A NEW BACTERIAL STALK ROT OF *BRASSICA*

A NEW stalk rot disease of *Brassica juncea* var. brown sarson appeared in epiphytotic proportion on a widely cultivated variety Varuna during the middle of October, 1979 in the Pali district of Rajasthan. Survey carried out during November in Roopawas and Mandia areas of the district showed that on average about 40% of the crop was affected although in isolated fields more than 60% of the plants were lost necessitating resowing or gap filling with healthy seedlings. Subsequent examination of the crops at Indian Agricultural Research Institute, New Delhi, also revealed the presence of this disease in fodder varieties of *Brassica* spp. The disease incidence was relatively more in crops sown during the first week of September than those planted later. Vigorously growing succulent plants due to an extra dose of nitrogen as well as those growing on poorly drained soil that remained moist suffered most.

The disease is characterized by the appearance of water soaked lesions at the collar region of plants which is usually accompanied by a white frothing. The lesions advance rapidly upwards affecting the tender branches. The foliage shows signs of water stress and withering. The pith tissues of the affected stem and branches become soft, pulpy and often produce dirty white ooze with foul smell. The infected collar region becomes sunken, turns buff white to pale brown in colour. Badly affected plant topples over at the basal region within a few days (Fig. 1).

Infected tissue gives out bacterial ooze from cut ends when mounted on a glass slide with a drop of water. Isolations from such tissues consistently yielded colonies of a bacterium on yeast glucose chalk agar (YGCA) plates within 24 hours at 28° C. Pure culture was established on nutrient agar (NA). For pathogenicity test, one month old healthy Varuna seedlings were wound-inoculated at the stem base with 48 hours growth of the bacterium and then incubated in a moist chamber. Suitable controls were kept. Water soaked lesions appeared within 3 days and typical stalk rot symptoms in another 7 days at 22–23° C and 100% relative humidity. Plants kept as control remained healthy. The bacterium was re-isolated and found to be the same as the original one.

Nutrient agar colonies were greyish, circular, translucent, shining, smooth, with raised centre and wavy margin. Slopes of YGCA, PDA (potato dextrose agar) and NA supplimented with dextrose supported better growth. Nutrient broth becomes uniformly turbid within 24 hours without formation of pellicle even on prolonged incubation.

The bacterium is Gram negative, rod shaped with blunt ends, capsulated, motile with peritrichous flagella. Acid but no gas is produced from glucose,

