EFFECTS OF SELECTED HERBICIDES AND PLANT HORMONES ON *PROTOTHECA WICKERHAMII*

PENELOPE A. HENNING AND R. E. LEE

Department of Botany and Microbiology, Witwatersrand University, Johannesburg, South Africa 2001

PROTOTHECA is most probably a colourless derivative of a green alga such as *Chlorella* (Davies, Spencer and Wakelin, 1964; El-Ani, 1967; Migaki, Garner and Imes, 1969). The organism lacks chloroplasts and pyrenoids and has adopted a heterotrophic mode of nutrition (Emmons, Binford and Utz, 1970). It is the cause of the infectious disease protothecosis in mammals (Davies *et al.*, 1964; Klintworth, Fetter and Nielsen, 1968; Frank *et al.*, 1969; van Kruiningen, Garner and Schiefer, 1969; Migaki *et al.*, 1969; Povey *et al.*, 1969; van Kruiningen, 1970). In dogs, abscesses are produced in the viscera with some spread to local lymph nodes (Povey *et al.*, 1969). The disease in cows produces a type of mastitis (Frank *et al.*, 1969; Migaki *et al.*, 1969). In man, protothecosis causes lesions in the skin, but spread to the lymph nodes has been reported (Davies *et al.*, 1964; Davies and Wilkinson, 1967; Klintworth *et al.*, 1968).

To date there is no known cure for protothecosis. Treatment *in vitro* such as irradiation, cytotoxic agents and antiprotozoal drugs show little apparent effect on the organism (Davies and Wilkinson, 1967). Furthermore, this pathogen does not respond to fungal or bacterial antibiotics administered to human patients (Davies *et al.*, 1964; Klintworth *et al.*, 1968; Mars *et al.*, 1971).

The use of herbicides and plant-growth regulators in the control of undesirable plants has increased in recent years. According to the available nitrogen source, it has been found that *P. zopfii* may be inhibited by 3-amino-1,2,4-triazole (Casselton, 1964, 1966 and 1967). In the present study the effect of different types of inhibitor on the growth of *P. wickerhamii* was studied in the hope of recognising a substance that might be of value in the control of protothecosis in mammals.

MATERIALS AND METHODS

The strain of *P. wickerhamii* used was obtained directly from a dermal lesion at the South African Institute for Medical Research (Mars *et al.*, 1971), and was maintained in a nutrient-broth medium consisting of beef extract 0.1% (w/v), yeast extract 0.2% (w/v), peptone 0.5% (w/v) and 10^{-5} M thiamine (Anderson, 1944). The cultures were grown at 32°C with a 12-h light-dark cycle of 2000 Lux light (Epel and Krauss, 1966; Emmons *et al.*, 1970). The herbicides selected for study were: maleic hydrazide (MH); 2,4,5-tri-chlorophenoxyacetic acid (2,4,5-T); 2-chloro-4,6-bis(ethylamino)-S-triazine (simazine); sodium chloroauric acid (gold salt), while the plant hormones used were: indolyl-3-acetic acid (IAA); indolyl-3-butyric acid

Received 11 Mar. 1975; accepted 5 June 1975.

FIG. 1.—Time course curves of *Prototheca wickerhamii* with different herbicides: □—□ = IAA, ○—○ = IBA, △—△ = IPA, ●—● = control.

(IBA); indolyl-3-propionic acid (IPA); naphthylacetic acid (NAA); kinetin; gibberellic acid (GA_3) and coconut milk. All the chemicals were dissolved or diluted in deionised water (Vance and Smith, 1969). They were neutralised with sodium hydroxide, autoclaved and added via sterile syringes to the cell cultures.

Cells of *P. wickerhamii* were grown initially in double-strength nutrient concentration. Addition of stock solution of herbicide to the test-tube gave the selected concentration of herbicide (μg per ml) and the normal nutrient concentration (Vance and Smith, 1969). Control tubes were made up with sterile deionised water. Forty-eight-hour cultures of *P. wickerhamii* were inoculated with herbicides and grown for a further 48 h. The cells were fixed in 2.3% glutaraldehyde and then counted in a hemacytometer.

