Bot. Bull. Acad. Sin. (1998) **39:** 81–86

(*Invited review paper*)

Chemical stimulation of sexual reproduction in *Phytophthora* and *Pythium*

Wen-Hsiung Ko

Department of Plant Pathology, Beaumont Agricultural Research Center, University of Hawaii at Manoa, Hilo, Hawaii 96720, U. S. A.

Keywords: Fatty acids; Oospore formation; Phospholipids; *Phytophthora*; Pythiaceous fungi; *Pythium*; Sexual reproduction; Sterols; Terpenoids; Vitamin A.

Contents

Introduction

The genera of *Phytophthora* and *Pythium* are two of the very destructive groups of plant pathogens in the world (Plaats-Niterink, 1981; Erwin and Ribeiro, 1996). They attack mainly mature plants and young seedlings, respectively, of forest, fruit, vegetable, flower, and ornamental species. These two groups of fungi are classified in the family Pythiaceae of the Oomycetes, which is excluded from the traditional "true fungi" of the kingdom Myceteae and included along with brown algae in the kingdom chromista according to the newer classification (Erwin and Ribeiro, 1996). One of the main reasons for such separation is that the major part of their life history is diploid whereas the other fungi are haploid.

Sexual reproduction in *Phytophthora* and *Pythium* results in production of thick-walled oospores each in an oogonium attached by single or multiple antheridia. The process is very important in the life cycle of these fungi because it provides not only a means of propagation and survival in nature but also a potential source of genetic variation. Some species of *Phytophthora* are homothallic and are capable of producing oospores by single isolate while others are heterothallic and require the presence of opposite mating types, known as A1 and A2, for sexual reproduction. The sexual behavior of heterothallic species of *Phytophthora* is different from all other known organisms in that sexual reproduction readily occurs even between morphologically and physiologically distinct species (Savage et al., 1968; Ko, 1980). Extensive research eventually revealed that the opposite mating type is needed for the production of a mating-type specific hormone to initiate sexual reproduction by selfing (Ko, 1978). Subsequently it was also found that, in *Phytophthora*, species are homothallic because they carry receptor(s) for hormone(s) produced by themselves (Ko, 1980a). In the interspecific crosses in *Phytophthora*, all the progeny are resulted from selfing induced by hormones (Chang and Ko, 1993), while in the intraspecific crosses both selfed and hybrid progeny are produced (Chang and Ko, 1990). Such hormonal beterothallism and homothallism represent a novel mode of sexual reproduction in the biological world (Ko, 1988). Recent evidence suggests that a similar phenomenon may also exist in *Pythium* (Guo and Ko, 1991). This review focuses on the nutritional requirement for sexual reproduction in *Phytophthora* and *Pythium*. These chemicals may be needed for hormone production and/or oospore formation after hormonal induction.

Discovery of Sterol Requirement for Sexual Reproduction in Pythiaceous Fungi

As early as 1937, Leonian and Lilly found that many species of *Phytophthora* and *Pythium* were not able to produce oospores on a defined medium consisting of essential salts and glucose unless an extract from peas was added (Leonian and Lilly, 1937). The stimulatory activity was also found in other seeds and various kinds of oils from plants and animals (Haskins et al., 1964; Klemmer and Lenney, 1965). In 1964, scientists from several institutes independently reported that sterols are required for sexual reproduction in *Phytophthora* (Harnish et al., 1964; Elliott et al., 1964; Hendrix, 1964; Leal et al., 1964) and *Pythium* (Haskin et al., 1964; Hendrix, 1964). Their observations have since been confirmed and expanded by researchers from other laboratories (Hendrix, 1970; Elliott, 1983). Although a number of species of *Phytophthora* and *Pythium* tested were able to produce oospores on basal medium supplemented with cholesterol or β-sitosterol (Tables 1 and 2), in both genera several species tested failed to produce oospores in the presence of sterols (Table 3).

