

involved in the formation of the spatulate prothallus. In some cases the growth was very irregular and such gametophytes differentiated more than one marginal meristems (Fig. 2). As a result of meristematic activity of marginal meristems at different loci, a large number of gametophytes became lobed structures before the onset of tuber formation. As shown in Fig. 3, the mature gametophyte was not cordate, there being no distinct apical meristem.

#### Formation of Tubers and Sex Organs

Tubers were consistently observed in all the cultures and served three purposes (i) perennation, (ii) vegetative reproduction and (iii) production of sex organs. One or more tubers could develop anywhere from the marginal cells of the gametophyte; the site of inception was demarcated by the differentiation of a large number of rhizoids around it. The marginal cells near the rhizoids divided in different planes (Fig. 4) and gave rise to radially symmetrical tubers (Fig. 5). Three-dimensional growth was, therefore, restricted to the formation of tubers; thus unlike other homosporous ferns, no central cushion was formed within the expanded portion of the gametophytes.

As regards the sites of sex organs, the antheridial formation was restricted to the proximal region of the tubers (Fig. 6). Occasionally they were observed on the surface of the gametophyte as well in close proximity of the tuber. Archegonia were present in the distal region of the tuber (Fig. 7). Such archegonia bearing tuber has earlier been described under the term "archegoniophore" by Goebel<sup>2</sup>.

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#### PEROXIDASE, PHENOLOXIDASE AND TOTAL PHENOLS IN THE SPINACH LEAVES INFECTED WITH *FUSARIUM EQUISETI*

The fungus *Fusarium equiseti* (Corda) Sacc. causes leaf spot disease in spinach (*Spinacia oleracea* L.). Young immature leaves (1 week old) do not develop lesions but only pinkish spots are formed due to infection. The mature leaves (2 weeks old) develop circular spots of about 0.5 cm diameter with a pinkish halo. Much older (4 weeks old) leaves which turned yellow at their senescent stage were severely damaged. Only mature and older leaves show a sunken lesion

area of dead tissue. Oxidative enzymes are known to play a role in the defence mechanism of plant tissues<sup>1-3</sup>. In view of this, the activities of peroxidase, phenoloxidase and total phenolic contents of spinach leaves under different stages of infection with *F. equiseti* are presented in this communication.

The leaf tissues from the healthy or infected areas avoiding dead tissue in the sunken lesion spot, were cut and put into chilled extractants. The fresh weight of each tissue was determined and separately homogenized in phosphate buffer (0.1 M) at pH 6.8 using a Virtis tissue homogenizer for 2 minutes at the maximum speed. The homogenate was centrifuged at 2,000 g at 4°C. The supernatant was made up to 25 ml with distilled water and used as enzyme source.

For peroxidase (E.C. 1.11.1.7) assay<sup>4</sup> 3 ml of 0.05 M guaiacol and 0.1 ml of tissue extract were taken in the colorimeter tube and the absorbance was adjusted to zero at 470 nm. Then 0.5 ml of 1% H<sub>2</sub>O<sub>2</sub> was added to the tube and the contents were quickly mixed. Change in absorbance was recorded every 10 seconds for a period of 3 minutes. In the phenoloxidase (E.C. 1.10.3.1) assay<sup>5</sup> 2 ml tissue extract and 3 ml of phosphate buffer (0.1 M; pH 6.0) were taken in a colorimeter tube, and its absorbance was adjusted to zero at 495 nm. Then 1 ml of 0.01 M catechol was added, contents were mixed and change in the absorbance was recorded every 10 seconds up to 3 minutes. Heated enzyme served as control and the activity of the enzymes was expressed as increase in absorbance  $\Delta A/\text{minute}/2 \text{ mg}$  fresh weight of leaf tissue. For the study of total phenols tissue slices were extracted in boiling 80% ethanol (1 g/10 ml), and estimation was made with Folin-ciocalteu reagent using catechol as the standard<sup>6</sup>.

There was nearly four fold increase in peroxidase and phenoloxidase activities in the immature leaves in the infected areas (Table I). The increase of enzyme activities in the immature leaves was, however, three-fourths due to infection, while the leaves at their senescent stage showed no appreciable increase. Though relatively little is known regarding the biological function of peroxidase<sup>6</sup>, its direct participation in the inhibition of fungal growth cannot be ruled out<sup>7,8</sup>. These enzymes bring about the oxidation of phenolic substances and the oxidation products could be toxic to the pathogen<sup>9</sup>, especially in younger leaves. There was also an age-dependent correlation in the increase in the total phenolic content after infection and inhibition of the pathogen. Some of the phenolic contents may serve as suitable substrates for phenoloxidase which is presumed to play a role in the defence mechanism<sup>10</sup>. The present investigation shows a rough correlation between enzyme activities and lesion size<sup>11</sup>, thus implicating these factors in symptom expression<sup>12</sup>.



TABLE I

*Peroxidase, phenoloxidase activities and total phenolic contents in the young, mature and old leaves of spinach infected with Fusarium equiseti*

Stage of leaf	Area of leaf	Peroxidase*	Phenoloxidase**	Total phenols***
Young green	Healthy	0.42±0.04	0.24±0.02	410±21
	Diseased	1.58±0.08	0.93±0.03	447±26
Mature green	Healthy	0.31±0.02	0.21±0.06	368±27
	Diseased	0.55±0.05	0.36±0.04	386±33
Old yellow	Healthy	0.21±0.03	0.14±0.02	289±21
	Diseased	0.24±0.01	0.16±0.02	286±27

\*  $\Delta A$  470 nm. \*\*  $\Delta A$  495 nm/minute/2 mg fresh wt. of leaf tissue. \*\*\* Catechol equivalents (mg)/g dry wt. of leaf tissue.

Mean  $\pm$  Standard deviation based on 6 sets of experiments.

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#### COLLECTERS ON THE COTYLEDONS OF IN VITRO RAISED SEEDLINGS OF WITCHWEED—*STRIGA ASIATICA* (L.) KUNTZE

UNDER ordinary conditions witchweed<sup>1</sup> seeds will not germinate unless it be in the presence of some stimulatory substance or a complex of such substances<sup>2</sup>. The putative host plants obviously secrete a stimulant that induces germination of the seeds of the parasite<sup>3-6</sup>. Using standard methods<sup>3-6</sup>, germination of pretreated seeds has been induced in petridishes in the medium of root exudates of host weeds. The methods employed are as follows. Firstly, pretreated seeds of *Striga* are sandwiched between discs of blotters and introduced on to the surface of the root mass by drilling a hole and then covered with soil. After 2 or 3 days, the discs can be retrieved, the sandwiches opened and germination counted (personal communication of Dr. C. Parker). The second method consists of preparation of witchweed seeds for germination in the host root exudate on one hand and collection of host root exudate on the other. Washed and dried seeds after surface sterilization with sodium hypochlorite (for 5 minutes) were sprinkled in between two moist filter papers in a petri plate and kept in darkness for 3 weeks. The collection of host root exudate involves raising of host seedlings from seed specially for the purpose, transplanting 2-6 each of them in 5 pots filled with manured garden soil. At appropriate ages of the respective weeds, they are knocked out from the pots, thoroughly washed and transferred to suitable containers with sterile distilled water, kept like that for 30 hours, allowing the root exudate to diffuse into the medium. The root eluate so collected is then dispensed into petri plates in which the pretreated seeds are maintained on the discs of filter-papers of diameter of 0.5 mm. Hosmani<sup>4</sup> cited that

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