# EFFECT OF FUNGICIDES ON PECTOLYTIC ENZYME ACTIVITY OF FUNGI

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SEVERAL fungitoxic chemicals have been found to inhibit the respiratory enzyme activity of various fungi, thus demobilizing them to become effective parasites.[1] While studying the effect of fungicides and antibiotics on pectolytic enzyme secretion of brown rot fungi, *Sclerotinia fructicola* (Wint.) Rehm. and *S. laxa* Ader. and Ruhl., it was observed that the fungicides, that were effective in inhibiting the secretion of pectolytic enzymes of these fungi *in vitro*, were also effective in checking the brown rot of sour cherries in the field.[2,3] It appeared that fungi which produced pectolytic enzymes and invalidated host by their action could be controlled by the fungicides which checked or reduced the production of such hydrolytic enzymes. In rot-inducing fungi the production of pectolytic enzyme complex is inevitable and the importance of the pectolytic enzymes in the host-parasite interaction has been definitely established.[4] In the present account the effect of five fungicides on the production and activity of pectolytic enzymes secreted by *Sclerotinia sclerotiorum* (Lib.) d. By. and *Botrytis allii* Munn. *in vitro*, and also the effect of these fungitoxic chemicals on rotting of tissues brought about by these fungi has been presented. The production of pectolytic enzymes by *S. sclerotiorum* and *B. allii* has already been demonstrated.[5,6]

As a part of the investigations, the concentrations of fungicides required to inhibit the mycelial growth of the two fungi was determined by poison-food-technique,[2] using Ashour's synthetic agar medium in order to find the $ED_{50}$ values of each fungicide against the two organisms. The fungicides used were: Cycloheximide—Beta-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide; Dodine—*N*-dodecylguanidine acetate; Difolatan—*N*-(1,1,2,2-tetrachloroethylsulfenyl)-*cis*-delta-4-cyclohexene-1, 2-dicarboximide; Nabam—disodium ethylenebis dithiocarbamate; and, Phaltan—*N*-trichloromethyl-thiophthalimide. All the chemicals were of technical grade of purity. For preparation of enzymic solutions, modified Ashour's synthetic medium containing $ED_{50}$ concentrations of each fungicide was used, and a suitable control without fungicide was also maintained. After growth of the two fungi on these media for 5 days at 25-27° C the mycelium was harvested, dried and weighed, and the filtrates examined for enzymic activity. The enzymic activity was determined quantitatively by maceration tests using 5 potato discs ($0{\cdot}5 \times 1{\cdot}0$ mm.) for each treatment.[7] The pH of the filtrates was adjusted to 5·0, as the enzymic activity has been found to be optimum at this pH.[5,6] The enzymic activity is expressed as the ratio between the dry mycelial weight (mg./ml. of the medium) and the time taken (minutes) for macerating the potato discs. All the experiments were repeated at least twice and the results are summarized in Table I.

**TABLE I**

*Pectolytic enzyme activity of the filtrates of* Sclerotinia sclerotiorum *and* Botrytis allii *grown for 5 days on Ashour's synthetic medium containing fungicides at $ED_{50}$ concentration*

| Ashour's medium containing fungicide | *Sclerotinia sclerotiorum* | | *Botrytis allii* | |
|---|---|---|---|---|
| | Fungicide concentration* (μg./ml.) | Enzymic activity | Fungicide concentration* (μg./ml.) | Enzymic activity |
| Cycloheximide .. | 6·5 | 1·24 | 5·0 | 0·89 |
| Difolatan .. | 10·5 | 0·31 | 8·5 | 0·26 |
| Dodine .. | 6·5 | 1·45 | 5·5 | 0·98 |
| Nabam .. | 13·5 | 2·46 | 10·5 | 2·10 |
| Phaltan .. | 12·5 | 0·21 | 9·0 | 0·13 |
| No fungicide .. | .. | 2·82 | .. | 2·24 |

* Average of three replicates tested twice. Fungicide concentration is the $ED_{50}$ value of each fungicide against the respective organism.