Different species appear to have different sterol requirements. Wilkinson and Millar (1981) reported the production of oospores by *Phytophthora megasperma* on synthetic medium supplemented with stigmasterol but not with cholesterol or β-sitosterol. However, for *Phytophthora cactorum* cholesterol, β-sitosterol and stigmasterol were all very effective in supporting oospore formation (Elliott, 1972). There are some indications that even among isolates of the same species, the ability to produce oospores in the presence of sterols may differ markedly. Among 10 isolates of *Phytophthora fragariae* tested, only three isolates were able to produce oospores on bean meal agar, and only one isolate showed an increase in oosproe production with β-sitosterol (Maas, 1972). However, it is not known if this isolate will produce oospores on basal medium amended with β-sitosterol. Response of sexual reproduction to sterols in a chemically defined medium among isolates of the same species remains to be investigated.

Nutrient contaminants in agar or carried over from the inoculum have a strong effect on the activity of sterols. Addition of 1.5% Bacto agar to liquid basal media containing β-sitosterol or cholesterol increased production of oospores by *P. cactorum* more than 7 and 11 fold, respectively (Ko and Ho, 1983). When highly purified SeaKem HGT-P agarose (Ho and Ko, 1980) was used to solidify the liquid media, the increased activity of sterols disappeared (Ko and Ho, 1983). A number of pythiaceous species reported to show sterol stimulation of sexual reproduction (Hunter et al., 1965; Hendrix, 1965) needs to be reevaluated because it was based on tests with agar media. To prevent the carry-over of nutrient contaminants, the inoculum should be obtained from culture grown on a basal agarose medium (Ko, 1985; 1986; Jee et al., 1997).

Table 1. Species of *Phytophthora* reported to have a sterol requirement for sexual reproduction.

Species	Reference
Homothallic	
P. cactorum	Leal et al., 1964; Elliott et al., 1964; Harnish et al., 1964
P. heveae	Leal et al., 1964; Harnish et al., 1964
P. sojae	Hendrix, 1964; Harnish et al., 1964
P. boehmeriae	Harnish et al., 1964
P. erythroseptica	Harnish et al., 1964
P. megasperma	Hunter et al., 1965
P. citricola	Hunter et al., 1965
P. ilicis	Hunter et al., 1965
P. porri	Hunter et al., 1965
Heterothallic (A1 + A2)	
P. capsici	Harnish et al., 1964
P. drechsleri	Harnish et al., 1964
P. palmivora	Harnish et al., 1964
P. parasitica	Harnish et al., 1964
P. cambivora	Leal et al., 1967
P. cinnamomi	Leal et al., 1967

Table 2. Species of *Pythium* reported to have a sterol requirement for sexual reproduction.

Species	Reference
Homothallic	
Py. periplocum	Hendrix, 1964
Py. acanthicum	Haskins et al., 1964; Child and Haskins, 1976
Py. ultimum	Kerwin and Duddles, 1989
Py. aphanidermatum	Hendrix, 1965
Py. paroecandrum	Hendrix, 1965
Py. spinosum	Hendrix, 1965
Py. vexans	Ko, 1965
Py. catenulatum	Child and Haskins, 1991
Py. sylvaticum	Child and Haskins, 1971
Heterothallic (paired)	
Py. catenulatum	Child and Haskins, 1971
Py. sylvaticum	Child and Haskins, 1971

Table 3. Species of *Phytophthora* and *Pythium* with sexual reproduction non-responsive to sterols.

