Pathogenicity test with slight wounding on the leaves with a culture of the pathogen (on PDA) produced lesion after 5 to 6 days. The fungus was reisolated from the infected leaves.

*Leaf spot of* Calanthe *sp.*

The disease occurred on the leaves of *Calanthe* sp. at Duliajan in December 1972. Symptom developed as light brown patches with dark brown borders covering extensive portion. The causal agent was identified as *Machrophomina phaseolina* (Tassi) Goid. (identification confirmed by C.M.I., IMI 511599).

*Blossom blight of* Spathoglottis pubescens *Lindl.*

This malady was observed during the last part of winter continuously for last three years (1976–1978) at Jorhat affecting usually petals and occasionally leaves. The causal fungus was identified as *Botrytis cineria* Pers. ex Pers. (a culture has been deposited to HCIO, New Delhi, ITCC 2105).

Pathogenicity tests with conidial suspension as well as mycelium (on PDA) produced infection after 2 days on petals and after 3 days on leaves in saturated atmosphere. The fungus could be reisolated from the infected parts. Detailed investigations of this disease are in progress which will be reported later. In describing orchid diseases, Duarte[1] mentioned *inter alia* about petal blight caused by the same fungus.

*Leaf blight of* Spathoglottis pubescens.

The disease, recorded at Jorhat in August 1972, manifested as linear light brown spots with brown margins on leaves which increased in size (occasionally exceeding 7 cm) and in severe cases the spots, although a few in number, might cover even two-third of the lamina blighting the tissues. The causal organism was identified as a species of *Sphaeropsis.*

Pycnidia developed as minute black dots, amphigenous, globose, brown, ostiolate, pseudoparenchymatous, (50–) 91–166 $\mu$ in diameter; conidia dilute coloured, elliptical ovoid or oval, occasionally one end may be irregularly narrower, (5–)5·8–10(–10·9) $\times$ (3·3–) 4·2–5·8 $\mu$ held together when extruded. Mycelium intercellular, brown, septate, constricted at septa, not uniformly wide.

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## A MODIFIED IMMUNIZATION SCHEDULE FOR RAPID DEVELOPMENT OF ANTIBODIES AGAINST WHOLE CELL *RHIZOBIUM* ANTIGEN

Serological methods have been used in studies on legume-*Rhizobium* symbiosis[1-6]. More recently, Gaur and Sen[6] advocated the adoption of this technique for the screening of efficient and dominant strains of rhizobia. This technique suffers from one disadvantage, *viz.*, a long immunization schedule (21 to 71 days) required for the development of antibody of high titre value[7-16]. Vincent[17] had advocated a rapid immunization schedule of 11 days which resulted in good antibody formation. However, the method and schedule did not find much favour with later workers, probably because it comprised four intravenous injections on four successive days. Dudman's[8] modified immunization schedule, mainly to avoid casualties, was of 47 days, duration. A need for the development of a rapid as well as safe immunization schedule was thus felt and hence, the present work was taken up.

Antigens of *Rhizobium* of green gram (M 1 and M 7), soybean (SB–16), pea (P–2) and Bengal gram (G–2) were prepared according to Vincent[18] and 1·0 ml of antigen mixed with 1·0 ml of Freund's adjuvant was injected intramusularly to the hind quarter of the rabbits. After 7 days, 1 ml of the respective antigens without adjuvant were introduced through the marginal ear vein of the rabbits. After an interval of 6 days, a second intravenous injection with an increased dose of 1·5 ml was given. After 5 days, *i.e.*, on the 18th day of the schedule, the animals were bled. Sera of the bloods were obtained and after diluting them in saline solution, titre values of the antisera were determined by the usual agglutination test[18] against their respective antigens. The results obtained are shown in Table I.

