. Ent. **40** : 814~817.
 CARTER, W. (1945) J. Econ. Ent. **38** : 335~338.
 DAY, M. F., H. IRZYKIEWICZ and A. MCKINNON (1952) Aust. J. Sci. Res. (B) **5** : 128~142.
 EDELMAN, J. and M. A. HALL (1964) Nature **201** : 296~297.
 FISHER, E. H., A. J. RIKER and T. C. ALLEN (1946) Phytopathology **36** : 504~523.
 LAWSON, F. R., G. B. LUCAS and N. S. HALL (1954) J. Econ. Ent. **47** : 749~752.
 MILES, P. W. (1958) J. Ins. Physiol. **2** : 338~347.
 MILES, P. W. (1959) J. Ins. Physiol. **3** : 243~255.
 NAITO, A. (1962) Proc. of the Kanto-Tosan plant protection society **9** : 55.
 NUORTEVA, P. (1956) Ann. ent. Fenn. **22** : 108~117.
 SAXENA, K. N. (1963) J. Ins. Physiol. **9** : 47~71.
 SMITH, K. M. (1920) Ann. Appl. Biol. **7** : 40~55.
 STOREY, H. H. (1939) Proc. Roy. Soc. Lond. (B) **113** : 463~485.

Preliminary Report on the Plant Virus Multiplication in the Leafhopper Vector Cells Grown *in Vitro*¹

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In recent years, work on plant virus-insect vector relationship has been greatly aided by studies at the tissue or cellular level. Tissue cultures of leafhopper vectors have been tried in this and other laboratories (VAGO and FLANDRE, 1963; MITSUHASHI and MARAMOROSCH, 1964; MITSUHASHI, 1965). These leafhopper tissue cultures were mostly primary cultures and cell lines have

not as yet been established from leafhopper tissues. Primary cultures of leafhopper embryonic tissues, however, can be maintained well enough to be utilized in the study of plant virus multiplication. The present paper describes the results obtained from preliminary experiments on the multiplication of the rice dwarf virus (RDV) in cells grown *in vitro* from green rice leafhopper embryos, *Nephotettix cincticeps* UHLER.

CULTIVATION OF VIRULIFEROUS EMBRYONIC TISSUES

RDV has been known to be transmitted from female adults of *N. cincticeps* to their progenies through ovaries (FUKUSHI, 1934). Viruliferous embryonic tissues were cultivated in the same

¹ *In vitro* で培養されたヨコバイ細胞での植物ウイルスの増殖 (予報) 三橋 淳 (農林省農業技術研究所 昆虫科)

Japanese Journal of Applied Entomology and Zoology, Vol. 9, No. 2, 1965.

(Received for publication, March 15, 1965)

manner as those of healthy embryonic tissues (MITSUHASHI, 1965). Cell migration and cell multiplication by mitoses occurred normally in explanted viruliferous tissues. Epithelial cells formed cell sheets in a large area around the explants. These cells contained more granules, compared with the identical type of cells obtained from healthy embryonic tissues. Considerable histolysis of explants occurred simultaneously with the development of cell sheets. A part of the developed epithelial cell sheet began to degenerate 40 days after the culture was set up. On the 27th day of cultivation, one culture was fixed for 1 minute in 1 per cent buffered osmium tetroxide solution and embedded in Epon 812 *in situ*. Electron microscopical examination showed the presence of RDV particles in the developed epithelial cell sheets (Fig. 1). Most of the RDV particles were arranged in lines and were accompanied by a sheath-like substructure. These RDV particles were found in almost all the cells developed from explants.

INOCULATION OF CELLS GROWN IN VITRO WITH RDV

Healthy embryonic tissue cultures of *N. cincticeps* were inoculated with RDV by bringing the virus material into the culture medium. The virus material was prepared from viruliferous female adults. The fat bodies which were aseptically taken from 5 viruliferous female adults, were homogenized in 0.5 ml of the culture medium, and the homogenates were centrifuged at 3,000 r. p. m. for 5 minutes. The clear part between the lipid layer and precipitate was used as the virus material. Fourteen days old cultures were used in the virus inoculation experiment. At this age of cultivation, the epithelial cell sheets were well formed, and all types of cells obtained from leafhopper embryonic tissues could be seen (MITSUHASHI, 1965). Inoculation of virus was carried out by simply replacing the culture medium with virus material. The inoculated culture was

maintained at 25°C for 24 hours with virus material, and then the virus material was thoroughly washed away by changing the culture medium several times.