Species	Reference
Phytophthora	
Homothallic	
P. fragariae	Harnish et al., 1964
P. colocasiae	Harnish et al., 1964
P. hibernalis	Harnish et al., 1964; Leal et al., 1967
P. syringae	Hunter et al., 1965; Leal et al., 1967
Heterothallic (A1 + A2)	
P. cinnamomi	Hunter et al., 1965
P. cryptogea	Hunter et al., 1965
P. parasitica	Ko and Ho, 1983
P. capsici	Ko, 1985
Pythium	
Homothallic	
Py. polytylum	Hendrix, 1965
Py. proliferum	Hendrix, 1965

Reevaluation of Essentiality of Sterols for Sexual Reproduction

Since the discovery of the stimulatory effect of sterols on oospore formation of *Phytophthora* and *Pythium*, these compounds have been considered essential for the production of sexual propagules. Moreover, the alleged essentiality of sterols for sexual reproduction in pythiaceous fungi has been frequently cited as factual (Smith and Berry, 1974; Barnett, 1976; Webster, 1980). Subsequently, Ko and Ho (1983) found that sterols were stimulatory to oospore formation of *P. cactorum* but not *Phytopthora parasitica* (A1 + A2), while phosphatidylcholin (lecithin) was stimulatory to the sexual reproduction of both species. According to the criteria of essentiality, an essential substance can not be replaced by any other substance (Bidwell, 1974; Naggle and Fritrz, 1976). It was, therefore, concluded that sterols are stimulatory to, but not essential for, sexual reproduction of *P. cactorum*. At that time it was not known whether lecithins are essential to *P. parasitica* as other chemicals were not tested. Chemical stimulation of sexual reproduction of another heterothallic species of *Phytophthora*, *P. capsici* (A1 + A2), was similar to that of *P. parasitica*. The process was stimulated by lecithins but not sterols (Ko, 1985). In the genus *Pythium*, sterols were also found to be stimulatory to, but not essential for, sexual reproduction in *Pythium aphanidermatum* because, in addition to sterols, lecithin, phosphatidylethanolamine (cephalin), and certain glycerides tested (such as dipalmitin and trilinolein) were also effective in supporting oospore formation (Ko, 1985; 1986). Another species of *Pythium* tested, *Pythium vexans,* behaved quite differently. It produced oospores in the presence of sterols but not lecithins (Ko, 1985). Whether sterols are essential for sexual reproduction in this fungus remains to be tested.

Nes (1987), and Kerwin and Duddles (1989) suggested that the stimulatory effect of phospholipids on sexual reproduction in pythiaceous fungi was caused by sterol contamination. However, sterols alone did not stimulate sexual reproduction in *P. parasitica* and *P. capsici*, but these fungi were stimulated by lecithin (Ko and Ho, 1983; Ko, 1985). Even when sterol-responsive *P. cactorum* was used as the test organism, sterol contamination still could not account for the stimulatory effect of lecithin. Assuming that sterols constitute the 1% of impurities in the natural lecithin used in these experiments, it has been estimated that these compounds would account for less than 2% of oospores produced in the presence of lecithin (Ko, 1995). Moreover, our recent GC-MS analysis of phospholipids failed to detect the presence of sterol contaminants, and further purification of phospholipids by column chromatography increased rather than decreased their activity (Jee et al., 1997). When cholesterol, at the concentration detected by Kerwin and Duddles (1989) in their phospholipid sample was added to basal medium containing purified phospholipid, the numbers of oospores produced by *P. cactorum* did not significantly increase or decrease (Jee et al., 1997). Therefore, sterols did not play any significant role in the stimulation of sexual reproduction in *P. cactorum* in these experiments.

Presence of Inhibitory Substances in Highly Purified Commercial Products

Kerwin and Duddles (1989) and Nes (1988) reported that the ability of lecithin to induce oospore formation in *P. cactorum* diminished after passage through aminopropyl or alumina columns, respectively, and assumed that such an effect was due to removal of sterol contaminants. However, when we used an aminoproyl column to remove neutral lipids such as sterols and fatty acids from the highly purified commercial phospholipids, instead of reduction in activity these compounds became more stimulatory to sexual reproduction of *P. cactorum* compared to untreated control (Jee et al., 1997). The activities of lecithin (99% pure) and cephalin (98% pure) were increased 47 and 2.8 fold, respectively, by this treatment.

