with a very small basal prolongation, enlargement (20 : 11) 0·63 the length of the segment and 1·81 × its maximum thickness, stem (9 : 5) 0·45 the length of the enlargement and 1·80 × its maximum thickness; fourth segment (30) with enlargement (20 : 12) 0·66 the length of the segment and 1·66 × its maximum thickness, stem (10 : 5) half the length of the enlargement and 2·00 × its maximum thickness; fifth segment (33) longer than fourth, enlargement (20 : 12) 0·66 the length of the segment and 1·66 × its maximum thickness, stem (13 : 5) 0·65 the length of the enlargement and 2·60 × its maximum thickness; sixth segment similar to fifth; seventh segment (35) longer than sixth; eighth, ninth and tenth segments similar to each other and as long as seventh; eleventh segment (30) shorter than tenth; twelfth segment (27) shorter than eleventh; penultimate segment (25) shorter than twelfth, enlargement (18 : 13), 0·72 the length of the segment, 1·40 × as long as thick, stem (7 : 3) 0·38 the length of the enlargement and 2·33 × its maximum thickness; terminal segment (19) shorter than penultimate, enlargement (19 : 8) with an apical nipple-like prolongation, length 2·37 × its maximum thickness. *Wing* : (50 : 23) hyaline, costa sparsely hairy, vein R_1 joining costa a little beyond the basal 1/4 of the wing, vein R_s present at an oblique angle, vein R_5 reaching costa before the apex of the wing, vein C_u forked. *Legs* : long, moderately hairy, metatarsus (7) shorter than terminal tarsal segment, second tarsal segment (52) longest of all, shorter than the following segments combined together (62) ; claw simple on all legs, not sharply bent at right angles; empodium rudimentary. *Genitalia* : light-brown, basal clasp segment (39 : 20) quadrate, with a small median triangular lobe, length nearly 2·00 × its maximum thickness; terminal clasp segment (25 : 9) short, stout, gradually tapering and ending in a tooth, extreme end of its lower margin with a few serrations, length 2·77 × its maximum thickness; dorsal plate broadly and deeply incised in the middle, lobes broadly rounded apically, pubescent; subdorsal plate shorter than dorsal, entire, broadly rounded apically; parameres as long as dorsal plate, moderately sclerotized, rest of the details as in figure; aedeagus (17 : 8) straight, broad basally, pubescent, weakly sclerotized, hairy, longer than dorsal plate, length a little more than 2·00 × its maximum thickness, truncate apically.

Female : Unknown.

Holotype : Male dissected and mounted on slide labelled as "at light, Fruit Research Centre, Aurangabad, Maharashtra, India, R. M. Sharma Coll., dated 11.viii.1976."

This species closely resembles *R. champakii* Grover, the only known species, but differs from it in the characters as indicated in the key :

Claw dentate on front legs, simple on hind legs, bent at right angles; empodium shorter than claw; subdorsal plate angulated apically, aedeagus short and rounded ..... *champakii* Grover ♂ ♀

Claw simple on all legs, not sharply bent at right angles; empodium rudimentary; subdorsal plate broadly rounded apically; aedeagus long, broad, truncate apically ..... *orientalis*, sp. nov. ♂

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Department of Zoology,
Marathwada University,
Aurangabad 431 004 (India),
June 4, 1979.

R. M. SHARMA.
S. N. RAO.

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PROTEASE ACTIVITY IN *BRASSICA JUNCEA* PLANTS INFECTED WITH *SCLEROTINIA SCLEROTIORUM*

WHITE ROT caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a very destructive disease and causes heavy economic losses to *Brassica juncea* crop in north-eastern India[1]. In view of this, various physio-pathological aspects of the disease were taken up and the present paper deals with the production of protease in susceptible and resistant cultivars of *B. juncea* plants at various intervals after inoculation with the isolates of *S. sclerotiorum*.