Changes in cells were recognized 24 hours after inoculation with virus. Granules appeared around nuclei at first, increased in number day after day, and finally filled the whole area of the cytoplasm (Fig. 2, A~D). This was followed by destruction of cells. Granulation was quite marked in the epithelial cells. In the epithelial cell sheets, granulation of cells started from the periphery and proceeded to the inner parts of the cell sheets. During the first 3 days after the inoculation of virus, mitoses could be seen at the periphery of the cell sheets, although all the cells were more or less granulated. With the advance of granulation, cells shrank and the cell sheets were destroyed. Explanted tissues themselves were also affected by the inoculation of virus. At the time of inoculation, they were contracting and this movement continued for a week after inoculation and then ceased. The cells of the explants were also filled with granules at the end of the first week after inoculation.

In the control experiments, the inoculant was prepared in the same manner as the preparation of virus material, but from 5 healthy female adults. When this non-viruliferous inoculant was brought into the culture medium, the cells and explants did not show marked changes like those observed in the virus inoculated culture, and

Fig. 1. RDV particles observed in the cells grown *in vitro* from the viruliferous embryonic tissues of *N. cincticeps*. M, mitochondria; N, nucleus; V, RDV particles. (X 24,000)
The electron micrograph was prepared by Dr. S. NAsu.

Fig. 2. Processes of granulation observed in a epithelial cell sheet after the inoculation of RDV (X 350). A, Before inoculation; B, 2 days after inoculation; C, 5 days after inoculation; D, 7 days after inoculation.

continued to grow.

Electron microscopical examination was carried out on an ultra-thin section of the inoculated culture on 9th day after the inoculation. Many small particles which might be considered as one of the forms of RDV could be seen in almost all of the cells (Figs. 3, 4). The size of the small particles was $30\text{ m}\mu$ in diameter. Normal RDV particles of about $70\text{ m}\mu$ in diameter were also seen, but not many.

DISCUSSION

The granulation observed in viruliferous embryonic tissue culture as well as in virus inoculated tissue culture seems to suggest the relation between virus multiplication and formation of granules. Similar granulation of cells was also observed when the leafhopper vector tissue cultures, *Macrostes fascifrons* and *Agallia constricta*, were inoculated with aster yellows virus and wound tumor virus respectively. Furthermore, the same was true when embryonic tissue cultures of *A. constricta* were inoculated with the Reo virus which is an animal virus and has similar structure and immunological properties to the wound tumor virus (MITSUHASHI, unpublished). On the other hand, similar granules were also seen when non-viruliferous cells began to degenerate. The granulation of cells might be, therefore, a secondary effect of virus multiplication in the cell.

In the viruliferous embryonic tissue culture, RDV particles were found in the long linear arrangement. Such a long chained virus alignment

was rarely observed in the cells of intact insects, and this may be one of the characteristics of virus multiplication in cultured cells, which take very flat form on the glass surface. In virus inoculated cells, small virus-like particles were found in abundance. The diameter of the small particles is $30\text{ m}\mu$, while that of normal RDV particles is $70\text{ m}\mu$ in diameter (FUKUSHI, SHIKATA, KIMURA and NEMOTO, 1960). From their geometrical arrangement, these small particles are most likely one of the form of RDV, although polymorphism of RDV or developmental stages of RDV particles are not known. These small particles are also found in the cells of intact viruliferous leafhoppers. The presence of three different sizes of virus particles was also reported in Japanese encephalitis virus (NISHIMURA, NOMURA and KITAOKA, 1964). Further electron microscopical studies as well as attempts to recover the activity of RDV from cultivated viruliferous cells are under way.

The author wishes to thank Dr. S. NASU of the National Institute of Agricultural Sciences for valuable suggestions and for the electron microscopy.

REFERENCES

- FUKUSHI, T. (1934) J. Fac. Agr. Hokkaido Imp. Univ. **37**: 41~164.
- FUKUSHI, T., E. SHIKATA, I. KIMURA and M. NEMOTO (1960) Proc. Japan Acad. **36**: 352~357.
- MITSUHASHI, J. (1965) Japan. J. Appl. Ent. Zool. **9**: 107~114.
- MITSUHASHI, J. and K. MARAMOROSCH (1964) Contr. Boyce Thompson Inst. **22**: 435~460.
- NISHIMURA, C., M. NOMURA and M. KITAOKA (1964) Abstracts of 12th Ann. Meet. Soc. Japan. Virologist (Tokyo, 1964): 38.
- VAGO, C. et O. FLANDRE (1963) Ann. Épiphyties **14**: 127~139.

Fig. 3. Small RDV-like particles observed in the cell grown *in vitro*, which was inoculated with RDV. V, small RDV-like particles. (X70,000). The electron micrograph was prepared by Dr. S. NASU.

Fig. 4. The same as Fig. 3. (X 50,000)