When highly purified commercial phospholipids were developed with a solvent system of acetone-chloroform-acetic acid-water on a thin layer chromatograph (TLC) plate, the major spot on TLC was R_F 0.2 for lecithin and R_F 0.35 for cephalin as indicated by the iodine stain. There was also a faint stain of unknown component at the origin in each case. When the unknown component was isolated from the TLC plate and added to the basal medium supplemented with purified lecithin or cephalin, growth of *P. cactorum* was completely inhibited (Jee et al., 1997). Addition of the unknown component to the phospholipid fraction of lecithin and cephalin caused a 50% and 100% inhibition of oospore production by *P. cactorum*, respectively. The 99% pure commercial lecithin from one of the shipments was partially inhibitory to the growth of *P. cactorum* on basal medium and was not stimulatory to oospore formation of the fungus. When it was dissolved in ether and then washed with deionized water or NaCl solution, the inhibitory effect disappeared, and the compound became strongly stimulatory to oospore formation of *P. cactorum*.

It is considered possible that the decrease of the stimulatory effect of lecithin after purification by column chromatography as reported by Kerwin and Duddles (1989) and Nes (1988) is due to the introduction of inhibitory substances during column treatment. The nature of the inhibitory substances present in the commercial products remains to be investigated. The inhibitors appear to be very polar. They could be the by-products formed during manufacture or could result from degradation or oxidation of the products.

Stimulation of Sexual Reproduction by Fatty Acids and Related Compounds after Removal of the Inhibitory Substances

The discovery of substances inhibitory to oospore formation of *P. cactorum* in highly purified commercial lecithin and cephalin suggests that there may be other chemicals stimulatory to sexual reproduction of *Phytophthora* and *Pythium* if they are purified just before the biosassay. A number of fatty acids and related compounds were, therefore, tested. We were pleasantly surprised to find that eight out of nine tested fatty acids stimulated oospore formation in *P. cactorum* after purification by TLC (Jee and Ko, 1997). From 243 to 10,250 oospores per 12 mm-disc of basal medium containing 100 mg of the fatty acid were produced. Among the fatty acids tested, palmitoleic acid was the most stimulatory to oospore formation followed by oleic acid, palmitic acid, and linoleic acid. Lauric acid, myristic acid, stearic acid, and linoleic acid were moderately stimulatory. Only arachidonic acid was not stimulatory. Without prepurification none of the tested fatty acids was stimulatory to oospore formation. Purified palmitoleic acid also stimulated *P. parasitica* to produce 2,700 oospores per basal medium disc containing 100 mg of the test chemical. The other purified fatty acids had no effect on oospore formation of *P. parasitica*.

Three hydrocarbons and five derivatives of hydrocarbons tested become slightly stimulatory to oospore formation in *P. cactorum* after purification by TLC (Jee and Ko, 1997). With or without TLC purification these compounds did not stimulate oospore formation of *P. parasitica*. After TLC purification, geranial and squalene were stimulatory to oospore formation of *P. cactorum* but not *P. parasitica*, while phytol, retinol (vitamin A) and vitamin A esters were stimulatory to oospore formation by both fungi.

The presence of inhibitory substances in commercially available fatty acids and terpenoids may explain the negative results previously reported on the effect of squalene (Elliott et al., 1964; Harnish, 1968; Nes et al., 1982), and certain fatty acids (Harnish, 1968; Ko, 1985) on sexual reproduction in *P. cactorum*. The reported inhibitory effect of linoleic acid on *Py. aphanidermatum* (Ko, 1986), and that of palmitic acid and oleic acid on *P. cactorum* (Ness, 1988), likewise could be caused by contaminants rather than the fatty acids themselves. The presence of inhibitory substances in highly purified commercial chemical products coupled with the unique ability of cholesterol and β-sitosterol even without purification to stimulate oospore formation by *P. cactorum* (Jee et al., 1997) may explain the early discovery of sterols and the near two-decade delay in the finding of other substances stimulatory to sexual reproduction in pythiaceous fungi. It is considered possible that more physiological activities may be found if the test chemicals are purified just before bioassay.