Experimental

Four isolates of *S. sclerotiorum* markedly differing in the degree of virulence, and different cultivars of *B. juncea* showing maximum and minimum disease reaction against these pathogens were used in this study. Preflowering plants of both the varieties were inoculated by agar disc method of Rai and Dhawan[2]. Infected plant parts were harvested after 5, 10 and 15 days of inoculation. Samples were homogenized with 0·1 M phosphate buffer (pH 7·0) in a ratio of 1 : 5 (w/v), strained and centrifuged at 600 rpm for 15 min.; supernatants were used as crude enzyme preparation.

Enzyme assay—Proteolytic activity was determined by modified method of Davis and Smith[3]. Reaction consisted of 2 volumes of 1% casein in 0·1 M phosphate buffer (pH 7·0), 1 volume of the same buffer

and 1 volume of the enzyme preparation was incubated at 30° C. Aliquots of 1·0 ml of reaction mixture were withdrawn after 2 hrs and immediately treated with 2 ml of ninhydrin reagent which stopped the reaction. Tubes were kept in boiling water bath for 20 min to develop the colour and were cooled to room temperature. Final volume was made up to 50 ml with 50% *n*-propanol and the colour intensity measured at 570 mμ. The enzyme activity was expressed as nanomoles of α-amino nitrogen released at 30° C per ml of reaction mixture in the specified time of reaction.

Results and Discussion

Production and role of protease in the development of fungal diseases has been critically discussed by various workers[4–6].

The results of the present study clearly indicate that all the isolates of *S. sclerotiorum* produce significant amount of protease in susceptible and resistant *B. juncea* plants (Table I). There was very little difference in the enzyme activity recorded in the case of different isolates of the pathogen. In the susceptible host, all the 4 isolates showed considerably good degree of activity from the 5th day; and subsequently only a slight increase in activity which suggest that this enzyme might be playing a secondary role in concert with other enzymes at various stages of pathogenesis. In the case of the resistant cultivar enzyme, the activity was lower than that of susceptible ones and the maximum activity was observed on 10th day after which a decrease in activity was recorded (Table I). Mahadevan and Chandramohan[7] have also reported a lower protease activity in resistant plants as compared to the susceptible ones in a wilt disease of cotton caused by *Fusarium oxysporum*. Recently Khare and Bompeix[8] have shown a high protease activity of *S. sclerotiorum in vitro* and *in vivo* and have found it to be related with the pathogenicity. It has also been related that this fungus can reduce the pH of the medium of the infected tissue to the value most favourable for the activity of their own proteases. In the case of the present disease also, there was a fall in the pH of the infected tissue with the advancement of the disease which might be favouring the activity of protease.

TABLE I

Protease activity (nano moles of aminonitrogen released/ml of reaction mixture/2 hr) in the tissue extract of susceptible and resistant B. juncea *plants after* 5, 10 *and* 15 *days after inoculation with* 4 *different isolates of* S. sclerotiorum

Isolates of *S. sclerotiorum*	Days after inoculation	Susceptible host tissue	Resistant host tissue
Ss–1 (MHV)	5	1202	1156
	10	1221	1173
	15	1246	1155
Ss–2 (WV)	5	1152	1083
	10	1161	1117
	15	1202	1055
Ss–3 (HV)	5	1086	1011
	10	1114	1098
	15	1139	951
Ss–4 (MV)	5	1180	1122
	10	1189	1133
	15	1221	1062

MHV—Most highly virulent; HV—Highly virulent; MV—Moderately virulent; WV—Weakly virulent.

Data presented in the table are average of 3 independent assays.

Note: Control sets showed no activity.

From the findings of the present study it appears that in white rot of *B. juncea*, the protease possibly causes the tissue maceration by degrading the structural wall protein and thus facilitating the spread and development of the pathogen.

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Department of Botany, (Miss) SHASHI DHAWAN,
Lucknow University,
Lucknow 226 007, India,
August 8, 1979.

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