It is clear from Table I that anti-M 1, M 7 (*Rhizobium* of *Vigna aureus*), SB–16 (*R. japonicum*), P–2 (*R. leguminosarum*) and G–2 (*Rhizobium* of *Cicer arietinum*) sera obtained were of high titre value resulting in firm agglutination at the highest dilution of 6,400 to 12,800 against their homologous antigens. Weak cross reactions between M 1 and M 7 against SB–16 and between P–2 and G–2 were also seen. Antisera, obtained are thus suitable for use in immunodiffusion test where a titre value of at least 3,200 is desirable.

Thus by following this immunization schedule antisera of *Rhizobium* of different species of sufficiently high titre can be obtained within 18 days of first injection, thus cutting short the total time required for this purpose by about half to one-third.

TABLE I

*Cross-agglutination reaction among different species of* Rhizobium

(Agglutination tubes kept at 70° C for 2 hrs and overnight at 4° C)

| Antigen | Highest dilution of antisera showing firm agglutination | | | | |
|---|---|---|---|---|---|
| | M I | M 7 | SB-16 | P-2 | G-2 |
| M I | 6,400 | 6,400 | 200 | .. | .. |
| M 7 | 12,800 | 12,800 | 400 | .. | .. |
| SB-16 | 400 | 400 | 12,800 | .. | .. |
| P-2 | .. | .. | .. | 12,800 | 1,600 |
| G-2 | .. | .. | .. | 1,600 | 6,400 |

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## NITROGEN FIXATION AND AMMONIA ASSIMILATION IN *AZOTOBACTER CHROOCOCCUM* ISOLATES FROM $C_3$ AND $C_4$ PLANTS

THE diazotroph, *Azotobacter*, still holds greater promise not only for fundamental studies on nitrogen fixation but also in the practical use as a biofertilizer[1,2]. Though of wider distribution in the tropical soils, *Azotobacter* colonizing the rhizosphere of certain tropical grasses are significant as they have been found to be more efficient in dinitrogen fixation[3,4]. Recently, from our laboratory it has been brought out that the isolates of *Azotobacter* from the rhizosphere of certain $C_4$ plants recorded more efficiency in nitrogen fixation than the isolates from $C_3$ plant species[5]. One of the reasons for the luxuriant and rank growth of the tropical grasses and weed plants is believed to be their asociation in their root system with more efficient nitrogen fixing organisms[6,7]. In the present paper, using the acetylene reduction technique, the N-fixing capacities of *Azotobacter chroococcum* isolates and the pathway of ammonia assimilation are reported.

The cultures of *A. chroococcum* (Table I) were isolated from the rhizosphere of the respective plant species and the pure cultures were maintained on Waksman medium No. 77 agar slopes. The isolates were grown under aerated conditions in N-free liquid medium for ten days at room temperature (28 ± 2° C) and the nitrogen content was estimated in a mikro-kjeldhal unit[3].

The enzyme nitrogenase, (nitrogen acceptor oxido-redudtase E.C.1.7·99·27) of the isolates was assayed in a Perkin Elmer Model F-33 gas chromatograph fitted with potapak–N column (80–100 mesh) and flame ionization detector[9]. The assay system consisted of 2·0 ml of the cell suspension, harvested from the early log phase growth of the organism in Waksman No. 77 broth devoid of calcium carbonate and washed in 0·1 M phosphate buffer (pH 7·0) in 20 ml vial. The air in the vessel was driven-off by flushing with a jet of nitrogen. Ten per cent acetylene was injected and the internal oxygen pressure was maintained at 0·2 atm. The vials were incubated for 24 h at 28° C after which 1 ml of the gas sample was analysed. The ethylene peaks were compared with a standard graph prepared with pure ethylene and the quantity of $C_2H_4$ formed calculated. The ammonia assimilatory pathway predominantly involved the coupled reaction catalysed by the dual enzymes, glutamine synthetase (GS, E.C 6.3.1.2) and glutamate synthase (GOGAT, E.C. 2.6.1.5.3) in most of the nitrogen fixing organisms[10,11]. With a view to estimating the activities of these enzymes early log phase cultures were harvested by centrifugation, washed in buffer, disrupted by sonica-