Do Pythiaceous Fungi Really Need an Exogenous Stimulatory Substance for Sexual Reproduction?

Prior to our discovery of the stimulatory effect of nonsterol substances on oospore formation, failure of pythiaceous fungi to produce sexual progeny on basal medium was believed to be due to their inability to synthesize the substances required for sexual reproduction (Elliott, 1983; Hendrix, 1970; Nes, 1987). Although incapable of synthesizing sterols, pythiaceous fungi are known to be able to synthesize various types of terpenoids, fatty acids, phospholipids, and other lipid compounds (Losel, 1988). *Phytophthora cinnamomi* has been shown to be capable of synthesizing geraniol and squalene from acetate (Wood and Gottlieb, 1978a; 1978b). The synthesis of phospholipids including lecithin and cephalin by *P. parasitica* and of fatty acids by *P. cactorum* have been reported by Hendrix and Rouser (1976) and Nes (1988), respectively. *Pythium ultimum* has also been reported to synthesize lecithin, cephalin, and fatty acids (Bowman and Mumma, 1967). Since these compounds are all stimulatory to the sexual reproduction of *P. cactorum* (Jee and Ko, 1997), it was considered possible that pythiaceous fungi are capable of synthesizing substances needed for their own sexual reproduction and that a certain stress factor is needed to trigger the process of sexual reproduction in these fungi growing on basal medium.

We, therefore, grew *P. cactorum* in liquid basal medium and obtained extracts from mycelium, culture filtrate, and basal medium separately. Although extracts from culture filtrate and basal medium were not stimulatory, the extract from mycelium was highly stimulatory to oospore formation of *P. cactorum* and *P. parasitica* (Jee and Ko, unpublished data). When mycelium of *P. cactorum* produced in liquid basal medium was transferred to nutrient-free water agarose, oospores were produced. The fungus also produced oospores on solid basal medium after a prolonged incubation period (Jee and Ko, unpublished data). These results show that the hypothesis is well founded. Contrary to the common belief, *P. cactorum* is capable of synthesizing substances needed for its sexual reproduction. However, a stress factor such as nutrient deprivation or aging is required to trigger the sexual process. This may explain why oospore formation by pythiaceous fungi on basal medium had not been reported previously.

Perspective

The discovery of stimulation of sexual reproduction in homothallic *P. cactorum* and heterothallic *P. parasitica* by non-sterol substances and the new revelation of *P. cactorum's* ability to synthesize substances needed for sexual reproduction broaden the research scope of the physiology of sexual reproduction in pythiaceous fungi. The exogenous stimulatory substances may serve as a signal, just like the stress factors, for initiation of the sexual reproduction process. If this is the case, addition of these substances should only shorten the time for oospore production and not change the amount of oospores produced on basal medium. On the other hand, these substances may serve as nutrients needed for the sexual reproduction process per se. In this case, adding these substances should increase the amount of oospores produced on basal medium. Each substance should then be tested to determine if it is needed for hormone production and/or oospore formation after hormone induction. Because of the disclosure of the presence of inhibitory substances in highly purified commercial chemical products and the availability of methods for their removal, numerous compounds await exploration for their physiological activities in pythiacious fungi and possibly other organisms as well.

Literature Cited

Barnett, J.H. 1976. Fundamentals of Mycology. Crane Russak & Co., New York.

Bidwell, R.G.S. 1974. Plant Physiology. Macmillan Publishing Co, New York.

Bowman, R.D. and R.O. Mumma. 1967. The lipids of *Pythium ultimum*. Biochem. Biophys. Acta **144:** 501–510.

Chang, T.T. and W.H. Ko. 1990. Resistance to fungicides and antibiotics in *Phytophthora parasitica*: Genetic nature and use in hybrid determination. Phytopathology **80:** 1414–1421.

Chang, T.T. and W.H. Ko. 1993. Evidence for absence of hybridization in crosses between *Phytophthora infestans* and *Phytophthora parasitica*. Mycol. Res. **97:** 675–678.