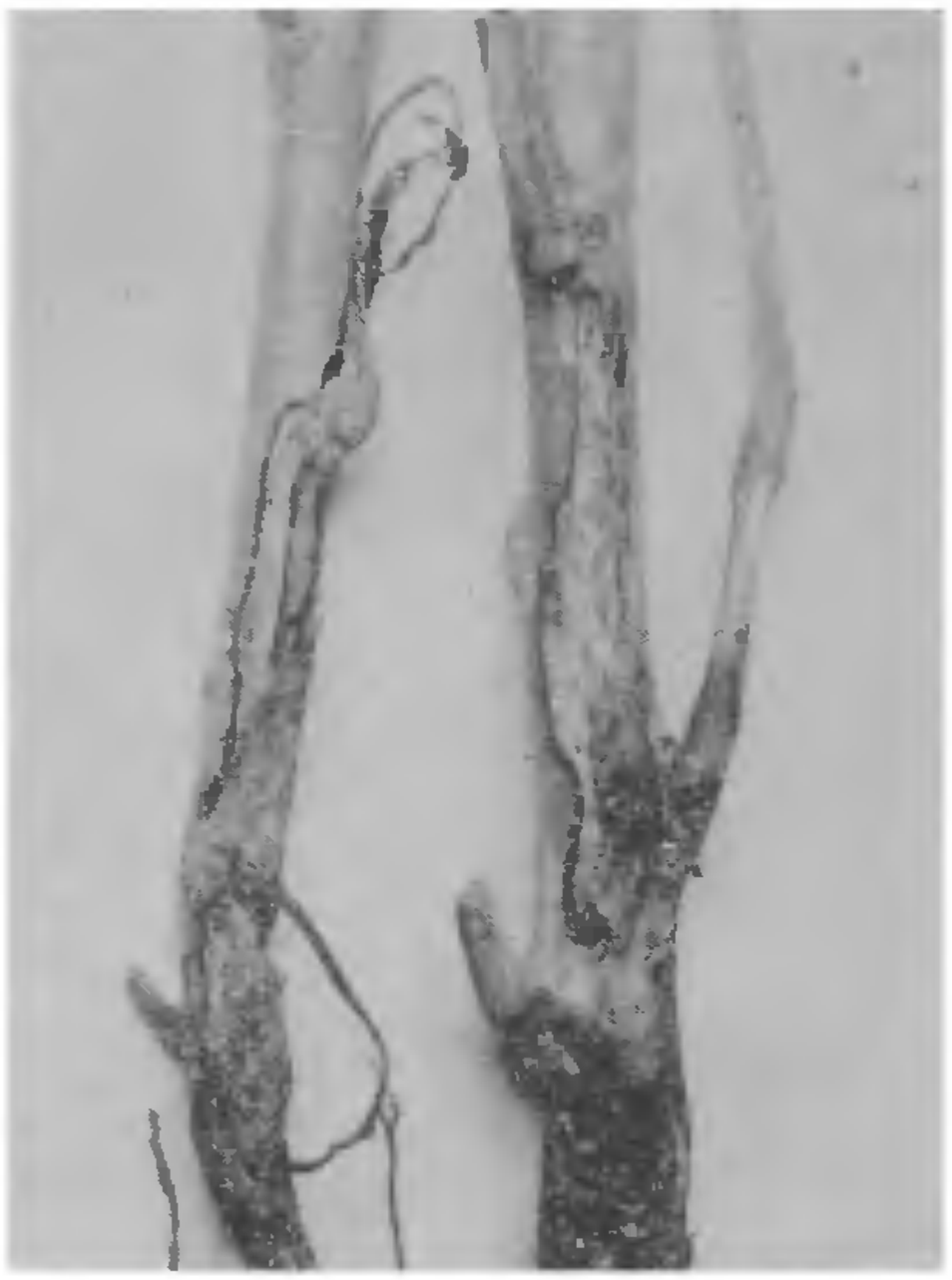

FIG. 1. Stalk rot of *Brassica juncea* (var. Varuna) incited by *Erwinia carotovora*.

arabinose, sucrose, lactose, cellobiose, meso-inositol, salicin, raffinose and galactose. Neither acid nor gas is produced with inulin on Dye's medium C[3]. It showed fermentative metabolism of glucose with Hugh and Leifson's medium[4]; aesculin, gelatin and sodium polypectate hydrolysed but not starch; produced ammonia and hydrogen sulphide from peptone water; Voges Proskeur test proved negative while methyl red and catalase tests were positive; nitrate was reduced to nitrite and asparagine utilized as a sole source of carbon and nitrogen. The bacterium did not produce fluorescence or green pigment on King's Medium B[5].

Beside its own host, the pathogen could infect *Nicotiana tabaccum*, *Lycoperisicon esculentum*, *Daucus carota*, *Brassica oleracea* var. *botrytis* on wound inoculations. It rooted 8 mm thick discs of potato and radish at 28° C and 100% R.H.

A black rot disease of cabbage, cauliflower, radish, turnip, knol-khol, mustard, and rai (*B. juncea*) caused by *Xanthomonas campestris* (Pammel) Dowson was described by Patel *et al.*[6] in 1949. The diseased leaves show prominent brown veins which, when extensive, bring about wilting of leaves. Such infected plants, if stripped, show dark brown bundles full of bacterial ooze. In the later stages of the disease many saprophytic organisms get in and bring about rotting and softness of the cabbage head[2,6]. Noticeable symptoms require 2 to 3 weeks to develop from the time of

infection in the seed bed. The black rot does not cause any disagreable odour. Satyavir *et al.*[7] also reported a bacterial rot of raya (*B. juncea*) which in the initial stage causes dark coloured streaks at the stem base. These streaks gradually girdle the stem, its tissues become soft and there is collapse of the plant. Leaves are also infected, the lower leaves being attacked first. A yellow fluid oozes out from the diseased tissues. Isolations yielded a yellow pigmented bacterium, which was identified as a species of *Xanthomonas*. Healthy plants become infected after 12–15 days following inoculation. No particulars about the inoculation method, characteristic properties of the bacterium and its host range have been furnished.