RESULTS

Using time-course curves, we discovered that there was a delay in the absorption or activity (or both) of the inhibitory chemicals (fig. 1). This delay (d) was used in a formula compounded to calculate the percentage inhibition of *P. wickerhamii*:

$$\text{percentage inhibition} = \frac{(C96 - C48 + d) - (T - C48 + d)}{(C96 - C48 + d)} \times \frac{100}{1}$$

where T = number of herbicide-treated cells present at 96 h, $C96$ = number of cells present in the control at 96 h, and $C48 + d$ = number of cells determined from the control after 48 h plus the delay (d).

Fig. 2 shows the results of experiments with herbicides in the concentration range 100–1000 μg per ml. The percentage inhibition with kinetin, which could be used only in the range 10–100 μg per ml because of its insolubility, and with coconut milk (percentage v/v) is shown in fig. 3. Fig. 3 also shows the trend of inhibition obtained with IAA, IBA and IPA at lower concentrations. Concentrations of MH, 2,4,5-T, NAA, GA_3 and simazine of 2000 μg per ml and 4000 μg per ml were also used to treat the cells.

FIG. 2.—Percentage inhibition of *P. wickerhamii* with herbicides at concentrations of 100–1000 $\mu\text{g/ml}$: $\square$ — $\square$ = IAA, $\circ$ — $\circ$ = IBA, $\triangle$ — $\triangle$ = IPA, ∇ — ∇ = MH, $\bullet$ — $\bullet$ = NAA, $\blacksquare$ — $\blacksquare$ = GA_3 , $\blacktriangle$ — $\blacktriangle$ = 2,4,5-T, $\square$ — $\square$ = simazine, ∇ — ∇ = gold salt.

FIG. 3.—Percentage inhibition of *P. wickerhamii* with herbicides at concentrations of 10–100 $\mu\text{g/ml}$: $\square$ — $\square$ = IAA, $\circ$ — $\circ$ = IBA, $\triangle$ — $\triangle$ = IPA, ∇ — ∇ = kinetin, $\bullet$ — $\bullet$ = coconut milk.

Time-course studies (fig. 1) indicated a levelling off in the increase in cell number after incubation for 10 h with the inhibitory herbicides. Fig. 2 shows that MH, NAA, 2,4,5-T, simazine, gold salt and GA_3 did not affect growth. IAA, IBA and IPA inhibited cell growth with maximum effect at 400 $\mu\text{g per ml}$ (fig. 2). Coconut milk caused stimulation of growth at the 50% (v/v) level, while kinetin had no effect (fig. 3). IAA, IBA and IPA, studied at the lower concentrations, affected cell growth at 60 $\mu\text{g per ml}$, the effect being accentuated with further increase in concentration (fig. 2). The general effect of these three chemicals was a steady increase in inhibition of *P. wickerhamii* from 60 $\mu\text{g per ml}$ to a maximum at 400 $\mu\text{g per ml}$. None of the other chemicals, at the higher concentrations used, produced any effects.

DISCUSSION

Vance and Smith (1969) were unable to inhibit *Chlorella pyrenoidosa* growth with 2,4,5-T and simazine at a concentration of 1000 $\mu\text{g per ml}$.

It is known that IAA is present in human urine, and is a normal metabolic product of tryptophan (Anderson, Shimkin and Leake, 1936; Armstrong and Robinson, 1954; Jepson, 1956). Anderson *et al.* (1936) found the LD50 in

mice given intraperitoneal injections of IAA to be 25 mg per kg, while the LD₅₀ of IBA and IPA was 100 mg per kg. The lower additional requirement of IAA to attain the LD₅₀ concentration may be accounted for by the presence of IAA in mammalian tissues as a product from tryptophan.

Detailed studies of the IAA concentration in human tissues are necessary before it can be ascertained whether cutaneous application of IAA, IBA or IPA will prove effective in the treatment of protothecosis. It is therefore suggested that the use of these compounds to cure this disease merits further investigation.