Child, J.J. and R.H. Haskins. 1971. Induction of sexuality in heterothallic *Pythium* spp. by cholesterol. Can. J. Bot. **49:** 329–332.

Child, J.J. and R.H. Haskins. 1976. Effect of inhibitors of sterol biosynthesis on growth and sexuality of *Pythium* and *Zygorhynchus*. Mycopathologia **59:** 91–93.

Elliott, C.G. 1972. Sterols and the production of oospores by *Phytophthora cactorum*. J. Gen. Microbiol. **72:** 321–327.

Elliott, C.G. 1983. Physiology of sexual reproduction in *Phytophthora*. *In* D.C. Erwin, S. Bartnicki-Garcia, and P.H. Tsao (eds.), Phytophthora, Its Biology, Taxonomy, Mycology, and Pathology, American Phytopathological Society, St. Paul, Minnesota, pp. 71–80.

Elliott, C.G., M.E. Hendrie, B.A. Knights, and W. Parker. 1964. A sterol growth factor requirement in a fungus. Nature **203:** 427–428.

Erwin, D.C. and O.K. Ribeiro. 1996. *Phytophthora* Diseases Worldwide. APS Press, St. Paul, Minnesota.

Guo, L.Y. and W.H. Ko. 1991. Hormonal regulation of sexual reproduction and mating type change in heterothallic *Pythium splendens.* Mycol. Res. **95:** 452–456.

Harnish, W.N. 1968. Effect of sterols in sexual reproduction of *Phytophthora cactorum*. Proc. W. Va. Acad. Sci. **40:** 99–104.

Harnish, W.H., L.A. Berg, and V.G. Lilly. 1964. Factors in lima bean and hemp seed required for oospore formation by species of *Phytophthora*. Phytopathology **54:** 895.

Haskins, R.H., A.P. Tulloch, and R.G. Micetich. 1964. Steroids and the stimulation of sexual reproduction of a species of *Pythium*. Can. J. Microbiol. **10:** 187–195.

Hendrix, J.W. 1964. Sterol induction of reproduction and stimulation of growth of *Pythium* and *Phytophthora*. Science **144:** 1028–1029.

Hendrix, J.W. 1970. Sterols in growth and reproduction of fungi. Annu. Rev. Phytopathol. **8:** 111–130.

Hendrix, J.W. and G. Rouser. 1976. Polar lipids of *Phytophthora parasitica* var. *nicotianae*, in comparison with those of selected other fungi. Mycologia **68:** 354–361.

Ho, W.H. and W.H. Ko. 1980. Agarose medium for bioassay of antimicrobiol subtances. Phytopathology **70:** 764–766.

Hunter, J.L., L.A. Berg, W.N. Harnish, and W.G. Merz. 1965. The nutritional requirements for sexual reproduction in some species of *Phytophthora*. W. Virg. Acad. Sci. **37:** 75–82.

Jee, H.J. and W.H. Ko. 1997. Stimulation of sexual reproduction of *Phytophthora cactorum* and *P. parasitica* by fatty acids and related compounds. Mycol. Res. **101:** 1140–1144.

Jee, H.J., C.S. Tang, and W.H. Ko. 1997. Stimulation of sexual reproduction in *Phytophthora cactorum* by phospholipids is not due to sterol contamination. Microbiology **143:** 1631–1638.

Kerwin, J.L. and N.D. Duddles. 1989. Reassessment of the role of phospholipids in sexual reproduction by sterol-auxotrophic fungi. J. Bacteriol. **171:** 3831–3839.

Klemmer, H.W. and J.F. Lenney. 1965. Lipids stimulating sexual reproduction and growth in pythiaceous fungi. Phytopathology **55:** 320–323.

Ko, W.H. 1978. Heterothallic *Phytophthora*: evidence for hormonal regulation of sexual reproduction. J. Gen. Microbiol. **107:** 15–18.

