The viability of the organism in different carriers was monitored at regular intervals thereafter, by plating dilutions of the different carriers on a modified Okon's medium[3] containing bromothymol blue (Lakshmi-Kumari *et al.*—unpublished).

The population of *Azospirillum* in different carriers reached a maximum by 15 days of incubation at room temperature with the exception of soil + FYM where it reached a maximum at 30 days (Table I). The combination of the soil and FYM gave higher (*Azospirillum* count than the individual carriers. Besides holding high moisture, the obvious virtues of FYM lie in its ability to improve the surface area and porosity of the carriers[5] when used in combination with soil so as to facilitate increased *Azospirillum* growth. Addition of FYM also enhances the organic matter content of the carrier which in turn is reflected in the survival of *Azospirillum* as well.

In most of the cases, the population of *Azospirillum* was maintained upto a level of $10^{10}$ in different carriers even after 120 days of storage except in the case of the soil, where the count was considerably reduced by 30 days of incubation at room temperature (Table I). However, it is interesting to note that the combination of soil and FYM helped to retain the population of *Azospirillum* ($400 \times 10^{\hat{}}$) even upto six months' storage. These results are in conformity with our earlier findings[2] on the survival of the organism in different carriers upto 31 weeks.

Our sincere thanks are due to Dr. N. S. Subba Rao, Head, Division of Microbiology, Indian Agricultural Research Institute, New Delhi, for suggesting the problem and encouragement provided during the course of the investigation.

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1. Albrecht, S. L., Okon, Y. and Burris, R. H., *Pl. Physiol.*, 1977, **60**, 528.
2. Lakshmi, V., Rao, A. S. N., Vijaya Lakshmi, K., Lakshmi-Kumari, M., Tilak, K. V. B. R. and Subba Rao, N. S., *Proc. Indian Acad. Sci.*, 1977, **86 B** (6), 397.
3. Okon, Y., Albrecht, S. L. and Burris, R. H., *Appl. Environ. Microbiol.*, 1977, **33**, 85.
4. Tarrand, J. J., Krieg, N. R. and Dobereiner, J., *Canad. J. Microbiol.*, 1978, **24**, 967.
5. Tilak, K. V. B. R. and Subba Rao, N. S., *Fertil. News.*, 1978, **23** (2), 25.

## BOLL ROT OF COTTON FROM AGRA

During September–November 1977, a large number of cotton bolls were found rotted in the fields exhibiting mycelial growth and discolouration. The present communication deals with the nature and extent of such boll rots.

For isolation of associated fungi, small bits of surface sterilized, diseased bolls were plated on PDA and Czepek's media and incubated for a week at 28° C (± 2° C). Five fungi, *viz.*, *Aspergillus flavus* Link, *A. niger* van Tieghem, *Cladosporium cladosporioides* (Fres) de Vries, *Cephalosporium roseogriseum* Saksena and *C. asperum* Marchal were found consistently associated with the diseased bits.

The pathogenicity tests were conducted with all the isolates in the laboratory and in the field. During field trials the atmsopheric temperature ranged between 22–23·5° C and relative humidity between 60–65%; whereas in the laboratory, the inoculated bolls were incubated at 60 (approximately near field level) and 90% relative humidity (approximately the average level available during rainy season).

The inoculations were made by three methods; (1) pricking the sterilized boll surface with a fine needle and immediately after the injury, the inoculations were made with each of the above fungi separately; (2) the respective fungi were inoculated on uninjured healthy boll surfaces; and (3) the bolls were pressed so that their valves were separated slightly at the top and immediately after, the inoculum was placed on the edge of the valves. Subsequently, the inoculated bolls, in each case were covered with sterilized polythene bags. In the field tests, however, the bags were removed after three days to provide the natural conditions. Ten replicates were used for each fungus under each treatment. Respective controls were maintained. Reisolations from inoculated bolls were made, following the standard procedures. The maintenance of relative humidity and calculation of per cent rot was done by the procedure and formula suggested by Prasad and Bilgrami[2].

Under field tests only *C. roseo-griseum* caused some rotting when inoculated on injured fruits but not on uninjured while others failed to infect.

In the laboratory tests, only *A. flavus* and *C. roseogriseum* caused 4 and 8·5% rot, respectively, at 60% relative humidity. However, at 90% relative humidity all the isolates except *C. cladosporioides* were pathogenic and displayed increased virulence as compared to that at 60% relative humidity. The differential symptoms and severity of rots induced by the respective pathogen at 90% relative humidity may be summarized as follows : (*a*) *A. flavus* produced dry, brown rots to the extent of 10 and 23% on uninjured and injured fruits respectively. *A. niger*

incited about 80% blackish soft rot through wound inoculations. (*b*) *C. asperum* decayed 46·3% tissue of injured fruits exhibiting dry brown colouration. The fruit valves were baldy damaged and the cotton fibres in contact of decaying valves turned brownish and brittle. (*c*) *C. roseo-griseum* was found to be highly virulent as it damaged 20·5% boll tissue even on uninjured fruits. The mycelium grew luxuriantly on the boll surface. When inoculated by either of the injury methods, the pathogen caused rotting of the whole fruit (100%) within the same period (8–10 days). The boll valves became very soft and turned bluish in colour. The inside cotton fibres were replaced by watery substance with abundant mycelium and spores. Even the seeds were found decayed. The rotting was so extensive that a bit of pressure was enough to crush the infected bolls.