In all cases the enzymic activity in the filtrates of *S. sclerotiorum* was more than that of *B. allii*. Very little macerating activity was found when Phaltan or Difolatan were incorporated into the medium as compared with other chemicals. With Nabam, however, the enzymic activity was higher in proportion to its dry mycelial weight. Although the mycelial yields from media containing Phaltan or Difolatan were relatively higher than those containing Cycloheximide, Dodine, or Nabam, yet the enzymic activity was least in the former case, indicating thereby that Phaltan and Difolatan inhibited the production of enzymes more than the other chemicals tested.

It was further observed that in the filtrates of the two fungi grown on normal medium with-

out any fungicides, the high pectolytic activity could be reduced with the addition of Phaltan or Difolatan and not so by other chemicals tested. In other words, Phaltan and Difolatan not only acted upon the two fungi in inhibiting the production of enzymes *in vitro*, but also inactivated or decomposed the enzymes after having been secreted by them. Complete inactivation of the enzymes in the culture filtrates was obtained at relatively higher concentrations of these fungicides.

Tests were also carried out to determine the efficacy of these fungicides on checking the rot production on potato tissues. Potato cylinders cut aseptically with cork-borer of the size of 30 mm. long and 10 mm. in diameter were inoculated with spore suspensions of the two fungi separately and kept in moist chambers at 25–27° C. After 24 hours of inoculations five potato cylinders were sprayed with each of the fungicide at $ED_{50}$ concentration. The time taken for complete rotting of cylinders was noted. In case of *S. sclerotiorum* the potato cylinders were rotted completely after 26, 25, 12, 14, and 8 days of inoculation when sprayed with Phaltan, Difolatan, Dodine, Cycloheximide, and Nabam respectively. In another case when the potato cylinders were first dipped for 30 minutes in the same fungicides and then inoculated with the two fungi, the time taken for complete rotting was 29, 26, 18, 20, and 9 days for Phaltan, Difolatan, Dodine, Cycloheximide, and Nabam treatments respectively. The unsprayed or untreated potato cylinders rotted completely in 5 days. With *B. allii* the time taken for complete rotting of the potato cylinders with the two treatments mentioned above was invariably 2 to 3 days more than that taken by *S. sclerotiorum* in all cases and the untreated tissues rotted in 7 days. In both cases the initiation of rotting of potato tissues was delayed when these were treated or sprayed with either Cycloheximide or Dodine. However, once the rotting was initiated in such treated tissues, the completion was brought about quickly.

On the basis of above results it can be said that fungicides like Phaltan and Difolatan, which are not so toxic like Cycloheximide or Dodine in inhibiting the mycelial growths of the two fungi, are effective in checking the rot development in the tissues by the invasion of these organisms. This may be attributed to the property of these fungitoxic chemicals to inhibit and inactivate the pectolytic enzyme activity of these fungi and thus disturb host-parasite interaction. It is envisaged that this property of various fungitoxic chemicals may be utilizable in controlling rot diseases in the field. The metabolic behaviour of such fungitoxicants to bring about inhibition or reduction of pectolytic enzyme activity still remains to be studied.

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## PLASTIC LASERS

THE Radio Corporation of America (RCA) Laboratories, Princeton, N.J., has announced the development and operation of the first plastic laser. The new laser consists of plastic fibres (each 15 in. long and about 20 times the diameter of a human hair) containing traces of the rare earth europium. To bring about the laser action the fibres are placed in a Dewar flask filled with liquid nitrogen, and exposed to intense flashes of ultra-violet light. The energy from the ultra-violet light is transmitted by the fibres to chelates, which absorb the energy and transfer it to the europium atoms, causing them to emit bright flashes of red light.

The fibres trap most of the light and force it to travel along their length. Each time such a flash occurs, it sweeps along the fibre and stimulates other flashes, all of which combine to create a single pulse of coherent light which bursts from the ends of the fibres with enormous intensity.—(*J. Frank. Inst.*, 1963, 275, 454.)