Ko, W.H. 1980a. Hormonal regulation of sexual reproduction in *Phytophthora*. J. Gen. Microbiol. **116:** 459–463.

Ko, W.H. 1980b. Sexuality, evolution and origin of *Phytophthora*. Plant Prot. Bull. (Taiwan) **22:** 141–151.

Ko, W.H. 1985. Stimulation of sexual reproduction of *Phytophthora cactorum* by phospholipids. J. Gen. Microbiol. **131:** 2591–2594.

Ko, W.H. 1986. Sexual reproduction of *Pythium aphanidermatum*: stimulation by phospholipids. Phytopathology **76:** 1159–1160.

Ko, W.H. 1988. Hormonal heterothallism and homothallism in *Phytophthora*. Annu. Rev. Phytopathol. **26:** 57–73.

Ko, W.H. and W.C. Ho. 1983. Reassessment of apparent sterol requirement for sexual reproduction in *Phytophthora*. Ann. Phytopathol. Soc. Japan **49:** 316–321.

Leal, J.A., J. Friend, and P. Holliday. 1964. A factor controlling sexual reproduction in *Phytophthora*. Nature **203:** 545–546.

Leal, J.A., M.E. Galleghy, and V.G. Lilly. 1967. The relation of the carbon-nitrogen ratio in the basal medium to sexual reproduction in species of *Phytophthora*. Mycologia **59:** 953–964.

Leonian, L.H. And V.G. Lilly. 1937. Partial purification of a vitamin-like substance which stimulates sexual reproduction in certain fungi. Am. J. Bot. **24:** 700–702.

Losel, D.M. 1988. Fungal lipids. *In* C. Ratledge and S.G. Wilkinson (eds.), Microbial Lipids. vol. 1, Academic Press, London.

Maas, J.L. 1972. Growth and reproduction in culture of ten *Phytophthora fragariae* races. Mycopathol. Mycol. Appl. **48:** 323–334.

Naggle, G.R. and G.J. Fritz. 1976. Introductory Plant Physiology. Prentice-Hall, Englewood Cliffs.

Nes, W.D. 1987. Biosynthesis and requirement for sterols in the growth and reproduction of Oomycetes. *In* G. Fuller and W.D. Nes (eds.), Ecology and Metabolism of Plant Lipids, American Chemical Society, Washington D.C., pp. 304–328.

Nes, W.D. 1988. Phytophthorols-novel lipids produced by

Phytophthora cactorum. Lipids **23:** 9–16.

Nes, W.D., G.A. Saunders, and E. Heftmann. 1982. Role of steroids and triterpenoids in the growth and reproduction of *Phytophthora cactorum*. Lipids **7:** 178–183.

Plaats-Niterink, A. J. van der. 1981. Monograph of the genus *Pythium*. Stud. Mycol. **21:** 1–242.

Savage, E.J., C.W. Clayton, J.H. Hunter, J.A. Brenneman, C Laviola, and M.E. Gallegly. 1968. Homothallism, heterothallism and interspecific hybridization in the genus *Phytophthora*. Phytopathology **58:** 1004–1021.

Smith, J.A. and D.R. Berry. 1974. An Introduction to Biochemistry of Fungal Development. Academic Press. New York.

Webster, J. 1980. Introduction to Fungi. Cambridge University Press, London.

Wilkinson, H.T. and R.L. Millar. 1981. Reproduction and growth of *Phytophthora megasperma* var. *megasperma* on a defined medium. Phytopathology **71:** 265.

Wood, S.G. and D. Gottlieb. 1978a. Evidence from mycelial studies for differences in the sterol biosynthetic pathway of *Rhizoctonia solani* and *Phytophthora cinnamomi*. Biochem. J. **170:** 343–354.

Wood, S.G. and D. Gottlieb. 1978b. Evidence from cell-free systems for differences in the sterol biosynthetic pathway of *Rhizoctonia solani* and *Phytophthora cinnamomi*. Biochem. J. **170:** 355–363.