These studies have demonstrated that the pathogens under study require high humidity and some sort of injury for producing the rots. The injury may be provided by insect feeding as has been demonstrated by Ashworth *et al.*[1] in relation to *A. flavus* and/or by the opening of the boll valves as has been found in the present study. The success of the pin-prick method employed presently, further support the role of insect feeding in boll rots. The fact, that the present pathogens more or less failed to incite rots in the field (av. temp. 23° C, av. R.H. 63%) and laboratory at 60% R.H., but showed increased virulence on injured and uninjured fruits at high humidity (90%) suggests that high humidity (80–100%) prevalent at the normal furiting season (August–September) at Agra in concert with injury, plays a very critical role in pathogenesis.

Only a few boll rot diseases of cotton have been reported from India. A dry pink rot caused by *Trichothecium roseum* and a black soft rot by *Rhizopus nigricans*, have recently been described from Maharashtra[3]; *A. flavus* rot has been recorded from abroad by Ashworth *et al.*[1] and Stephensen and Russell[4]. The rots caused by *Cephalosporium asperum* and *C. roseo-griseum* have not so far been recorded in India or elsewhere.

The authors are grateful to the farmers of the locality for supplying the diseased specimens, to Dr. M. N. Gupta, Professor and Head, Department of Botany, Agra College, Agra, for his inspiration.

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*January* 9, 1979.

1. Ashworth, L. J. Jr., Rice, R. E., MacMeans, J. L. and Brown, F. M., *Phytopath.*, 1971, **61**, 488.
2. Prasad, S. S. and Bilgrami, R. S., *Indian Phytopath.*, 1973, **26**, 517.
3. Rao, V. G., *Maharashtra Vidhya Mandir Patrika*, 1974, 9, 121.
4. Stephenson, L. W. and Russell, J. E., *Phytopath.* 1974, **64**, 1502.

## EFFECT OF ANTIBIOTIC (BENZYL PENICILLIN) ON PROTOCEREBRAL NEUROSECRETORY ACTIVITY OF THE MILLIPEDE *GONOPLECTUS MALAYUS* (CARL)

SOME aspects of neurosecretory system of the millipede *G. malayus* have been described earlier[9–11]. In the present work an attempt has been made to study the effect of Benzyl penicillin on the neurosecretory activity of this millipede. Penicillin is one of the most important and widely used antibiotic in the world. Medical practitioners often use this antibiotic for the treatment of cerebro-spinal and other diseases, therefore, it was thought opportune to see what happens at the cellular (neurosecretory cells) level.

Adults of *G. malayus* were locally collected for the experiment. For each replicate (5 individuals), millipedes were divided into two lots, one as experimental and the other as control. A vial of five lacs units of Benzyl penicillin (Alembic Chemical Works Co. Ltd., Baroda, India) was diluted to 10 cc. with distilled water. The solution was tested with the animal and it has been confirmed that millipedes show no allergy with the antibiotic. A minimum dose of 0·5 cc solution was injected directly in the haemocoel of the first lot of animals and the control was injected with the same amount (0·5 cc) of distilled water. Observations were recorded after half an hour. The brain along with neurohaemal organs were dissected, embedded in paraffin (m.p. 56–60° C) and the serial sections were cut at 7 to 10 $\mu$ in transverse and longitudinal planes. Observations were made by applying neurosecretory staining techniques[1–3]. For assessing the neurosecretory activity, the diameter of the nuclei of neurosecretory cells were calibrated.

The effect of antibiotic becomes significant within 30 minutes. The millipedes injected with antibiotic show greater amount of discharge of NSM, in comparison with the control. The nuclei of the neurosecretory cells of the treated millipedes displayed enlargement over those of control millipedes (Figs. 1, 2) and scanty NSM was spotted in their pericarya and axons. Presence of vacuoles in the neurosecretory cells of treated millipedes indicate that neurosecretory cells are extremely active and probably the transport and release of NSM is faster than the synthesis[6]. The tracts of transport of NSM have been observed packed with neurosecretory products (Fig. 3).

Numerous abnormalities like—hematuria, pyuria, elevation of serum creatinine, ECG, abnormalities suggestive of myocarditis, thrombo phelebitis, etc.,