However, considering the pattern of flagellation, staining reactions and physiological and biochemical characteristics mentioned above as well as the rapidity with which it causes foul smelling rots on *Brassica* and other host species tested, the bacterium is identified as a species of *Erwinia* belonging to carotovora group[1,2] inciting stalk rot of *Brassica*. Perusal of literature shows that this disease has not been reported so far from anywhere.

We are grateful to Dr. V. V. Chenulu, Professor and Head, Division of Mycology and Plant Pathology, I.A.R.I., New Delhi, for providing necessary facilities and encouragements. Sincere thanks are also due to Mr. K. S. Jhala, Joint Director of Agriculture, Jodhpur (Rajasthan), for help in surveying the *Brassica* fields.

Division of Mycology and
Plant Pathology, I.A.R.I.,
New Delhi 110 012,
January 24, 1980.

T. P. Bhowmik.
B. M. Trivedi.

1. Buchanan, R. E. and Gibbons, N. E., *Bergey's Manual of Determinative Bacteriology*, 8th ed., Williams and Wilkins Co., Baltimore, 1974.
2. Dowson, W. D., *Plant Diseases Due to Bacteria*, Cambridge University Press, 1957.
3. Dye, D. W., *New Zeland J. Sci.*, 1962, **5**, 393.
4. Hugh, R. and Leifson, E., *J. Bact.*, 1953, **66**, 24.
5. King, E. D., Ward, M. K. and Raney, D. E., *J. Lab. Clin. Med.*, 1954, **44**, 301.
6. Patel, M. K., Abhyankar, S. G. and Kulkarni, Y. S. K., *Indian Phytopath.*, 1949, **2**, 58.
7. Satyavir, Kaushik, C. D. and Chand, J. N., *PANS*, 1973, **19**, 46.

EVIDENCE OF COMPLEMENT FIXING ANTIBODIES AGAINST EQUINE RHINOPNEUMONITIS VIRUS IN ARMY HORSES AND MULES

Serological surveys against Equine Herpes Virus 1 (EHVI) also known as Equine Rhinopneumonitis Virus (ERV)[1] have been conducted in several countries[2–7]. In India the incidence of ERV infection was first reported at Saharanpur in aborted foetuses, on the basis of histological lesions[8]. Subsequently Kentucky D-strain of ERV was isolated at Hissar, in suckling hamsters[9]. Abortions in some mares have been reported recently from Hissar and a few other places in the country[10]. However, systematic survey has not been undertaken to assess the extent of its endemicity in India. The present report is a preliminary study on the detection of complement fixing (CF) antibodies to ERV among army horses and mules.

Four hundred and thirtyeight serum samples of 333 horses and 105 mules, received in this laboratory for screening against glanders, were simultaneously processed for the detection of CF antibodies to ERV. The sera were inactivated at 56° C for 30 minutes in a water-bath. Hamster adapted lyophilized antigen (EHVI Kentucky D Strain), positive and negative sera against ERV were obtained from Dr. T. Shimizu (National Institute of Animal Health, Japan). The antigen was reconstituted with distilled water, mixed with an equal volume of 1·8% sodium chloride solution and then centrifuged at 10,000 r.p.m. for 20 minutes. The supernatant was titrated for determining CF units of the antigen. Hyper-immune serum against ERV and antisheep erythrocyte haemolysin were raised in the laboratory in rabbits. Pooled serum complement from ten guinea pigs was titrated before each run of the test proper. Two units each of complement (100% haemolytic unit) and antigen were employed in the test. Complement fixation test (CFT) was carried out in microtitre plates using Veronal buffer as diluent[11,12]. The highest dilution of the serum showing +++ (60 to 80% CF reaction) indicated evidence for the presence of CF antibodies.

Table I indicates that 47 (10·73%) of 438 serum samples were positive for CF antibodies. Out of 333 horse sera, 44 (13·21%), and out of 105 mule sera, only 3 (2·86%) were found to show the presence of CF antibodies against ERV. CF titres obtained were 1 : 16 in 2 (0·60%), 1 : 8 in 12 (3·60%) and 1 : 4 in 30 (9·01%) of positive horse serum samples. Mule sera demonstrated high anticomplementary activity, with CF reaction in an insignificant number of samples.

It has been elucidated that a 50% CF reaction indicates a significant CF titre[8]. In our studies, however,