SUMMARY

Prototheca wickerhamii was treated *in vitro* with 11 different herbicides and plant hormones. Growth was inhibited by indolyl-3-acetic acid, indolyl-3-butyric acid and indolyl-3-propionic acid at 400 µg per ml. Coconut milk was stimulatory.

The authors would like to thank Dr A. R. Rabson of the South African Institute for Medical Research for supplying the culture. We are grateful to Professor C. F. Cresswell for provision of facilities for this work.

REFERENCES

- ANDERSON, E. H. 1944. Nature of the growth factor for the colourless alga *Prototheca zopfii*. *J. gen. Physiol.*, **28**, 287.
- ANDERSON, H. H., SHIMKIN, M. B. AND LEAKE, C. D. 1936. Acute intraperitoneal toxicity of some plant growth substances for mice. *Proc. Soc. exp. Biol. Med.*, **34**, 138.
- ARMSTRONG, M. D. AND ROBINSON, K. S. 1954. On the excretion of indole derivatives in phenylketonuria. *Archs Biochem. Biophys.*, **52**, 287.
- CASSELTON, P. J. 1964. Reversal by histidine of the inhibition of *Prototheca* growth due to 3-amino-1,2,4-triazole. *Nature, Lond.*, **204**, 93.
- CASSELTON, P. J. 1966. Further observations on the inhibition of *Prototheca zopfii* growth by 3-amino-1,2,4-triazole. *Physiol. Plant.*, **19**, 411.
- CASSELTON, P. J. 1967. Influence of the nitrogen source on the adenine reversal of 3-amino-1,2,4-triazole inhibition of *Prototheca zopfii* growth. *Can. J. Microbiol.*, **13**, 1564.
- DAVIES, R. R., SPENCER, H. AND WAKELIN, P. O. 1964. A case of human protothecosis. *Trans. R. Soc. trop. Med. Hyg.*, **58**, 448.
- DAVIES, R. R. AND WILKINSON, J. L. 1967. Human protothecosis: supplementary studies. *Ann. trop. Med. Parasit.*, **61**, 112.
- EL-ANI, A. S. 1967. Life cycle and variation of *Prototheca wickerhamii*. *Science, N.Y.*, **156**, 1501.
- EMMONS, C. W., BINFORD, C. H. AND UTZ, J. P. 1970. Medical mycology, 2nd ed., Philadelphia, p. 457.
- EPEL, B. AND KRAUSS, R. W. 1966. The inhibitory effect of light on growth of *Prototheca zopfii* Krüger. *Biochim. biophys. Acta*, **120**, 73.
- FRANK, N., FERGUSON, L. C., CROSS, R. F. AND REDMAN, D. R. 1969. *Prototheca*, a cause of bovine mastitis. *Am. J. vet. Res.*, **30**, 1785.
- JEPSON, J. B. 1956. Indolylacetyl-glutamine and other indole metabolites in Hartnup disease. *Biochem. J.*, **64**, 14p.
- KLINTWORTH, G. K., FETTER, B. F. AND NIELSEN, H. S. 1968. Protothecosis, an algal infection: report of a case in man. *J. med. Microbiol.*, **1**, 211.

- KRUININGEN, H. J. VAN 1970. Protothecal enterocolitis in a dog. *J. Am. vet. med. Ass.*, **157**, 56.
- KRUININGEN, H. J. VAN, GARNER, F. M. AND SCHIEFER, B. 1969. Protothecosis in a dog. *Pathologia vet.*, **6**, 348.
- MARS, P. W., RABSON, A. R., RIPPEY, J. J. AND AJELLO, L. 1971. Cutaneous protothecosis. *Br. J. Derm.*, **85**, suppl. 7, 76.
- MIGAKI, G., GARNER, F. M. AND IMES, G. D. 1969. Bovine protothecosis. A report of three cases. *Pathologia vet.*, **6**, 444.
- POVEY, R. C., AUSTWICK, P. K. C., PEARSON, H. AND SMITH, K. C. 1969. A case of protothecosis in a dog. *Pathologia vet.*, **6**, 396.
- VANCE, B. D. AND SMITH, D. L. 1969. Effects of five herbicides on three green algae. *Tex. J. Sci.*, **20**, 329.