Annulment of Sterol-Induced Sexual Reproduction by Estradiol in Pythium periplocum

Abstract. *Estradiol prevented cholesterol-induced sexual reproduction by the fungus* Pythium periplocum. *Inhibition by estradiol was partial at* 10^{-6}M *and complete at* 10^{-5}M, *the same concentrations at which cholesterol and β-sitosterol were initially and maximally active. Higher concentrations of estradiol were required for growth inhibition than for inhibition of reproduction.*

The requirement for exogenous 3β-hydroxy sterols for sexual or zoosporangial reproduction by fungi of the plant pathogenic family Pythiaceae has been established (*1*). The exogenous sterol requirement is apparently due to the inability of these fungi to synthesize sterols (*2*). In studying the relationship between steroid structure and activity in the induction of sexual reproduction, we noticed that estradiol ($\Delta^{1,3,5(10)}$-estriene-3,17β-diol) failed to support reproduction when given as the sole steroid in the medium, and also prevented reproduction of *Pythium periplocum* in a medium containing cholesterol.

We used the same isolate of *P. periplocum* that we used when we first reported sterol-induced reproduction of pythiaceous fungi (*3*) and in subsequent studies (*4*). The agar and methods were similar to those used previously (*4*), except that the medium contained only 0.8 g of glucose and 0.3 g of $NaNO_3$, per liter. Steriods were introduced into the autoclaved medium as dimethylformamide (DMF) solutions by placing the tips of long-tipped measuring pipets into the medium as it was being rapidly stirred. Since DMF is miscible with water, the steroids remained in solution at low concentrations and were suspended as fine colloids at concentrations exceeding their solubility in water. After inoculation, plates were incubated in paper bags at 20° or 25°C. They were exposed to light for 10 minutes after 2 or 3 days for a linear measurement of growth from the inoculation point. Oospores were counted 16 days after inoculation. All oospores in all focal planes at a magnification of 150 × (field area, 1.84 mm²) about 1 cm from the inoculation point were counted.

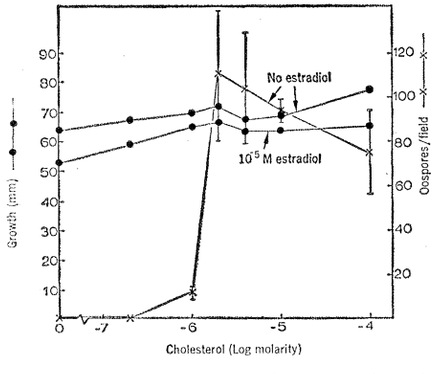

Fig. 1. Effect of cholesterol on growth and reproduction of *Pythium periplocum* in the presence and absence of estradiol. Values are means of data from four or five plates. Least significant difference at 95 percent confidence level for growth data is 3.1 mm; least significant difference at 99 percent confidence level is 4.2 mm. Brackets for reproduction data enclose standard errors. No reproduction occurred in the presence of $10^{-5}M$ estradiol at any concentration of cholesterol tested.

Oospore formation was induced by $10^{-6}M$ cholesterol, and maximum induction activity was observed consistently with $10^{-5}M$ (Fig. 1). The response to β-sitosterol was similar. Sexual reproduction was prevented by $10^{-5}M$ estradiol even at cholesterol concentrations up to tenfold that required for maximum oospore production (Fig. 1). Sexual reproduction was reduced by $10^{-6}M$ estradiol and was almost eliminated at $3.3 \times 10^{-6}M$ (Fig. 2).

Estradiol inhibition of reproduction could be reversed to a limited extent. Reproduction was almost eliminated by $2.5 \times 10^{-6}M$ estradiol in media containing $10^{-5}M$ β-sitosterol, but reproduction at $10^{-4}M$ β-sitosterol was 25 percent that of the estradiol-free control. Likewise, at $10^{-6}M$ estradiol, reproduction was greater in the presence of $2.5 \times 10^{-5}M$ cholesterol than at $2.5 \times 10^{-6}M$ cholesterol (Fig. 2). We have never observed reproduction at any sterol concentration when estradiol concentrations were $10^{-5}M$ or higher.

Growth was not affected in the same manner as reproduction. Much higher concentrations of estradiol were required to reduce growth significantly than were required to prevent reproduction (Fig. 2). While $10^{-5}M$ estradiol reduced growth by 10 to 20 percent, stimulation by cholesterol still occurred, even at cholesterol concentrations too low to support reproduction (Fig. 1). Thus the inhibition of reproduction cannot be credited to growth inhibition.

The mechanism by which sterols induce reproduction in pythiaceous fungi is not known. The prevention of sterol-induced reproduction in *P. periplocum* by polyene antibiotics (*4*) suggests a membrane or permeability role, since polyene antibiotics interfere with a membrane function of sterols (*5*).

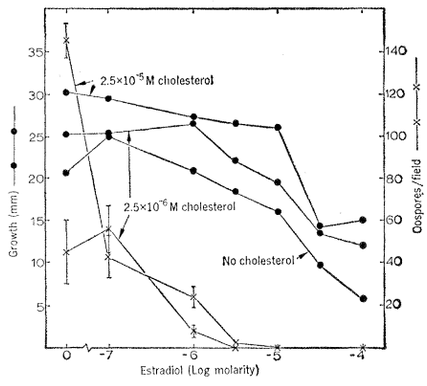

Fig. 2. Effect of estradiol concentration on growth and reproduction of *Pythium periplocum* in the presence and absence of cholesterol. Values are means of four or five plates. Least significant difference at 95 percent confidence level for growth data is 2.1 mm; least significant difference at 99 percent confidence level is 2.8 mm. Brackets for reproduction data enclose standard errors.

Whether or not permeability is involved, it may be significant that estradiol is polar at both ends of the molecule, whereas sterols active in inducing reproduction have a nonpolar hydrocarbon chain at the 17-position rather than a hydroxyl group.

Although numerous steroid effects on growth and metabolism of microorganisms have been described (*6*), this and the early report by Plumb and Durrell (*7*) that estrogen inhibits zygospore formation by *Rhizopus nigricans* appear to be the only reports of estrogen inhibition of microbial reproduction not involving growth. This phenomenon may be analogous to mammalian sterility caused by large doses of estrogen (*8*).

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References and Notes

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